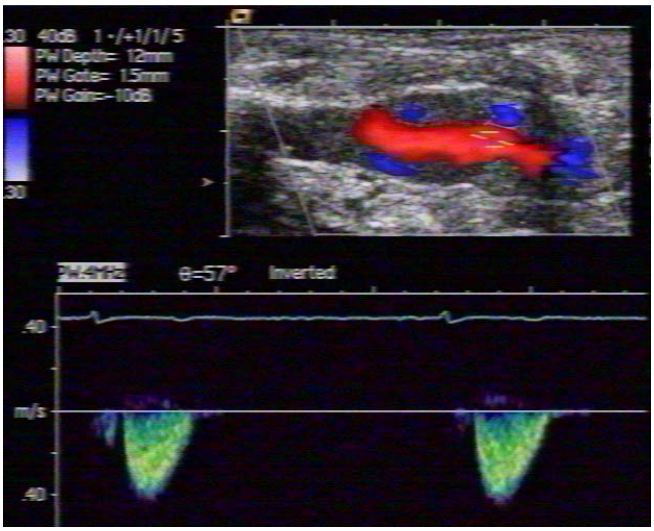
Cardiovascular Physiology Of Fish

SYMPOSIUM PROCEEDINGS



Kurt Gamperl
Tony Farrell
Don M[ac]Kinlay

International Congress on the Biology of Fish
University of British Columbia, Vancouver, CANADA

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International Standard Book Number (ISBN) 1-894337-26-3

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PREFACE

In adult fishes, as in other vertebrates, the cardiovascular system is critical to performance and survival because it ensures adequate oxygen and nutrient delivery to the tissues, and removes carbon dioxide and other metabolic wastes. Fish cardiovascular morphology/physiology has been an extremely active area of research over the past two decades, and our understanding of this organ system's design, function, control and importance continues to grow as new research tools become available, and novel or integrative research approaches are utilized. This symposium brought together an international group of scientists who presented data and actively participated in discussions on wide range of topics. These topics ranged from molecular evolution of cardiac proteins, through the regulation of cardiac, arterial and venous function, to effects of catch-and-release angling and mode of life on cardiac function. Issues such as the anoxic myocardium, the influences of beta-adrenergic stimulation, angiotensin II, steroids and adenosine on the cardiovascular system, and hemodynamic assessments of cerebral blood flow and ventricular dynamics were covered. The range of fish species covered was equally broad, and included hagfish and tuna, icefish and desert fish, dogfish and toadfish, lungfish and neotropical fishes, and of course rainbow trout, eels, cod and flounder. The technical approaches were remarkably diverse, and included mutant proteins, patch-clamp studies on isolated cells, isolated tissues and organs, *in vivo* studies and non-invasive measurements. This symposium truly reflected the diversity, ingenuity and excitement of the researchers in this fast growing field.

You may have missed a wonderful symposium, but you can enjoy these abstracts and plan on coming to the next symposium in 2004.

Symposium Organizers:

Kurt Gamperl, Memorial University, St. Johns, Newfoundland, Canada

Tony Farrell, Simon Fraser University, Burnaby, B.C., Canada

Don MacKinlay, Fisheries & Oceans Canada, Vancouver, Canada

CONGRESS ACKNOWLEDGEMENTS

This Symposium is part of the International Congress on the Biology of Fish, held at the University of British Columbia in Vancouver B.C., Canada on July 22-25, 2002.

The sponsors included:

- Fisheries and Oceans Canada (DFO)
- US Department of Agriculture
- US Geological Service
- University of British Columbia Fisheries Centre
- National Research Council Institute for Marine Biosciences
- Vancouver Aquarium Marine Science Centre

The main organizers of the Congress, on behalf of the Physiology Section of the American Fisheries Society, were Don MacKinlay of DFO (overall chair, local arrangements, program and proceedings) and Rosemary Pura of UBC Conferences and Accommodation (facility arrangements, registration and housing). Thanks to Karin Howard for assistance with Proceedings editing and word-processing; to Anne Martin for assistance with the web pages; and to Cammi MacKinlay for assistance with social events.

I would like to extend a sincere 'thank you' to the many organizers and contributors who took the time to prepare a written submission for these proceedings. Your efforts are very much appreciated.

Don MacKinlay
Congress Chair

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REGULATION OF BRAIN TEMPERATURE IN YELLOWFIN TUNA:

EVIDENCE OF ALTERATIONS IN BLOOD FLOW

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Introduction

Tunas are regional endotherms, maintaining elevated temperatures in deep red swimming muscles by way of vascular counter-current heat exchangers (*retia mirabilia*) that trap metabolically produced heat (Carey and Teal, 1966). Many tunas also warm the viscera and brain with associated *retia*. These *retia* allow the tissues to be kept at temperatures higher than the surrounding water, enhancing metabolic capacity, but also act as effective insulators, slowing the rate of heat loss during forays into cooler waters. The yellowfin tuna *Thunnus albacares* is a tropical-subtropical species inhabiting the surface waters, but periodically makes rapid "dives" through the thermocline in search of prey, encountering water temperatures as much as 10°C cooler (Holland et al., 1990). Tunas of the genus *Thunnus* possess a carotid *rete* in the blood supply to the eye and brain, which can act as a thermal barrier (Linthicum and Carey, 1972). The objective of this study was to measure the ability of the carotid *rete* to insulate the brain of yellowfin tuna from rapid changes in environmental temperatures.

Materials and Methods

Yellowfin tuna (0.7 – 1.5 kg) were anesthetized and implanted with a thermocouple through the pineal foramen of the skull to record brain temperature, and exposed to step changes in ambient temperature (from 25 to 15°C and then back to 25°C). The effectiveness of the carotid heat exchanger in affecting heat flux was examined by calculating the thermal rate coefficient (k) from the rates of brain temperature change (Brill et al., 1994). To test for nervous control of *retial* efficiency, several tuna were injected I.M. with

bretylium tosylate (10 mg kg^{-1}) to abolish the effects of adrenergic nerves on the circulatory system, and the step temperature change was repeated.

Results and Discussion

Excess brain temperatures in the yellowfin were not significant (a few tenths of a degree above ambient). However, rates of brain cooling (k = 0.154) during drops in ambient temperature ($T_{water}<T_{brain}$) were significantly lower than rates of heating (k = 0.232) when water temperature was increased ($T_{water}>T_{brain}$) (Table 1). This difference indicates alterations in the effectiveness of the heat-exchanger to reduce heat loss during exposure to colder waters, and enhance heat gain upon return to warm waters. On average, the brain warmed about 50% faster than it cooled.

Table 1. Thermal rate coefficients [k, °C min^{-1} (°C gradient)$^{-1}$] for brain of yellowfin tuna during step changes in ambient temperature (25°C → 15°C → 25°C).

	Control k values			Bretylium Treatment k values		
Fish No.	Cooling	Heating	Heating / Cooling	Cooling	Heating	Heating / Cooling
1	0.108	0.185	1.71			
2	0.154	0.193	1.25			
3	0.307	0.458	1.49			
4	0.110	0.181	1.65	0.180	0.175	0.97
5	0.109	0.178	1.63	0.184	0.149	0.81
6	0.157	0.229	1.46	0.226	0.208	0.92
7	0.134	0.199	1.49			
Mean	**0.154**	**0.232**	**1.53**	**0.197**	**0.177**	**0.90**
S.E.	0.027	0.038	0.06	0.015	0.017	0.05

Similar modulation of heat transfer is seen in the deep red myotomal muscle of yellowfin tuna, although the effectiveness of the muscle *retia* is greater (lower k value when cooling) than we found for the carotid *rete* (Dewar et al., 1994). It has been hypothesized that temperature could be regulated in tissues with *retial* blood supply by shunting of blood either through the *rete* or partially by-passing it *via* alternate circulatory pathways and thereby altering the efficiency of the heat-exchange system (Linthicum and Carey, 1972). Following blockage of adrenergic nervous control of the circulation with bretylium, alterations of heat

transfer to the brain were eliminated (Table 1), suggesting active nervous control of heat-exchanger efficiency to regulate brain temperature.

Although we did not measure significant excess brain temperatures during steady-state conditions, we did find that the carotid *rete* was effective in minimizing heat loss, which would protect the brain (and perhaps eyes) during rapid dives into cooler waters. The heat-exchanger could then be turned "off" so that rapid warming could occur upon return to warm surface waters, with the overall effect of regulating more stable, and warmer, brain temperatures in the face of fluctuating environmental temperatures.

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Acknowledgements

Financial support provided by Hawaii Pacific University's Trustee's Scholarly Endeavors Program.

NON-INVASIVE HEMODYNAMIC ASSESSMENT OF SYSTOLIC AND DIASTOLIC FUNCTION OF THE TOADFISH HEART.

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Introduction

The hemodynamic study of cardiac flows allows for the evaluation of important parameters of cardiac performance. Conventionally, cardiac hemodynamics have been obtained by invasive catheterization techniques. However, with the development of non-invasive techniques such as Doppler echocardiography, it is now possible to study intracardiac flows without the disadvantages of interventive approaches.

The systolic phase of the cardiac cycle is responsible for ventricular contraction and for generation of the pressures that are required to pump and distribute blood to systemic circulation. Further, the study of systolic function provides information on how efficiently the heart distributes oxygen to the whole body. Diastolic function is ultimately responsible for ventricular filling, and determines end-diastolic volume (EDV). In fish species, diastolic function is particularly important since EDV is the major determinant of cardiac output (Olson, 1998).

In lower vertebrates, there are a very few studies which used a non-invasive hemodynamic approach (Coucelo et al., 1996; Lai et al., 1996 and 1998). For

example, in fish, most available data on the hemodynamics of ventricular ejection flow come from isolated perfused heart preparations, and *in vivo* studies are limited to Doppler or Transonic® (electromagnetic) flow probes fitted around to the ventral aorta.

The aim of this study was to use Doppler echocardiography to perform a non-invasive study of the systolic and diastolic function of the Lusitanian toadfish (*Halobatrachus didactylus*) heart.

Material and Methods

Specimens of the Lusitanian toadfish (*Halobatrachus didactylus*) were caught at Ria Formosa lagoon (south cost of Portugal). The studies were performed in 14 male specimens with body weight of 270-940 g, and echocardiographic examinations were conducted on anaesthetised animals, as described elsewhere (Coucelo et al., 2000).

Spectral records of systolic ventricular flow velocity were obtained by Doppler echocardiography and were used to determine maximum velocity, corresponding to peak of ejection flow, and various systolic time intervals (STI): pre-ejection period, ventricular ejection time, acceleration time, deceleration time and total electromechanic systole. The peak flow velocity was converted into the pressure gradient between the ventricle and bulbus, according to Bernoulli equation. Stroke volume was calculated by multiplying the cross sectional area of the ventricle-bulbus orifice and the time velocity integral of the systolic ejection flow. The Doppler velocity spectral records of ventricular filling flow, were used to determine maximum velocity, of both early (E wave) and late (A wave) ventricular filling, E/A ratio and several diastolic time intervals (DTI): isovolumetric relaxation time, early filling period, diastasis, atrial contraction period and total diastole. STI and DTI were also calculated as a percentage of the R-R interval, and were correlated with basal heart rate (HR) for each specimen studied.

All presented values are averages of at least five measurements in different cardiac cycles. The significant level used was 5% (α=0.05).

Results and Discussion

Doppler echocardiography velocity spectral records of ejection ventricular flow presented a typical spectrum, characterised by two phases: rapid acceleration and slow deceleration. Peak flow velocity varied between 0.13 and 0.25 m/s, and the pressure gradient between the ventricle and the bulbus, ranged between 0.07 and 0.25 mmHg. This pressure gradient is very low, reflecting the bulbus's elastic properties, which absorbs the kinetic energy of cardiac ejection as potential energy in their walls, and ensure a positive pressure for arterial run-off during ventricular diastole (Olson, 1998).

Using the described approach, stroke volume and cardiac output were calculated independent of ventricular shape, and averaged of 0.245±0.066 ml/kg and 9.9±2.0 ml/min/kg, respectively. These values for the for *H. didactylus* heart, are in the range of values reported using invasive methods for other fish species with 100 % trabecular myocardium and low ventricular mass.

The study of STI revealed temporal relations between several electromechanical events of the cardiac cycle and HR. The shorter intervals (pre-ejection period and acceleration time) were independent of HR, while the longer intervals (deceleration time, ventricular ejection period and total systole) negatively correlated with HR. These results suggest that, in this specie, increases in HR are achieved by cutting off diastole, by shortening diastasis and increasing ventricular filling velocity.

Doppler velocity records of ventricle filling showed a typical biphasic pattern, characterized by two waves: an E-wave (early filling), with peak velocities of 4.8-25.0 cm/s, and an A-wave (atrial contraction), with peak velocities that ranged from 4.7-33.9 cm/s. The E/A ratio presented two patterns, depending on HR ($p<0.05$): for individuals with high values (>40 bpm), E/A ratio was <1, corresponding to low E-wave and high A-wave velocities. In contrast, individuals with low HR values (<20 bpm), the profile was inverse with an E/A ratio >1. Thus, the present study shows that ventricular relaxation contributes significantly to ventricular filling in *H. didactylus*, and that ventricular filling is highly dependant on HR (especially on duration of isovolumic relaxation and early filling)>

In conclusion, this work demonstrates that Doppler echocardiography is a powerful tool for studying systolic and diastolic function of the fish heart, and that it can be used to study cardiac performance in a variety of physiopathological situations.**References**

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VOLUME CHANGE AND VENOUS FUNCTION IN HAGFISHES

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Central venous pressure is the major determinant of cardiac output in all vertebrate animals, through the Frank-Starling effect. Autonomic nervous control and the actions of circulating hormones then fine tune the heart's performance. In a "fish" circulation the blood returning to the heart has passed at least two vascular resistance beds in series and its kinetic energy is low. Venous pressures, therefore are low. The importance of *vis a fronté* filling of the heart, driven by negative pressures within the pericardial cavity, is thus particularly significant in teleost and elasmobranch fishes. Johnsson et al. (1996) demonstrated that even in the absence of a rigid pericardium cutting the connective tissues investing the portal heart of the hagfish *Eptatretus cirrhatus* changed heart rate.

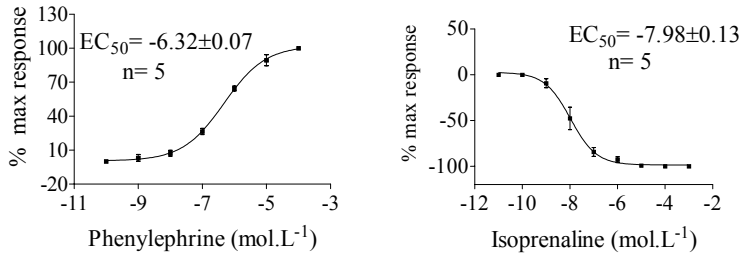


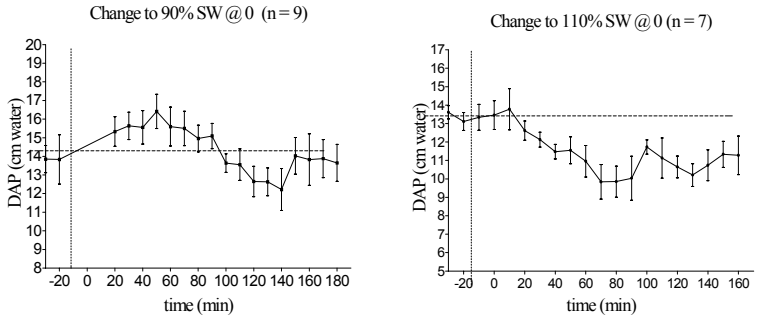
Fig. 1. Cumulative concentration-response curves, supra-intestinal vein.

Though smooth muscle is a small component in fish veins, there is sufficient data from teleosts, at least, to demonstrate the potential for active vasomotion in these vessels (Zhang et al., 1998). They are not merely passive conduit vessels. Hagfish veins react to a variety of pharmacological agents, including catecholamines (Fig 1).

Hagfishes are osmoconformers, with an ionic composition of their extracellular fluids which is little different from seawater. Their arterial blood pressures are the lowest of any chordate animal and venous pressures

are low, but positive. Pressures in the supraintestinal vein averaged 0.70±0.12 cm H_2O and those in the posterior cardinal vein averaged 0.37±0.08 cm H_2O (n = 15). Hagfishes are unlikely to experience hypertonic media, but may risk volume loading if they venture into estuaries or consume teleost fish in their diet. We used volume loading (90% seawater) and volume depletion (110%) seawater to stress the vascular system of *E. cirrhatus* and monitor changes in arterial and venous pressures *via* indwelling cannulae. In the absence of any passive compensating mechanisms or active volume regulation, we predicted that arterial and venous blood presssures would rise and remain at a value at least 10% greater than pre-loading and would decrease by at least 10% in dehydrated animals.

24 hours after transfer to 90% seawater dorsal aortic blood presssure was 12.94±1.69 compared to a pre-treatment value of 14.45±1.32 (n =3). In the volume loaded animals there seemed to be an extremely rapid adjustment in pressures, with compensation occuring within the first three hours of osmotic challenge (Fig 2).



Fig, 2 Dorsal aortic pressure changes on transfer to 90% or 110% seawater.

Judged by their behavioural responses, hagfishes tolerated hydration better than they tolerated dehydration. They were much more active in 110% seawater, exhibiting knotting behaviour. Toop and Evans (1993) found that *Myxine glutinosa* were able to compensate for volume loading, but not for the volume decrease in 115% SW. 24 hours after transfer to 110% seawater mean dorsal aortic pressure was 11.43 cm H_2O compared to their 100% value of 13.38±0.05 (n = 6). Pressures in the posterior cardinal vein also fell in animals exposed to 110% seawater. Given the variability in resting venous pressures and the difficulty of measuring them reliably, it might seem optimistic to expect to record a change with a 10% change in blood volume. However, the volume change should have its effect on the

stressed volume in the veins and therefore the predicted pressure change would be greater than 10%.

Blood volume as a percentage of body volume is greater in hagfishes than other chordates ((Forster et al., 2001) and the venous system is compartmentalised, with numerous separate sinuses. Arterial "pressure relief valves" were first described by (Cole, 1913). It is easy to envisage movement of blood out of the central circulation and into the sinus system in volume loaded animals as arterial pressures rise. It is more difficult to conceive of a system that restores blood volume once lost. That the blood volume is large will not buffer an osmoconforming animal from volume changes and there is evidence that blood osmotic pressure changes rapidly on hydration and dehydration.

These experiments were approved by the Animal Ethics Committee of the University of Canterbury.

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COMPARISON OF HEART RATE IN FISHES: COLD, TEMPERATE SEA WATER VERSUS WARM, DESERT RIVERS

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Introduction

Globally, fish are found in a wide spectrum of water temperatures, ranging from cold sea water (-2 C, Scholander et al. 1954) to the hot desert springs, streams and rivers with temperatures above 30 C (Deacon & Minckley 1974, Soltz & Naiman 1978). Because of being eurythermic organisms, all functions of fishes are effected by ambient water temperature. Research on fishes in the past few decades have shown that heart rate is a good indicator of reactions in fish (Kanwisher et al. 1972, Holand 1987). Fish react physiologically, not only to physical and emotional stimuli, but also to environmental factors such as changes in water temperature.

Due to modern technology we can now study the heart rate and electrocardiogram (EKG) of free swimming fishes (Kanwisher et al. 1972). From coastal waters in Norway we have found correlation between heart rate of fish and water temperatures, but these temperatures rarely exceed 20 C. No studies of heart rate in fishes inhabiting cool (20-25 C), to warm (25-30 C), to hot (> 30 C) waters have been conducted. Likewise, the effect on the heart of extreme diurnal temperature changes in desert rivers is not known. Therefore, we were interested in comparing heart activity of fishes living in the cold Norwegian seawater to fishes living in warm desert rivers in Arizona. The diversity of fish species is low in the

southwestern United States (Minckley 1973, Sublette et al. 1990, Rinne & Stefferud 1998), however their adaptations to environmental conditions are great (Deacon & Minckley 1974, Rinne & Stefferud 1997). With the ever-increasing impact of humans, these adaptations to rather harsh environments is of great comparative importance. Although important, basic data on the response of the heart rate in fishes living in hot desert streams in arid deserts is lacking. We decided to investigate the heart rate of two desert species from the Verde River, Arizona, USA because of their subjection to and adaptation for flooding, drought, and characteristic elevated water temperatures,. Our primary objectives were: 1) to observe and define heart rate/temperature relationships of the two desert fish species in the field and laboratory, 2) to compare these rates with those of a cold temperature marine fish species, and 3) to suggest possible management implications from results of study.

Materials and Methods

Research animals

The species chosen for this comparative study were: 1) cod, *Gadus morhua (Gadiformes: Gadidae)* caught in sea waters in the Trondheimsfjord, Norway, and 2) Sonora sucker*, Catostomus insignis (Cypriniformes: Catostomidae),* and roundtail chub, *Gila robusta (Cypriniformes: Cyprinidae)* captured in a large desert river (Verde River) in Arizona, USA (Stefferud & Rinne 1995).

Experimental design

Cod were held in large tanks of 5 m^3 where the water temperature was controlled by an electric heater and kept near temperature of capture (7 C). The water was approximately one meter in depth. Temperature stratification was avoided by aeration with a small compressor injecting air at the bottom of the tank. Prior to experiment initiation, experimental animals were allowed to acclimatize thermally (5 C) and behaviorally in the tanks for two weeks after
capture. Data were collected from three individual cod varying from 450 to 650 mm total length (TL).

Initial (1994) experiments in the desert Southwest were conducted, *in situ,* in net cages and in small stream enclosures on the Verde River, near Camp Verde, Arizona. To refine definition of the relationship of increasing water temperature and heart rate, subsequent (1997), controlled experiments were conducted in

artificial raceways in the laboratory, Flagstaff, Arizona. Roundtail chub and Sonora sucker were captured in the Verde River and held in cages and/or exclosures for several days to acclimate behaviorally, thermally (10-15 C), and to restrained conditions. For laboratory studies, captured fishes were transported live in oxygenated coolers and similarly permitted to acclimate for several days at 15 C before transmitter implantation. Sample size was one sucker and one chub in 1994, two chubs and two suckers in 1995, and three suckers and two chubs in 1997. Experimental individuals used for the desert experiments ranged from 230 to 500 mm TL.

Fish were retained and monitored in net cages (1.5 X 1.0 X 3.0 m, .06 cm mesh) in the Verde River in October 1994 and in July 1995 . In 1995, two additional fish (one chub and one sucker) were released after tagging and allowed to swim freely in a small enclosure (ca. 10 m long) in a side channel of the Verde River. One sucker was released to swim freely in the river. Prior to taking measurements, both in the river and the laboratory, fish were allowed
to behaviorally stabilize for 24 hours. Because of the effect on heart rates as personnel approached the experimental site, longer electronic cables (20 m) were used between the hydrophone (which was permanently placed in the water near experimental fish) and the receiving/recording equipment to avoid the effects on heart rate of personnel approaching the research site.

Laboratory fish were allowed to re-stabilize their heart rate for more than 12 hours to acclimate after each temperature change before heart rates were recorded at a new environmental temperature. This procedure allowed fish to re-stabilize their heart rate, prior to recording heart rate/ temperature (ambient water temperatures) relationship data. In the laboratory, the receiving and recording equipment was installed in adjacent rooms to avoid disturbances from human activity. In laboratory experiments in Flagstaff, artificial, circular raceways (Frigid Units, Inc.) were filled to a depth of 25 cm with Verde River water, oxygenated by agitating chiller units set at their highest temperature (15 C) and water was then circulated by small, 1/3 hp pumps. Industrial heating units capable of 15,000 btu's per hour were inserted into raceways to facilitate rate of temperature elevation. All experiments began at 12-13 C and ceased upon loss of equilibrium (ecological death) of individual fish. Heart rate was continuously measured as described above. Raceway water temperature was monitored with a continuous-recording Hydrolab sonde unit.

Transmitters

Acoustical heart transmitters were developed at SINTEF to be used on the desert cypriniform fishes. Similar transmitters have been used successfully on cod from the North sea (Mohus and Holand 1983). However, in the study of desert fishes, it was important to construct a transmitter which was smaller in size, but had a battery life of at least 10 days. The smaller-sized transmitter used on the smallest chub (230 mm, TL) from the Verde River was cylindrical in shape, measured 8 mm OD, 18 mm long and weighed 1.7 g in air and 0.8 g in water. For the larger suckers and chubs, transmitters were 11 mm OD, 35 mm long, and weighed 5.8 g in air and 2.6 g in water. Transmitters used on codfish in studies in Norway were similar in size to the larger (35 mm) units used for the cypriniform species.

Heart rate signals

The EKG signal that is generated by the heart muscles, is detected by means of two electrodes. The reference electrode is normally attached to the transmitter housing, while the "active" electrode is soldered to the end of an insulated wire, protruding from the transmitter. Normally, the transmitter is placed within the stomach of the experimental fish with the reference electrode in contact with the stomach wall. The insulated wire containing the active electrode extends throughout the esophagus and the gill cavity. The electrode is then inserted through the skin and positioned close to the pericardium (Figure 1a). To secure the electrode, the electrode wire is sutured to the skin, close to the point of insertion. However, in the Sonora sucker, the transmitter could not be placed in the stomach because of the specific anatomy of the species. This species, unlike the predaceous cod and chub, is an omnivore and its esophagus and stomach morphology were not compatible to such implantation. The transmitter, therefore, was sutured to the back of the sucker (Figure 1b).

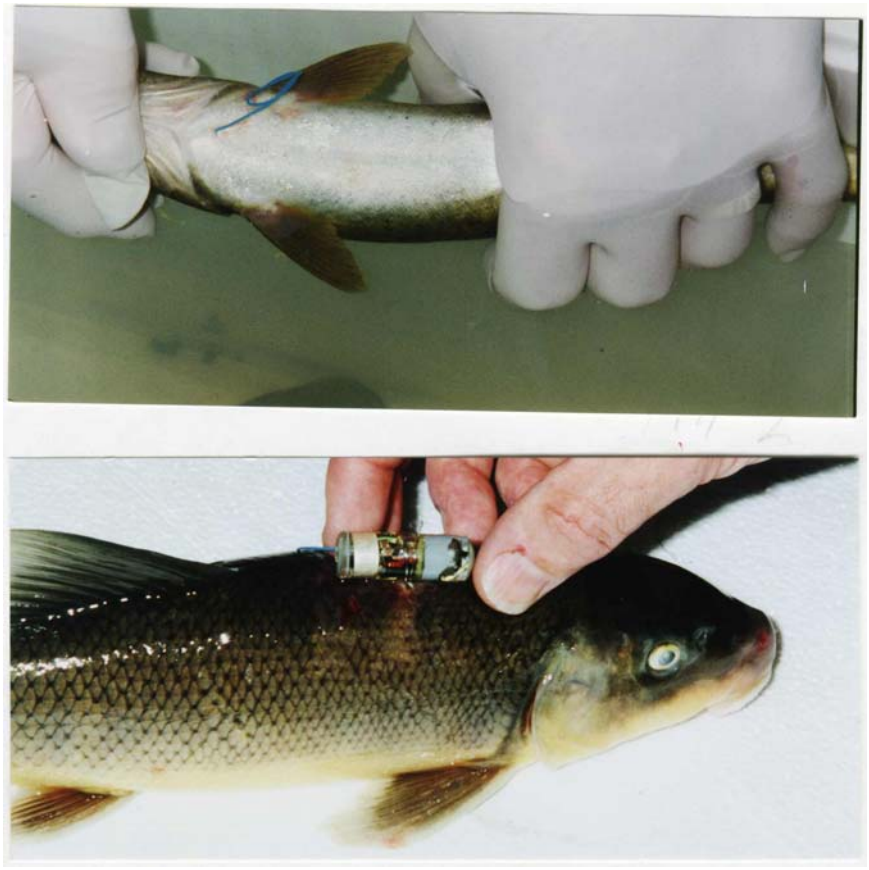

Figure 1. Photo showing normal implantation of electrode on a) a roundtail chub, Gila robusta, and b) attachment of a transmitter to the back of a Sonora sucker, Catostomus insignis.

The EKG signal causes a frequency modulation (FM) of the continuous carrier frequency of the transmitter. The modulated signal is received by a hydrophone and an acoustical receiver also developed and constructed at SINTEF. The audio signal from the receiver may be recorded for later processing, or used for manual counting of the heart beat signal on site. Normally, to determine the heart rate, heart beats were counted and averaged over a period of 30 seconds.

Results

The summary of heart rates measured in fishes (three suckers and three chubs in the Verde River in July, 1995 are shown in Figure 2. During maximum elevated water temperature and diurnal variations, temperature at 0.5 m depth varied up to 8 C daily (July 14). Considerably greater variation in water temperature occurred during the duration of experimentation, July 11 to 17 (Figure 3). There were no obvious differences between heart rates recorded at similar temperatures for fish swimming freely in the 10 m enclosures in the Verde River compared to those restrained in net cages.

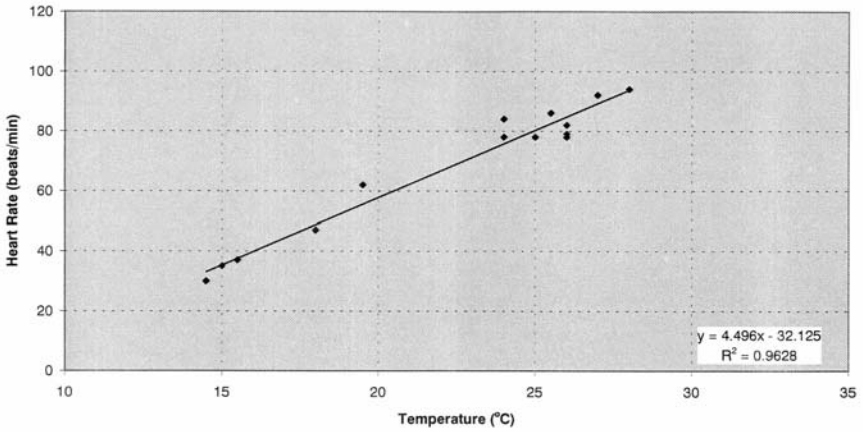

Figure 2. Heart rate and temperatures relationships measured in two desert cypriniform species in the Verde River, 1995.

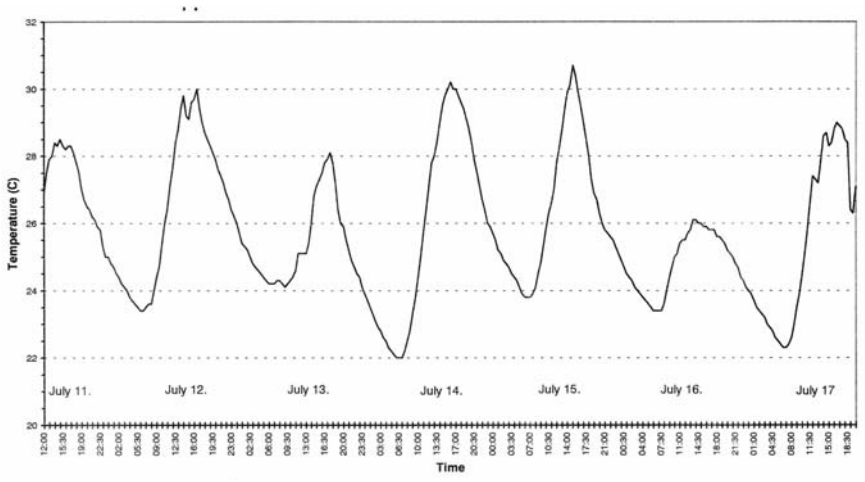

Figure 3. Diel temperature variations at the experimental site in the Verde River measured at approximately 0.5 m depth in July 1995.

Finally, a summary of heart rates measured under laboratory conditions with controlled temperatures in 1997 is shown in Figure 4. First, the relationships measured in the field and laboratory were markedly similar and not significantly different ($P < 0.05$, DF 4). Coefficients of determination (R^2) were high for both the field (0.96) and the laboratory (0.92) relationships. Heart rates reached a high of 95 at 27 C in the field experiments (Figure 3) and a markedly high value of 117 for one of the Sonora sucker at 33 C in laboratory experiments (Figure 5). Individual rates of four the five individual fish are shown in Figure 6.

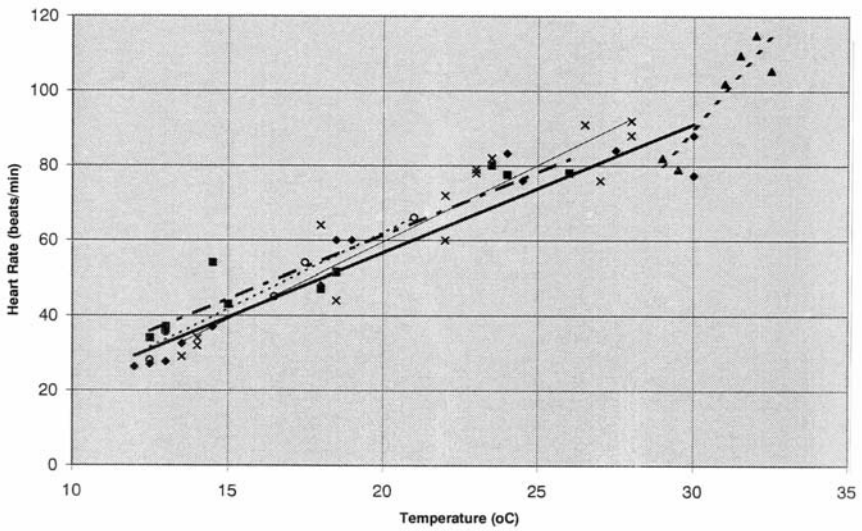

Figure 4. Summary of the recordings of heart rates of five native cypriniforms relative to temperature as measured under controlled laboratory conditions, 1997.

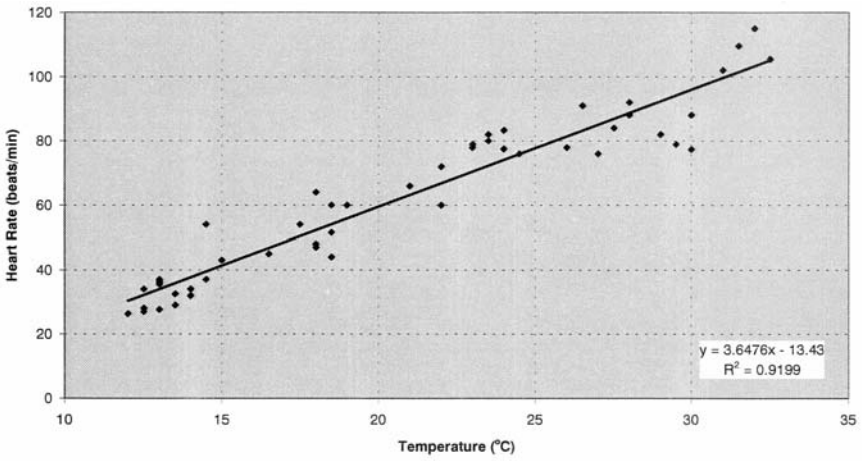

Figure 5. Heart rate/temperature relationships of two native individual cypriniform fishes monitored in the laboratory, 1997.

For comparison, a temperature/heart rate conversion relationship for cod under laboratory conditions in Norway are shown in Figure 6. Heart rates never exceeded 70 beats per minute for this coldwater species, however, the heart rate/temperature relationship was similar to desert cypriniform species, but was slightly lower at similar points on the plotted relationship line. Coefficient of determination was again significantly high ($R^2 = 0.89$).

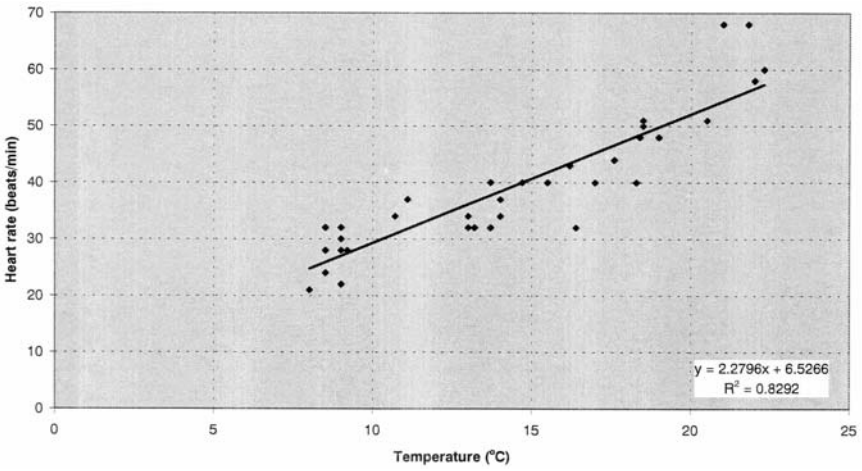

Figure 6. Heart rate and temperature relationships of cod measured under laboratory conditions in Norway.

Discussions and conclusions

Results of field and laboratory experimentation on the two native, cypriniform species do not show any significant difference in heart rate versus temperature between the two species (Figure 4). Further, there is a good resemblance between this relationship on data collected from free swimming fish in the river and that

collected under laboratory conditions (Figure 5).

Water temperature in the Verde River may surpass 30 C daily (Figure 3). We hypothesize that these desert fishes may even survive at temperatures up to 35 C. However, all three fish exposed to water temperatures of 33-34 C in the laboratory for over 24 hours suffered mortality. Such sustained, diel, elevated temperatures do not normally occur in the wild (Figure 3). If such high temperatures should occur in the upper water layer, fish may normally have the capability to move to cooler bottom waters and areas of cooler water strata resulting from intra-gravel flow. However, entrapment in a shallow, disconnected pool during drought, or flow reduction resulting from irrigational diversion removal or pumping of river corridor, subterraneous aquifers may result in mortality--even in time periods of less than 24 hours.

In field experiments, suckers and chubs were caught, fitted with transmitters, and released in enclosures in the river in an area about a meter in maximum depth. Also, the area of enclosure was immediately below a high gradient riffle (Rinne and Stefferud 1996) where elevated water temperature may be mitigated by evaporational cooling. In addition, some thermal stratification in pools of greater than a meter in depth most probably occurs. Cooling of waters during evening hours (Figure 4) further provides desert fish species, such as the sucker and chub, ample time for re-stabilizing body temperatures and removal of physiological (cardio) stress.

Unlike the warm to hot conditions sustained by cypriniform species, cod experience temperature variations that are very slow and annual variations that are small compared to desert aquatic systems. North temperate sea waters vary less than 15 C annually. Water temperature in desert systems such as the Verde River vary half that amount on a diel basis (Figure 3) and perhaps more in other desert environments. By comparison, although heart rate/temperature relationships are similar between this Gadiform species and the two cypriniform species the rate is somewhat lower in the former. Nevertheless, even though cod and desert fish are generally living in different temperature and salinity regimes, there is a remarkable similarity between heart rate and temperature change in these members of three families of fishes (Figures 5 and 6). The main differences again, are that; 1) the maximum heart rate of cod (60-70 beats/min) is only about half that of the two cypriniform species, and 2) the rate of change versus temperature change is somewhat higher in the two desert fishes we examined (Figures 5 and 6).

Summary and Management implications

A comparison of heart rate/temperature relationships was made among fishes inhabiting warm to hot desert aquatic environments in the desert Southwest (USA) and cold temperate sea waters of Norway. Two species of Cypriniform and one Gadiform (cod) were compared. Relationships were markedly similar between the two orders and three families (and species) of fish, however, the cod displayed both a slower and lesser response to elevation in water temperatures. Maximum heart rate reached almost 120 beats per minute for one species of Cypriniform at 30 C. By comparison, cod attained half that rate in 20 C water. Heart rate and temperature relationships for the two native desert species studied, and perhaps other desert fish species, have management implications relative to urbanization, associated water demand and watershed management of a large, yet free-flowing desert river.

Relationships established between heart rate and elevated water temperatures have some management implications for these two, and perhaps other desert fish species. The Verde River, although yet one of the few free-flowing systems in the Southwest (Stefferud & Rinne 1995), is set in a perilous state (Rinne 1999). As with other desert rivers, damming, irrigation diversion and groundwater pumping have and potentially may markedly alter quantity and quality of water in time and space (Rinne et al. 1998). The only dam on the upper Verde is Sullivan, which is almost completely filled with sediment and retains only periodic surface water. Damming is not currently a threat to water quality in the upper Verde River (Rinne et al. 1998, Rinne et al. in press). However, the other two anthropogenic factors could potentially affect flow and water quality conditions including temperature.

In the Verde River headwaters wells are being established at an ever-increasing rate (Rinne et al. in press, Wirt and Hjalmarson 2002, Neary and Rinne 2001). Neary & Rinne (1997) documented an increase in base flow in the Verde over the past two decades, attributable, in part, to the wettest period in the Southwest over the past 1,000 years. In addition, irrigational pumping for agriculture in the headwaters has declined in that same time period. On the other hand, urbanization of this area is proceeding at an unprecedented rate. Yavapai County, which encompasses the Verde River headwaters, is currently one of the fastest growing rural area in the United States (Rinne et al. in press). Current population of the Prescott/Prescott Valley/Chino Valley is 43,000, but is projected to be 135,000 by the year 2040--a 300+% increase in human population.

Water demand will parallel these projected human populations. Already, increased pumping of ground water, indicated by the number of new wells for domestic use has occurred over the past two decades in the county. These increasing groundwater removal rates have a high probability of eventually reducing flows in the Verde River to intermittent levels. Under reduced flow regimes, elevated water temperatures and reduced dissolved oxygen during summer have a high probability of occurring. Concentration of fish into pools combined with accompanying changes in water quality may becomes lethal for not only suckers and chubs in the river, but all other fish species.

Irrigational diversions of river water is most intensive in the municipality complex of Cottonwood, Clarkdale, and Camp Verde in the Middle Verde Valley (Rinne et al. 1998); pumping of the river aquifer is very high in this reach. Rinne et al. (1998) documented a dramatic change in fish community structure in this reach of river, unexplainable by flow regimes alone. Change in quality of water may be responsible, in part, however, the combination of increased pumping in the headwaters and downstream in the middle Verde Valley could result in markedly reduced flows in both reaches of the river in the future. Reduced surface flows potentially will be accompanied by sustained, elevated water temperatures to the levels (>30 C) where mortality resulted in Sonora sucker and chub in the laboratory.

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INTERFACING CARDIAC PHYSIOLOGY WITH FISHERIES MANAGEMENT THROUGH STUDIES OF CATCH-AND-RELEASE ANGLING EFFECTS ON FRESHWATER RECREATIONAL FISHES

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Abstract

In this paper we outline the utility of cardiovascular monitoring techniques that have been historically restricted to "basic" research, for more management and conservation oriented applications. We focus on the potential contributions to fisheries management through a series of examples and case studies on catch-and-release angling, although this approach also has merit for other fisheries management applications. We provide an overview of published accounts to date, and advocate a comprehensive approach that incorporates real-time cardiac monitoring with other methods of assessment commonly used in catch-and-release angling assessment (i.e., blood and muscle biochemistry, injury and mortality). Fisheries managers can use data generated using this methodology to enact guidelines and regulations for the development of sustainable recreational fisheries.

Background on Basic Research in Cardiovascular Physiology

To date, most studies of cardiovascular physiology have focused principally on comparative and environmental physiology and evolutionary biology. These studies have provided a strong framework for understanding basic cardiac physiology in a variety of fish species (e.g., Satchell 1991). However, the tools used for generating data for basic studies also have the potential to provide answers and insights into issues of more applied nature. Indeed, because of the strong relationship between cardiac parameters and metabolic rate, cardiovascular parameters can yield information in real time on response of fish to different stressors including those of anthropogenic origin.

Cardiac Monitoring Techniques

Methodologies employed by cardiovascular physiologists that have relevance to fisheries management include the assessment of cardiac output using Doppler or transonic flow probes and the determination of heart rate using transmitters. Measurement of cardiac output involves the placement of a probe around the ventral aorta of fish. The probe permits the measurement of cardiac output, and its two components, heart rate and stroke volume. The cardiac output apparatus requires that the fish is hard-wired (i.e, tethered) limiting the field use of this technique. For field assessments in free-swimming fish, heart rate telemetry can be used, however, it does not measure cardiac output or stroke volume. Only measuring heart rate in some species can be limiting in that for many species of fish, heart rate is rather static, whereas stroke volume varies widely in response to different stressors (see Thorarensen et al. 1996).

Assessment of Catch-and-Release Angling

To date, there are four published accounts that link cardiovascular physiology to catch-and-release angling management. The first (Anderson et al. 1998) used heart rate transmitters to monitor the response of Atlantic salmon (*Salmo salar*) to angling at different water temperatures. Cardiac disturbance and mortality rates were more extreme at higher water temperatures. Schreer et al. (2001) measured cardiac output of smallmouth bass (*Micropterus dolomieu*) while simulating either brief or exhaustive angling over three water temperatures and determined that angling duration affects cardiac recovery time. In a study of rock bass (*Ambloplites rupestris*), Cooke et al. (2001) reported that different terminal tackle (i.e., live bait versus lures, barbed versus barbless hooks) resulted in different difficulties in removing hooks that subsequently affected air

exposure durations. Consequently, rock bass held out of water for longer periods experienced longer cardiac recovery times (assessed by cardiac output in the laboratory) than those exposed to air only briefly. A forthcoming report by Cooke et al. (In Press a) presents data on the cardiac response of both smallmouth bass and largemouth bass (*Micropterus salmoides*) to a variety of angling related disturbances including air exposure, livewell densities, simulated tournaments, and use of livewell conditioners. Monitoring real-time cardiac disturbance also permitted the estimation of oxygen consumption demands. This approach provides the only reliable technique for assessing livewell oxygen demand in situ and can be used to develop livewell operating guidelines to maintain oxygen at tolerable levels.

Preliminary research by our group that coupled laboratory derived studies of cardiac output (using Doppler flow probes) with field derived studies of heart rate (using ultrasonic transmitters) on largemouth bass has provided understanding of the benefits and limitations of field and laboratory studies. In the field, recovery patterns were somewhat obscured by a suite of influences such as movement, social interaction with other fish, predation risk, and variation in thermal environment. In the laboratory, recovery patterns were more distinct. These data illustrate the value in conducting comprehensive assessments that incorporate measurements from both laboratory and field. To date, heart rate appears to be the best indicator of post-angling disturbance and recovery in free-swimming fish (Anderson et al. 1998).

Collectively, all of these studies outlined above use techniques developed for basic cardiovascular physiology and apply them to pressing fisheries management issues. All of these papers conclude with a series of management recommendations that are being disseminated by outdoor media, conservation organizations, and government (e.g., focus on enhanced survival, and minimizing sublethal disturbances.

Conclusions and Prospectus

We are not in any way questioning the importance of basic research; instead, we are advocating expanding the application of conventional "basic" research tools and techniques to address more applied issues in fisheries science. Similarly, Comroe and Dripps (1976) showed the value of basic cardiovascular science to the advent of some of today's medical breakthroughs (i.e., the development of the electrocardiogram). Sometimes basic studies can simply be viewed in different contexts to yield information that is of use in more applied instances.

Some of the cardiovascular measurements that we advocate are complimentary to blood and muscle biochemistry; fields that have been integrated with applied physiology for some time. There is no doubt that the science and management of catch-and-release angling will benefit from more comprehensive studies that incorporate several different techniques for monitoring physiological disturbance and recovery. Although we have focused largely on freshwater fishes in this paper, this approach also has merit in marine environments (Cooke et al. In Press b). The application of techniques in cardiovascular physiology to address issues in aquaculture, environmental monitoring and other aspects of fisheries management is also possible.

Acknowledgements

We would like to thank the numerous individuals that have contributed to the development of the ideas presented here. We also thank the following funding agencies and organizations for supporting aspects of this research; University of Waterloo, Queen's University, Illinois Natural History Survey, University of Illinois, and the Natural Sciences and Engineering Research Council.

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EVIDENCES OF A FUNCTIONAL SARCOPLASMIC RETICULUM UNDER PHYSIOLOGICAL CONDITIONS IN NEOTROPICAL FISH

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

Most of the studies in teleost cardiac myocites have shown that E-C coupling is a ryanodine-insensitive mechanism at physiological frequencies and temperatures, implying an important role for transarcolemmal calcium movement. Nevertheless, there are some indications of an increased functional importance of the cardiac sarcoplasmic reticulum (SR) in trout at high experimental temperatures and low contraction frequencies (Keen *et al*. 1994) and in "athletic" fish, like tunas (Keen *et al*., 1992, Aho & Vornanen, 1998). Different results were obtained by Shiels & Farrell (1997), that found a significant Ca^{2+} contribution from the SR in trout at physiological pacing frequencies and by Hove-Madsen et al. (2001), that demonstrated a relevant contribution of the SR (~ 40% of total Ca^{2+} required to activate contraction) in this species, independently of the experimental temperature. However, little is known about tropical species.
In order to understand the role of the sarcoplasmic reticulum (SR) and extracellular calcium concentration in the contractile myocardium system, experiments *in vivo* and *in vitro* were conducted with 4 tropical teleost species from different habitats and distinct modes of life: the traira, *Hoplias malabaricus*, a sedentary fresh water fish characteristic of lentic environments, the armored catfish, *Hypostomus regani*, a sedentary species

living in fast-stream rivers, the pacu, *Piaractus mesopotamicus*, a migratory freshwater species from lotic environments and the curimbata, *Prochilodus scrofa*, an "athletic" freshwater fish. The results were compared to previous studies conducted with the embore, *Bathygobius soporator*, a teleost inhabiting tide pools (Rantin *et al*., 1998) and the Nile tilapia, *Oreochromis niloticus*, (Costa *et al*., 2000).

Material and Methods

In vivo heart rate (f_H - bpm) was obtained by electrocardiography. ECG electrodes were placed in a ventral position between the gills and the heart and in a ventral position close to the pelvic fins. A reference electrode was located in the water of the experimental chamber. The electrode set was connected to a universal coupler of a Narco recorder. After a recovery period of 12 h, the f_H was measured at 25 ^oC. For the *in vitro* preparations, pairs of strips with a thickness of maximally 1 mm were excised from the ventricle. The preparations were connected to a NARCO F-60 isometric force transducer with surgical silk and placed around a platinum electrode. The temperature of the muscle bath was maintained at 25 ^oC. The maximal capacity of hearts to develop force was assessed trough the addition of Ca^{2+} to bathing medium. The force development upon the first stimulation following a rest period was determined at 25 ^oC to examine the capacity for the storage of intracellular Ca^{2+} during resting period. After a steady-state condition at 12 bpm was achieved, stimulation ceased for a period of 5 min. Force development of the first contraction following the resting period was compared to the last contraction in a steady-state train. These experiments were conducted with and without 10 μM ryanodine in the medium.

Results and Discussion

All the species showed resting heart rate (f_H) values similar to those exhibited by temperate species (ranging from 30 to 70 bpm), differing from *B. soporator*, which presented f_H values similar to those of mammals (~ 150 bpm at 25 ^oC and 300 bpm at 40 ^oC). Increasing extracellular Ca^{2+} concentration caused a significant increase of force contraction (Fc), which reaches maximum values at 8.5 mM Ca^{2+} for all the experimental species (figure 1).

In *B. soporator* (Rantin *et al*., 1998) and *O. niloticus* (Costa *et al*., 2000), the post rest potentiation was not influenced by ryanodine (figure 1), an inhibitor of the SR, indicating that this organelle is not important to the excitation-contraction coupling irrespective of temperature (25 and 40 ^oC). This is an interesting result since both fish, particularly *B. soporator*, are frequently subjected to acute changes in temperatures. Furthermore, *B.*

soporator presents very high f_H values, suggestive of a functional SR. On the other hand, the post rest potentiation of *H. malabaricus*, *H. regani*, *P. mesopotamicus* and *P. scrofa* (figure 1) was strongly inhibited by ryanodine at both physiological temperature (25 ^{o}C) and stimulation frequency (12 bpm). At these experimental conditions, *H. malabaricus*, *P. mesopotamicus* and *P. scrofa* hearts derive a large portion (60%, 30% and 25 %, respectively) of activator calcium from intracellular stores (figure 2). Curiously, *H. malabaricus* is the most sedentary species in this group. These results indicate that other factors than high temperatures and activity can determine the level of functionality of the sarcoplasmic reticulum in tropical fish.

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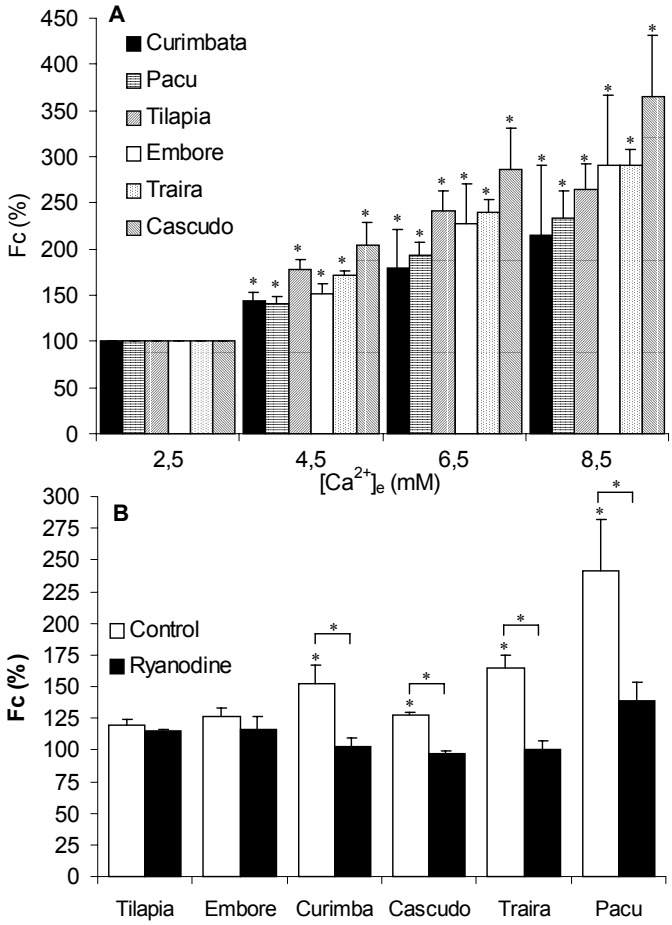

Figure 1. **A**. The effect of extracellular Ca^{2+} on twitch force (Fc - % of the initial values) of cardiac muscle of each species. **B**. Twitch force of the first contraction following a rest period. Mean values ± S.E.

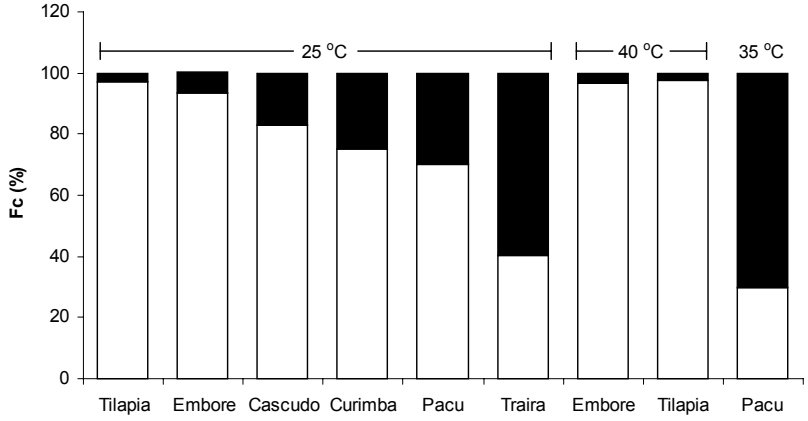

Figure 2. Estimated values of activator calcium derived from the influx across the sarcolemma (☒) and SR stores (⊟) at different experimental temperatures.

SALMONID AND ICEFISH CARDIAC TROPONIN C: A STUDY IN MOLECULAR EVOLUTION

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EXTENDED ABSTRACT ONLY- DO NOT CITE

As the temperature of a heart is lowered its ability to function decreases which is due in part to a reduction in contractile element sensitivity for intracellular Ca^{2+}. If the temperature of a mammalian heart were to be lowered to that of a temperate teleost (7-20 ^{o}C) the ability to generate adequate rates of contractile force to pump blood around the body (Churcott et al., 1994) is compromised. One adaptation of the rainbow trout cardiac myocyte allowing function at low temperatures is the comparatively high sensitivity of its contractile element for Ca^{2+} (Churcott et al., 1994). Myocyte contraction is triggered when Ca^{2+} enters the cell, following membrane depolarization, and binds to the protein cardiac troponin C (cTnC), a component protein of the thin filament of the contractile element. Because of its role in sensing intracellular Ca^{2+}, cTnC is a logical place to begin looking for the mechanism responsible for the high Ca^{2+} sensitivity of trout cardiac myocytes that allows for adequate cardiac function at low temperatures.

Cloning and sequencing of cTnC from the rainbow trout, *Oncorhynchus mykiss*, reveals that of the 161 amino acids that compose cTnC there are 13 differences between the sequences of salmonid cTnC (ScTnC) and mammalian cTnC (McTnC) (Moyes, et al., 1996). We have demonstrated that this difference in sequence has functional consequences (figure 1) (Gillis et al., 2000). Ca^{2+} binding was measured by titrating the recombinant proteins with Ca^{2+} while monitoring a fluorescent probe engineered into the protein. The results demonstrate that ScTnC exhibits more than twice the affinity for Ca^{2+} as McTnC under a wide variety of temperatures and pH values (Gillis et al., 2000). These

data demonstrate that the higher Ca^{2+} sensitivity of trout cardiac myocytes is due to the specific cTnC isoform that they express.

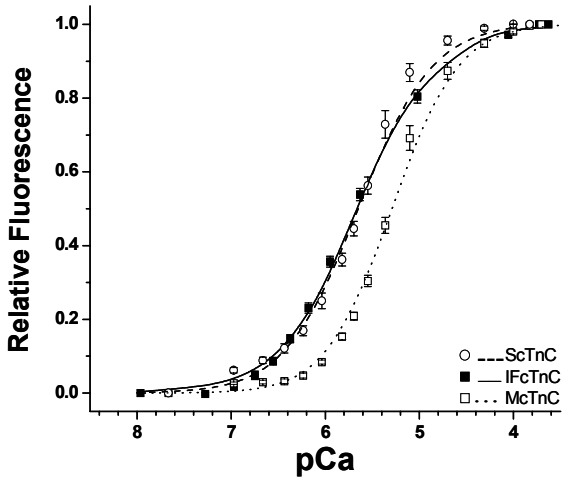

Figure 1. Difference in Ca^{2+} sensitivity between ScTnC, IFcTnC, and McTnC measured through titration of fluorescence at 21.0 ºC, pH 7.0. The curves generated by fitting the data with the Hill equation have been added to the figures for comparison against the data points.

To identify the ScTnC amino acids responsible for its higher Ca^{2+} affinity, a number of ScTnC and McTnC mutants were made in which the amino acid sequence of the proteins were altered through site-directed mutagenesis. The results demonstrate that the glutamine and aspartate at residues 29 and 30 in ScTnC are responsible for the high Ca^{2+} affinity. When glutamine and aspartate at positions 29 and 30 are replaced in a ScTnC mutant with leucine and glycine, the residues present at positions 29 and 30 in McTnC, the Ca^{2+} affinity of the protein is decreased to that of McTnC. Residues 29 and 30 are located in a region of the protein that allosterically impacts Site II the Ca^{2+} binding site that initiates the conformation change in the thin filament when Ca^{2+} binds to it.

Glutamine and aspartate are also present as residues 29 and 30 in all known cTnC sequences from fish. cTnC has been cloned from the following: icefish: *Chaenocephalus aceratus* (Yang et al., 2000), white sucker, *Catostomus*

commersoni (Yang et al., 2000), goldfish, *Carassius auratus* (Yang et al., 2000), yellow perch, *Perca flavescens* (Yang et al., 2000), yellowfin tuna, *Thunnus albacares* (Yang et al., 2000), and lamprey, *Entosphenus japonicus* (Yuasa et al., 1998). The maintenance of glutamine and aspartate at residue 29 and 30 in all of these fish cTnCs strongly suggests that these residues are critical to protein function (Gillis, et al., 2002).

Evidence from mitochondrial DNA suggests that icefish diverged as a species at least 1 million years ago (Bargelloni, et al., 1994). During this time they have been geographically isolated in waters that remain below 0^{o}C throughout the year suggesting that there has been enough time for icefish cTnC (IFcTnC) to have evolved to optimally function at subzero temperatures. While there are 8 sequence differences between IFcTnC and ScTnC glutamine and aspartate are present at residues 29 and 30 (Yang et al., 2000). To determine the Ca^{2+} affinity of IFcTnC, the protein was expressed and its function characterized. The results demonstrate that there are no differences between IFcTnC and ScTnC when measured at the same temperature (figure 1). This result corroborates that it is glutamine and aspartate at residues 29 and 30 that are responsible for high Ca^{2+} affinity. However, this result raises the question of how cardiac function is maintained in icefish at subzero temperatures. We have demonstrated that the Ca^{2+} affinity of ScTnC and McTnC increases when pH is increased according to the alpha-stat relationship (Gillis et al., 2000). A 0.3 pH increase increased the affinity of ScTnC and McTnC by 1.4 fold at 21^{o}C (Gillis et al., 2000). Alpha-stat regulation occurs in poikilotherms when temperatures decrease to keep relative alkalinity (OH^{-}/H^{+}) constant. This change equals approximately -0.019 pH units/^{o}C. We think that it is the effect of pH on IFcTnC that is responsible for maintaining cardiac function at sub-zero temperatures. The pH of IFcTnC at –1.8 ^{o}C is expected to be approximately 0.3 pH units higher than that of ScTnC at 14^{o}C. This higher pH would help maintain the Ca^{2+} affinity of IFcTnC at an adequate level allowing for cardiac function.

By identifying the amino acid residues responsible for the high Ca^{2+} affinity of ScTnC, this work has made significant contributions to our understanding of the molecular adaptations that allow fish cardiac myocytes to remain functional at low temperatures. Additionally, by demonstrating that there are no functional differences between ScTnC and IFcTnC the results suggest that it is the effect of pH on the affinity of IFcTnC that allows for cardiac function at sub-zero temperatures.

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Acknowledgments

This work was funded by the Heart and Stroke Foundation of Canada and NSERC of Canada.

PRECONDITIONING AND ANOXIA TOLERANCE OF THE TROUT HEART: NON-ADDITIVE EFFECTS AND THE ASSESSMENT OF MYOCARDIAL DAMAGE.

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

Although interspecific variation in myocardial hypoxia-tolerance has been extensively studied in fishes (Driedzic and Gesser, 1994), intraspecific differences have received little attention. In general, rainbow trout are considered to be a hypoxia-sensitive species. However, in recent years, we have identified groups of rainbow trout that vary greatly in their hypoxia-tolerance, and have used these fish to examine the relationship between inherent hypoxia-tolerance and preconditioning. Preconditioning is a phenomenon whereby a brief insult or stimulus to the heart greatly diminishes myocardial damage following a subsequent prolonged bout of hypoxia and/or ischemia, and the mammalian literature suggests that only hypoxia-sensitive hearts can be preconditioned; ie. these mechanisms of myocardial hypoxic protection are not additive.

This talk will focus on preconditioning experiments performed on hypoxia-sensitive trout hearts at Simon Fraser University (SFU) (Gamperl *et al*., 2001) and on hypoxia-tolerant hearts at both Portland State University and SFU, and will incorporate recent data that questions whether both loss of function and cell death (irreversible damage) can be used as criteria to assess myocardial damage following severe hypoxic/anoxic exposure in fishes.

Methods

In situ heart preparations (Farrell *et al*., 1986) at 10°C were used to determine myocardial hypoxia tolerance in three stocks of hatchery-reared rainbow trout, and to examine whether hearts from each stock could be preconditioned. In these experiments, the preconditioning stimulus was one or two brief (5 min) anoxic periods, and functional damage was assessed by comparing cardiac performance after and prior to experimental manipulations.

Irreversible myocardial damage (cell death) was evaluated by a number of techniques including: 1) measurement of creatine kinase (CK) activity, lactate dehydrogenase (LDH) activity and [myoglobin] in the perfusate being pumped by the *in situ* hearts at 5 min to 1h post-anoxia; 2) measurement of myocardial LDH activity in *in situ* hearts following reperfusion; and 3) incubation of small (4-6 mg) myocardial pieces with MTT (3-[4,5]-dimethylthiazol-2,5-diphenyltetrazolium bromide).

Results and Discussion

In the three groups of trout examined, there was a significant degree of variation in inherent-anoxia tolerance. Maximum cardiac function in the hearts used by Gamperl *et al.* (2001) was reduced by approx. 20-25% after just 15 minutes of anoxia (with only 5 min at physiological output pressure, 50 cm H_20). In contrast, comparable reductions in myocardial function could only be achieved at PSU, and more recently at SFU, by significantly increasing both the duration of anoxia (to 20 or 30 min) and the amount of work performed by the heart during anoxia (by maintaining output pressure at 50 cm H_20 and/or attempting to maintain resting a cardiac output of 16 ml min^{-1} kg^{-1}). Thus, a significant amount of intra-specific variation in myocardial hypoxia tolerance exists between populations (groups) of hatchery-reared rainbow trout. Although the reasons for this are unknown, water quality during rearing is likely to play a predominant role.

Of the three stocks of rainbow trout examined, it was clear that only the hypoxia-sensitive hearts used by Gamperl et al. (2001) could be preconditioned by anoxic pre-exposure. For example, although these authors report that preconditioning completely eliminated the myocardial dysfunction associated with 15 min of anoxia, the hypoxia-tolerant hearts tested at PSU were not protected, and appeared to be damaged further, by an identical preconditioning stimulus (Fig. 1). These results agree with studies on neonatal mammals (e.g.

Ostadal *et al*., 1999; Baker *et al*., 1999) which show that hearts with an innate level of myocardial hypoxia tolerance cannot be preconditioned (i.e. the protection afforded by inherent hypoxia-tolerance and preconditioning are not additive), and suggest that the cellular pathways and/or effectors that confer protection against ischemic/hypoxic damage are already maximally stimulated in hypoxia tolerant hearts.

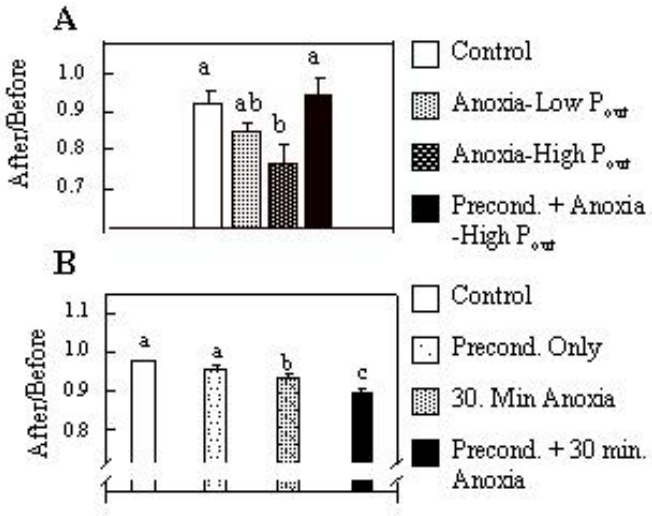

Figure 1. Effect of 5 min. of anoxic pre-exposure (preconditioning) on the recovery of maximum stroke volume in the hypoxia-sensitive hearts from Gamperl *et al*. (2001) (**A**) and the hypoxia-tolerant hearts used at PSU (**B**). For the hearts used in Gamperl *et al*. (2001), the main anoxic period was only 15 min. In both experiments, control hearts were only exposed to normoxic perfusate. Dissimilar letters indicate significant differences between groups ($P < 0.05$, one-way ANOVA). N = 7 - 9

To this point, none of the biochemical markers of myocardial damage that we have used has proven satisfactory for quantifying the degree of irreversible cell death in *in situ* hearts following anoxic exposure: LDH, CK and myoglobin were not detectable in the perfusate; no significant differences in myocardial LDH levels were found in trout hearts exposed to 0, 10, 20 or 30 min. of anoxia (maximum degree of functional damage approx. 20%) (Fig. 2A); and

preliminary experiments with MTT failed to reveal any differences in tissue damage between control and anoxia-exposed hearts. However, these experiments (Fig. 2B) with MTT staining of myocardial pieces incubated under various conditions suggest that the MTT assay works, and that trout myocardial cells may not die/become irreversibly damaged for some time even when exposed to prolonged (> 5 hrs) anoxia.

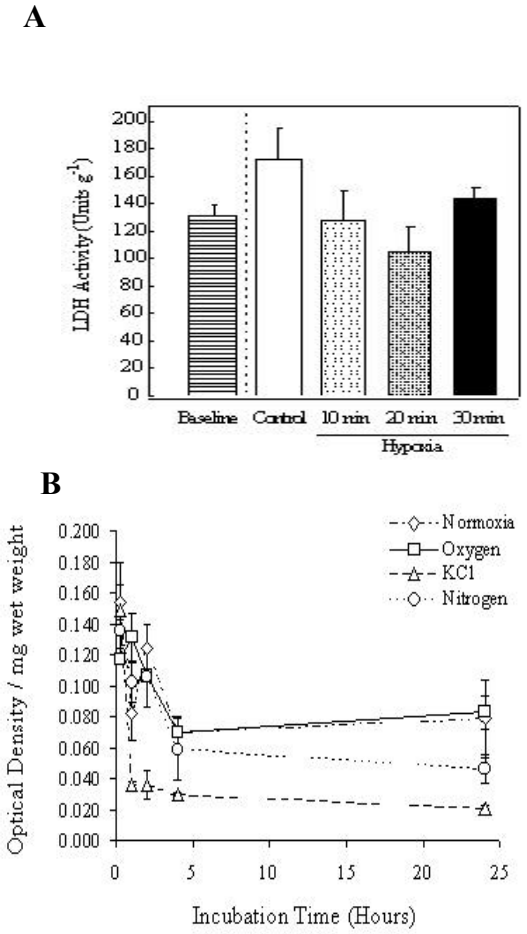

Figure 2. Biochemical assessment of myocardial cell death (irreversible damage) in the trout heart. (**A**) LDH activity in trout hearts exposed to

normoxia, or anoxia of increasing duration (N = 7 – 8). (**B**) MTT staining in myocardial pieces [4 – 6 mg; N = 1 (3 sub-samples per group] exposed to saturated KCl for 10 min, or exposed to various levels of perfusate oxygenation. Note: Since the loss of MTT staining in the 3 non-KCl groups was identical during the first 4 h of incubation, we believe this represents mechanical damage related to cutting the myocardium into such small pieces.

When combined, our experiments suggest that: 1) the trout heart is merely "stunned" following brief periods of anoxic (severe hypoxic) exposure; and 2) that myocardial cell death only results following anoxic/severe hypoxic periods of several hours. These results are in contrast to those obtained with the mammalian heart, but not surprising given that trout generally live in cool water and that most of the trout heart is perfused with venous blood whose PO_2 can be reduced to < 10 mm Hg during intense exercise or aquatic hypoxia.

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Acknowledgements

This work was supported by a American Heart Fund grant to A.K. Gamperl (Ref. # 9960208Z), an Natural Sciences and Engineering Research Council of Canada operating grant to A.P. Farrell, and a Danish Research Council grant to Tobias Wang.

EFFECTS OF STEROIDS AND THEIR ANTAGONISTS ON THE CARDIAC PERFORMANCE OF RAINBOW TROUT (*ONCORYNCHUS MYKISS)*

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EXTENDED ABSTRACT ONLY- DO NOT CITE

Positive effects of steroid hormones and their antagonists on cardiac tissue in mammals are well known. For example, Valencia *et al.* (1989) demonstrated positive inotropism in rat atrium after addition of testosterone and dihydrotestosterone; this effect was blocked by antiandrogens. However, the acute effects of steroids in teleost cardiac tissue have not been studied. The goal of this research was to investigate the effects of steroids on contractile performance of cardiac tissue from rainbow trout. We also tested whether steroid effects involve activation of transcription or protein synthesis.

Experimental Animals

Immature male and female (251 ± 9 g; 27.2 ± 3.4 cm) and sexually mature male (1216 ± 348 g; 44.2 ± 4.2 cm) rainbow trout were obtained from Clear Springs Foods, Inc. (Buhl, Idaho).

Ventricle strip preparation

Fish (n = 6 for each treatment) were killed by a sharp blow to the head, the ventricle was excised and placed in ice-cold teleost Ringers solution (pH 7.6). Four uniform strips were cut from each ventricle (dry weight = 1.5 ± 0.5 mg).

Each strip was tied with surgical silk and attached to a Kent isometric transducer, bathed in Ringers at 14°C, and gassed with 99.5% O_2 / 0.5% CO_2 in tissue baths. Strips were stimulated to contract with maximum voltage (60V) at 0.5 Hz and the length of each strip was adjusted to produce maximal twitch force. After equilibration for 1 h, physiological levels of androgens (T, 11KT) and cortisol dissolved in ethanol were administered and recordings were taken continuously for 30 min. Pre-treatment (10 min) with flutamide and RU486, (inhibitors of androgen and cortisol receptors, respectively) were used to determine if steroid effects involved specific receptors. In addition, cycloheximide or actinomycin D were used to determine whether steroid effects were non-genomic. Cholesterol was used to ensure that cardiac performance did not improve with general steroid administration. Twitch force development was measured using the Biopac MP100 system and Acknowledge software. Changes in twitch force development were normalized (%) based on initial equilibration.

Data analysis

Differences between control and steroid treated strips were assessed by ANOVA ($p < 0.05$).

Results

Both androgens (T, 11KT) increased contractile force 40% within 10 min in strips from male rainbow trout. However, this effect was not seen in female rainbow trout. In males, flutamide effectively blocked the increases induced by T and 11KT (Figure 1A). Cortisol increased contractile force in both males (40%) and females (20%) within a similar time. RU486 blocked cortisol effects in both males and females (Figure 1B). The optimal concentration for increasing contractile force was 0.03μM for the sex hormones and 0.01mM cortisol.

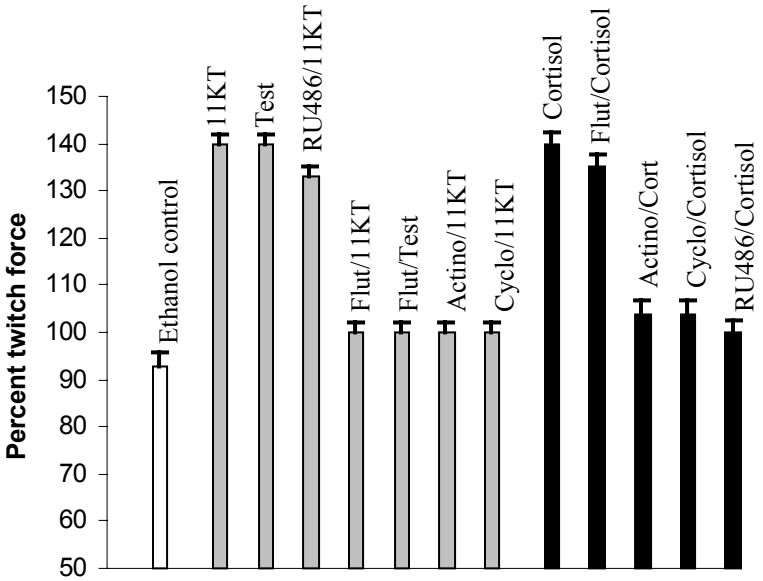

Fig. 1A. Percent of original twitch force (+SE) in ventricle strips from **male rainbow trout** after exposure to steroids and steroid antagonists (n = 6 for each treatment). 11-ketotestosterone (11KT), Testosterone (Test), Flutamide (Flut), Actinomycin (Actino), Cycloheximide (Cyclo).

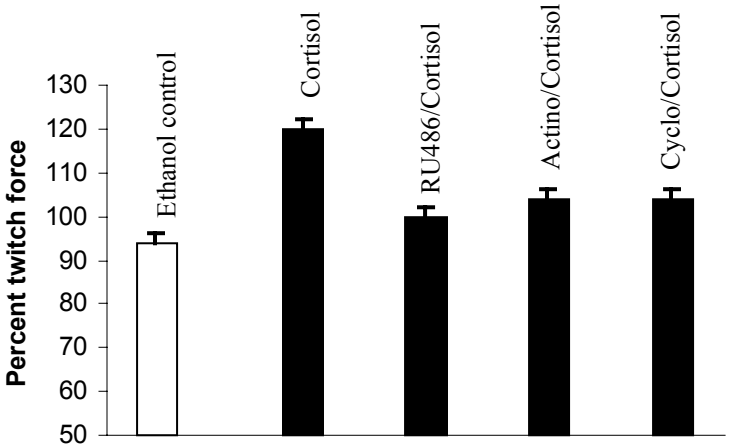

Fig. 1B. Percent of original twitch force in ventricle strips from **female rainbow trout** after exposure to cortisol and steroid antagonists (n = 6 for each treatment). 11KT or T strips were not significantly different from ethanol control. Actinomycin (Actino), Cycloheximide (Cyclo).

However, flutamide had no effects on cortisol treated strips, and RU486 did not affect T or 11KT treated strips. Actinomycin D or cycloheximide almost completely inhibited the increased twitch force observed after treatment with T, 11KT or cortisol. Cholesterol had no effects on contractile performance.

These results demonstrate that physiological concentrations of specific steroids can enhance ventricular contractility in rainbow trout. In males, testosterone, 11KT, and cortisol each act through their own receptors and may involve transcription and translation. Cortisol had similar effects in females. Although effects are realized within 10 min, it appears that the improved inotropism may involve transcription or protein synthesis. Ultimately, steroid mediated improvements in cardiac performance might enhance swimming activity during migration, spawning or other stressful periods.

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Acknowledgements

The work described was supported by a grant from the Idaho State University Graduate Student Research and Scholarship Committee (681-006-01).

We extend a special thanks to Scott Williams at Clear Springs Foods Inc. (Buhl, ID).

THE INOTROPIC INFLUENCE OF ANGIOTENSIN II ON THE WORKING HEART OF THE EEL (*Anguilla anguilla*): PARACRINE ASPECTS AND SUBCELLULAR MECHANISMS

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Angiotensin II (Ang II) not only is a pluripotential hormone but is also an autocrine-paracrine cardiac factor modulating short- (i.e. inotropism) and long-term (i.e. hypertrophy) adaptations of the heart. While a wide range of species-dependent variations is apparent in the inotropic effects of Ang II in mammalian myocardia, there is a lack of information regarding the direct influence of this hormone on the mechanical performance of the fish heart.

Therefore, experiments were carried out in fresh water eels (*Anguilla anguilla*) using an isolated working heart preparation, previously set up in our lab (Imbrogno et al., 2001), to explore whether Ang II exerted direct inotropic effects and to clarify the underlying mechanisms. Ang II ($10^{-11} \div 10^{-7}$ M) elicited negative chronotropic influence and caused on the electrically paced preparations a cardio-suppressive action, decreasing stroke volume (SV) and

stroke work (SW). This negative inotropic effect was abrogated by a selective AT_1 subtype antagonist, CV 11974 (10^{-7} M), specific for non mammalian vertebrates, while it was not antagonized by the mammalian AT_1 antagonist losartan or the AT_2 antagonist CGP42112. The Ang II-mediated inotropism was abolished by exposure to either pertussis toxin (PTx; 10^{-11} M) or atropine (10^{-6} M), indicating an involvement of the Gi protein system and the muscarinic cholinergic receptors, respectively. In contrast, it was not affected by pre-treatment with the adrenergic receptor antagonists propanolol, sotalol and phentolamine.

Nitric oxide (NO) is an important modulator of the mechanical performance in the eel heart (Imbrogno et al., 2001). Using donors (L-arginine) and inhibitors (L-NMMA, L-NIO) of nitric oxide synthase (NOS), as well as hemoglobin as NO scavenger, we have demonstrated the obligatory role of the nitrergic signalling in mediating the Ang II response. Pretreatment with either ODQ (10^{-6} M), a specific inhibitor of soluble guanylate cyclase (GC) or the inhibitor of the cGMP-activated protein kinase (PKG) KT5328 (10^{-7} M) abolished the Ang II-mediated inotropism, indicating that the cGMP-PKG component is a crucial target of the NO signal-transduction pathway.

Interestingly, while NO remarkably modulates the Frank-Starling response of the eel heart (Imbrogno et al., 2001), Ang II *per se* did not affect this mechanism. This stresses the importance of the *local* cardio-suppressive action of Ang II in regulating cardiac performance in the eel heart without deterioration of its intrinsic eterometric modulation.

In the eel heart, the endocardial endothelium (EE) is a major source of NO (Imbrogno et al., 2001). The functional integrity of the EE is a prerequisite for mediating intracavitary Ang II-mediated inotropic signals, since these are abolished by pre-treatment of the EE with Triton X-100. This emphasises the autocrine-paracrine role of the EE in the control of fish heart function.

In conclusion, these results provide the first evidence in fish that endoluminal Ang II exerts a direct cardio-suppressive action on the mechanical performance of the heart via an EE interaction, thereby activating G protein coupled-AT_1 type receptors which trigger a NO-cGMP-protein kinase G signalling transduction pathway. The cardio-depressive action of Ang II does not influence the Frank-Starling response of the heart. These data, together with the involvement of the muscarinic receptors in mediating the Ang II inotropic stimulation, suggest that the EE caveolae (i.e. the plasma invaginations located at or near the plasma

membrane) can be attractive candidates as the domains in which the tonic-phase Ang II-NO signalling is generated.

Reference

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SWITCH FROM VOLUME PUMP TO PRESSURE PUMP

VENTRICLE: A MORPHODYNAMIC STUDY IN THE EUROPEAN

EEL (*Anguilla anguilla)*

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Due to its structural and functional plasticity the fish heart ventricle adapts to volume and pressure hemodynamic loads by distinct chamber remodellings (Tota and Gattuso, 1996). This can be best illustrated by ranking elasmobranchs and teleosts on the basis of the relative contribution of pressure and volume to the stroke work. The spectrum of cardiac dynamic patterns obtained allows to distinguish between heart ventricles producing mainly pressure work (prototype: tuna) and those producing mainly volume work (prototype: Antarctic icefish) (Tota and Gattuso, 1996). This adaptational flexibility of the ventricle chamber is also illustrated by some fish species that either by growing or under

particular environmental challenges can move from one ventricular pattern to another. As a paradigm of this point we have studied cardiac rearrangements in fresh water eels (*Anguilla anguilla*). Changes in mechanical cardiac performance and heart morphology relative to body growth were analysed in juvenile (96.8 ± 27.5 g body weight, mean ± SD) and adult (656 ± 12 g body weight, mean ± SD) fish. Cardiac performance, assessed using an *in vitro* isolated working heart preparation (Imbrogno et al., 2001), showed similar Frank-Starling responses in the two groups. However, while the adult eel hearts were able to maintain stroke volumes (SV) up to an afterload of 6 kPa, the juvenile fish showed a rapid decrease of SV when afterload was increased above 3 kPa. Thus, the adult fish appears better suited to maintain cardiac output in relation to increased outout pressure. Increases of either the preload or the afterload did not significantly alter heart rate in the two groups. Morphometric evaluation was achieved by histology, transmission electron microscopy and image analysis. Both juvenile and adult show a typical mixed type of ventricle (compacta + spongiosa). The ventricular growth was achieved by increasing both the compacta and the spongiosa without a corresponding increase of the ventricular lumen. In fact, the surface area of the lacunary spaces was relatively lower in the adult ventricle with respect to the juvenile. The whole adult ventricle was characterised by a higher number of smaller myocytes than the juvenile counterpart. Thus, hyperplasia of the existing myocytes can be considered a major mechanism for the increase of the ventricular mass. In contrast to the adult, in the juvenile ventricle the compacta showed a lower vascular supply, as indicated by the significantly higher myocardium/vessel ratio. The ventricular myocardial growth is paralleled by enlargements of both the myofibrillar and the mitochondrial compartments.

In conclusion, the morphodynamic analysis of juvenile and adult hearts of *Anguilla anguilla* illustrates how the ventricle pump is able to operate in a very flexible manner in relation to the growth of this teleost. On the basis of its mechanical behaviour the juvenile heart ventricle, also due to its relatively larger lacunary space, is better designed to produce volume work. In contrast, the adult ventricle appears better suited to produce pressure work. This shift from volume pump to pressure pump ventricle is accompanied both in the compacta and spongiosa by myocardial remodelling partly due to hyperplasia, together with enlargement of the myofibrillar and mitochondrial compartments. The adult heart ventricle exhibits also an increased vascularization to match the enhanced contractile demands.

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CARDIAC FUNCTION AND CALCIUM MANAGEMENT IN THE SOUTH AMERICAN LUNGFISH, *Lepidosiren paradoxa*

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EXTENDED ABSTRACT ONLY-DO NOT CITE

Introduction

Contractile mechanisms (i.e., actin-myosin interaction and its regulation by calcium) appear to be similar in all vertebrate hearts (Driedzic and Gesser, 1994). However, anatomical and ultra-structural distinctions between hearts of different species underlie important physiological differences, particularly to the regulation of calcium delivery to contractile apparatus (Tibbits et al., 1992).

Among these differences, the putative contribution of the sarcoplasmic reticulum (SR) calcium stores to excitation-contraction coupling (E-C coupling) has a profound impact on calcium management, since this organella is able to reduce significantly difusional distances and accelerates both contraction and relaxation. In most lower vertebrates studied, the main source of calcium activator is the extracellular space by its influx across the sarcolemma on a beat to beat basis, although the functional relevance of the cardiac SR in supplying calcium varies with temperature, stimulation frequency and species (Bers, 2001).

Material and Methods

Active lungfish were obtained from water filled clay pits in Brazilian Pantanal area and acclimated at 25 ^{o}C. Pairs of ventricle strips ($\phi \cong 1$ mm) were excised from the ventricle and placed into a bathing medium containing (in mM) 100 NaCl, 5 KCl, 1.2 $MgSO_4$, 1.5 NaH_2PO_4, 27 $NaHCO_3$, 2.5 $CaCl_2$ and 10 glucose and bubbled with a gas mixture of 98% O_2 and 2% CO_2 throughout the experiment. Preparations were connected to an isometric force transducer and to a stimulator delivering electrical square pulses with a voltage 50% above that of the threshold value. Twitch tension was allowed to stabilize for about 30 min at 0.2 Hz before each protocol.

In order to analyze the main source of calcium-activator, contraction frequency was increased at different temperatures (15, 25 and 35 ^{o}C) in Control preparations (physiological calcium concentration - 2.5 mM), High Calcium (supraphysiological calcium concentration - 10.5 mM) and Ryanodine (2.5 mM of calcium + 10 μM of ryanodine). When applied, ryanodine was added to the medium at least 40 min before changes in frequency. Moreover, *in vitro* frequencies were also compared to *in vivo* heart rate obtained to *L. paradoxa* by Costa et al. (2002).

Results

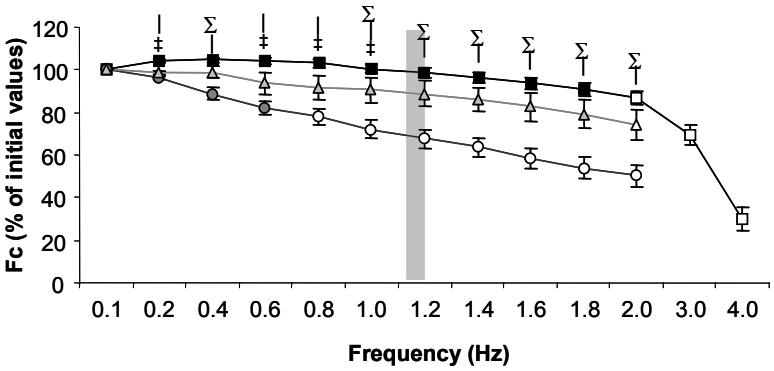
The dashed area in figure 1A indicates that the twitch force (Fc) of control preparations showed a significant increase ($p < 0.05$) when physiologically relevant stimulation frequencies (0.29 ± 0.01 Hz) were reached at 15 ^{o}C. However, when treated with ryanodine, the strips could not contract regularly to reach the *in vivo* frequency range (force-frequency relationship shifted to the right), while it was observed a decrease in force development (relationship shifted downwards) at this stimulation range when strips were tested in a supraphysiological calcium concentration.

At acclimation temperature (25 ^{o}C), when force-frequency responses were analyzed at frequencies inside the *in vivo* frequency range (0.54 ± 0.03 Hz; dashed area in figure 1B), ryanodine treatment resulted in a significant reduction ($p < 0.05$) in twitch force, which was further reduced in response to exposure to a supraphysiological calcium concentration.

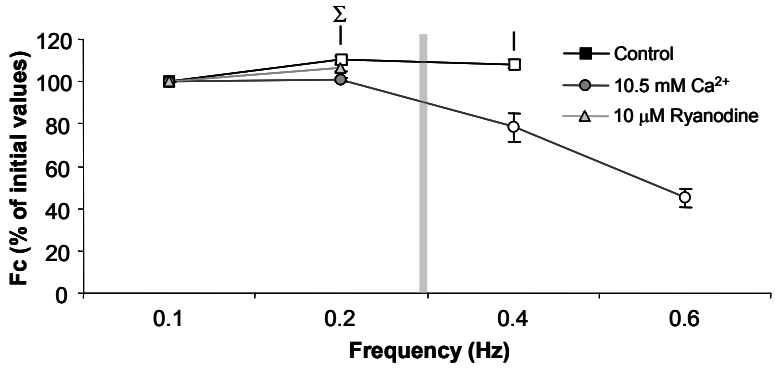
Finally, when the inotropic responses are analyzed at frequencies inside the *in vivo* range (1.17 ± 0.03 Hz; dashed area in figure 3) at the highest

temperature (35 °C), the force-frequency relationship was shifted downwards only in response to increases in extracellular calcium, while ryanodine had no effect on force development at physiologically relevant frequencies at 35 °C.

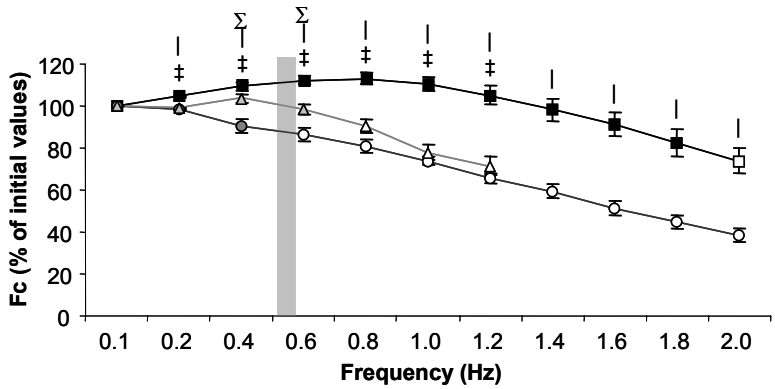
Figure 1 - Effect of increases in stimulation frequency on twitch force (Fc - % of initial values) of ventricle strips from *L. paradoxa* at different experimental conditions (Control: 2.5 mM of calcium and without ryanodine; 10.5 mM of Calcium; 10 µM of Ryanodine) at 15 °C (n = 10; A), 25 °C (n = 10; B) and 35 °C (n = 10; C). For each treatment, opens symbols indicate a significant difference ($p < 0.05$) in relation to the Fc observed initially (at 0.1 Hz). ⊣ - Control ≠ 10.5 mM of Calcium; ‡ - Control ≠ 10 µM of Ryanodine; Y - 10.5 mM of Calcium ≠ 10 µM of Ryanodine in the same frequency ($p < 0,05$). The dashed area indicates the heart frequency observed *in vivo* at each temperature by Costa et al., 2002. Mean values ± 1 S.E.M.



A


Σ
120
100
80
60
40
20
0
Fc (% of initial values)
0.1
0.2
0.4
0.6
Frequency (Hz)
Control
10.5 mM Ca2+
10 μM Ryanodine

B


Σ
Σ
‡
120
100
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40
20
0
Fc (% of initial values)
0.1
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
Frequency (Hz)

C

Discussion

The results indicate that the species, in spite of having a potentially functional and anatomically well-developed SR (Hochachaka and Hulbert, 1978), this organella probably presents a slower calcium-cycling capacity, which does not allow it to play a functional role at higher heart frequencies that are usually observed *in vivo* in response to increases in temperature.

However, when temperature is decreased, SR plays a central role in calcium regulation, since it can be observed a temperature-dependent decrease in chronotropism. Furthermore, this organella becomes essential to cardiac performance maintenance when the lowest temperature (15 ^{o}C) is reached, compensating the temperature-dependent decrease in Na^{+}/Ca^{2+} exchanger activity (Costa et al., 2002). These results contrast with those described to most fish already studied, in which ryanodine does not have any effect on force development when physiological frequencies and/or temperatures are considered (Shiels and Farrell, 1997).

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Acknowlegements

This work was supported by a doctoral grant from FAPESP to Monica J. Costa (# 98/11846-6).

ASSESSMENT OF VENTRICULAR FUNCTION IN RAINBOW TROUT: COUPLING OF ELECTROCARDIOGRAPHY AND ECHOCARDIOGRAPHY

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EXTENDED ABSTRACT ONLY- DO NOT CITE

Introduction

Male rainbow trout (*Oncorhyncus mykiss*) experience pronounced cardiovascular changes during sexual maturity. Relative ventricle mass [(ventricle weight/body weight) X 100)] can nearly triple, and compliance and distensibility of the bulbus arteriosus is reduced, probably in response to increased mineralization of its elastic medial layer (Heidel *et al.* 1997). These animals also become hypertensive (Clark and Rodnick 1999). Until now, direct measurement of how these changes influence cardiac performance and blood flow dynamics has remained unexplored. The objective of the present study was to characterize cardiovascular changes in male trout during sexual maturation using echocardiography, Doppler imaging, and electrocardiography.

Methods

We used high-resolution, 2-dimensional echocardiography to conduct longitudinal studies of ventricle morphometry and distensibility of the bulbus arteriosus in the same males at peak sexual maturity and in a post mature (gonadally-regressed) state, 5 months later. Immature males and females were included as controls. All animals were anesthetized with MS-222 and kept at 14°C during studies. Using simultaneously recorded ECG, we measured ventricular size at diastole and systole, using ECG landmarks as cardiac cycle references. Similarly, the lumen dimensions of the bulbus arteriosus were measured at fully distended and fully relaxed states. The change in area (from 2-dimensional images) provided an index of distensibility Further, we compared three volume flow/stroke volume estimates derived from our echocardiographic studies: 1) Valve aperture time analysis (VATA), 2) Velocity-time integral (VTI), and 3) Ventricular dimension analysis (VDA) and compared them with published values.

VATA was performed on both atrioventricular and ventriculobulbar valves. This analysis requires accurate measurements of valve diameter, blood velocity through the valve aperture, and duration of valve aperture opening. To calculate VATA, we use the equation: $V_{flow} = A_{valve} \times D_{flow} \times V_{blood}$, where V_{flow} is the volume flow (cm^3/s), or stroke volume, A_{valve} is the cross-sectional area (cm^2) of the valve aperture, D_{flow} is the duration (s) of flow through the valve, and V_{blood} is the mean velocity (cm/s) of blood moving through the valve. We measured maximum diameter of valve aperatures using long-axis images and real-time color Doppler imaging, from which we calculated valve cross-sectional area. We also obtained both velocity and flow duration directly from the Doppler profile, a graphical representation of flow velocity versus time. VTI was measured directly from Doppler profiles of both atrioventricular and ventriculobulbar valves. The VTI is simply the area under the velocity versus time plot taken at a particular valve and provides a relative measure of valvular flow. VDA requires accurate ventricular area/volume measurements from long-axis (sagittal) images. Comparing diastolic versus systolic ventricular area/volume provided an estimate of stroke volume.

Results

The simultaneous use of echocardiography, Doppler imaging, and electrocardiography provides opportunities for noninvasive, *in vivo* studies on

cardiac function in anesthetized fish. As predicted, sexually-mature rainbow males had stiffer bulbus arteriosi than immature male or female trout. Quantifying bulbus arteriosus stiffness and its implications on cardiovascular hemodynamics will be discussed in detail during our presentation. Estimates derived from VATA correspond to previously reported values from trout (Franklin and Davie 1992) and will also be highlighted.

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A COMPARISON OF MYOCARDIAL ß-ADRENORECEPTOR DENSITY AND LIGAND BINDING AFFINITY AMONG SELECTED TROPICAL FISHES

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EXTENDED ABSTRACT ONLY – DO NOT CITE

In fish, the β-adrenoreceptor (β-AR) signaling pathway is known to mediate the cardiac actions of adrenaline primarily via receptors of the β_2 subtype (Ask et al., 1980; Gamperl et al., 1994). Temperature acclimation can alter the response of the heart to adrenaline and some of this change has been attributed to a temperature-dependent change in cell surface β-AR density. Olsson et al. (2000) found that B_{max} and K_d differed among a limited number of tropical, temperate and Antarctic teleost fish species alluding to the possibility that intrinsic differences in β-AR density and binding affinity may exist among species adapted to different temperatures. The purpose of this study was to examine interspecific variation in myocardial β-adrenoreceptor density (B_{max}) and binding affinity (K_d) for ventricular tissue in 7 previously unstudied species of tropical fish.

Materials and Methods

Quantification of ß-Adrenoreceptors

Cell surface ß-adrenoreceptor density and binding affinity were determined for ventricular punches, using a tritiated ligand technique (Watson-Wright et al., 1989; Gamperl et al., 1994). Ventricular tissue punches were incubated with various concentrations (0.05-3.5nM) of the hydrophilic $ß_2$-adrenoreceptor ligand [^{3}H] CGP-12177.

The mass of individual ventricles determined the degree of replication for the binding assays. Assays were replicated up to six times and consisted of 6-8 replicates for each ligand concentration.

Data Analysis

Where only one binding curve was performed, mean values ± SEM are presented for the replicates at each ligand concentration. Where more than one ligand binding curve was performed, mean values ± SEM are presented for all specimens. Binding parameters were determined using a Scatchard plot (Zivin and Waud, 1982). Protein content of representative punches was determined using a Bradford protein assay so that B_{max} could be expressed as fmol mg protein^{-1}.

Results

B_{max} values ranged from 19.5 to 52.8 ± 8.0 fmol mg protein^{-1} (Figure 1). The highest B_{max} values were observed in marble goby, blue spotted fantail ray, sleeper, and snakehead. B_{max} was significantly higher than rainbow trout in blue spotted fantail ray and marble goby (Figure 2).

Ligand binding affinity (K_d) varied from 0.19 ± 0 .02 to 1.05 ± 0.11 nm among the 8 species (Figure 1). K_d of both African catfish and Blue spotted fantail ray was significantly higher when compared with rainbow trout ($p < 0$ .05, Figure 2).

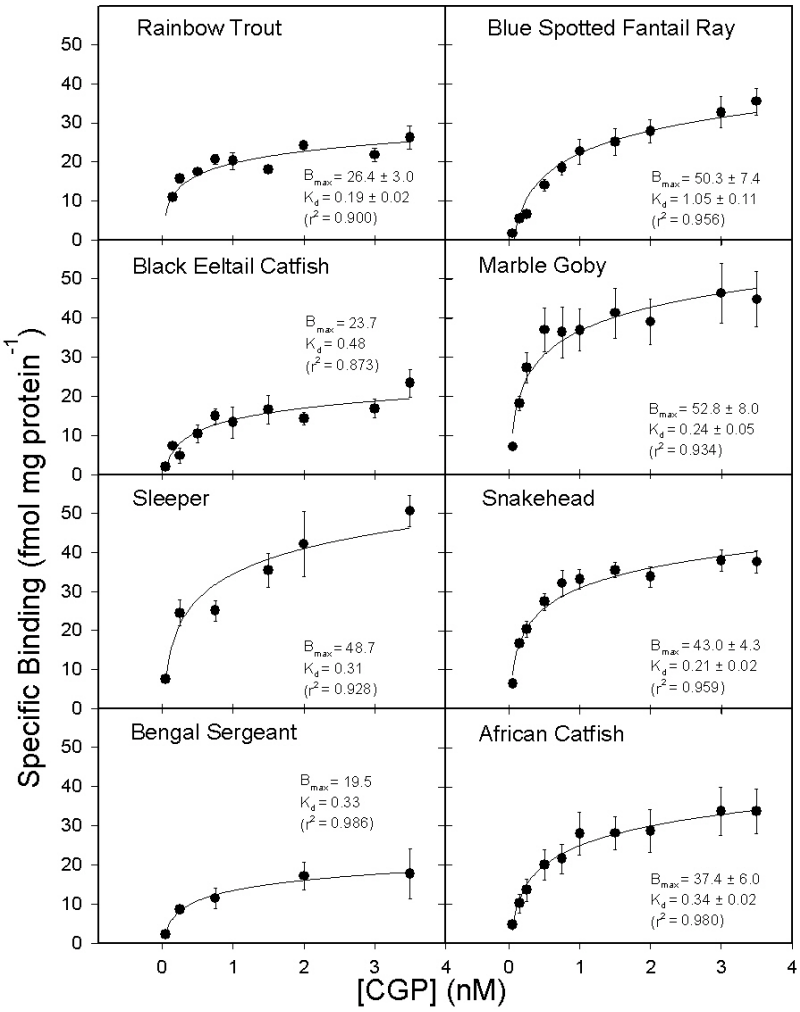

Figure 1 - Specific binding of [^{3}H]CGP-12177 to ventricular β-adrenoreceptors in rainbow trout (N=6), blue spotted fantail ray (N=6), black eeltail catfish (N=1, 10 hearts pooled), marble goby (N=6, 19 hearts), sleeper (N=1, 10 hearts),

snakehead (N=6, 17 hearts), Bengal sergeant (N=1, 10 hearts) and African catfish (N=6, 15 hearts). The B_{max} (fmol mg protein^{-1}), K_d (nM) and r^2 values for each graph are indicated. Values are mean ± SEM.

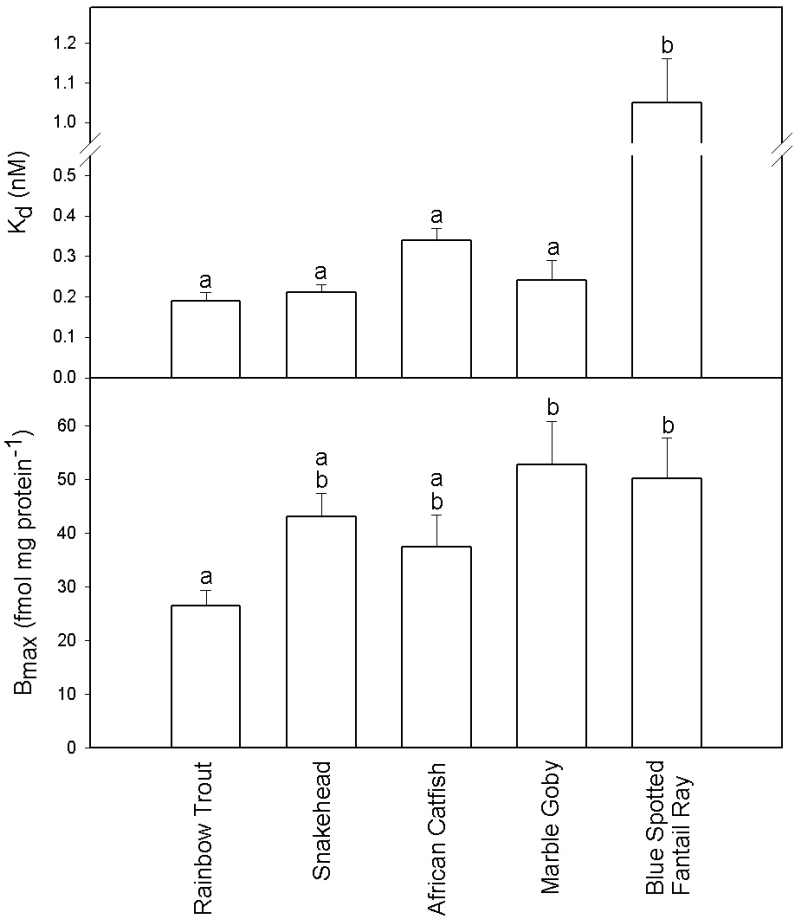

Figure 2 - ^{3}[H]CGP-12177 dissociation constant (K_d) and ventricular β-adrenoreceptor density (B_{max}). Values are presented as mean ± SEM. Dissimilar letters denote significant differences at $P<0.05$. N=6 for all species.

Conclusions

We demonstrated variation between species of over twofold in B_{max} and almost sevenfold in K_d. Our results for rainbow trout compare favorably with earlier studies giving us confidence in the methods utilized to characterize myocardial β-AR density and binding affinity.

Tropical Marine Elasmobranchs

Blue spotted fantail ray is the first elasmobranch in which β-AR density and binding affinity have been characterized. The B_{max} value for this species was similar to those of the other tropical species we studied. This significantly lower binding affinity observed may be due to variation in β-AR subtypes between teleosts and elasmobranchs.

Tropical Marine Teleosts

Due to the small size of the tropical marine ventricles we were only able to generate 1 binding curve for each species. In this regard the results can only be considered a guideline as to the true values of β–AR density and binding affinity. Neither B_{max} or K_d differed significantly from rainbow trout.

Tropical Freshwater Teleosts

β-AR densities for snakehead, African catfish, and marble goby were all observed to be greater than the rainbow trout. Nevertheless, due to the large standard errors present in the values of the tropical species, the difference was only statistically significant in the case of the marble goby ($P < 0.05$).

The present results suggest that B_{max} is higher in freshwater, but not marine, tropical species. However, Olsson et al. (2000) reported high B_{max} values for both marine tropical species (mahimahi = 46.9, skipjack tuna = 41.3) and marine temperate species (sockeye salmon = 47.5). As of yet, no clear phylogenetic or environmental pattern of β-AR values is evident.

Acknowledgements

Thank you to Nellie Yee and Dr. Kurt Gamperl for their assistance in performing the receptor binding assay. This study was funded in part by research grants to A.P.F and undergraduate support to L.M.H. from the National Sciences and Engineering Research Council of Canada.

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ARE FISH PRIMARILY STROKE VOLUME-MODULATORS? NOT ACCORDING TO CENTRARCHIDS

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Abstract

In most animals, physical or chemical stressors usually elicit an increase in metabolic rate and consequently an increase in cardiac output and one or both of its components, heart rate and stroke volume. While mammals and birds primarily increase cardiac output through the elevation of heart rate (frequency modulation), fish are generally thought to increase cardiac output principally through changes in stroke volume (volume modulation). It has even been suggested that among vertebrates there is an evolutionary trend from volume-modulated to frequency-modulated cardiac output. Within fish, only a few species have been regarded as frequency-modulators including the highly active tuna and the Antarctic nototheniids. In an effort to expand our comprehension of cardiac function in fish, we collected data on several species from two common families, Salmonidae and Centrarchidae, under various conditions. Our preliminary findings indicate that frequency modulation is more prevalent than previously thought especially among centrarchids.

Introduction

Recovery from burst exercise, specifically dealing with oxygen debt and lactate clearance, results in an increased metabolic rate and consequently an increase in cardiac output (CO) and one or both of its components, heart rate (HR) and stroke volume (SV) (Farrell and Jones, 1992). While mammals, birds, reptiles, and amphibians primarily increase CO by elevating HR (i.e., frequency modulation), fish are unique among vertebrates increasing CO primarily through the elevation of SV (i.e., volume modulation) (Farrell, 1991; Farrell and Jones, 1992; Thorarensen et al., 1996). The highly active and evolutionarily advanced tuna have typically been considered the main exception, increasing HR by 2-fold over resting values (Farrell, 1991). Consequently, it has been suggested that there is an evolutionary trend from volume-modulated to frequency-modulated CO in fish (Farrell, 1991). However, recent work in our laboratories has demonstrated that frequency modulation among fish may not be as rare as previously thought.

Methods

We exposed fish to various perturbations that resulted in increased metabolic rate and therefore elevated CO. Our test subjects were fish from several species within the families Salmonidae and Centrarchidae. Brown trout (*Salmo trutta*), Atlantic salmon (*Salmo salar*), largemouth bass (*Micropterus salmoides*), and rock bass (*Ambloplites rupestris*) were chased by hand to elicit burst swimming for 30 to 150 sec and until the fish was completely fatigued (non-responsive, loss of equilibrium) (see Cooke et al., 2001). Immediately following this event, fish were exposed to air for 10 sec to 10 min. The centrarchids were held by the lower lip and supported on the ventral surface while salmonids were held out of water in a wetted sling. Rainbow trout (*Oncorhynchus mykiss*) were electroshocked at various settings for 2 to 32 sec. Smallmouth bass (*Micropterus dolomieu*) were swum in a Blazka-type respirometer and exposed to elevated velocities to elicit burst swimming for 20 to 180 sec and until the fish would no longer swim and/or had lost equilibrium (see Schreer et al., 2001).

Detailed descriptions of surgical procedures and general measurement theory are provided in Schreer et al. (2001). Briefly, in all cases fish were anesthetized and a flexible silicone cuff-type Doppler flow probe (subminiature 20 MHz piezoelectric transducer: Iowa Doppler Products, Iowa City, IA, USA) was placed around the ventral aorta. Cardiac output was monitored and recorded by

a hard-wired flowmeter (545C-4 Directional Pulsed Doppler Flowmeter: Bioengineering, The University of Iowa, Iowa City, IA, USA) and a digital strip-chart recorder (LabVIEW, Version 4.0.1, National Instruments Corporation, Austin, TX, USA). Cardiac output was calculated by averaging the flow index per unit time and HR was determined by counting peaks. The quotient of CO divided by HR yielded SV. Where possible, actual flow rates were calculated directly through a postmortem calibration. Following surgery fish were allowed to recover for at least 12 hr. Blood flow was monitored for at least 1 hr prior to experimentation and for at least 5 hr following the perturbation.

Results and Discussion

There is no simple answer to the question of whether fish are primarily volume-modulators. Salmonids are perhaps the most well studied family of fish and our studies on various salmonids indicate that indeed most species are volume-modulators (Figure 1).

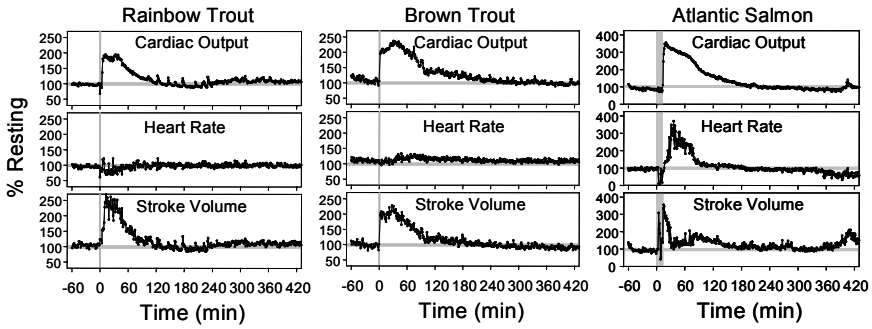

Figure 1. Example cardiac traces for salmonids exposed to various perturbations. All values are percent resting (100%) and the perturbation always begins at time 0. The grey lines indicate the approximate duration of the perturbation (vertical) and the 100% resting value (horizontal). The rainbow trout was held at 12˚C and electroshocked with a pulsed DC electrofisher for 8 sec at 100 V, 80-8 Hz (frequency decreased over 2 sec), and 2 ms (pulse width). The brown trout was held at 12˚C, chased by hand for 1 min, and exposed to air in a wetted sling for 10 sec. The Atlantic salmon was held at 5˚C, chased by hand for 1 min, and exposed to air in a wetted sling for 10 min.

However, among the centrarchids, frequency modulation is apparently quite common (Figure 2). This puts centrarchids in the same company as tuna and Antarctic nototheniids (Farrell, 1991; Farrell and Jones, 1992; Farrell, 1996). In the tuna frequency modulation has been explained by their high activity levels with standard metabolic rates approaching that of mammals (Farrell, 1991). As well, tuna, like mammals, have large ventricles that can beat faster and may have relatively fixed stroke volumes which are normally near maximum values (Farrell, 1991). Similarly, Antarctic nototheniids also have large ventricles and high stroke volumes at resting levels (Farrell, 1996). Centrarchids are generally an active group of fish (above ~10˚C), but their activity levels are still not comparable to that of tuna. It has been hypothesized that frequency modulation (in addition to a suite of other physiological and anatomical adaptations, e.g. coronary circulation) in centrarchids may have evolved in concert with parental care provided by the males (Cooke, *unpublished data*). This type of cardiac control may allow centrarchids to provide protracted and heightened care to developing offspring and to respond quickly to predator threats.

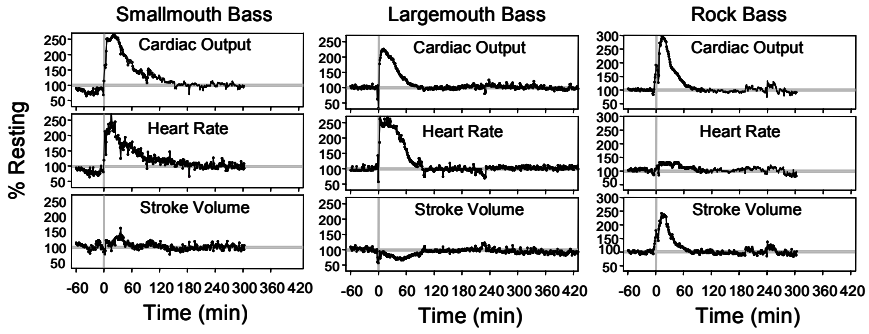

Figure 2. Example cardiac traces for centrarchids exposed to various perturbations. All values are percent resting (100%) and the perturbation always begins at time 0. The grey lines indicate the approximate duration of the perturbation (vertical) and the 100% resting value (horizontal). The smallmouth bass was held at 12˚C and exposed to a series velocity burst for 3 min in a Blazka-type respirometer. The largemouth bass and the rock bass were held at 16˚C, chased by hand for 150 and 30 sec, and exposed to air for 150 and 30 sec, respectively.

It is important to note, however, that even within a family of fish or within a specific experiment, results are not always consistent. Atlantic salmon have been shown to increase HR only slightly following angling (Anderson et al., 1998) which is consistent with our results for rainbow trout and brown trout. However, when Atlantic salmon were chased by hand and exposed to air they showed a strong increase in HR (Figure 1). The rock bass were also anomalous among the centrachids showing very little change in HR (Figure 2). As previously mentioned this may be due to the amount of paternal parental care as rock bass generally provide very little relative to largemouth bass and smallmouth bass (Cooke, *unpublished data*).

In considering trends in the relative contributions of HR and SV to CO it is important to consider the type of stressor. In a limited number of cases, it has been suggested that following anaerobic, burst exercise, increases in CO were due to elevated HR (Lucas et al., 1991; Farrell and Jones, 1992). However, HR in electroshocked rainbow trout and in brown trout chased and exposed to air increased very little as compared to the >100% increase in HR for smallmouth bass and largemouth bass post-simulated angling (Figure 2). All of these events would likely result in anaerobic activity (Mitton and McDonald, 1994; Kieffer, 2000).

An additional factor to be considered in the interpretation of the relative contributions of HR and SV to increased CO is recovery time following the surgical procedure. In Atlantic cod, *Gadus morhua*, HR remains elevated for up to 8 to 10 days following surgery (Webber et al., 1998). Therefore, if the relative contributions of HR and SV to increases in CO during exercise are examined within this recovery period, elevated post-surgery resting HR values may prevent typical increases in HR from being observed. However, even though all the fish in the present study were only allowed to recover for greater than 12, but less than 24 hr following surgery, considerable increases in HR were observed for several species especially within the centrarchids. Therefore, it is highly unlikely that these fish had abnormally high resting HRs. Further, we have held smallmouth bass and largemouth bass for extended periods of time (1 to 2 weeks) and not observed any further decrease in cardiac parameters after the 12 hr recovery period (Schreer and Cooke, *In press*; Cooke, *unpublished data*).

In conclusion, as with most biological questions, there is no clear answer as to whether fish are primarily volume-modulators. It is important to note that in our studies we have only examined CO, HR, and SV, and not other physiological or

anatomical variables that also play important roles in determining the relative contributions of HR and SV to changes in CO. However, the findings in this study indicate frequency modulation may be more prevalent than previously thought, at least among the centrarchids, and more work is needed to fully describe and understand evolutionary trends in cardiac function.

Acknowledgements

This work was supported by the University of Waterloo, the Waterloo Biotelemetry Institute (R. McKinley), the Illinois Natural History Survey, the University of Illinois, and the Natural Sciences and Engineering Research Council of Canada. Field and laboratory assistance was provided by Larry de Koning, Carly O'Brien, Amy Dick, Pauline Bloom, Andrea Buckman, Kate Deters, Wendy McCaul, and Karen Dunmall. This paper benefited from editorial comments by Jennifer Lapierre and Geoff Yunker. JFS thanks XOQ.

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EFFECTS OF ADENOSINE IN THE BRANCHIAL CIRCULATION OF *A. anguilla* AND *S. acanthias*: INVOLVEMENT OF NO

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EXTENDED ABSTRACT ONLY: DO NOT CITE

Adenosine (AD) is a potent vasoactive modulator that in mammals dilates systemic, coronary and cerebral vasculatures (Collis, 1989), whereas can exert a constrictor actions in several lung vessel preparations (Lippton et al., 1982). In fish, the scant data indicate variable vasomotive actions of AD, ranging from vasodilation in hagfish (*Myxine glutinosa*) gills (Axelsson et al., 1990) to vasoconstriction in the gills of *Oreochromis niloticus* (Okafor and Oduleye (1986) and rainbow trout (*Oncorhynchus mykiss*) (Sundin and Nilsson, 1996). In the swim bladder vessels of *Anguilla anguilla* (Schwerte et al., 1999) and in the intact coronary tree of the trout (*Oncorhynchus mykiss*) (Mustafa and Agnisola, 1998), AD exerts vasodilation. In mammals, the cardiovascular effects of AD are primarily mediated by A_1 and A_2 receptors, the former causing vasoconstriction and the latter inducing vasodilatation (Ralevic et al., 1998). In the ventral aorta of the dogfish *Squalus acanthias*, in which both receptors have been detected, the A_1 elicited contraction while the A_2 mediated relaxation (Evans, 1992).

Endothelial cells modulate vascular tone via the release of many substances such as nitric oxide (NO), the typical vasodilator in mammals. In fish, both NO and

AD induce atypical effects compared to mammals. Since there is very little information on the role of NO and its involvement in mediating the actions of AD in fish, we have analysed this question in the branchial vasculature of the elasmobranch *Squalus acanthias* and the teleost *Anguilla anguilla.*

An isolated perfused head (*S. acanthias*) and a branchial basket preparation (*A. anguilla*), set up according to the method described by Perry et al. (1982) were used. The preparations were perfused with physiological saline using a peristaltic pump with pulsatile flow and a compliant system (syringe). Afferent perfusion pressure was measured via a pressure transducer connected through a T-tube placed immediately before the input cannula, and its output displayed on a rectilinear pen recorder.

Infusion of the dogfish preparation with saline containing increasing concentrations of adenosine (AD) induced a diphasic effect: AD at lower doses (nano-micromolar range) caused a vasoconstrictory response while at higher doses (millimolar range) caused vasodilation. The curve generated in the eel preparation showed the same profile. However, in the eel the vasoconstrictory response started at much lower concentrations (picomolar range), while the vasodilatory response appeared at the same concentration range observed in the dogfish. In both preparations, the vasoconstrictory and vasodilatory effects were abolished by theophylline (theo). Theo is the classic xanthine inhibitor but it shows weak affinity and subtype selectivity for the AD receptors. We have also used three antagonists of A_1 and A_2 receptors subtypes that in mammals have shown high degree of potency and selectivity, i.e. CPT and DPCPX (antagonists of A_1 receptors) and DMPX (antagonist of A_2 receptors). In the dogfish preparation, exposure to either DPCPX or DMPX elicited a significant vasoconstriction, suggesting removal of an AD-mediated vasodilatory tone. In contrast, in the eel preparation all antagonists had no effect on input pressure. In both preparations, all antagonists (10^{-5} M) blocked both the vasocostrictory and the vasodilatory effects of AD.

To study the putative involvement of the NO/cGMP system in the AD responses, we tested a specific inhibitor of NOS, L-NIO, and a specific soluble guanylate cyclase (sGC) blocker, ODQ. In both dogfish and eel preparations L-NIO abrogated all vasomotor effects of AD, whereas ODQ blocked the AD-mediated vasoconstriction without affecting the vasorelaxant response. These data indicate that the vasoconstrictory response of AD is mediated by a NO-cGMP-dependent mechanism, while the vasorelaxant response is cGMP-independent. In agreement with this, when both preparations were exposed to a

stable analogue of cGMP, 8-Br cGMP, they exhibited a dose-dependent vasoconstriction. Finally, by using the NO donor SIN-1 to test the effect of exogenous NO, we showed a dose-dependent vasoconstrictory effect that in turn was completely blocked by ODQ.
On the whole, these results confirm the branchial NO-cGMP vasoconstrictory tone previously detected in the eel by us (Pellegrino et al., 2002) and provide compelling evidence that the vasoactive role of AD in the branchial circulation of *S. acanthias* and *A. anguilla* involves a NO-cGMP signalling,

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MITOCHONDRIAL ATP-SENSITIVE K^+ CHANNELS (K_{ATP}) INFLUENCE FORCE DEVELOPMENT AND ANOXIC CONTRACTILITY IN A FLATFISH, YELLOWTAIL FLOUNDER (*Limanda ferruginea*), BUT NOT ATLANTIC COD (*Gadus morhua*) HEART.

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Flatfishes often inhabit hypoxic waters and are known to exhibit substantial tolerance to cardiac acidosis and hypoxia. Studies on flounder suggest that flatfish have an atypical cardiovascular response to hypoxia. Winter flounder subjected to hypoxia exhibit increased cardiac output with no bradycardia (Cech et al., 1977). In isolated ventricular muscle from the European flounder, cardiac force development is initially potentiated following exposure to acidosis, before slowly declining over time (Gesser and Poupa, 1979). Gesser and Poupa have proposed that intracellular acidosis may trigger a release of stored mitochondrial Ca^{2+}, subsequently enhancing force production.

Adenosine 5'-triphosphate sensitive potassium (K_{ATP}) channels have been identified in goldfish hearts (Ganim et al., 1998). K_{ATP} channels are activated by a decline in energy status and are most likely to affect cardiac function throughout periods of impaired ATP production, such as hypoxia. In mammalian heart K_{ATP} channels exist on the sarcolemmal membrane (sK_{ATP}) and inner

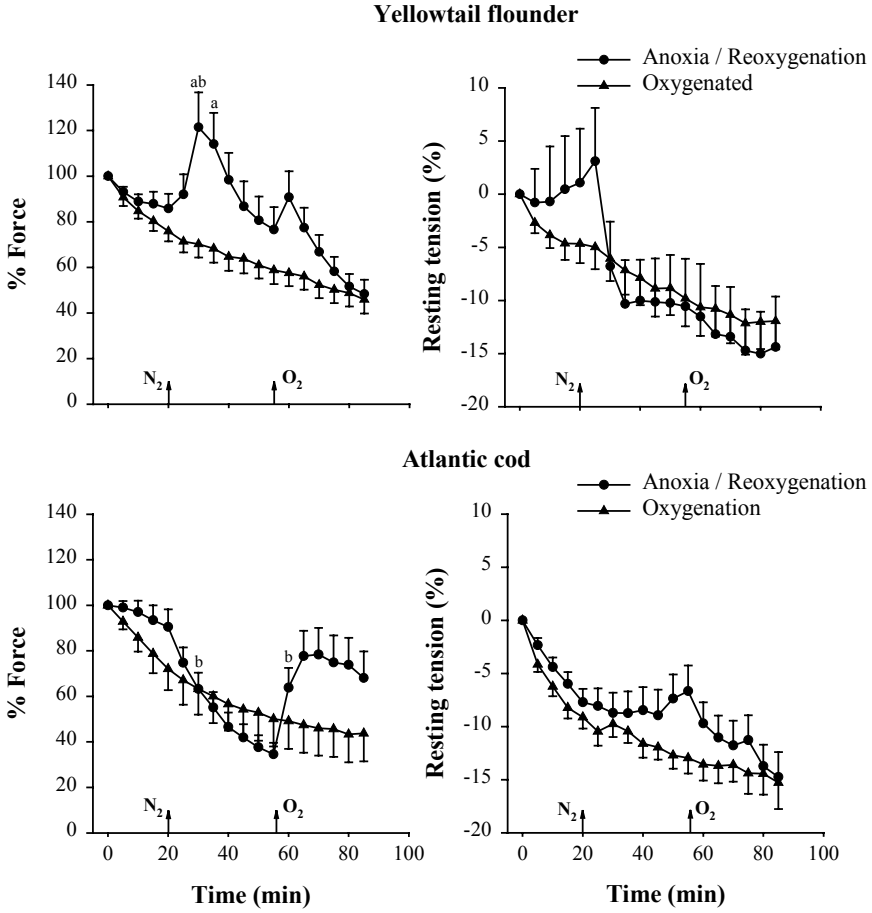
mitochondrial membrane (mK_{ATP}) and their activity has been linked with the cardioprotection afforded by preconditioning. sK_{ATP} channels facilitate cellular K^+ efflux while mK_{ATP} channels allow mitochondrial K^+ influx.

The objective of this study was to evaluate the possible involvement of K_{ATP} channels in hypoxic force potentiation in the yellowtail flounder heart, and to investigate their role in cardiac performance during anoxia and reoxygenation. Atlantic cod were chosen for comparisons as this species is considered to have poor cardiac anoxia tolerance. The contribution of K_{ATP} channels to heart performance during anoxia and reoxygenation was studied using isometrically contracting ventricular muscle preparations and pharmacological agents targeting sarcolemmal and mK_{ATP} channel activity.

Results

Force production in ventricular muscle from flounder is potentiated at the onset of anoxia, while force immediately declines in cod preparations (Fig. 1). Data on flounder preparations treated with agents to alter K_{ATP} activity are presented in Figure 2. Glibenclamide, a general K_{ATP} blocker, impaired oxygenated force development in flounder heart but was without effect on cod (data not shown). The mK_{ATP} specific blocker 5-hydroxydecanoic acid (5HD) improved oxygenated force production in yellowtail flounder heart without influencing contractility during anoxia or reoxygenation. The specific mK_{ATP} agonist diazoxide tended to preserve resting tension and significantly eliminated anoxic force potentiation in flounder preparations. Neither 5HD nor diazoxide affected contractility in cod preparations (data not shown).

Figure 1. Twitch force and resting tension for ventricular preparations from yellowtail flounder and Atlantic cod exposed to oxygenated conditions (flounder n = 6, cod n = 5) and to 35 min of anoxia followed by reoxygenation (n = 10, both species). a - significant difference between treatments. b - significant change from measurements within the treatment taken 5 min earlier.



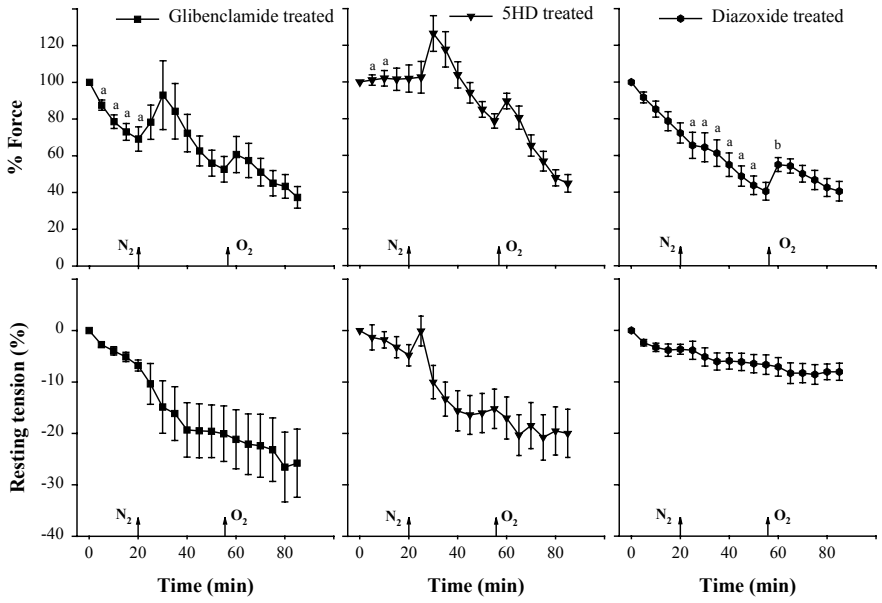
Discussion

Results suggest the presence of mK_{ATP} channels in ventricular muscle of yellowtail flounder. Under anoxic conditions, diazoxide eliminated the transient elevation in force development and stabilised resting tension. Acute activation of mK_{ATP} channels with diazoxide has been shown to depolarise the inner mitochondrial membrane in rat heart, leading to a rapid reduction in mitochondrial Ca^{2+} and inhibited Ca^{2+} uptake (Holmuhamedov et al., 1999). We suggest that in flounder heart, diazoxide releases mitochondrial Ca^{2+} more slowly than in rat heart due to the low temperature (6 °C) utilised in this experiment. Following a period of diazoxide treatment, mitochondrial Ca^{2+} content should already be reduced, so that when subjected to anoxia, force potentiation resulting from a bolus release of Ca^{2+} would be eliminated. If diazoxide does trigger a gradual release of mitochondrial Ca^{2+} to the cytoplasm, it may act to protect $[Ca^{2+}{}_i]$ during anoxia and overcome a net loss in activity, leading to the observed preservation of resting tension in diazoxide treated preparations.

Altering K_{ATP} channel activity in cod ventricle strips did not affect force development or resting tension under any of the conditions tested (data not shown). The data suggest that Atlantic cod do not have cardiac K_{ATP} channels sensitive to the pharmacological agents used, or that all of the factors needed to alter channel activity are not present in this tissue. Evolutionary differences within teleost fishes, and between fish and mammals may influence the sensitivity of K_{ATP} channels to pharmacological manipulation.

This study provides evidence for the presence of K_{ATP} channels in a fish heart and their potential importance in the control of cardiac function. The novel effects of mK_{ATP} channel modulators in yellowtail flounder heart imply differences exist in this channel's function over those known for mammalian systems. The influence of channel modulation on contractility revealed by this study also suggests a prospective role for these channels in excitation-contraction coupling in the fish heart.

Figure 2. Twitch force and resting tension for ventricular preparations from yellowtail flounder subjected to anoxia and reoxygenation and treated with agents to affect K_{ATP} channel activity. **A**: glibenclamide treated (n = 8). **B**: diazoxide treated (n = 5). **C**: 5HD treated (n = 6). *a*, significant difference between pharmacological treatment and appropriate control (untreated or vehicle DMSO treated). *b*, significant change from measurements within the treatment taken 5 min before hand.



Acknowledgements

Research was supported by the Natural Sciences and Engineering Research Council (NSERC) (W.R.D.). T.J.M. was supported by an NSERC postgraduate fellowship. We would like to thank Dr. T. Avery for statistical advice.

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EFFECT OF VANADATE OLIGOMERS ON THE LUSITANIAN TOADFISH HEART *(Halobatrachus didactylus)*: FUNCTIONAL NONINVASIVE ANALYSIS

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Introduction

Vanadium effects on biological systems have became an important area of research due to the continuous increase of vanadium in the environment and to the pharmacological effects of some of its forms (Barceloux, 1999).

In biological, systems vanadium exists mainly in the oxidation states +4 (vanadyl) and +5 (vanadate) (Barceloux, 1999). Previous studies have identified several physiopathological effects of vanadate on the cardiovascular system, such as negative inotropy and essential hypertension (Carmignani et al., 1998). Recently, it was suggested that different vanadate species, namely monomeric and decameric species, may contribute differently to oxidative stress and to histopathologic changes on cardiac tissue (Aureliano et al., 2002; Borges et al., 2002), which may be related to cardiac dysfunction.

In order to explore this hypothesis, systolic and diastolic functions of Lusitanian toadfish heart were analysed, using echocardiography, after acute *in vivo* exposure to "metavanadate" or "decavanadate" solutions.

Material and Methods

Specimens of the Lusitanian toadfish (n=10, body weight ranging 362-983 g) were caught at Ria Formosa lagoon (south coast of Portugal) and were divided in two groups: Meta and Deca, injected i.p. with 5 mM "metavanadate" or "decavanadate" solutions (1ml/Kg), respectively. Echocardiographic examinations were performed on anaesthetised animals at day 0 (control), 1 and 7 after metal intoxication, as described elsewhere (Coucelo et al., 1996).

Spectral records of systolic ventricular flow velocity were obtained by Doppler echocardiography and were used to determine: 1) maximum velocity (m/s), corresponding to peak of ejection flow; and 2) systolic time intervals: including pre ejection period, ventricular ejection time, acceleration time, deceleration time and total electromechanic systole. The Bernoulli equation was used to convert peak flow velocity into the pressure difference between the ventricle and the bulbus arteriosus. The Doppler velocity spectral records of ventricular filling flow, were used to determine maximum velocity (m/s), of both early (E wave) and late (A wave) ventricular filling and several diastolic time intervals: isovolumetric relaxation time, early filling period, diastasis, late filling period and total diastole.

All reported values are averages of at least five measurements in different cardiac cycles, and represent the % difference between values for control fish (day 0), and values for fish injected with "metavandate" or "decavandate"

Results and Discussion

"Metavandate" significantly reduced the systolic flow peak velocity ($p<0.05$), after both 1 and 7 days (Figure 1). However, decavanadate" only reduced cardiac function after 1 day. By day 7, peak systolic flow was not different from that in control fish. The pressure gradient between the ventricle and bulbus and stroke volume varied in accordance with ventricular systolic peak velocity.

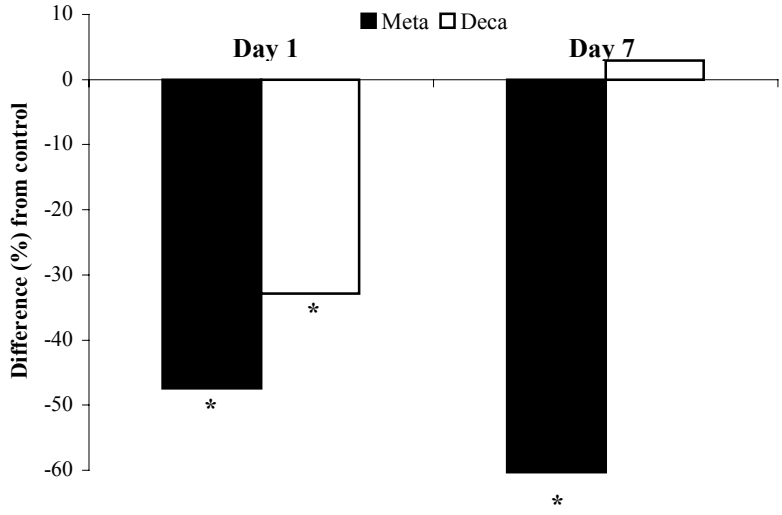

Figure 1. Effect of "metavanadate" or "decavanadate" solutions (5 mM) on systolic peak flow velocity at 1 and 7 days-post-injection; * $p<0.05$.

The maximum velocities of ventricular filling were also changed in the presence of vanadium: E and A waves velocities diminished significantly after 1 day in both groups, and after 7 days in just the Meta group (Figure 2). These results indicate a reduced ventricle filling, which may be related to decreased stroke volume. On other hand, the reduction of both E and A waves indicates a deficient ventricular relaxation and atrial contraction.

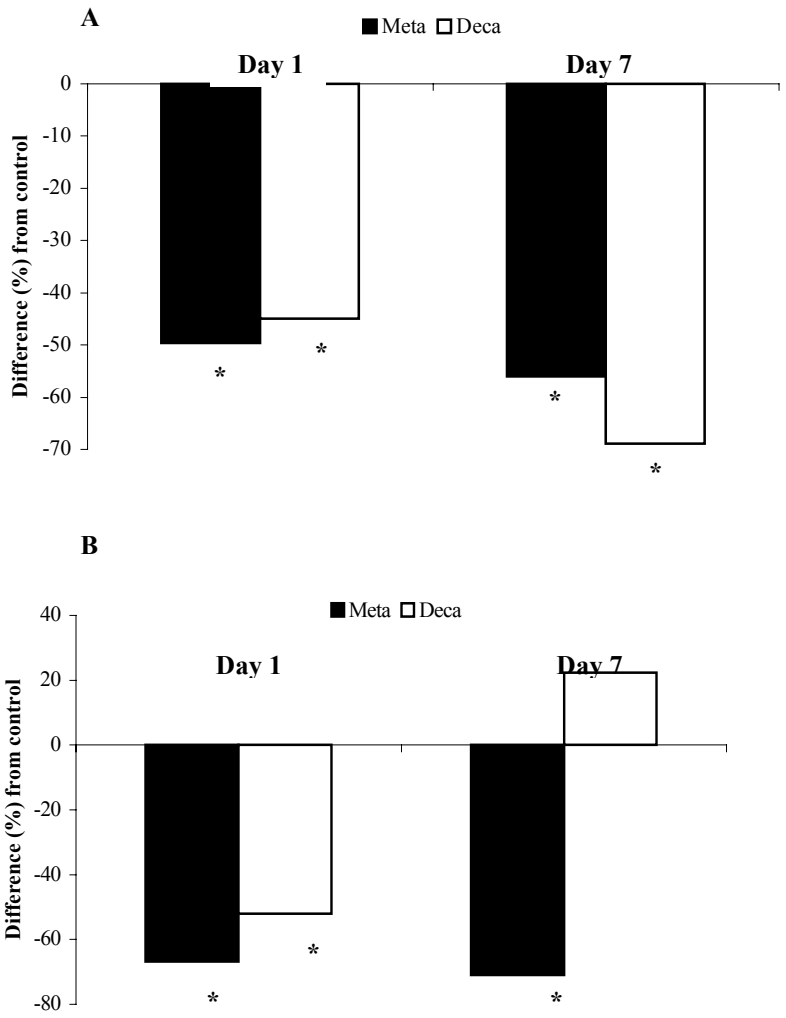

Figure 2. Effect of "metavandate" and "decavandate" (5 mM) exposure on diastolic flow peak velocity during the E (A) and A (B) waves; * $p<0.05$.

Although the vanadium species used affected flow velocities, there was no evidence that systolic or diastolic time intervals or heart rate were affected by vanadium exposure.

In conclusion, vanadium significantly affects toadfish cardiac performance. The results obtained suggest that different vanadate species present in vanadium (V) solutions, may contribute to vanadium toxicity by decreasing ventricular filling flow and inducing diastolic ventricle dysfunction. Both ventricle relaxation and atrial contraction are depressed in the presence of vanadate compounds.

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ß-ADRENERGIC STIMULATION MODULATES THE RELATIONSHIP BETWEEN INTRACELLULAR Ca^{2+} AND SR Ca^{2+} LOADING IN TROUT ATRIAL MYOCYTES

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Introduction

In the teleost heart, chronotropic and inotropic effects of β-adrenergic stimulation have been described *in vivo, in situ* and in multicellular preparatinos (1). Furthermore, the stimulatory effect of the beta adrenergic agonist isoproterenol on L-type Ca^{2+} current has been described in a few teleost species (2). The influence of β-adrenergic stimulation on Ca^{2+} removal from the cytosol has, however, not been examined in the teleost heart. The aim of the present work was therefore to examine the effect of β-adrenergic stimulation on intracellular Ca^{2+} transients and Ca^{2+} uptake in the sarcoplasmic reticulum (SR).

Methods

Long depolarizations cause a steady tonic contraction and induce SR Ca^{2+} uptake in trout atrial myocytes and rapid caffeine (CAF) applications at –80 mV before and after a long depolarization can be used to determine SR Ca^{2+} loading (3). To determine the relationship between intracellular [Ca^{2+}] and SR Ca^{2+} uptake, simultaneous measurements of ionic currents and intracellular [Ca^{2+}] ($[Ca]_i$) were done in trout atrial myocytes. Depolarizations to different membrane potentials were used to induce SR Ca^{2+} uptake and rapid CAF applications were used to measure it.

Results

Simultaneous measurements of $[Ca]_i$ and ionic currents showed an elevated $[Ca]_i$ throughout the depolarization, and the SR Ca uptake rate was 120±18 μM/s at +50 mV in control conditions. In order to evaluate the effect of the β-adrenergic agonist isoproterenol (ISO) on L-type Ca^{2+} current and the amplitude of the intracellular Ca^{2+} transient, repetitive 200 ms depolarizations from –80 to 0 mV were used. With this protocol ISO increased L-type current (I_{Ca}) to 191±29% of the control value and the Ca^{2+} transient to 141±10% of control. Using a 3 s depolarization to +50 mV, ISO increased the SR Ca uptake rate slightly to 132±29 μM/s. In contrast the average $[Ca]_i$ during a 3s depolarization decreased from 1.36±0.16 μM in control to 0.89±0.10 μM with ISO. Plotting Ca^{2+} uptake at different membrane potentials against the corresponding $[Ca]_i$ resulted in a shift in the $[Ca^{2+}]_i$, where the SR uptake rate was 50% of the rate at +50 mV, from 0.70 to 0.52 μM. Lowering of the extracellular $[Ca^{2+}]$ from 1.8 to 0.3 or 0.5 mM reduced the stimulatory effect of ISO on L-type Ca^{2+} current. The inhibitory effect of lowering the extracellular Ca^{2+} was even more pronounced on SR Ca^{2+} uptake, resulting in a shift of +26 mV in the relationship between mambrane potential and SR Ca^{2+} uptake. Furthermore, ISO in the presence of low extracellular $[Ca^{2+}]$ caused a significant reduction of the SR Ca^{2+} loading during a 10s depolarization at all membrane potentials above –30 mV, when compared with control conditions.

Conclusions

These data suggest that NCX plays a central role in the regulation of SR Ca^{2+} loading in trout atrial myocytes and that ISO has a stimulatory effect on the SR Ca^{2+} pump that is not caused by an increased $[Ca]_i$ during depolarization. As phospholamban has not been characterized in trout atrial myocytes, it remains to be determined if the SR Ca^{2+} pump in this species is modulated by a direct phosphorylation of the pump or through phospholamban phosphorylation as described in mammalian species.

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ON-LINE MEASUREMENT OF VENOUS P_{O2}

IN EXERCISING RAINBOW TROUT

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EXTENDED ABSTRACT ONLY – DO NOT CITE

The vertebrate heart needs oxygen to survive. For fish such as trout and salmon, oxygen is delivered to the heart through two routes: a coronary circulation to the outer myocardium directly from the gills and a cardiac circulation which delivers partially deoxygenated venous blood to the inner myocardium of the heart (Davie and Farrell, 1991). Of these two circulations the oxygenation of cardiac tissue is more reliant on the venous blood returning to the heart. When fish exercise and due to increases in cardiac output and ventral aortic pressure, the oxygen needs of the heart increase. This, in association with increased oxygen extraction from the blood perfusing locomotory muscles could cause an imbalance in the supply and demand relationship at the heart, i.e. a compromise in the O_2 delivery to the heart when its demand is at its greatest.

Previously, examination of oxygen delivery to the heart has involved monitoring venous Po_2 via either extracorporeal circulation, which limits the locomotory capabilities of the fish, or blood sampling through a cannula, which does not allow for fine temporal resolution of Po_2 changes. In this study we utilized a fibre-optic micro-optode that once implanted in a blood vessel allowed us to monitor venous Po_2 on-line with a response time of < 3 s, with limited interference to the movement of the fish.

Methods

Experiments were conducted at two acclimation temperatures, according to seasonal ground water temperature (spring, 6-10ºC, N=6; summer, 13-15 ºC, N=5). Rainbow trout (840 ± 122 g) were anesthetized and the optode was implanted into the ductus Cuvier. Venous Po_2 values are recorded every 1 s. Post-surgery fish were placed into a Brett-type respirometer for a 2-h recovery period that was followed by critical speed (U_{crit}) test used to habituate the fish to the respirometer and testing protocol. Fish were then given an overnight recovery and a second U_{crit} was preformed (all results reported are for the second U_{crit} test).

Results

Venous Po_2 during swimming was very labile, as abrupt changes in venous Po_2 can be seen as a reaction to the fish struggling (Fig. 1). Along with these temporary changes in venous Po_2, there was a general decrease associated with increased swimming speed. For cold-acclimated fish the threshold was reached prior to the fish quitting swimming (86-96 % of U_{crit}) and even the most severe struggles rarely decreases venous Po_2 below the threshold. For warm-acclimated fish the venous Po_2 threshold at U_{crit} was less apparent and was significantly higher at U_{crit} (28.9 ± 3.5 torr) than the threshold for cold-acclimated fish (15.3 ± 3.7 torr) (Fig. 2); as well it is significantly higher for all points beyond 50% of U_{crit}.

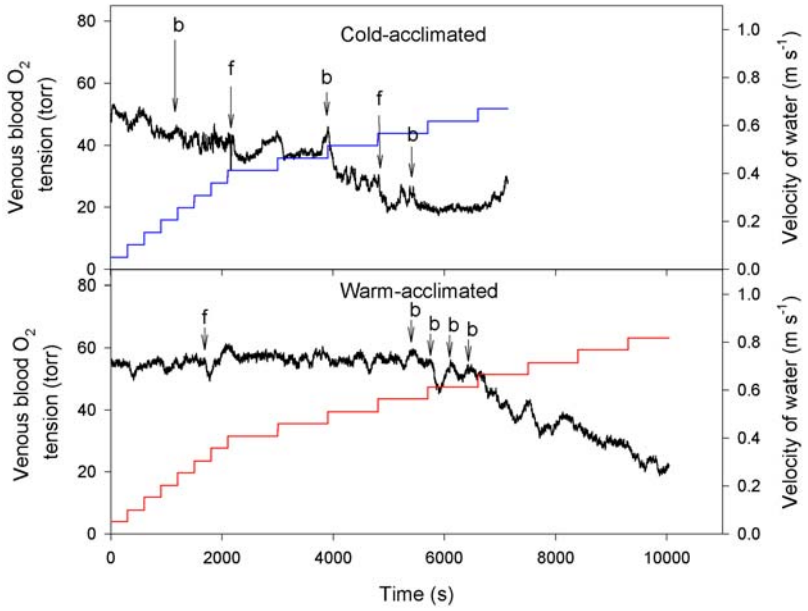

Fig. 1. Individual, on-line venous Po_2 during an U_{crit} test. "b" and "f" represent bursting and fighting events during steady state swimming.

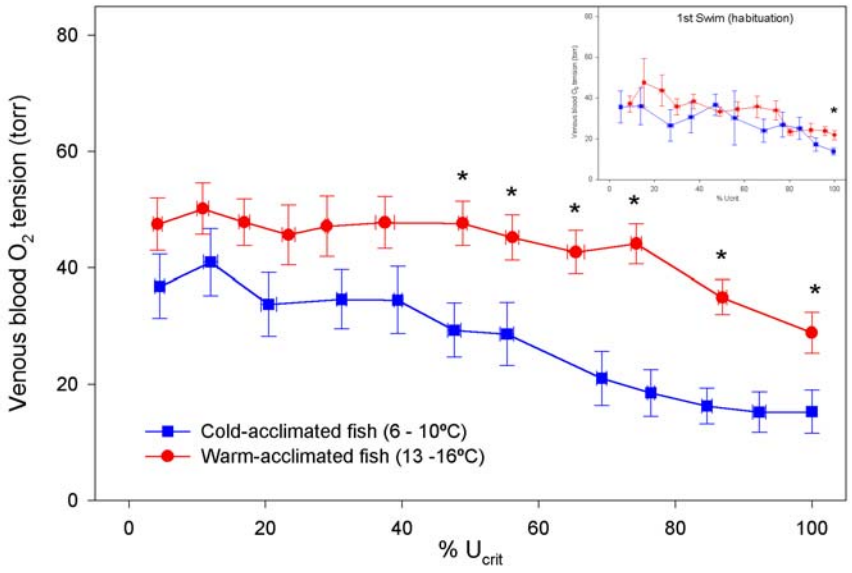

Fig. 2. Mean venous Po_2 for cold- and warm-acclimated fish, derived from periods of steady state swimming at each step of a critical speed test, expressed as a percentage of U_{crit}. * represents significant differences between cold-acclimated and warm-acclimated fish ($P<0.05$). **Inset**: Results for the initial habituation swim test.

Discussion and Conclusions

Oxygen delivery to the heart via the venous blood can be estimated using a hemoglobin-oxygen (Hb-O_2) dissociation curve (Thomas et al., 1994), [Hb] of rainbow trout (Gallaugher et al., 1995) and cardiac output during exercise (Thorarensen et al., 1996), which is assumed to increase 2.5 fold. As venous Po_2 decreases to threshold from routine and titrates down the Hb-O_2 curve, the increase in cardiac output will compensate for the decreased oxygen content in the blood allowing the oxygen delivery to the heart via venous blood to remain constant. An increase in temperature will shift the Hb-O_2 curve to the right, therefore making it necessary for the venous Po_2 threshold to increase, which is in accordance with the findings of this study (Fig. 3).

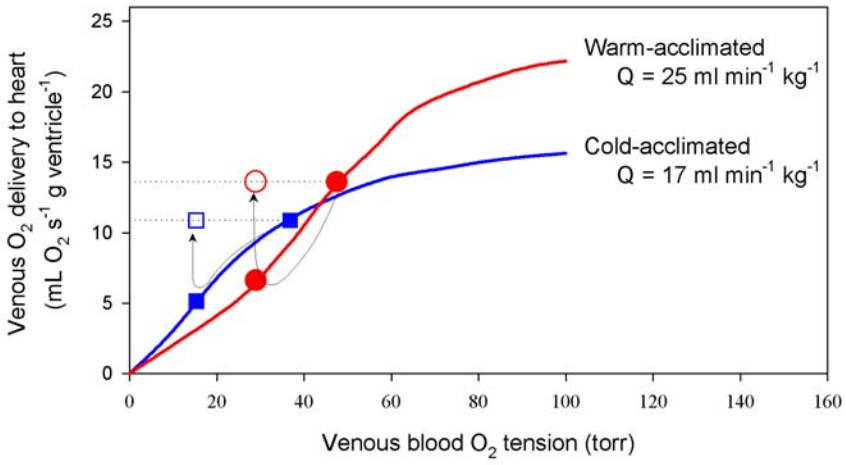

Fig. 3. Venous O_2 delivery curve to the heart using routine and exercise P_{VO2} and Q.

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