Fish Cardiovascular Physiology: Plasticity in Design and Function

SYMPOSIUM PROCEEDINGS

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International Congress on the Biology of Fish

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PREFACE

Fish cardiovascular function/physiology has been an extremely active area of research over the past 25 years, and our knowledge of how this organ system adapts to evolutionary forces or environmental challenges continues to increase. The design of the fish cardiovascular system shows significant inter-specific variation. In addition, intra-specific modifications in cardiovascular morphology, biochemistry and physiology are evident during development, when fish are exposed to changes in environmentally relevant parameters such as temperature, hypoxia and food availability, or when fish are forced to meet increased demands associated with training, elevated activity and maturation. In this symposium, there are 14 presentations that highlight specific aspects of cardiovascular plasticity in a wide range of fish species (from flounder to bluefin tuna), and that clearly demonstrate the multi-level (molecular, cellular and organ) nature of the control of, or adaptations in, this organ system. This is the 5th symposium on cardiovascular physiology organized for the International Congress on the Biology of Fish, and nicely illustrates the diversity of technical approaches and research models that will be required to provide a comprehensive picture of how the fish's heart, vasculature, blood cells and associated organs will respond to challenges associated with natural perturbations or anthropomorphic induced alterations in the environment.

We trust that you will find these abstracts interesting, and hope you will plan on contributing to the next symposium on fish cardiovascular physiology (St. John's, Newfoundland, Canada: July 18-23, 2006).

Symposium Organizers:

Kurt Gamperl, Memorial University of Newfoundland
Holly Shiels, University of Manchester
Don MacKinlay, Fisheries and Oceans Canada

CONGRESS ACKNOWLEDGEMENTS

This volume is part of the Proceedings of the 6th International Congress on the Biology of Fish, held in Manaus, Brazil in August, 2004. Ten years have passed since the first meeting in this series was held in Vancouver, BC, Canada. Subsequent meetings were in San Francisco, California; Baltimore, Maryland; Aberdeen, Scotland; and again in Vancouver, Canada. From those meetings, colleagues from over 30 countries have contributed more than 2,500 papers to the Proceedings of over 80 Congress Symposia, all available for free viewing on the internet.

We would like to extend our sincere thanks to the many people who helped us organize the facilities and program for this 6th Congress.

The local arrangements team worked very hard to make this Congress a success. The leaders of those efforts were Vera Almeida Val, Adriana Chippari-Gomes, Nivia Pires Lopes and Maria de Nazare Paula Silva (Local Arrangements); Marcelo Perlingeiro (Executive Secretary) and Maria Angelica Laredo (Fund Raising). The enormous contribution of time and effort that was required has led to an unforgettable experience for the participants, thanks to the imagination, determination and dedication of this team.

Many sponsors helped ensure the success of the meeting through both monetary and in-kind contributions, including: Fundação Djalma Batista, Honda, Merse, Cometais, Turkys Aquarium, Banco da Amazônia, Banco do Brasil, FUCAPI, SEBRAE/AM, IDAM/SEPROR, FAPEAM, SECT-AM, SUFRAMA, PETROBRÁS, CAPES, FINEP, CNPq, the Physiology Section of the American Fisheries Society, UFAM - Federal University of Amazonas, Fisheries and Oceans Canada and INPA - National Institute for Research in the Amazon.

Travel arrangements were ably handled by Atlantic Corporate Travel (special thanks to Maria Espinosa) and Orcal Planet, and the venue for the meeting was the spectacular Tropical Hotel Conference Center in Manaus.

The Student Travel Award Committee of the Physiology Section of the American Fisheries Society, led by Michael Redding, evaluated 65 applications from 15 countries and awarded 40 Travel Grants, after an ambitious and trying fund-raising effort. Special thanks must go the US Department of Agriculture, the US Geological Survey, US National Science Foundation and the World

Fisheries Congress for providing funds. In addition, the American Fisheries Society contributed books to be used as prizes for the best student papers.

The editorial team compiled the short abstracts into an abstract book and formatted and compiled the papers for the Symposium Proceedings. Thanks to Karin Howard, Christie MacKinlay, Anne Martin, Callan MacKinlay and Marcelo Perlingeiro.

In particular, we would like to extend a sincere 'thank you' to the organizers of the individual scientific Symposia and their many contributors who took the time to prepare a written submission for these proceedings. Their efforts are very much appreciated. We hope that their participation will result in new insights, new collaborations and new lines of research, leading to new papers to be presented at the 2006 Congress in St. John's, Newfoundland.

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MECHANISMS RESPONSIBLE

FOR THE ENHANCED CARDIAC PERFORMANCE

OF WINTER FLOUNDER (*Pleuronectes americanus*)

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Introduction

Despite their abundance and diversity (Fletcher, 1975; Moyle and Cech, 1996), flatfish cardiovascular biology/physiology has not been extensively studied, and much of the published data on cardiac function may be inaccurate due to the use of indirect measurements and/or techniques (e.g. the Fick principle). Recently, Joaquim *et al*. (*in press*) performed the first direct *in vivo* measurements of cardiac function in winter flounder and found that maximum stroke volume (S_V) is extremely high in this species (1.5 ml g ventricle^{-1}, 10ºC) compared with other teleosts. To examine the factors that contribute to the high S_V in this species, *in situ* Starling curves and power curves, and *in vitro* pressure-volume curves were determined for the winter flounder (*Pleuronectes americanus*), Atlantic cod (*Gadus morhua*) and Atlantic salmon (*Salmo salar*).

Material and Methods

Wild winter flounder were captured in Conception Bay (Newfoundland), while hatchery-reared cod and salmon were obtained from a cage-site operation (Bay D'Espoir) and the Ocean Sciences Centre (OSC), respectively. All fishes were acclimated at 8 to 10 ± 1ºC for at least 4 weeks prior to experimentation.

In situ heart preparations at 8 to 10ºC were performed as previously described by Farrell *et al*. (1982) for sea-raven, but adapted for the flounder, cod and salmon.

To obtain pressure-volume curves (Forster and Farrell, 1994) the heart (without pericardium) was dissected free from the animal, and pressure-volume curves were generated for the atrium, ventricle and bulbus arteriosus of the 3 species. In addition, atrial:ventricular (A:V) and bulbus:ventricular (B:V) mass ratios were calculated to examine if the size of the heart chambers or their relative size influences the shape of the pressure-volume curves.

After ln transforming the data for the Starling and pressure-volume curves, ANCOVA was used to test for homogeneity of slopes between species ($p<0.05$; SPSS Software). Maximum power values were obtained by fitting a 3rd order power curve (SigmaPlot Software) to the data of each fish. Differences in maximum power output (P_H), cardiac output (Q), heart rate (f_H), stroke volume (S_V) and A:V and B:V mass ratios between species were assessed by ANOVAs and pairise Tukey tests (SPSS Software, $p<0.05$).

Results and Discussion

In situ maximum Q was not significantly between the three species, averaging 63 ml min^{-1} kg^{-1}. However, because of the small size of the flounder heart (RVM 0.05%), the maximum S_V achieved by the winter flounder was significantly higher (2.2 ± 0.1 ml g^{-1} ventricle) as compared with the Atlantic cod (1.7 ± 0.2) and Atlantic salmon (1.4 ± 0.1) (Fig.1A). The maximum P_H of the flounder heart (7.6 ± 0.3 mW g^{-1}) was significantly lower than the salmon (9.7 ± 0.5 mW g^{-1}), but surprisingly similar to the cod (7.8 ± 0.6 mW g^{-1}) (Fig.1B).

Cod and salmon hearts could generate *in vivo* resting levels of Q at negative filling pressures (P_{in}), whereas the flounder heart required a positive P_{in} of 0.4 cm H_2O to achieve resting Q. However, fewer increments in P_{in} were required by the flounder heart to achieve elevated levels of S_V. For instance, to achieve a S_V of 1.4 ml g^{-1} ventricle, the flounder heart only needed a P_{in} increase of 1.9 cm H_2O, whereas cod and salmon hearts required P_{in} increases of 2.9 and 6.9 cm H_2O, respectively. These data show that the high S_V values (in ml g

ventricle^{-1}) measured for the flounder are partly related to an enhanced sensitivity to filling pressure. This conclusion is supported by the pressure-volume curves, which indicate that the flounder's atrium, ventricle and bulbus are significantly more compliant when compared to the cod and salmon (Fig.2).

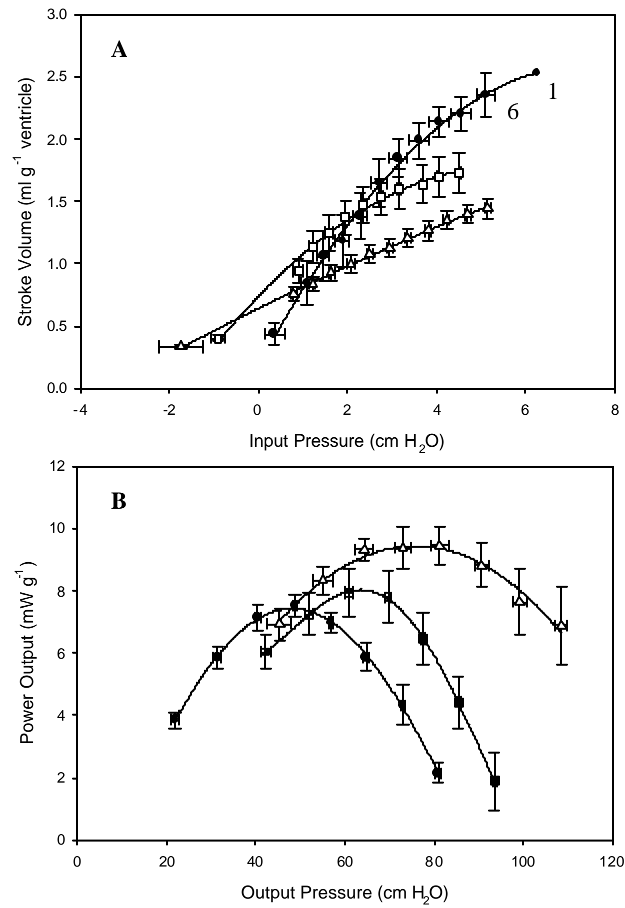

Figure 1. Starling curves (A) and Power Curves (B) for the winter flounder (?), Atlantic cod (?) and Atlantic salmon

(?) at 8 ºC. N= 7-8, except when numbers appear next t data point.

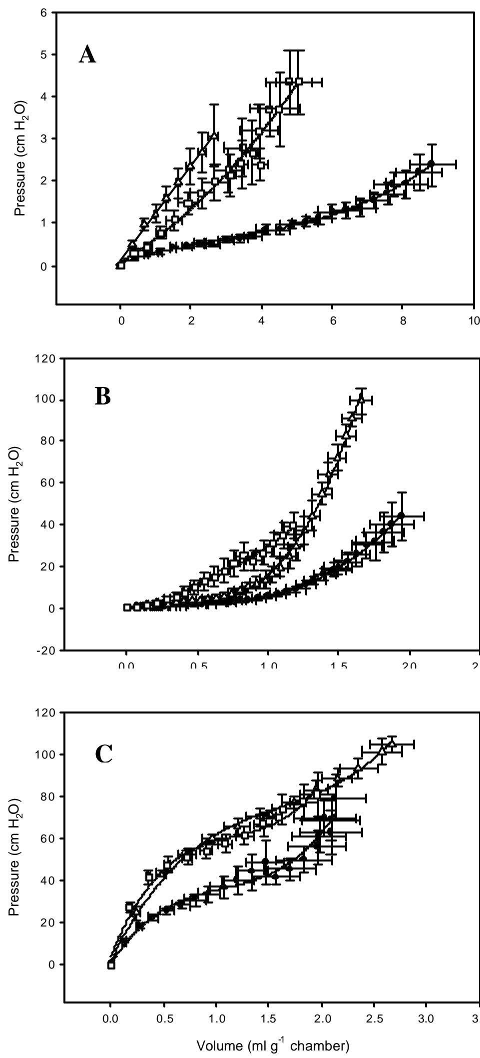

Figure 2. Pressure-volume curves for the atrium (A), ventricle (B) and bulbus (C) of the winter flounder (?), Atlantic cod (?) and Atlantic salmon (?) at 8-10 ºC. N= 6, except salmon ventricle where N= 2.

Although, the flounder's A:V ratio (0.22) was comparable to the cod (0.21) and the salmon (0.18), the flounder's B:V ratio (0.59) was significantly higher (cod 0.37; salmon 0.22). In fact, the high B:V mass ratio may be partially responsible with the low arterial pressures reported for the flounder.

In conclusion, the S_V measured in winter flounder (per g of ventricle) is extremely high. This high S_V is related to 1) a pronounced Starling curve; 2) more compliant heart chambers; and 3) a high B:V mass ratio. Our data support the *in vivo* data of Joaquim *et al.* (*in press*) and others, which show that the cardiovascular system of flatfish is a high volume, low pressure design. Further, these data suggest that the pericardium, and thus *vis a fronte* filling, may not be important for cardiac function in flatfishes.

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CARDIORESPIRATORY MODIFICATIONS, AND LIMITATIONS,

IN GROWTH HORMONE TRANSGENIC

ATLANTIC SALMON (*Salmo salar*)

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

In recent years, there has been a great deal of interest in growth hormone (GH) transgenic fish, and how their physiology differs from their non-transgenic counterparts. However, early studies were performed using fish with multiple copies of the GH gene, which often led to physical deformities and poor performance (Farrell et al., 1997). Further, the results of more recent studies are hard to interpret because transgenic fish and their non-transgenic conspecifics often have different environmental histories. This study provides the most comprehensive examination of the cardiorespiratory physiology of GH transgenic salmon (including measurements of maximum cardiac function), and uses a stable line of fish reared in a shared tank with controls (at 10^0C, for 4 – 6 months). This "common garden" experiment eliminates exogenous environmental variables, leaving the transgene's effects as the sole determinant of any measured physiological differences.

Results

Body morphometrics were not different between control and transgenic salmon, and surprisingly no difference was observed in gill surface area. Standard and routine oxygen consumption (measured in an 81L Blazka respirometer) were significantly higher (by 20% and 18%) in the transgenics, however, maximum oxygen consumption was not different (Table 1). Thus, transgenic fish had a 29% lower absolute metabolic scope and a 19% lower factorial metabolic scope, and this lowered scope was associated with an 11% reduction in critical swimming speed (U_{crit}).

Table 1. Routine, standard, and maximal oxygen consumption (ml min^{-1} kg^{-1}), U_{crit} (BL sec^{-1}), and factorial and absolute metabolic scope for transgenic and control Atlantic salmon at 10^0C. Values represent means ± 1 standard error. In all cases a one-way ANOVA was used to assess significance. * indicates a significant difference ($p<0.05$).

	Control (n=8)	Transgenic (n=8)	Ratio (Con/Trans)
Routine	46.4 ± 2.1	58.1 ± 4.4*	0.81
Standard	64.5 ± 3.9	78.2 ± 4.7*	0.83
Maximum	418.2 ± 18.6	379.5 ± 25.3	1.10
Absolute Scope	354.9 ± 19.1	286.0 ± 16.8*	1.24
Factorial Scope	9.13 ± 0.49	6.51 ± 0.58*	1.40
U_{crit}	2.2 ± 0.1	2.0 ± 0.1*	1.11

Plasma cortisol, norepinephrine, and epinephrine levels, before and after a 45 second net stress, were measured as indices of the fish's stress response. Resting plasma cortisol and epinephrine levels were not different between transgenic and control salmon (cortisol: 11.6 ± 2.3 vs. 12.1 ± 1.7 ng ml^{-1}; and epinephrine: 5.8 ± 1.7 vs. 3.3 ± 0.6 nM). However, the transgenics exhibited a significantly higher resting norepinephrine level than the controls (1.7 ± 0.3 vs. 4.3 ± 0.8 nM). Interestingly, the transgenic salmon had a significantly lower post-stress cortisol response (17.8 ± 1.3 vs. 24.7 ± 2.3 ng ml^{-1}), but significantly greater post-stress epinephrine (20.6 ± 2.8 vs. 12.3 ± 2.1 nM) and norepinephrine (8.9 ±

0.7 vs. 5.0 ± 0.8 nM) levels compared to the controls. Resting and post-stress haematocrit and mean cellular haemoglobin content were not different between groups, however the transgenics exhibited a slightly greater post-stress haemoglobin concentration (7.3 ± 0.3 vs. 6.6 ± 0.2 g dL^{-1}). Finally, the erythrocytes of transgenic salmon had a 3% shorter perimeter, and were 8% more compact (i.e. more oblong), but showed no difference in total surface area.

The transgenics had significantly higher citrate synthase activity in their heart muscle (0.129 ± 0.002 vs. 0.117 ± 0.002 units · g $protein^{-1}$), and greater cytochrome c oxidase activity in their red muscle (0.152 ± 0.001 vs. 0.145 ± 0.002 units · g $protein^{-1}$). In addition, their white muscle protein content was significantly higher than controls (147.8 ± 0.4 vs. 141.9 ± 0.7 mg g wet $tissue^{-1}$).

Finally, an *in-situ* heart preparation (Farrell et al., 1986) was used to measure the maximum cardiac performance in both groups (Table 2). Transgenic salmon had a 22% greater relative ventricular mass, and their *in-situ* hearts exhibited a significantly greater heart rate (by 7%). This greater heart size and frequency of contraction allowed them to achieve a 16% greater maximum cardiac output (measured in ml min^{-1} kg^{-1}). However, maximum power output (mW g $vent^{-1}$), % ventricular compact myocardium (46.5 ± 1.1 vs. 44.8 ± 1.0 %), and mass specific cardiac output (in ml min^{-1} g $ventricle^{-1}$) were not different between the two groups.

Discussion

This research identified numerous physiological differences between transgenic and non-transgenic salmon including increased routine/standard metabolism, higher enzyme activities, and lowered U_{crit} and metabolic scope, that are consistent with past studies on GH transgenic fish. However, we provide the first data on altered stress hormone levels, and on substantial differences in heart performance/morphology. These data: 1) suggest that elevated cardiac function is linked directly to the higher growth or indirectly with the increased feeding (swimming) activity of GH transgenic salmon; and 2) indicate that this increased cardiac performance was only a function of a larger heart, not remodelling of myocyte physiology. This latter result is consistent with recent work comparing cardiac physiology between wild vs. domesticated steelhead, and fed vs. starved cod (Gamperl and Farrell, in press).

Table 2. Heart morphometrics and maximum cardiac performance in transgenic and control Atlantic salmon. Values represent means ± 1 standard error. An ANCOVA, with body mass as a covariate, was used to examine differences in % compact myocardium. One-way ANOVAs were used to compare all other parameters. * indicates a significant difference ($p<0.05$).

	Controls N=8	Transgenics N=7	Con/Trans Ratio
Body mass (g)	595 ± 21	577 ± 21	1.03
Heart Morphometrics			
RVM	0.069 ± 0.002	0.089 ± 0.002*	0.77
% Compact Myocardium	44.8 ± 1.0	46.5 ± 1.1	0.96
Maximum Performance			
Q (ml min^{-1} kg^{-1})	63.8 ± 1.9	75.54 ± 2.8*	0.85
SV (ml kg^{-1})	0.93 ± 0.03	1.03 ± 0.05	0.91
Heart Rate (BPM)	69 ± 1	74 ± 2*	0.93
Q (ml min^{-1} g $vent^{-1}$)	96.0 ± 4.4	95.7 ± 1.9	1.00
SV (ml g $vent^{-1}$)	1.39 ± 0.09	1.3 ± 0.04	1.07
Power (mW g $vent^{-1}$)	9.5 ± 0.7	9.6 ± 0.5*	0.84

Interestingly, although many aspects of the oxygen uptake-transport-utilization pathway had been upregulated, gill surface area was not enhanced in the GH transgenic salmon. We suggest that the lack of an increase in gill surface area constrained the maximum performance capacity of these fast growing fish (ie. max. O_2 consumption is diffusion limited in GH fish). This conclusion is consistent with Pauly (1998), who provides a comprehensive analysis of the interrelationships between growth rate, swimming capacity, and gill surface area in fishes.

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MECHANISMS OF MYOCARDIAL HYPOXIA TOLERANCE,

AND PRECONDITIONING,

IN THE ATLANTIC COD (*Gadus morhua).*

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Introduction

Information on myocardial hypoxia tolerance is only available for a few marine species (e.g. see Driedzic and Gesser, 1994; Fritsche and Nilsson, 1989), and there is limited data on the cellular mechanisms that mediate the fish heart's ability to tolerate oxygen deprivation. Further, although hypoxic preconditioning has been demonstrated in rainbow trout heart, Gamperl et al. (2001) suggested that the phenomenon of preconditioning may be limited to the compact myocardium which is normally perfused with highly oxygenated arterial blood supplied by the coronary artery. The Atlantic cod heart possesses only spongy myocardium which is perfused by venous blood of low oxygen content, and an in situ heart preparation has recently been developed for this species. Thus, we determined the hypoxia tolerance of in situ cod hearts, investigated the role of protein kinase C (PKC) and sarcolemmal ATP-sensitive K+ channels in mediating hypoxia tolerance, and examined whether the cod heart can be preconditioned.

Materials and Methods

Atlantic cod reared at the Ocean Sciences Centre (OSC), or cod that were reared at the OSC and subsequently spent approximately 18

months in sea cages at Hermitage Bay (Newfoundland), were used in these experiments. These fish were held in 4000 L tanks at the Ocean Sciences Centre at 12 ± 1ºC for a minimum of 2 weeks prior to experiments, and fed chopped herring or commercial pellets every 2nd day.

In situ heart preparations were performed as previously described by Farrell *et al*. (1986), with a number of small modifications. Adrenaline was not added to the perfusate in these experiments because: 1) preliminary experiments showed that it is not required for the long-term viability of the cod heart; and 2) adrenergic stimulation of the hearts could confound the preconditioning experiments. In all experiments, severe hypoxia (PO_2 ~ 5 mm Hg) was induced by perfusing hearts with saline that was bubbled with N_2 for at least 2 hours, and the effects of hypoxia, pharmacological blockers and preconditioning was examined by comparing maximum cardiac performance prior to (Q_{max1}) and after experimental manipulation (Q_{max2}).

Results And Discussion

Hypoxia Tolerance of the Cod Heart

Maximum cardiac output (Q_{max}) prior to hypoxia ranged from 55 to 60 ml min^{-1} g^{-1} ventricle (~ 80 ml min^{-1} kg^{-1}), and decreased by 14.1 % after 10 min. of severe hypoxia, by 20.4 % after 15 min. of severe hypoxia, and by 21.9 % after 20 min. of severe hypoxia. However, because 2 out of 8 fish exposed to 20 min. of hypoxia failed to recover, 15 min. of severe hypoxia was used in subsequent experiments. This degree of hypoxia tolerance is similar to that measured in hypoxia-sensitive trout (Gamperl *et al*., 2001).

Role of PKC and Sarcolemmal K_{ATP} Channels in Myocardial Hypoxia Tolerance

This experiment examined the role of both sarcolemmal K_{ATP} channels and PKC in the hypoxia tolerance of Atlantic cod hearts. *In situ* cod hearts were exposed to one of 6 treatments: (a) control treatment with oxygenated perfusion; (b) 15 min. of hypoxia with no pharmacological agents (c) the PKC inhibitor chelerythrine (5 µmol L^{-1}) with no hypoxic period; (d) the K_{ATP} channel inhibitor BDM (100 µmol L^{-1}) with no hypoxic period; (e) the PKC inhibitor chelerythrine, prior to and during

a 15 minute hypoxic period; and (f) the K_{ATP} channel inhibitor BDM, prior to and during a 15 minute hypoxic period.

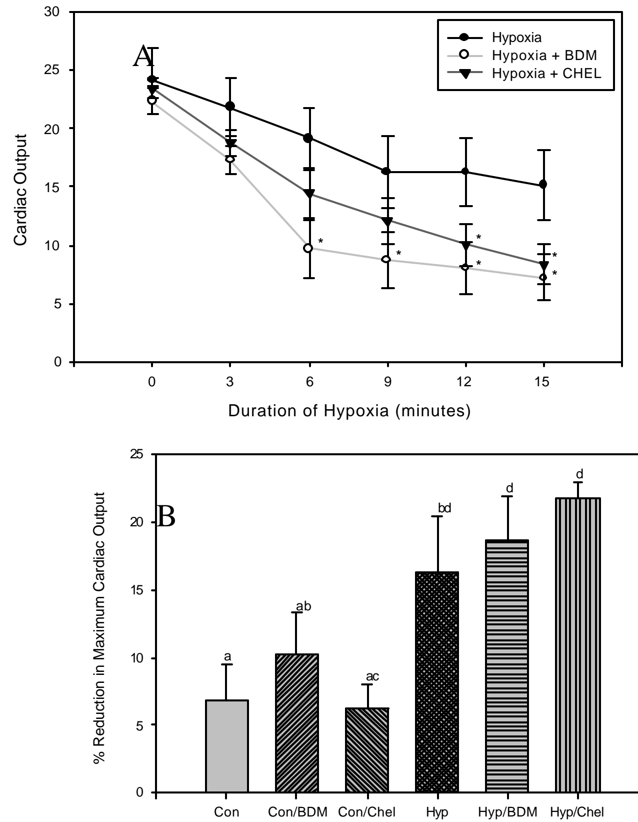

Figure 1. A. Decreases in cardiac output (Q, ml min^{-1} kg^{-1}) during 15 min. of severe hypoxia. Repeated measures ANOVA followed by one-way ANOVAs at each time interval showed that there were significant differences between the hypoxia (N=8) and hypoxia + BDM (N=9) treatments after 6 min., whereas differences between hypoxia and hypoxia + CHEL (N=9) were observed after 12 min. B. Percent reduction in maximum Q (between Q_{max1} and Q_{max2}). Dissimilar letters indicate a significant

difference ($p<0.05$) between treatments, as determined by a one-way ANOVA.

Cardiac function decreased to a greater extent when the pharmacologically blocked hearts were exposed to 15 min of hypoxia. Cardiac output fell by 38% in hearts exposed to hypoxia alone, and by 68% and 64% in hearts exposed to hypoxia + BDM and hypoxia + CHEL, respectively (Fig. 1A). Surprisingly, however, there were no significant differences in post-hypoxic maximum cardiac function (Q or Vs) between the 3 hypoxic groups (Fig. 1B). These data indicate that although both sarcolemmal KATP channel and PKC blockade influence cardiac function during hypoxia, they do not affect the ability of the heart to recover from an acute hypoxic episode. Our finding that sarcolemmal KATP channels are important for fish myocardial function during hypoxia supports the study of Cameron et al. (2003). Further, these data are the first to report a similar role for PKC in fish.

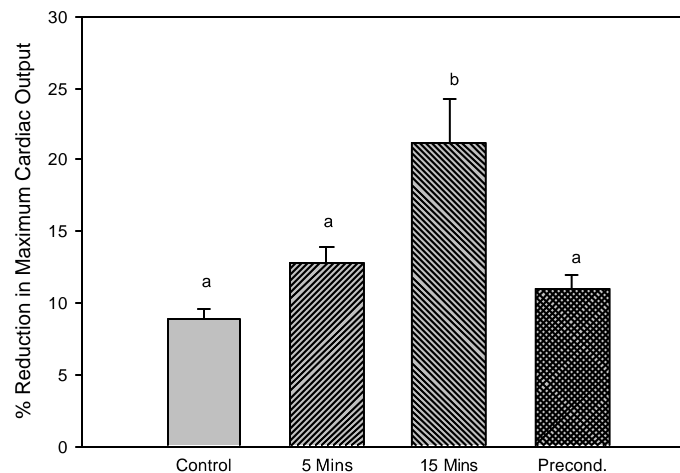

Figure 2. Effect of hypoxic pre-exposure (preconditioning) on the recovery of maximum *in situ* cardiac performance (Q_{max2} relative to Q_{max1}) in Atlantic cod. Dissimilar letters indicate significant differences ($p<0.05$) between treatment groups (N=8).

Can the Cod Heat Be Preconditioned?

In situ cod hearts were exposed to one of 4 treatments: (a) control, oxygenated perfusion; (b) 5 min. of severe hypoxia; (c) 15 min. of severe hypoxia; and (d) 5 min. of severe hypoxia with 15 min. of reperfusion, followed by 15 min. of severe hypoxia (preconditioning). The group that underwent 15 min. of severe hypoxia experienced a 21% decrease in Q_{max} as compared with 8.4% and 14% for the control and 5 min. of hypoxia groups, respectively. Hypoxic pre-exposure reduced the decrease in Q_{max} following 15 min. of severe hypoxia to 11.7% (Figure 2), indicating that the cod heart, which is exposed entirely of spongy myocardium and is perfused by poorly oxygenated venous blood, can be preconditioned.

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CARDIOVASCULAR CONTROL AND FELXIBILITY DURING EARLY DEVELOPMENT IN ZEBRAFISH (*DANIO RERIO*)

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Introduction

The cardiovascular system is the first organ system to function during vertebrate development, but for tiny animals like zebrafish the physiological importance of the early onset of cardiac activity is not yet understood. Among other possible functions this is believed to be related to hormonal communication, osmoregulation and haemodynamic force generation, which may be important for angiogenesis and also for cardiogenesis (Hove et al. 2003). For these and possible other functions we hypothesize that the maintenance of a stable blood pressure is imperative. This hypothesis includes that typical extrinsic and intrinsic mechanism controlling heart performance should be implemented early during development. Several antihypertensive control mechanisms are possibly involved in cardiac regulation:

- Frank–Starling-mediated reductions in stroke volume and cardiac output
- Differences in plasma concentration of vascular mediators in response to increased/decreased shear stress or blood velocity working indirectly via altered pre- and afterload due to changes in vessel diameters affecting arterial pressure
- Differences in catecholamine plasma concentration

In the present study we analyzed the possible mechanisms that may contribute to cardiovascular control in early developmental stages.

Methods and Results

In anaesthetized and immobilized larvae of *Danio rerio* (2 – 9 days post fertilization, dpf) we analyzed the time schedule of the appearance of adrenergic receptors. Injections of 1000 nl isoproterenol (ß-adrenergic agonist, 10^{-2}M) into the extravascular space close to the ventricle led to an immediate increase in heart rate beginning already at 3 dpf, while stroke volume remained unchanged. With phenylephrine (ß-adrenergic agonist, 10^{-2}M) no changes in all parameters could be were observed. Incubation with propranolol (ß-adrenergic antagonist, 10^{-2}M) led to a significant decrease in heart rate with a small but non-significant increase in stroke volume (3-9 dpf).

The ability to adopt cardiac performance in the face of a blood volume change was assessed in anaesthetized and immobilized *Danio rerio* (3 – 9 days post fertilization, dpf) by withdrawal (2 nl) of blood. Starting at 4 dpf in all stages a withdrawal of approximately 2 nl blood led to an increase in heart rate, while stroke volume decreased significantly (from 6-9 dpf). An overview of these results is shown in table 1.

Conclusions

The results of the present study show that baroreceptors, intrinsic control mechanisms like the Frank-Starling Mechanism and also humoral control mechanisms appear very early during development (3 – 6 days post fertilization) in zebrafish larvae. Similar experiments were performed in *Xenopus laevis* by Warburton and Fritsche (2000). They described the ability of stage 49–51 (Nieuwkoop and Faber) larvae to correct a volume-load-induced hypertension, but found no evidence for a baroreflex in these early stages. Vascular reactivity appears to be present very early in developing lower vertebrates: zebrafish (7 dpf, nitric oxide) and *Xenopus* leavis (nitric oxide and endothelin, stage NF 50-53) (Fritsche, Schwerte, and Pelster 2000; Schwerte, Printz, and Fritsche 2002). Receptors sensing hypoxic conditions and inducing a change in cardiac activity were shown to be present as early as 3 or 4 dpf in zebrafish larvae (Jacob et al. 2002). The early presence of these control mechanisms strongly supports the hypothesis that the early function of the cardiovascular control system is a

possible prerequisite for its own maturation. To test this hypothesis we are currently working with cardiac mutants and pharmacological attempts to investigate possible changes in angiogenesis and erythropoesis (in zebrafish) with a disturbed cardiovascular performance.

Parts of this study were funded by a grant of the University of Innsbruck (to TS) and a grant of the Fonds zur Förderung der wissenschaftlichen Forschung Austria (FWF P16272-B06 to TS).

	Stage (days post fertilization (dpf)								
	2	3	4	5	6	7	8	9	
Isoproterenol HR SV CO	→ → ↑	↑ → →	↑ → →	↑ ↓ →	↑ ↓ →	↑ ↓ →	↑ ↓ →	↑ ↓ →	
Incubation Phenylephrine; all parameters	→	→	→	→	→	→	→	→	
Incubation Propranolol HR SV CO	→ → →	↓ ↑ →	↓ ↑ →	↓ ↑ ↑	↓ ↑ ↑	↓ ↑ ↑	↓ ↑ ↑	↓ ↑ ↑	
2 nl withdrawal of venous return HR SV CO	→ → →	→ → →	↑ → ↑	↑ → ↑	↑ ↓ ↑	↑ ↓ ↑	↑ ↓ ↑	↑ ↓ ↑	

Table 1: Overview of changes in hear trate (HR), stroke volume (SV) and cardiac output (CO) resulting from application of isoproterenol (ß-adrenergic agonist, 10^{-2}M), incubation with phenylephrine (ß-adrenergic agonist, 10^{-2}M), propranolol (ß-adrenergic antagonist, 10^{-2}M) and volu me deload of venous return (2nl from the sinus venosus)

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THE INFLUENCE OF HEART PERFORMANCE

ON CARDIOVASCULAR PARAMETERS

IN DEVELOPING ZEBRAFIS H (DANIO RERIO)

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EXTENDED ABSTRACT ONLY - DO NOT CITE

The first organ that functions during larval development is the cardiovascular system. Zebrafish larvae acquire the oxygen by bulk diffusion until about 12dpf (Pelster and Burggren, 1996; Jacob et al. 2002), therefore the cardiovascular system obviously is not only needed for the transport of oxygen. Among other things also the distribution of nutritions and hormones appear to be important, and the immune system may in part also depend on convective transport. Thus, control over the cardiovascular system should be established quite early during development. It is known that zebrafish larvae are responsive to adrenaline beginning at 3dpf and respond according to the Frank-Starling mechanism at 5dpf (Schwerte et al., personal communication). Fritsche et al. (2000) described observations of vascular reactivity against NO starting at 7dpf old zebrafish larvae. It is also already known that there is a link between blood pressure and/or shear stress and angiogenesis as well as cardiogenesis (Hove et al., 2003)

If shear stress is connected to angiogenesis it might be possible that alterations in cardiac output and blood flow may cause changes in vascularization of tissues. Another interesting aspect would be the question which of the various parameters connected to cardiovascular performance or depending on sufficient blood supply would be most important for controlling cardiovascular activity? An overview of our current knowledge of short and medium term regulation of cardiovascular activity in larvae is shown in figure 1.

A reduced cardiac performance can be observed in numerous mutants (e.g. *breakdance* - *bre*), it can also be induced by pharmacons (e.g. Quinidine). Using high speed video imaging enables us to monitor the contracting heart and the developing vessel system of the transparent zebrafish larvae.

Bre-zebrafish larvae typically show an atrioventricular block, i.e. the atrium contracts twice while the ventriclecontracts only once. The expression of this 2:1 rhythm is not constant, but it depends on the upbringing temperature and the age of the larvae. At lower temperature a 1:1 heart beat often is observed, but heart rate is significantly lower than that of wildtype zebrafish. The same is true for older larvae. Remarkable is the temperature insensitivity during 2:1 contraction. Larvae raised at 25°C, 28°C or 31°C have a ventricular contraction rate of about 80 bpm, irrespective of the temperature. If beating in the 1:1 rhythm temperature sensitivity of heart rate even appears to be reversed. Animals raised at 31°C have a lower heart rate in the 1:1 rhythm than animals raised at 25 °C. Blood pressure in *bre* – mutants is reduced compared to wild type animals.

An artificial reduction in heart rate to 50% can be triggered by incubation of larvae with the pharmacon Quinidine, a blocker of K^+-channels. Zebrafish larvae incubated in Quinidine create a very low enddiastolic volume. This combined with the low heart rate caused a significantly reduced cardiac output. Another consequence of Quinidine was the dilatation of larger vessels (Caldwell et al., 1983). Thus, a decreased blood pressure can be expected, and was confirmed by Mariano et al., 1992 who also mentioned an a-adrenergic neurotransmission blocking.

Comparing *bre*, Quinidine incubated and wildtype zebrafish larvae a different blood allocation is seen but no significant changes in the main vascularization pattern could be detected. Thus, the basic development of vessels (at least of the major vessels) appears to be independent of the blood pressure. Currently we are focussing on blood vessels that develop in the angiogenetic processes. This might be a more potent field to find blood pressure related changes in the construction of the vascular bed.

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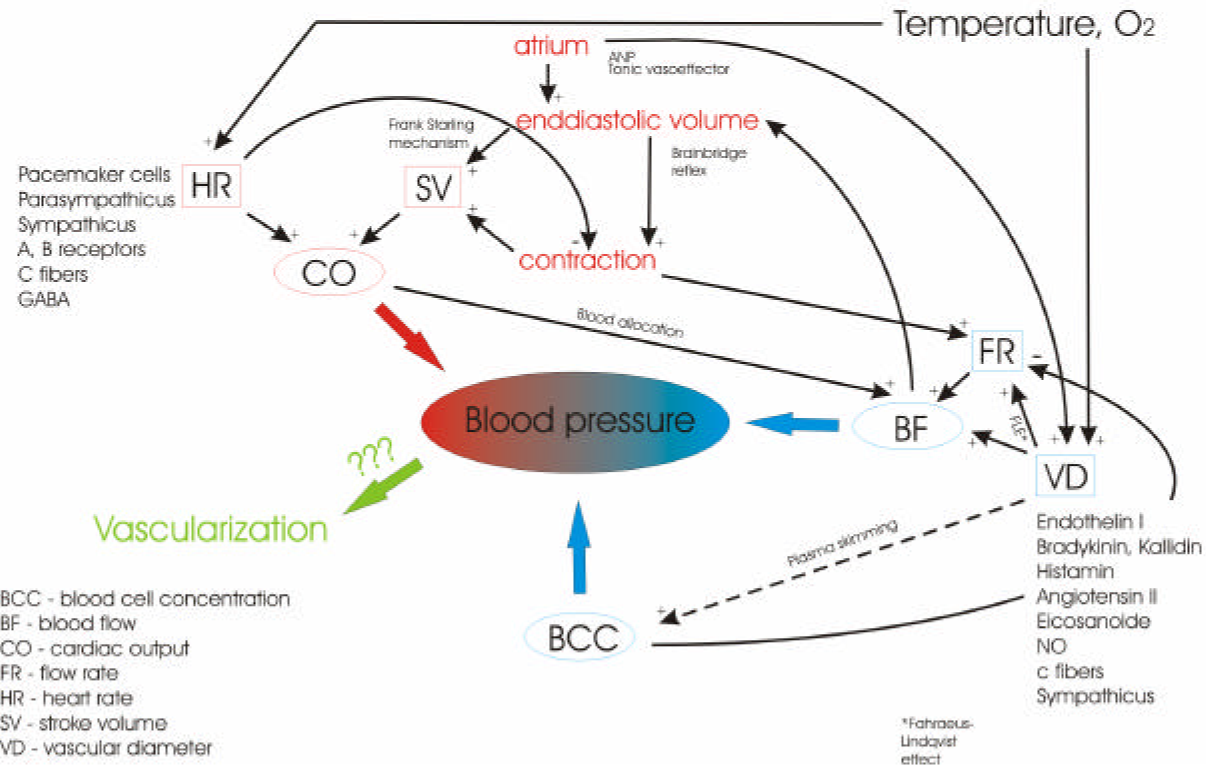

Figure 1: Short and medium

THE RELATIONSHIP BETWEEN STRESS PROTEIN (HSP) EXPRESSION AND METABOLISM IN THE HEART OF THE RAINBOW TROUT

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Introduction

Heat shock proteins (hsps) are a ubiquitous, highly conserved family of proteins, present within cells at constitutive levels, and upon exposure to stress are induced in order to provide protection against cellular insult (Feder and Hofmann, 1999, review). As molecular chaperones, hsps are able to bind to denatured or unfolded proteins thereby providing cytoprotective benefits to the cell so that it may better cope with the stress (Hartl, 1996). The molecular response of hsps has been described previously, however, information pertaining to how this induction of hsps benefits the intact organism is still lacking. The rainbow trout, (*Oncorhynchus mykiss*) is an ideal organism for studying the cellular stress response. Environmental stresses such as heat shock, have been shown to upregulate the expression of hsps in tissues of the trout (i.e. Currie et al., 2000). Also, with fluctuating water temperatures and oxygen availability in aquatic habitats, possessing an intricate cellular stress response system could be critical for survival.

In mammals, it is thought that hsps protect the mitochondrial integrity and energetic status of cells during oxidative stress (Polla et al., 1996). Furthermore, recent evidence suggests that the upregulation of hsps correspond with improved cardiac function (Sammut and Harrison, 2003). Our objectives for this study

were to investigate the possible targets of hsp protection within the heart of the rainbow trout by correlating expression profiles of certain hsps with metabolic changes that occur during an acute temperature stress. Expression profiles for hsps were determined following *in vivo* experiments where trout were subjected to an acute heat shock. Primary cell cultures of ventricular cardiomyocytes were also established in order to look at the *in vitro* expression of hsps and metabolic alterations that occur during heat shock. Through comparing the *in vivo* and *in vitro* stress response, possible specific metabolic roles for hsps will be determined in tissues of the rainbow trout.

Materials and Methods

Rainbow trout (n=44) were subjected to an *in vivo* heat shock of 3°C/hour, beginning at 13°C (ambient temperature) up to 25°C. Fish were maintained at this elevated temperature for one hour and then the water was allowed to cool naturally back to ambient temperature. Fish were sampled over the course of the heat shock and into recovery (0, 1, 2 and 24 h). Hearts were sampled to measure hsp70, hsp90 and hsp30 expression by immunoblotting, along with metabolic parameters including heart [ATP] and [lactate]. In order to further elucidate possible targets for hsp protection within the heart of the trout, similar experiments will be conducted using a cell model. A protocol for isolating ventricular cardiomyocytes has already been established. Experiments are underway to correlate hsp expression with metabolic indices in these ventricular cardiomyocytes.

Results and Discussion

Hsp70 was significantly induced in the heart of the rainbow trout following a 1 h heat shock at 25°C (Fig. 1). This corresponds with other studies that have shown hsps are induced at a set point that is determined in part by the magnitude of the stress and thermal history of the organism (Currie et al., 2000). Levels of this stress protein remain elevated into the recovery period. In contrast, hsp90 was not induced in the heart. Given that hsp90 is involved in many signal transduction pathways and has many important roles during normal cell functioning, this stress protein may have a more critical constitutive role in the heart.

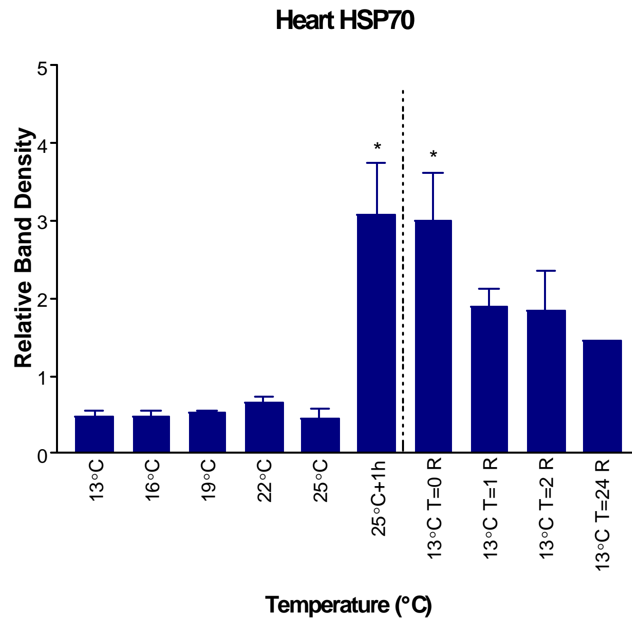

Figure 1. Quantification of hsp70 in the heart of rainbow trout subjected to an acute heat shock (3ºC/h) or during a recovery period following the temperature stress. Values represent hsp70 expression relative to a standard. The asterisks indicate a significant difference from control (13ºC) ($p<0.05$; one-way ANOVA). All values represent the mean + SEM.

The *in vivo* heat shock did not significantly alter ATP levels in the heart (Fig. 2), although a slight decline at 25ºC was observed. Lactate, an indicator of anaerobic metabolism was also measured in the heart. Again, no significant alteration in this metabolite was noted over the course of the heat shock or into recovery. The heart of the rainbow trout derives most of its energy from aerobic metabolism, however if available lactate may also be a preferred fuel source.

I have shown that during an *in vivo* heat shock, hsps are upregulated in the heart indicating the initiation of the cellular stress response and this most likely confers a certain degree of protection during exposure to the stress. The energetic status of the heart is maintained over the period of the stress and into recovery. With metabolism maintained throughout this heat stress, it is tempting

to speculate that hsps protect aspects of metabolic function. Experiments are in progress to determine specific cellular targets for hsp protection within this tissue using primary cell cultures of ventricular cardiomyocytes and pharmacological inhibition of hsps.

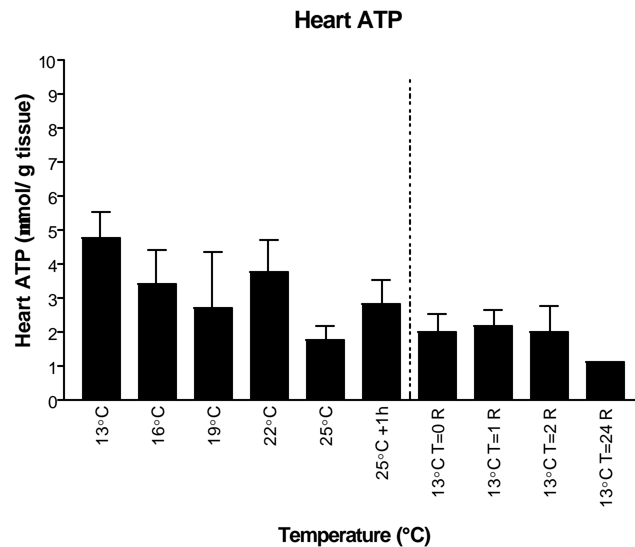

Figure 2. Heart ATP concentrations from rainbow trout exposed to an acute heat shock (3ºC/h) or during a recovery period. All values represent the mean +SEM ($p>0.05$; one -way ANOVA).

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TEMPERATURE SENSITIVITY AND E-C COUPLING

IN TUNA HEARTS

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Introduction

Tunas are remarkable among teleost fishes for their high metabolic rates and ability to conserve metabolic heat. High metabolic rates in tunas are supported by large hearts capable of high maximal heart rates and high cardiac outputs (Block and Stevens 2001). Tropical tunas such as yellowfin (*Thunnus albacares*) maintain only a small elevation above ambient temperatures, while temperate species such as bluefin tunas display large thermal gradients between peritoneal cavity, swimming muscle and ambient temperatures, exceeding 20°C in large adult fish in cold waters. However, all tuna species have hearts which operate at or near ambient temperature as they receive lumenal blood which has been cooled in the countercurrent *retia mirabilia* and coronary flow from the gills. This raises the possibility that the temperature sensitivity of cardiac performance limits the tuna's thermal niche in the wild.

Acoustic tracking studies in the eastern Pacific indicate that yellowfin tuna predominately inhabit waters of 17°C or warmer, with only occasional brief dives into colder waters. In contrast, Pacific bluefin tuna (*Thunnus orientalis*) of similar size encounter surface waters as cold as 12°C and dive into waters as cold as 5°C in the western Pacific (Kitagawa 2000). Swimming yellowfin tuna exhibit a drop in cardiac output at low temperatures, with a decrease in heart rate accompanied by a rise in stroke volume (Korsmeyer et al., 1997). However, physiological data on working bluefin tuna hearts have been lacking. In this study, we used an *in situ* heart preparation to measure cardiac performance, including heart rate, stroke volume, and cardiac output in Pacific bluefin tuna and yellowfin tuna across a range of temperatures likely to be encountered in the wild (Blank et al., 2002, 2004).

Methods

The effects of temperature on heart function of Pacific bluefin tuna (mass 6.75 ± 0.18 kg) and yellowfin tuna (mass 3.16 ± 0.38 kg) were studied using an *in situ* heart preparation. One albacore tuna (mass 5.30 kg) was also studied. Fish were euthanized, the peritoneum was opened, and cannulae were inserted into the sinus venosus and ventral aorta in order to perfuse the heart with oxygenated Ringer's solution. The fish was then transferred to a large saline bath and connected to an in-line flow probe and input and output pressure transducers hooked to a PowerLab system. Temperature was adjusted by changing the temperature of the bath and perfusate simulaneously, and input and output pressures were adjusted to maximize cardiac output and power output at each temperature. Values for heart rate, stroke volume, cardiac output, and cardiac power output were obtained from stable sections of 5 to 6 beats of recorded data at each temperature.

Results and Discussion

Decreasing temperature induced pronounced bradycardia (Fig 1) and a drop in cardiac output in all tuna species examined. Heart rates and cardiac outputs of bluefin tuna were significantly higher than those of yellowfin tuna at temperatures from 2 to 15°C and showed a significantly lower temperature dependence (Fig 2). Maximal stroke volumes were similar in the two species and were not significantly affected by temperature. Heart rate, stroke volume, and cardiac output recorded in one albacore tuna were qualitatively similar to values measured in bluefin.

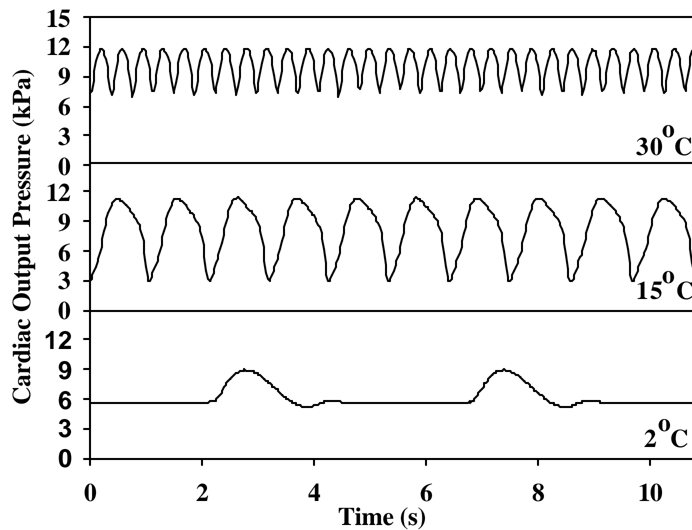

Fig 1. Heart rate from a Pacific bluefin tuna heart perfused with Ringer's solution in situ. Cardiac output pressure data from a single fish at 30°C (top), 15°C (middle), and 2°C (bottom) are shown.

In situ heart preparations indicate that bluefin tuna hearts are more cold-tolerant than those of yellowfin tunas, as demonstrated by a lower temperature sensitivity of heart rate and cardiac output and higher values of heart rate. This difference in temperature sensitivity of the heart may play a role in their different thermal niches in the wild. Recent studies of SR Ca^{2+} uptake and SR Ca^{2+} ATPase levels indicate that differences in SR calcium cycling are likely to play a role in the observed differences in thermal tolerance of yellowfin and bluefin tuna hearts.

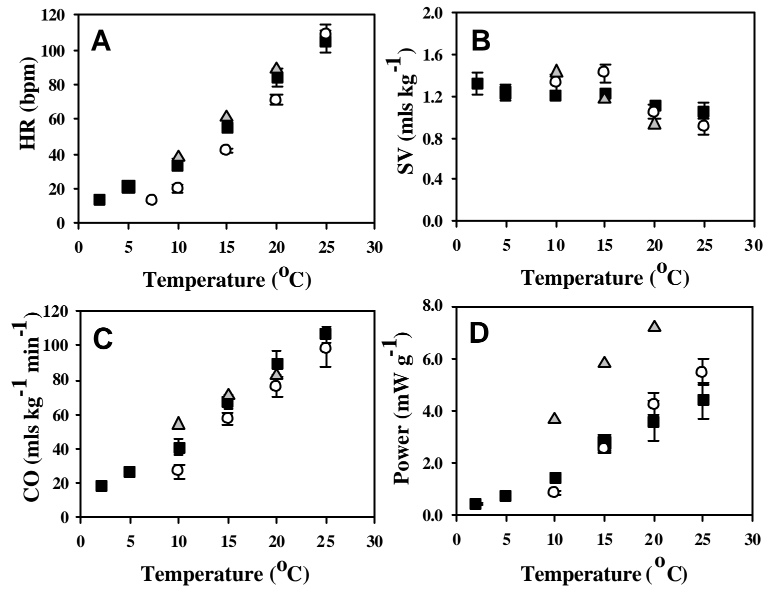

Fig 2. Comparison of cardiac performance *in situ* in three tuna species. Maximal values of cardiac parameters recorded in spontaneously beating hearts of albacore tuna (gray triangles), bluefin tuna (black squares), and yellowfin tuna (white diamonds) are presented. A. Heart rate B. Stroke Volume C. Cardiac Output D. Power output.

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THE INFLUENCE OF ENVIRONMENTAL PO_2 ON HEMOGLOBIN OXYGEN SATURATION IN DEVELOPING ZEBRAFISH, *DANIO RERIO*

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Introduction

Several studies performed on *Danio rerio, Xenopus laevis* or *Ambystoma mexicanum* suggested that during early larval development of lower vertebrates convective blood flow is not essential to supply oxygen to tissues and therefore in early developmental stages respiration of lower vertebrates is cutaneous respiration.

This appears to be especially true for the zebrafish, because their gills form relatively late in development and even then initially appear to be involved in ionoregulatory aspects, rather than in the uptake of oxygen (Rombough, 2002). Consequently, nicely developed vascular networks such as a large yolk sac represent an effective surface area for gas exchange in early developmental stages. Nevertheless, it is quite obvious that beyond a certain body mass convective oxygen transport must come into play, but data on the oxygenation status of the larvae during the time of cutaneous respiration is still missing.

If convective oxygen transport contributes to the oxygen supply to tissues, loading and unloading of the blood must be detectable, i.e. partly deoxygenated blood and thus partly deoxygenated hemoglobin must be found in central parts of the body.

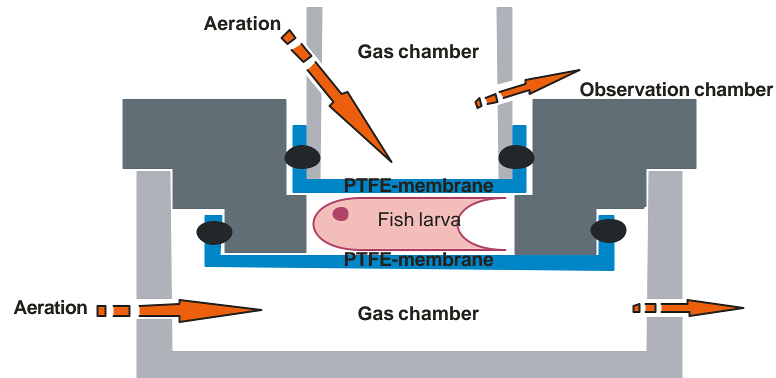

Fig. 1: Scheme of the aeration chamber with an embedded zebrafish larva

The present study was therefore set out to test the hypothesis that tissue oxygenation of zebrafish larvae can be improved by hyperoxic exposure. Tissue oxygenation was assessed by determination of hemoglobin oxygen saturation *in vivo* by combining video imaging techniques with spectrophotometrical analysis of hemoglobin light absorption.

Methodology

For the analysis of hemoglobin oxygen saturation a slightly anaesthetized fish larva was kept between PTFE-membranes in a modified incubation chamber (Fig. 1).

The larva was laid into a drop of anesthetics onto the lower PTFE-membrane. By lowering a cylindrical top with a second PTFE-membrane the animal was enclosed between the two membranes in a sandwich-like position. Aeration of the larva was carried out by directing temperature controlled and humidified air (or gas) from a gas-mixing device directly onto the two PTFE-membranes.

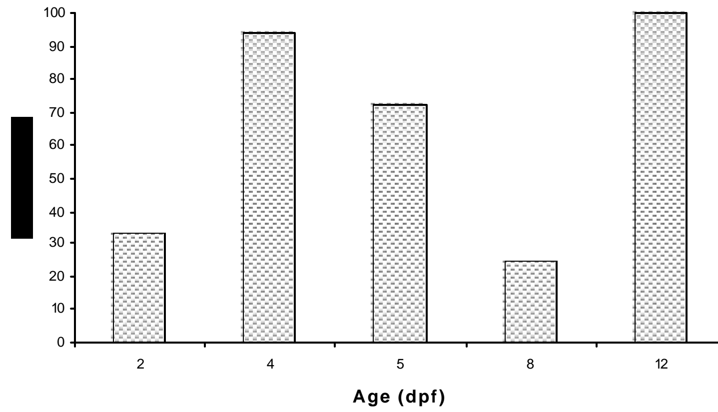

Fig. 2a: Number of larvae (in % of total larvae analyzed) showing partial deoxygenation of red blood cells under normoxic conditions (6 = n = 18)

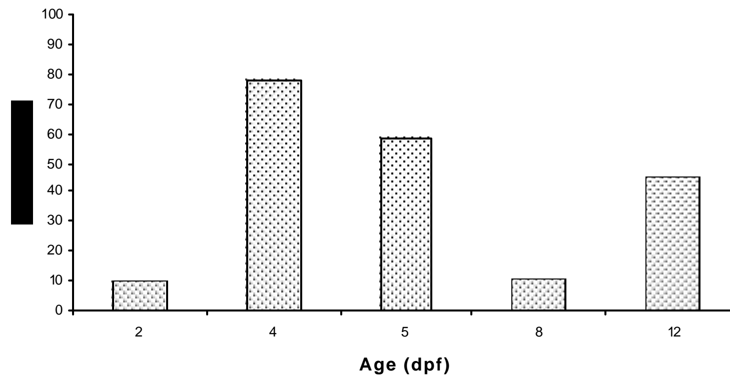

Fig. 2b: Possible increase of red blood cell oxygen saturation level due to hyperoxygenation

The measurement of hemoglobin oxygen saturation was carried out by irradiating the embedded larva with light corresponding to the maximum absorption peaks of fully oxygenated blood (413 nm), fully deoxygenated blood (431 nm) and the position of the isosbestic point (421 nm).

In order to connect the measured absorption values of the blood to oxygen partial pressure red blood cell (RBC) absorption values were determined in relation to the oxygen partial pressure in the fluid.

Results

Hemoglobin oxygen saturation

The comparison of hemoglobin oxygen saturation determined under normoxic conditions with values obtained under hyperoxic conditions revealed several periods of partially deoxygenated venous return, especially at 4 dpf, 5 dpf and 12 dpf (Fig. 2a and 2b).

Heart rate

At 2 dpf and 3 dpf, heart rate obtained under normoxic and hyperoxic conditions did not differ significantly. Beginning at 4dpf, hyperoxia significantly lowered heart rate of zebrafish larvae during almost the whole period of investigation until 12 dpf. At 9 dpf and 12dpf, heart rate measured under hyperoxic conditions was also lower than under normoxic conditions, but this difference was not significant.

Conclusions

The results of the present study indicate that bulk diffusion does not prevent partial deoxygenation of the blood in the ventricle, which contains partly deoxygenated blood at 4 dpf, 5 dpf and also at 12 dpf.

During these periods, acute hyperoxia significantly improved the larvae's inner environment in terms of oxygen saturation. Nevertheless, measurements of oxygen consumption at this time do not provide any indication for a switch to anaerobic metabolism (Pelster and Burggren, 1996).

In zebrafish the lowest blood oxygen saturation was detected at 4 dpf and 5 dpf, when yolk sac degradation was largely completed. A reduction of the gas exchange area would probably result in a change in the balance between oxygen supply and oxygen consumption and thus could be responsible for a partial deoxygenation of the blood. This seems especially conceivable as zebrafish are tropical fish and therefore with 25°C – 30°C typically experience higher temperatures than for example trout larvae, typically exposed to 5°C – 15°C, in which the richly vascularized yolk sac had no significant effect on gas exchange (Rombough, 1998).

The partial deoxygenation of zebrafish blood at 12 dpf is mainly caused by a significant increase in the rate of oxygen consumption, while the surface area of the gills mainly responsible for the gas exchange is only just starting to develop (Rombough, 2002). It therefore indicates that at this time oxygen is not only taken up by bulk diffusion, but also removed from the blood. This confirms the conclusion that at this point in development hemoglobin becomes necessary for oxygen transport.

Acknowledgements

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EFFECTS OF CELL VOLUME ON ERYTHROCYTE CIRCULATION

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EXTENDED ABSTRACT ONLY- DO NOT CITE

Introduction

Triploid fish provide a model to study the physiological importance of cell volume. Due to their extra nuclear material, triploid cells are larger than diploid cells; however, fish size is similar because of a reduced cell count in triploid tissues. The triploid cardiovascular system is particularly interesting because of the possible effects of cell volume on red blood cell circulation. Triploid red blood cells likely experience greater resistance compared to diploid cells passing through constrictive regions such as the microvasculature. However, it is possible that cellular compensations such as increased membrane deformability and reduced cytoplasmic viscosity allow for maintenance of a diploid-like resistance to triploid red blood cell passage.

The objective of this study was to investigate the effects of cell size on the passage of red blood cells through a restrictive area. The larger triploid red blood cells were expected to experience greater resistance compared to diploid cells which would be exhibited through a slower passage time and increased damage to red blood cells.

Methods

The passage of triploid brook trout (*Salvelinus fontinalis*) red blood cells through restrictive Nucleopore membranes was assessed by measuring the

filtration time of 5ml blood samples and cell damage due to filtration. The Nucleopore membranes contained pores with an average diameter of 8 µm, compared to a diploid red blood cell major axis of approximately 10 µm and a slightly greater diameter of triploid red blood cells (Benfey, 1999). Cell damage was estimated by comparing haematocrit values, red blood cell counts, and the percent of irregular-shaped red blood cells before and after filtration.

Results

The mean filtration times for diploid and triploid blood samples were 9.13 s (±1.72) and 3.80 s (±3.73), respectively (Figure 1). A Student's t test showed these values to be significantly different ($p < 0.05$). This suggests that triploid red blood cells passing through a constrictive area experience greater resistance than diploid cells.

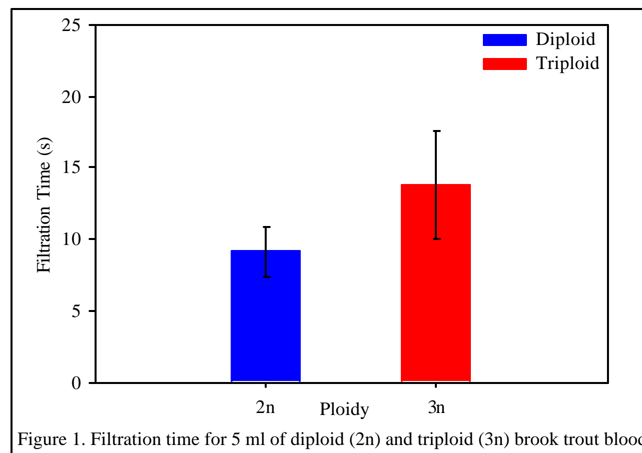

Figure 1. Filtration time for 5 ml of diploid (2n) and triploid (3n) brook trout blood.

Estimates of change in haematocrit, red blood cell count, and percent irregular red blood cells revealed no significant trends in cell damage. The mean reduction in haematocrit was 3 % (±1) for both diploids and triploids. The mean percent reduction in red blood cell counts for diploids and triploids was 1.6 % (±9.1) and 2.2 % (±2.8) respectively. The mean percent increase in percent irregular red blood cells was 44 % (±18) and 46 % (±12) for diploids and triploids respectively. It is difficult to draw conclusions about cell damage due to the large amount of variability present in haematocrit, red blood cell count and irregular red blood cell measurements.

Conclusions

The cardiovascular fitness of a fish depends upon the efficient circulation of blood to deliver oxygen to the metabolizing tissues. Results suggest that their larger size may affect the circulation of triploid red blood cells and thus the cardiovascular fitness of triploid fish.

The slower passage of triploid blood through the microvasculature likely affects the oxygen delivery capacity of the blood. Past research has found the oxygen carrying capacity, as reflected by total blood haemoglobin concentration and haematocrit, of diploid and triploid fish to be similar (Benfey, 1999). Thus a decrease in blood flow rate would impair oxygen delivery to metabolizing tissues in triploid fish. On the other hand, the slower passage of blood through the gills and capillaries may compensate for reduced oxygen diffusion rates into and out of triploid red blood cells due to their greater volume and subsequent reduced surface area to volume ratio.

Longer filtration time for triploid red blood cells is a reflection of increased resistance to flow due to the larger triploid cell size. Increased vascular resistance exerts greater demands on the heart muscle, consequently increasing the metabolic demand of blood circulation. The resting oxygen consumption rate of triploid fish appears to be similar to that of diploid fish (Hyndman *et al*., 2003), therefore the increased metabolic investment required for circulation is likely at the expense of other basal processes. The negative impacts of high resistance to blood flow may be offset by increased plasma mixing, which is thought to facilitate oxygen and metabolite exchange, at the gills (Nilsson *et al*., 1995) and capillaries.

Though, under the observed conditions, clear differences between diploid and triploid red blood cell passage were demonstrated, this study has not considered the importance of the blood vessels to blood flow characteristics.

Possibly the larger size of triploid red blood cells brings about a corresponding increase in blood vessel dimensions. Although blood vessel dimensions of triploid fish have yet to be measured, capillaries of triploid salamanders have been found to be of similar dimensions to those of diploids (Davison, 1959). This suggests a lack of compensation in triploids to accommodate for the circulation of their larger red blood cells.

Further research on the passage of blood through diploid and triploid fish vasculature (both *in vitro* and *in vivo*) is necessary to investigate the ability of the vascular tissue to accommodate for larger triploid red blood cell size.

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TEMPERATURE DEPENDENCE OF THE Ca^{2+}-ATPase (SERCA2)

IN THE VENTRICLES OF TUNA AND MACKEREL

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EXTEND ABSTRACT ONLY - DO NOT CITE

Introduction

Among the Scombridae family, tunas are unique in having high metabolic rates and systemic endothermy. In addition to warming their swimming muscles, temperate tunas have counter-current heat exchangers in the viscera, brain and eyes (Block BA and Stevens ED**,** 2001), that allow tunas to maintain the highly aerobic tissues of the brain, eyes, skeletal muscle and viscera above ambient water temperature (Block BA et al., 2001).

On the contrary, the hearts of all tunas operate at ambient temperature, as they pump blood that has been cooled by the counter current heat exchangers and receive their coronary circulation directly from the gills. How the cardiovascular system of tunas is capable of maintaining function across the wide range of temperatures in the ocean remains unknown.

A key factor influencing rates of ventricular contraction and relaxation is the cycling of Ca^{2+} ions into and out of the cytoplasm of the cardiac cells. For most of the fish species studied so far, extracellular Ca^{2+} rather than intracellular Ca^{2+} (sarcoplasmic reticulum) is the major source of activator Ca^{2+} for cardiac contraction (Farrell AP**.** 1991). However, fish with more active lifestyles, like

salmonids and tunas, have evolved SR stores, increasing the contribution of intracellular Ca^{2+} compartments (Shiels HA. et al., 1999).

Material and Methods

Pacific bluefin tuna (*Thunnus orientalis*), 13.7 ± 1.4 kg, albacore tuna (*Thunnus alalunga*), 8.4 ± 1.1 kg, yellowfin tuna (*Thunnus albacares*), 16.8 ± 4.5 kg, and Pacific mackerel (*Scomber japonicus*) with 0.333 ± 0.03 kg were caught on hook-and-line off the coast of California. The fish were euthanized and the hearts were immediately removed. Ventricles were sliced into small pieces, freeze-clamped and stored in liquid nitrogen. The microssomal enriched fraction retain the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2) and was isolated by differential centrifugation using a modified protocol of Harigaya and Schwartz (1969).

For measurement of Ca^{2+} uptake, 0.6mg/ml (tunas) or 1 mg/ml (mackerel) of microsomes were added to a temperature controlled cuvette containing 50 mM Mops-Tris pH 7.0, 100 mM KCl, 1 mM $MgCl_2$, 10 mM sodium azide, 10 mM potassium-oxalate, 5 mM creatine phosphate, 10 μg/ml creatine kinase (as an ATP regenerating system) and 1.5 μM Ca^{2+} sensitive fluorescent dye fura-2. Ca^{2+} uptake was stimulated by the addition of 1.5 mM MgATP.

Microsomal preparations from bluefin, yellowfin, albacore and mackerel ventricles were separated by SDS-PAGE. For western blot analysis, proteins were transferred to PVDF membranes and probed with a polyclonal antibody specific to cardiac SERCA2 (Morrissette JM et al., 2002)

Results

Comparing the rate of Ca^{2+}-uptake into the vesicles catalyzed by SERCA2 from different scombridae's species, at the same temperature (25°C), we found that pacific bluefin tuna ventricles has the highest activity among this family (fig.1, Table I). Measuring the rate of Ca^{2+}-uptake into the vesicles at temperatures similar to those reached by the tunas on the open ocean, bluefin tuna showed the highest rate of Ca^{2+}-uptake at all temperatures tested, followed by albacore and then yellowfin (Fig.1A).

A polyclonal antibody raised against a conserved cardiac SERCA2 protein sequence (47) was used to identify and quantify the amount of Ca^{2+}ATPase from the different microsomal preparations (Figure 1B).

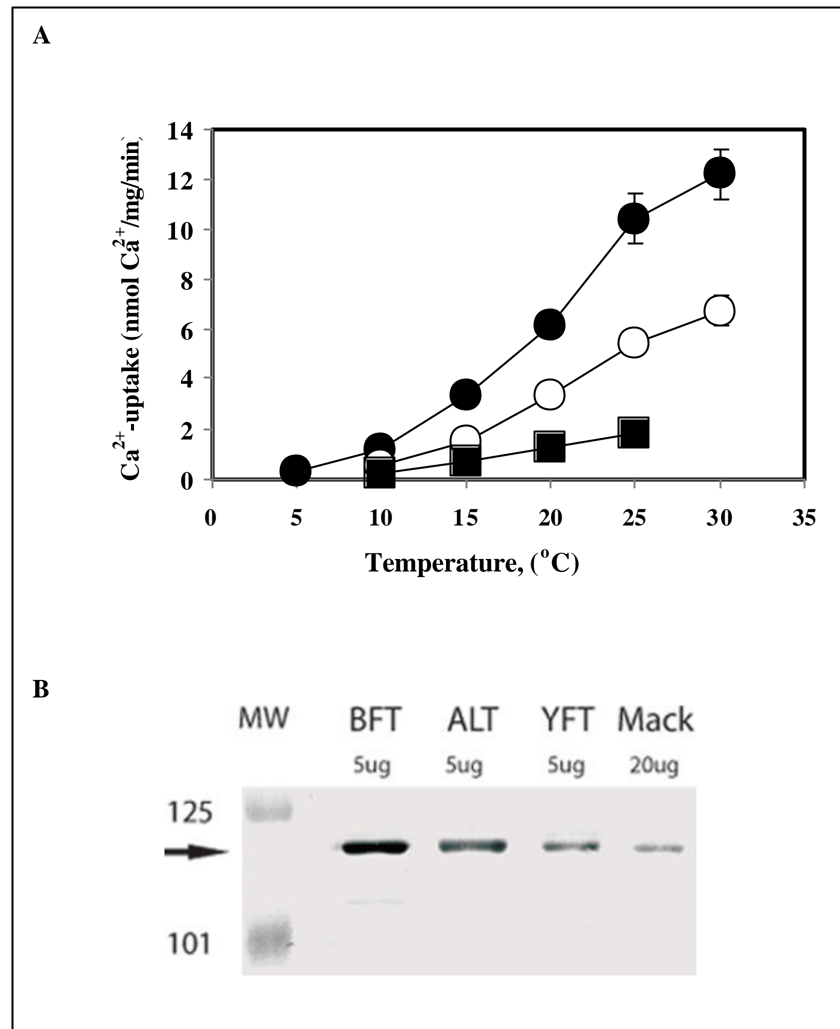

Figure 1. Temperature dependence of Ca^{2+}-uptake in ventricular SR microsomes (A) and SERCA2 Western blot from different Scombridae species (B). In (A) Symbols represent: Pacific bluefin(●); albacore(○) and

yellowfin(■). In (B) BFT, Pacific bluefin tuna; ALT, albacore tuna; YFT, yellowfin tuna; Mack, Pacific mackerel. The numbers above each lane indicate the micrograms of protein used. The arrow indicates the SERCA pump with an estimated M.W. of 110 kDa.

Table I. Comparative rate of Ca^{2+}-uptake catalyzed by the SERCA from different Scombridae fish ventricles microsomes.

Fish	Rate of Ca^{2+}-uptake (nmol Ca^{2+}/mg/min)
Bluefin tuna	10.424 ± 2.033 (n=4)
Albacore tuna	5.422 ± 0.444 (n=4)
Yellowfin tuna	1.793 ± 0.205 (n=3)
Mackerel	0.573 ± 0.042 (n=3)

Values are mean ±S.E and n= the number of experiments made with preparations from at least three individual fish.

Conclusions

Measurements of oxalate-supported Ca^{2+} uptake in SR enriched ventricular vesicles, indicated that tunas were capable of sustaining a rate of Ca^{2+}-uptake that was significantly higher than the mackerel. Among tunas, the cold tolerant bluefin had the highest rates of SR Ca^{2+} uptake.

Western blots reveal that increased SERCA2 protein content is associated with the higher Ca^{2+} uptake seen in bluefin ventricles compared to albacore, yellowfin and mackerel. We hypothesize that a key step in the evolution of high heart rate and high metabolic rate in tunas is increased activity of the SERCA2 enzyme. We also suggest that high levels of SERCA2 in bluefin tuna hearts may be important for retaining cardiac function at cold temperatures.

Acknowledgements

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THE ROLE OF INWARD RECTIFIER POTASSIUM CURRENT IN THE REGULATION OF ACTION POTENTIAL DURATION IN FISH CARDIAC MYOCYTES

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Introduction

The force and time course of cardiac contraction of the fish heart are closely correlated with duration of action potential (Vornanen, 1989) (Figure 1). Therefore, factors that determine action potential duration are important in determining the force and frequency response of the heart, i.e. its pump function.

Inward rectifier potassium current (I_{K1}) current is traditionally thought to maintain negative resting membrane potential close to the equilibrium potential of potassium ions and to contribute to the final phase of action potential repolarisation. More recent studies suggest that it might be active during the plateau phase and participate in the regulation of action potential duration (Ishihara et al. 2002). The repolarising outward current of the inward rectifier channels is limited by voltage-dependent block of intracellular Mg^{2+} and polyamines (spermine, spermidine, putrescine), and therefore small changes in the intracellular concentration of these regulators determinate effectiveness of the I_{K1} as a repolarising current and regulator of action potential duration. This study examines the mechanism of temperature-dependent regulation of the I_{K1}.

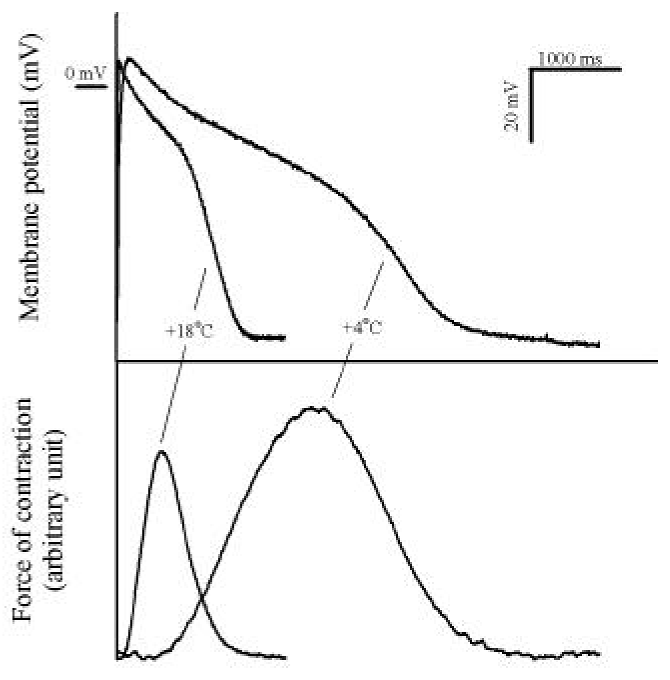

Figure 1. Action potentials and simultaneously recorded contractions of the ventricular muscle of the crucian carp heart at +4° and +18°C.

Methods

Experiments were conducted on enzymatically isolated ventricular myocytes of the crucian carp (*Carassius carassius*) and rainbow trout (*Oncorhynchus mykiss*) heart using the whole-cell patch-clamp technique. Cold-acclimated (+4°) carp and trout were used in the experiments.

Results and Discussion

By alternating the recording between current clamp and voltage clamp modes in the same myocyte we could demonstrate that in ventricular myocytes of cold acclimated (+4°C) crucian carp, acute heat stress increases outward flow of

potassium ions via I_{K1} and causes the temperature-dependent shortening of action potential duration (Figure 2) (Paajanen & Vornanen 2003).

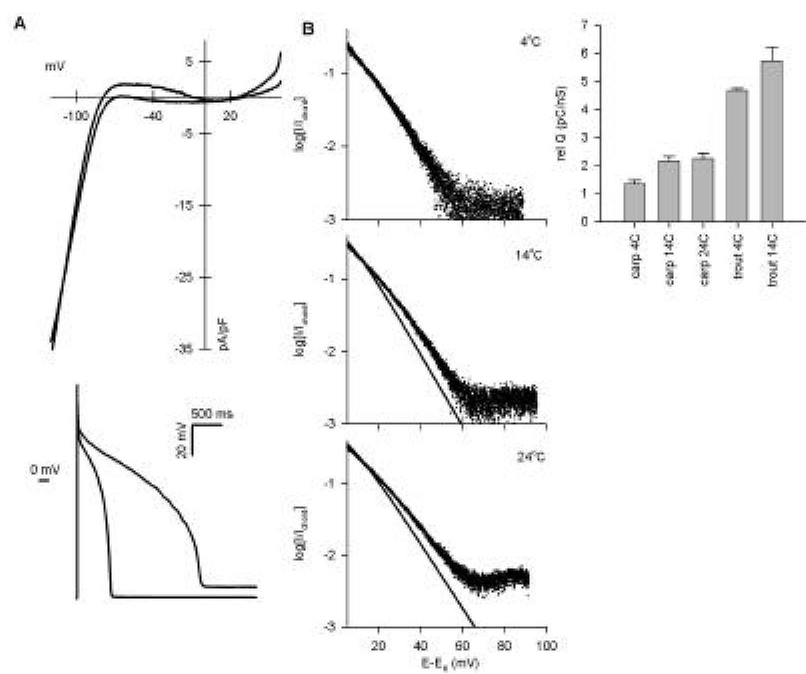

Figure 2. Effects of acute temperature changes on I_{K1} and action potential duration in fish cardiac myocytes. A) I_{K1} and action potentials recorded from the same myocyte of the crucian carp heart at +5° and 14°C. B) Heat stress increases the number of open channels at depolarising voltages (right) and the charge (rel Q) transferred in outward direction through the inwardly rectifying channels (left). The increasing deviation from the theoretical current-voltage relation (solid line) indicates an increase in the number of open channels.

The mechanism that specifically increases the outward current and shorten the action potential at high temperatures has three characteristics: i) Voltage-dependence of the channel block is shifted to more positive values (more open channels), ii) The channel block is incomplete at all voltages, and ii) The voltage

dependent block is less steep at depolarising voltages indicating quantitative/qualitative changes in blocking molecule(s).

Heat stress exerts similar effect on the I_{K1} of both rainbow trout and crucian carp cardiac myocytes suggesting that this mechanism is widely used in fish and possibly other ectotherms in temperature-dependent regulation of action potential duration. The two species differ, however, in regard to cardiac polyamine levels and magnitude of the outward I_{K1}. In comparison to carp myocytes, trout myocytes have less polyamines and therefore more open channels at depolarising voltages. The more powerful I_{K1} of the trout heart may partly explain the shorter action potential of trout ventricular myocytes (APD_{90} = 1.0 and 2.4 in trout and carp, respectively). To conclude, the inward rectifier potassium current is an effective repolarising current in fish cardiac myocytes and able to regulate action potential duration in temperature-dependent manner. Thus, it is likely to play a significant role in excitability and contractility of the fish heart under acute heat stress.

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Acknowledgements

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THERMAL PLASTICITY OF DELAYED RECTIFIER POTASSIUM CURRENT (I_{Kr}) AND FAST SODIUM CURRENT (I_{Na}) OF THE RAINBOW TROUT HEART

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Introduction

The electrical excitability of the cardiac myocytes is determined by sarcolemmal ion currents which flow through ion specific channels. Depolarisation of the sarcolemma is initiated by the sodium current (I_{Na}) which largely determines electrical excitability of the myocytes and conduction velocity of the cardiac action potential. The duration of action potential is regulated by different potassium currents among them the rapid component of the delayed rectifier (I_{Kr}). Since function of the ion channels is dependent on temperature, low temperatures are expected to reduce sarcolemmal ion currents and therefore compromise excitability and conductivity of the cardiac myocytes. The aim of the current study was to examine whether cardiac myocytes of the rainbow trout heart show compensatory changes in the function of sodium and potassium currents in order to achieve partial independence from seasonal temperature changes.

Material and methods

Rainbow trout were acclimated at either 4EC (cold-acclimated, CA) or 18EC (warm-acclimated, WA) for a minimum of 4 weeks before the experiments were done. Sarcolemmal ion currents were measured from enzymatically isolated cardiac myocytes with the whole-cell patch-clamp technique. Experimental

temperatures were 4EC and 11EC for CA trout and 11EC and 18EC for WA trout, respectively.

Results

Sodium current

The sodium current of trout cardiac myocytes was half-maximally inhibited by tetrodotoxin (TTX) at the concentration of 2 nM. Thus, unlike sodium channels of the mammalian heart, the I_{Na} of the fish cardiac myocytes is highly sensitive to TTX. The density of I_{Na} was similar in atrial and ventricular myocytes. In contrast, half-voltage for steady-state activation was more negative and inactivation kinetics was slower in atrial than ventricular myocytes.

Effects of thermal acclimation on I_{Na} were studied only in ventricular myocytes.

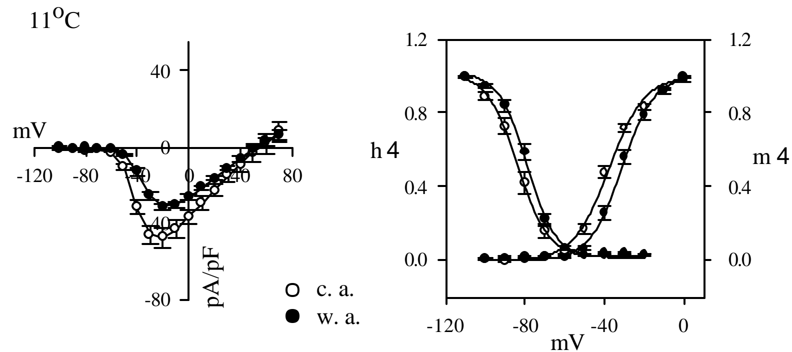

Figure 1. The I_{Na} of the trout ventricular myocytes from CA and WA fish at 11°C. The current-voltage relationship is at left and the steady-state activation and inactivation at right.

Cold-acclimation increased the density of I_{Na} suggesting a positive thermal compensation for the propagation velocity of the cardiac action potential. The half-voltage of the steady-state activation curve of trout I_{Na} was shifted about 6 mV to more negative voltages by cold acclimation (Figure 1). This will probably lower the stimulus threshold for action potentials and further improves cardiac

excitability in the cold. Furthermore, the kinetics of inactivation was faster in ventricular myocytes of CA than WA trout.

Delayed rectifier potassium current

The delayed rectifier potassium current is the major potassium current of the trout atrial cells, while ventricular cells have in addition to the I_{Kr} also a large background inward rectifier potassium current (I_{K1}) (Vornanen et al., 2002). The density of the I_{Kr} was much larger in atrial than ventricular myocytes of the trout heart. The *in situ* hybridization indicated that there was similar difference between atrium and ventricle in the level of mRNA of the respective ion channel gene, the trout ERG (trout ether a-go-go related gene). In both atrial and ventricular myocytes, acclimation to cold increased the density of the I_{Kr} but did not change the kinetics of the current. Inhibition of the I_{Kr} with a specific ERG channel blocker, 2 ΦM E-4031, caused marked prolongation of action potential duration in both WA and CA trout indicating the central role of this potassium current in the regulation of AP duration of the trout heart (Figure 2).

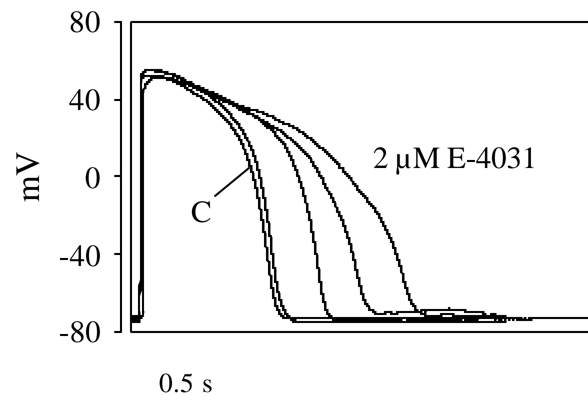

Figure 2. Inhibition of the I_{Kr} with 2 µM E-4031, a specific blocker of the rapid delayed rectifier potassium channels, prolongs the action potential of the rainbow trout ventricle.

Conclusions

These results indicate that both sodium current and delayed rectifier potassium current of the trout heart show plasticity under thermal stress. The changes in I_{Na} tend to maintain adequate excitability in the cold, while increased density of the I_{Kr} will prevent excessive lengthening of action potential duration in the cold.

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PLASTICITY IN EXCITATION-CONTRACTION COUPLING IN CARDIAC MYOCYTES FROM RAINBOW TROUT

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

Because fish are ectotherms they exhibit a degree of plasticity in excitation-contraction coupling which allows their hearts to function over a range of environmental conditions. We have investigated the role played by the cardiac sarcoplasmic reticulum (SR) in contributing to this plasticity in rainbow trout ventricle.

Methods

Our aim in this study was to better understand the role of the trout SR during e-c coupling by investigating temporal and spatial co-ordination of the Ca^{2+} transient and the occurrence of Ca^{2+} sparks in intact, contracting trout ventricular myocytes using confocal microscopy. Ca^{2+} sparks and Ca^{2+} transients have not been reported for fish hearts. Therefore, in addition to trout, experiments were conducted on rat ventricular myocytes as a positive control. Myocytes from Wistar rats and rainbow trout were loaded with 4-10 µM Fluo 4 and were examined with repetitive line scans (1,000 lines of 512 pixels, 4-7 ms intervals) across the width of the cell.

Results and Conclusions

Ca^{2+} transients were readily observed in both trout and rat but each showed different temporal and spatial characteristics. Ca^{2+} sparks were present in 80% of the rat myocytes under control conditions but none were observed in trout myocytes. Spark-like events were observed in a very small number (2.3%) of the trout myocytes after agonist stimulation. These results suggest a limited role for the SR in trout ventricular myocytes. The results will be discussed as they relate to plasticity in trout cardiac design.

Acknowledments

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COMPARATIVE ASPECTS OF SERCA2 TEMPERATURE DEPENDENCE IN THE ATRIUM OF ENDOTHERMIC FISHES: SALMON SHARK AND BLUFIN TUNA

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Introduction

Endothermy in fish and sharks is rare but two families (Lamnidae and Scombridae) have representatives that maintain elevated tissue temperatures in the brain, viscera and swimming muscles (Bernal et al., 2001). Both salmon sharks and bluefin tuna have convergent morphologies and physiology that permit the conservation of metabolic heat. These fish and sharks are active epipelagic predators that display evolutionary convergence for a number of morphological and physiological properties. These fish reduce conductance between the body and the sea with counter-current heat exchangers in the viscera, brain and body musculature. Specializations for their active lifestyles include numerous convergent characters including cardio-respiratory characteristics that ensure high rates of oxygen delivery to sustain elevated aerobic metabolism. While the bluefin tunas and salmon sharks maintain elevated internal body temperatures the heart operates at ambient temperatures. The characteristics of maintaining a warm body and a cold heart are rare among vertebrates.

Cardiac contraction is highly dependent on the free intracellular calcium concentration. The Ca^{2+}-ATPase (SERCA2) enzyme is responsible for removal of Ca^{2+} from the cytosol, which lowers the intracellular $[Ca^{2+}]$ and allows the relaxation of the cardiac muscle. Sarcoplasmic reticulum function measured in fish and mammals indicates there is higher calcium uptake in atrium than ventricle (Luss et al., 1999). In bluefin tuna, a prior study has shown that the ventricle SERCA2 activity is highly temperature dependent (Landeira et al. 2004). In this study, SERCA2 activity of atrium was examined in response to temperature in bluefin tunas (*Thunnus thynnus*) and salmon sharks (*Lamna ditropis*). For comparison, Western blot analysis was also examined in the closely related mako (*Isurus oxyrinchus*) and more distantly related thresher shark (*Alopias vulpinus*).

Methods

For atrium microsomal isolation, the fishes were euthanized and the hearts were immediately removed. Atriums were sliced into small pieces, freeze-clamped and stored in liquid nitrogen. Atrium SR-enriched vesicle isolation was prepared by a protocol described in Landeira et al. (2003). Ca^{2+} uptake was measured with a calcium sensitive dye (fura-2) method described in Liu et al. (1997). Atrial microsomal fractions (30μg protein) were separated by electrophoresis on 7.5% SDS-PAGE for gels and Western blot analysis.

Results

Gel electrophoresis of vesicles derived from all of fish atrium tested indicates that most of the microsomal vesicles protein migrated as 110kDa band characteristic of the SERCA2 enzyme. Western blot analysis shows that the single 110 kDa band reacts with a polyclonal antibody raised against a conserved cardiac SERCA2 protein sequence (Fig. 1).

The atrial microsomal vesicles derived from salmon shark and bluefin tuna accumulate Ca^{2+} and hydrolyze ATP at all temperature tested. The uptake rates increase with the temperature and the fastest rates of uptake were found at 30°C for bluefin and salmon sharks. At 25°C the salmon shark Ca^{2+} uptake rate was 5 times higher than tuna (Fig 2). Salmon shark (Laminidae) atrium has higher atrium SERCA2 activity at all temperatures tested when compared with bluefin tuna (Scombridae). These results indicate a high rate of expression of the

SERCA2 protein, and a higher capacity for Ca^{2+} uptake in the salmon shark atrium sarcoplasmic reticulum. This characteristic can produce faster recovery from cardiac muscle contraction in the salmon shark at all temperatures tested.

MM mako thresher s. shark BFT

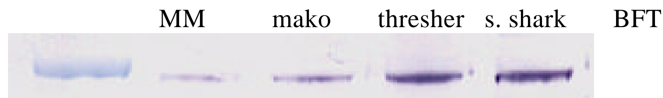

Figure. 1. Western blot analysis of microsomes from atria of different fishes. MM, molecular marker (110kDa); mako (*Isurus oxyrinchus*); thresher (*Alopias vulpinus*); salmon shark (*Lamna ditropis*); BFT bluefin tuna (*Thunnus thynnus*)

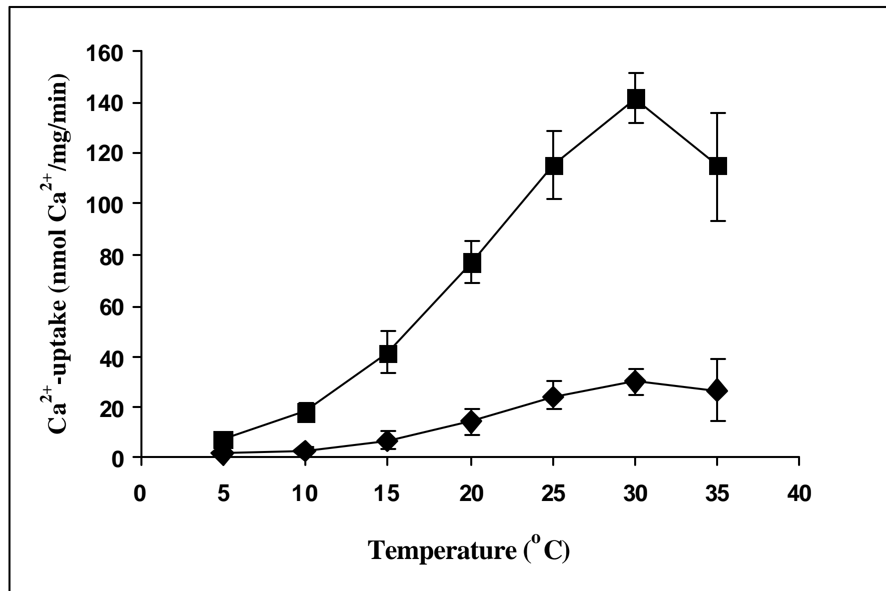

Figure. 2. Temperature dependence of Ca^{2+} uptake (SERCA2) in atrial sarcoplasmic reticulum (SR) microsomes from salmon shark (¦) and bluefin tuna (?). Values represent mean ± SE of 3 or 4 individual fish.

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