

Fish cardiorespiratory physiology in an era of climate change¹

A.P. Farrell, E.J. Eliason, E. Sandblom, and T.D. Clark

Abstract: This review examines selected areas of cardiovascular physiology where there have been impressive gains of knowledge and indicates fertile areas for future research. Because arterial blood is usually fully saturated with oxygen, increasing cardiac output is the only means for transferring substantially more oxygen to tissues. Consequently, any behavioural or environmental change that alters oxygen uptake typically involves a change in cardiac output, which in fishes can amount to a threefold change. During exercise, not all fishes necessarily have the same ability as salmonids to increase cardiac output by increasing stroke volume; they rely more on increases in heart rate instead. The benefits associated with increasing cardiac output via stroke volume or heart rate are unclear. Regardless, all fishes examined so far show an exquisite cardiac sensitivity to filling pressure and the cellular basis for this heightened cardiac stretch sensitivity in fish is being unraveled. Even so, a fully integrated picture of cardiovascular functioning in fishes is hampered by a dearth of studies on venous circulatory control. Potent positive cardiac inotropy involves stimulation of sarcolemmal β -adrenoceptors, which increases the peak trans-sarcolemmal current for calcium and the intracellular calcium transient available for binding to troponin C. However, adrenergic sensitivity is temperature-dependent in part through effects on membrane currents and receptor density. The membrane currents contributing to the pacemaker action potential are also being studied but remain a prime area for further study. Why maximum heart rate is limited to a low rate in most fishes compared with similar-sized mammals, even when Q_{10} effects are considered, remains a mystery. Fish hearts have up to three oxygen supply routes. The degree of coronary capillarization circulation is of primary importance to the compact myocardium, unlike the spongy myocardium, where venous oxygen partial pressure appears to be the critical factor in terms of oxygen delivery. Air-breathing fishes can boost the venous oxygen content and oxygen partial pressure by taking an air breath, thereby providing a third myocardial oxygen supply route that perhaps compensates for the potentially precarious supply to the spongy myocardium during hypoxia and exercise. In addition to venous hypoxemia, acidemia and hyperkalemia can accompany exhaustive exercise and acute warming, perhaps impairing the heart were it not for a cardiac protection mechanism afforded by β -adrenergic stimulation. With warming, however, a mismatch between an animal's demand for oxygen (a Q_{10} effect) and the capacity of the circulatory and ventilatory systems to deliver this oxygen develops beyond an optimum temperature. At temperature extremes in salmon, it is proposed that detrimental changes in venous blood composition, coupled with a breakdown of the cardiac protective mechanism, is a potential mechanism to explain the decline in maximum and cardiac arrhythmias that are observed. Furthermore, the fall off in scope for heart rate and cardiac output is used to explain the decrease in aerobic scope above the optimum temperature, which may then explain the field observation that adult sockeye salmon (*Oncorhynchus nerka* (Walbaum in Artedi, 1792)) have difficulty migrating to their spawning area at temperatures above their optimum. Such mechanistic linkages to lifetime fitness, whether they are cardiovascular or not, should assist with predictions in this era of global climate change.

Résumé : Notre rétrospective examine certains domaines choisis de la physiologie cardiovasculaire qui ont connu un avancement impressionnant des connaissances et elle signale des avenues prometteuses pour la recherche future. Parce que le sang artériel est en général complètement saturé en oxygène, le seul moyen de transférer plus d'oxygène aux tissus est d'augmenter le débit cardiaque. En conséquence, tout changement comportemental ou environnemental qui modifie l'apport d'oxygène implique typiquement une variation du débit cardiaque, ce qui chez les poissons peut représenter un changement de l'ordre de trois fois. Durant l'exercice, tous les poissons ne possèdent pas nécessairement la capacité qu'ont les salmonidés d'accroître le débit cardiaque en augmentant le volume systolique; ils utilisent plutôt une accélération du rythme cardiaque. Les bénéfices associés à l'augmentation du débit cardiaque par le débit systolique ou par le rythme car-

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A.P. Farrell,² E.J. Eliason, E. Sandblom, and T.D. Clark. Department of Zoology and Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC V6T 1Z4, Canada.

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²Corresponding author (e-mail: farrellt@interchange.ubc.ca).

diacque ne sont pas clairs. Néanmoins, tous les poissons examinés à ce jour montrent une très grande sensibilité à la pression de remplissage; la base cellulaire de cette sensibilité accrue à l'étirement cardiaque chez les poissons est en train d'être élucidée. Malgré tout, l'obtention d'une image totalement intégrée de la fonction cardiovasculaire chez les poissons est retardée par le manque d'études sur le contrôle circulatoire veineux. La puissante inotropie positive du cœur implique la stimulation des récepteurs β -adrénergiques du sarcolemme, qui augmente le courant de pointe du calcium à travers le sarcolemme et le calcium intracellulaire transitoire disponible pour la fixation à la troponine C. Cependant, la sensibilité adrénérgique est fonction de la température en partie par les effets sur les courants membranaires et la densité des récepteurs. Les courants membranaires contribuant au potentiel d'action du centre d'automatisme cardiaque font aussi l'objet d'études, mais restent toujours des sujets de premier ordre pour des travaux futurs. C'est encore un mystère de savoir pourquoi le rythme cardiaque maximal de la plupart des poissons est confiné à des valeurs basses, par comparaison à celui des mammifères de taille semblable, même après correction pour les effets du Q_{10} . Les cœurs de poissons bénéficient de jusqu'à trois voies d'approvisionnement en oxygène. Le degré de circulation dans la capillarisation coronaire est de première importance dans le myocarde compact; au contraire, dans le myocarde spongieux, la pression partielle d'oxygène veineux semble être le facteur critique en ce qui concerne l'apport d'oxygène. Les poissons à respiration aérienne peuvent faire augmenter le contenu en oxygène et la pression partielle d'oxygène veineux par une aspiration d'air, procurant ainsi une troisième voie d'approvisionnement du myocarde en oxygène, ce qui compense peut-être l'apport potentiellement fragile au myocarde spongieux durant l'hypoxie et l'exercice. En plus de l'hypoxémie veineuse, l'acidémie et l'hyperkaliémie peuvent accompagner un exercice épuisant et un réchauffement aigu, ce qui affaiblirait peut-être le cœur si ce n'était du mécanisme de protection cardiaque apporté par la stimulation β -adrénergique. Dans le cas du réchauffement cependant, il se développe au-delà d'une température optimale un mésappariement entre la demande d'oxygène de l'animal (un effet du Q_{10}) et la capacité des systèmes de circulation et de ventilation à fournir cet oxygène. Chez le saumon, aux températures extrêmes, nous croyons que les changements nuisibles dans la composition du sang veineux, combinés à une panne du processus de protection cardiaque, peuvent être un mécanisme pour expliquer le déclin du débit cardiaque maximal et les arythmies cardiaques que l'on observe. De plus, la chute de l'étendue des débit et rythme cardiaques sert à expliquer la diminution de la portée aérobie au-delà de la température optimale, ce qui peut expliquer à son tour les observations de terrain qui indiquent que les saumons rouges (*Oncorhynchus nerka* (Walbaum in Artedi, 1792)) adultes ont du mal à migrer vers leur site de fraie aux températures supérieures à l'optimum. Ces liens mécanistes à la fitness de la vie entière, qu'ils soient cardiovasculaires ou non, devraient aider à formuler des prédictions dans la période actuelle de changement climatique global.

[Traduit par la Rédaction]

Introduction

Some 20 years ago, a review on fish cardiovascular physiology appeared in this journal (Farrell 1984). At the time there were just two relevant chapters in the treatise *Fish Physiology*, one on the circulatory system (Randall 1970) and the other on the respiratory and circulatory systems during exercise (Jones and Randall 1978). Much has advanced since then. By 1992, the field of fish cardiovascular physiology required 13 chapters in a two-volume issue of *Fish Physiology* (edited by W.S. Hoar, D.J. Randall, and A.P. Farrell). Today, the breadth of information on cardiovascular physiology in fishes lies well beyond the scope of any single review. Indeed, whole chapters on the specialized cardiovascular systems of tunas (family Scombridae), polar fishes, and primitive fishes have appeared in recent volumes of *Fish Physiology*. Consequently, this review visits selected areas of cardiovascular physiology where there have been impressive gains of knowledge. It also indicates fertile areas for future research and revisits some earlier prognostications (Farrell 1991).

To ensure that the circulatory system is placed squarely within its primary role, the review begins and closes by considering oxygen delivery; anaerobic energy production is not a viable option for long-term survival in more than a few fish species and has been considered in depth elsewhere (Farrell and Stecyk 2007; Richards et al. 2009). The closing section also indicates how cardiorespiratory physiologists

might provide a meaningful contribution to the challenge of understanding global climate change, especially with respect to the warming trends that are impacting aquatic ecosystems. The reasoning for selecting this particular application of cardiovascular physiology is rather simple.

Some 60 years ago, a direct linkage was made between a fish's thermal tolerance and the fish's ability to consume oxygen (Fry 1947). Subsequently, a similar association was made between thermal optima for oxygen consumption, swimming performance, and cardiac performance (Brett 1971). Today, the concept of oxygen-limited thermal tolerance has been consolidated for a range of fishes, including some tropical and polar species, and even some aquatic invertebrates, and there is an increasing likelihood that the cardiovascular system limits tissue oxygen delivery (Farrell 1997; Taylor et al. 1997; Frederich and Pörtner 2000; Farrell 2002; Pörtner 2002; Gollock et al. 2006; Franklin et al. 2007; Pörtner and Knust 2007; Pörtner and Farrell 2008; Munday et al. 2008).

Throughout this review there is a bias towards salmonids because this is the group of fishes for which we have the greatest knowledge in this field. Nevertheless, other fish groups are well represented where information exists. Indeed, fish cardiovascular physiologists continue to place their discoveries into a broad evolutionary framework and in the process have become far less reliant on mammalian-based information. The ultimate research challenge is to dis-

cover to what degree the generalities presented here apply to the >25 000 species of fish, about half of all vertebrates.

Determinants of oxygen delivery to tissues

The Fick equation, established nearly a century ago, mathematically describes the central role of the circulatory system in delivering oxygen to tissues. It states that oxygen consumption by tissues can be estimated from the product of cardiac output (\dot{V}_b) and the difference between the oxygen content of arterial and venous blood (C_{aO_2} and C_{vO_2} , respectively), which is termed the arterio-venous oxygen difference (C_{a-vO_2}). Thus, according to the Fick equation, any change in oxygen uptake ($\dot{V}O_2$) must be accounted for by some combination of change in \dot{V}_b and C_{a-vO_2} .

In this regard, the specific contribution made by the heart can be mathematically expressed as the arterial oxygen transfer (T_{aO_2}): $T_{aO_2} = \dot{V}_b \times C_{aO_2}$. The primary means for transferring more oxygen to tissues is by increasing \dot{V}_b because arterial blood is usually fully saturated with oxygen (C_{aO_2} being determined primarily by the concentration of hemoglobin in the blood ([Hb]) and its percent oxygen saturation). Increases in T_{aO_2} are possible through changes in [Hb], typically resulting from splenic release of red blood cells as an acute response and manufacture of new red blood cells (erythropoiesis) as an acclimatory response. However, alterations in [Hb] tend to be small and variable across species (see review by Gallagher and Farrell 1998) relative to those for \dot{V}_b . Consequently, any behavioural or environmental change that alters $\dot{V}O_2$ typically requires a change in \dot{V}_b .

For fishes, it is well established that large increases in \dot{V}_b occur in situations where tissue oxygen demand increases, such as during aerobic exercise, after feeding and with increased temperature. For example, up to a threefold increase in \dot{V}_b has been measured in salmonids during prolonged swimming activity (i.e., a critical swimming speed test) (Kiceniuk and Jones 1977), and up to a twofold increase has been measured after a large meal (Farrell et al. 2001). Similarly, \dot{V}_b can double with a 10 °C increase in water temperature (i.e., a Q_{10} of 2; Gollock et al. 2006; Clark et al. 2008a). Nevertheless, the increase for $\dot{V}O_2$ in all of these situations exceeds that for T_{aO_2} because C_{a-vO_2} increases as C_{vO_2} decreases with increased tissue oxygen extraction. An increase in C_{a-vO_2} is possible because tissues routinely extract as little as a third of the oxygen contained in arterial blood (Clark et al. 2008a). Consequently, a sixfold increase in $\dot{V}O_2$ can be achieved during exercise in salmonids by simply coupling a threefold increase in \dot{V}_b with a twofold increase in C_{a-vO_2} .

Regulating cardiac performance

\dot{V}_b may increase in fishes through independent increases in cardiac stroke volume and heart rate (tachycardia). Both stroke volume and heart rate have intrinsic capacities, as well as extrinsic control mechanisms. Salmonids almost triple stroke volume during strenuous swimming activity, while increased heart rate is less important (Kiceniuk and Jones 1977). Even so, the relative contributions of stroke volume and heart rate towards a given increase in \dot{V}_b vary substantially among fishes and the type of circulatory chal-

lenge. During exercise, not all fishes necessarily have the same ability as salmonids to increase stroke volume, relying more on increases in heart rate (Cooke et al. 2003; Clark et al. 2005; Sandblom et al. 2005; Clark and Seymour 2006). Furthermore, during digestion in rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792)), for example, the postprandial increase in \dot{V}_b occurs largely via tachycardia and, as a result, a strong relationship exists between heart rate and $\dot{V}O_2$ (Eliason et al. 2008). The benefit associated with increasing via heart rate or stroke volume has not been fully characterized for fishes. Myocardial oxygen consumption per unit of mechanical work increases with heart rate in the dog ventricle, i.e., efficiency progressively decreases with increasing heart rate (Berglund et al. 1958). In contrast, cardiac efficiency of the myocardium of rainbow trout has an optimum at an intermediate pacing frequency (Harwood et al. 2002). Furthermore, in theory, a heart with a higher stroke volume (and large radius) has to create a greater wall tension to generate a similar blood pressure as a heart with a smaller stroke volume (Laplace's law of the heart). Further examination of these trade-offs for fish hearts would be worthwhile.

Changes in stroke volume and heart rate can also oppose each other with no change in \dot{V}_b . For example, during environmental hypoxia, reflex bradycardia, which is a response unique to fishes among the vertebrate world, is often associated with increased stroke volume and maintained \dot{V}_b (Sandblom and Axelsson 2005; Perry and Desforges 2006; Gamperl and Driedzic 2009). The possible benefits of reflex bradycardia in fishes are considered elsewhere (Farrell 2007a).

Regulating cardiac stroke volume

Intrinsic control of cardiac contractility

Critical to proper long-term cardiac functioning is that all the blood received by the heart from the central veins should be pumped out into the ventral aorta, i.e., the volume of blood entering the ventricle during diastole equals the stroke volume leaving the ventricle during systole. This functional coupling of filling and emptying is possible because of the intrinsic Frank–Starling mechanism, which links cardiac stretch with the resultant force of cardiac contraction at the cellular level. The fundamental importance of this intrinsic mechanism is evident because it is common to all vertebrate hearts examined to date. Even the primitive hagfish (*Eptatretus cirrhatius* (Forster in Bloch and Schneider, 1801)), which has no cardiac innervation for external cardiac control, can more than double stroke volume with increased cardiac filling pressure (Forster 1989; Johnsson and Axelsson 1996). The relationship between cardiac stroke volume and cardiac filling pressure is called a Starling curve.

When Starling curves for fishes are compared with mammals, all fishes examined so far show an exquisite cardiac sensitivity to filling pressure. Stroke volume can be doubled with just a 0.02 kPa increase in filling pressure. In contrast, mammalian hearts require larger increases in filling pressure to generate smaller increases (<50%) in stroke volume and they also operate at filling pressures that exceed those seen in fishes (Rothe 1983). To explain the cellular basis for this heightened cardiac stretch sensitivity in fish, a pivotal dis-

covery has been that the functional peak of the sarcomere length–tension relationship occurs at a longer sarcomere length in rainbow trout than in rat (Shiels and White 2008). In fact, the elastic protein titin may be critical in this regard because of its contribution to the passive tension of the myocardium. Titin, which anchors myofilaments at the Z-line and acts as a spring, has isoforms that differ in their passive properties and contributions to cardiac filling (Fukuda and Granzier 2005).

Cardiac filling (hence stroke volume) depends primarily on the total energy (= kinetic energy + potential energy) contained in central venous blood relative to that in the atrium. When \dot{V}_b increases in mammals, up to 50% of the total energy in systemic venous blood exists as kinetic energy (Burton 1972). The kinetic energy of the venous blood in fishes has not been estimated to our knowledge. Consequently, we still use venous blood pressure (= potential energy) as a surrogate for the total energy driving cardiac filling.

Given the exquisite sensitivity of the fish heart to venous blood pressure, the dearth of studies on control of the venous side of the fish circulatory system have prevented a fully integrated picture of cardiovascular functioning in fishes from emerging. Indeed, important differences have emerged in terms of venous pressure control for rainbow trout and dogfish (*Squalus acanthias* L., 1758). In rainbow trout, α -adrenergic venoconstriction increases venous blood pressure and mobilizes blood volume from capacious veins during exercise and hypoxia (Sandblom and Axelsson 2006; Sandblom et al. 2006b). However, this control mechanism may be absent in dogfish during hypoxia (Sandblom et al. 2009), although venoconstriction occurs in dogfish following injection of adrenaline and phenylephrine (Sandblom et al. 2006a). Furthermore, Minerick et al. (2003) used blood infusions and withdrawals in rainbow trout to illustrate that changes in venous pressure can entrain cardiac output with a time lag of just a few seconds. Venous control mechanisms deserve further attention, especially in primitive fishes.

The subambient venous blood pressures reported for certain fishes at rest, particularly among elasmobranchs, reduce the transmural pressure across the venular vessel wall (Sandblom et al. 2009). Under such situations, the heart must suck venous blood into its chambers (vis-a-fronte cardiac filling). The importance of vis-a-fronte cardiac filling in dogfish and rainbow trout is unequivocal, as pericardectomy increases venous blood pressure in dogfish (Sandblom et al. 2006a) and shifts the Starling curve to the right in trout (Farrell et al. 1988b; but see Minerick et al. 2003). This is not to say that vis-a-tergo filling (or perhaps kinetic energy) is unimportant in these species. Careful inspection of Starling curves (Farrell et al. 1988b; Franklin and Davie 1992b, 1993) clearly shows that, even when the pericardium is intact, positive venous filling pressures are needed to generate maximum stroke volume. Thus, as stroke volume increases when rainbow trout move from resting to exercising states, they likely shift from a vis-a-fronte to a vis-a-tergo cardiac filling mode. Perhaps during vertebrate evolution the shift away from vis-a-fronte cardiac filling was associated with an increased complexity for neural control of veins and venous return (Sandblom et al. 2009). To combat hydrostatic

pressure effects on veins in terrestrial vertebrates (i.e., the gravitational forces acting on a column of blood, increasing the transmural pressure acting across a blood vessel wall), a necessary evolutionary sophistication was the evolution of parietal valves in veins, which are absent (and perhaps not essential) in fishes (Satchell 1991). Thus, air emersion could create problems of venous pooling for fish, as (unrealistically) demonstrated by the massive pooling in the tail of a hagfish when it is briefly held upright in air (A.P. Farrell, personal observation). Consequently, the study of venous control during terrestrial air-breathing in fishes (e.g., in mudskippers (genus *Periophthalmus* Bloch and Schneider, 1801)) might be revealing in this regard.

Noninvasive echocardiography (high-frequency sound waves) has proven effective in producing high-resolution two-dimensional images of fish hearts, which have provided information on blood velocity and flow patterns through the heart (including chamber filling patterns; Lai et al. 1990, 1996, 1998, 2004; Coucelo et al. 1996; Cotter et al. 2008) and on cardiac chamber shapes and dimensions (including stroke volume and ventricular performance; Franklin and Davie 1992a, 1992b; Coucelo et al. 2000; Claireaux et al. 2005). However, this type of imaging necessitates anaesthesia and restraint of the fish. Therefore, given the acute sensitivity of the heart to venous pressure and of venous pressure to gravitational forces and extramural pressure, the results must be interpreted with great care. A consistent result from these collective works is that end-systolic volume of the fish ventricle is very small (Franklin and Davie 1992b; Coucelo et al. 2000), unlike that of the mammalian heart. Also atrial filling can occupy a substantial portion (40%–77%) of the cardiac cycle. Atrial filling can be monophasic as required for a purely vis-a-tergo mechanism (e.g., Minerick et al. 2003), biphasic owing to a vis-a-fronte effect from ventricular contraction (see above, e.g., Lai et al. 1990), and triphasic as reported for the leopard shark (*Triakis semifasciata* Girard, 1855) (Lai et al. 1990). The third wave of atrial filling likely involves sinus contraction, but a corresponding V-wave in the ECG has not been detected to confirm this possibility. Even so, such an association may be difficult to reveal given that the sinus venosus has a limited amount of cardiac muscle and only relatively small contraction is needed to significantly boost a subambient venous pressures.

In mammalian hearts, the majority of ventricular filling is driven by the positive venous blood pressure and kinetic energy in the central veins, and atrial contraction only “tops up” the ventricle. The precise role of atrial contraction in filling of the ventricle of fishes is still being debated among the aforementioned studies. Cotter et al. (2008) suggest that ventricular filling is primarily by atrial systole in rainbow trout. This result contrasts with several studies in various teleost and elasmobranch species that demonstrate a biphasic ventricular filling pattern (Lai et al. 1990, 1996, 1998, 2004; Coucelo et al. 1996), consisting of an early direct venous inflow followed by a late phase, which follows the P-wave (atrial contraction). The relative proportion of early to late phase filling (as indicated by the ratio of velocity profiles) suggests that the late phase is at least as large if not greater than the early phase. Consequently, atrial contraction in fishes has a more prominent role in setting the end-diastolic volume of the ventricle and hence cardiac stroke volume

than in mammals. Simply put, the thin-walled atrium is a preamplifier in terms of cardiac blood pressure development, while the much thicker-walled ventricle is the primary amplifier, setting systolic blood pressure in the ventral aorta. Atrial volume can also exceed that of the ventricle, which may allow the atrium to play the role of a variable volume reservoir within a relatively stiff pericardial cavity (Forster and Farrell 1994).

Ventricular anatomy sets an upper dimensional limit to cardiac stroke volume. Even so, ventricular mass shows an order of magnitude genetic variability among fish species (0.05%–0.4% of body mass), as well as an extraordinary environmental and physiological plasticity within a species (see Gamperl and Farrell 2004). Phenotypic plasticity can in some cases result in as much as a threefold increase in relative ventricular mass (rM_v). Stimuli known to cause cardiac hypertrophy in fishes include anemia (McClelland et al. 2005; Simonot and Farrell 2007), cold acclimation (Goolish 1987; Farrell et al. 1988a; Pelouch and Vornanen 1996), and male sexual maturation (Franklin and Davie 1992a; Clark and Rodnick 1999). This cardiac remodeling is apparently functional, even though Gamperl and Farrell (2004) make only vague reference to “increased functional capacity”. The increase in rM_v in mature male salmonids through ventricular hypertrophy is suggested to be required with the associated hypervolemia and hypertension (Clark and Rodnick 1999; Clark et al. 2009). Perhaps, the circulatory changes (increased blood volume and cardiac afterload) drive the cardiac remodeling.

The well-established interspecific range for rM_v among fishes reflects a tremendous genetic diversity for both maximum stroke volume (set in part by maximum end-diastolic volume) and arterial blood pressure (set in part by ventricular wall thickness). Benthic fish species with a rM_v around 0.05%, such as winter flounder (*Pseudopleuronectes americanus* (Walbaum, 1792)) and shorthorn sculpin (*Myoxocephalus scorpius* (L., 1758)), have a higher maximum stroke volume but lower arterial blood pressure than rainbow trout. Athletic fishes, such as salmonids and tunas, have a higher rM_v (between 0.1% and 0.4%) (Tota et al. 1983; Farrell 1996). However, the rM_v of 0.4% for Antarctic icefishes (family Channichthyidae) is associated with the largest resting stroke volume reported for a fish and a very low arterial blood pressure (Axelsson 2005). Despite such fascinating examples of phenotypic plasticity and genetic differences in cardiac anatomy, understanding how these differences integrate into fish cardiovascular function remains in its infancy.

Extrinsic control of cardiac contractility

The strength of a cardiac contraction is controlled by numerous extrinsic factors (cardiac nerves, hormones, and paracrine agents), which shift the position of the Starling curve relative to filling pressure as a result of inotropic actions, making the heart more or less sensitive to filling pressure. The diverse array of inotropic agents and their cellular transduction mechanisms have been summarized elsewhere (Farrar and Rodnick 2004; Imbrogno et al. 2006; Olson and Farrell 2006; Nobata et al. 2008). The most potent of the positive inotropic agents involve stimulation of β -adrenoceptors on the sarcolemmal membrane. Stimulation of these receptors increases the peak current for calcium (I_{Ca}) (Coyne et al.

2000; Kim et al. 2000; Shiels et al. 2003), and this increase in calcium influx increases the calcium available for binding to troponin C, thereby increasing the potential for crossbridge cycling between actin and myosin.

Still missing in an era of reductionist biology is an integrated picture of the interplay among various effector agents towards the overall control of the cardiovascular system. A good example of how effector system interplay may be critical in fishes is illustrated by inotropic agents that improve cardiac contractility when end-systolic volume is very small. Thus, unless more blood is mobilized towards the heart to increase end-diastolic volume, likely through venular control mechanisms, stimulation of cardiac contractility alone has little effect on the resultant stroke volume.

Among known negative inotropic agents, the variation of venous blood composition may be underappreciated, despite a longstanding knowledge of the actions of acidemia, hyperkalemia and hypoxemia on fish hearts (Farrell 1984; Driedzic and Gesser 1994; Nielsen and Gesser 2001). This situation is surprising for two reasons. First, a state of combined venous acidemia, hyperkalemia and hypoxemia is commonly found after exhaustive exercise (Milligan 1996; Holk and Lykkeboe 1998; Milligan et al. 2000), with acute warming (Clark et al. 2008a; Steinhausen et al. 2008), and during environmental hypoxia. Second, most if not all of the fish myocardium has intimate contact with venous blood (see below) and so these alterations in venous blood composition have a tremendous potential to negatively impact cardiac performance.

Emerging is the need to consider the potential for inotropic effector interactions. For example, when oxygen cannot diffuse fast enough into the cardiomyocyte to meet oxygen demands of cardiac work (see below), a threshold oxygen partial pressure (PO_2) will exist. Such thresholds are demonstrated easily in perfused heart preparations by progressively titrating down the PO_2 of the perfusate until maximum cardiac performance collapses (Hanson et al. 2006). Low extracellular pH and elevated K^+ then increase the threshold PO_2 for maximum cardiac work, as might be expected given their individual negative inotropic effects. Thus, during or following states of severe exercise, when the heart needs to function maximally, such interactions would be problematic were it not for the fact that β -adrenergic stimulation protects cardiac inotropy in rainbow trout, restoring the normal PO_2 threshold (Hanson et al. 2006). However, while this adrenergic protective mechanism is functional at 10 °C, it breaks down at an elevated acclimation temperature, resulting in an abnormally high PO_2 threshold at 18 °C (Hanson and Farrell 2007). Reduced adrenergic protection may be due to a reduced stimulation of sarcolemmal calcium influx during cardiac contraction that might accompany the decrease in cardiac sarcolemmal β -adrenoceptor density seen in warm-acclimated trout (Keen et al. 1993). Indeed, the response of I_{Ca} to adrenaline is proportionately lower at 7 versus 21 °C (Shiels et al. 2003). Because temperature clearly has a major influence on the qualitative and quantitative natures of cardiac control mechanisms in fishes and other ectothermic vertebrates (Ask 1981, 1983), it deserves greater attention in the future.

Regulating heart rate

The primary determinant of heart rate in all fish is the intrinsic pacemaker rhythm, which is set by the spontaneous discharges from a small number of specialized cardiac cells located near the sino-atrial junction. Pacemaker potentials (the steady depolarization of the resting membrane potential) and some of the membrane currents contributing to the pacemaker action potential have been recorded in rainbow trout and European plaice (*Pleuronectes platessa* L., 1758) (Harper et al. 1995; Vornanen 1998; Haverinen and Vornanen 2007). The role of the cardiac pacemaker current (I_f) in setting the pacemaker potential and its modulation by cAMP (e.g., Accili et al. 2002) are prime areas for study.

The pacemaker rhythm is inherently modulated by temperature (Harper et al. 1995), as well as by extrinsic humoral and neural chronotropic effectors. The idea that cardiac stretch is a major intrinsic modulator of heart rate in fishes (i.e., beyond a few beats per minute) has been put aside (Farrell and Jones 1992), even for hagfish (Johnsson and Axelsson 1996; Johnsson et al. 1996).

Temperature effects on heart rate

Temperature is a major modulator of intrinsic heart rate, with Q_{10} values typically around 2 for acute temperature changes (Gollock et al. 2006; Clark et al. 2008a). Temperature acclimation can reset the pacemaker to a lower rate with warm acclimation (Graham and Farrell 1989; Haverinen and Vornanen 2007). While the mechanism for resetting the intrinsic pacemaker rate is not fully understood, it has the effect of at least partially restoring the scope for heart rate given that there is an upper limit to heart rate. In the lower range of thermal tolerance, maximum heart rate may depend on acclimation temperature, but with so few studies of temperature effects during maximal aerobic exercise or during recovery from exhaustive exercise (two situations where cardiac performance may be maximal), a full understanding of how temperature affects maximum heart rate in fishes is unclear at this time. Maximum heart rate can also show thermal independence, a feature that could easily vary between stenothermal and eurythermal species. For example, heart rate reached a maximum of ~ 105 beats \cdot min $^{-1}$ with acute warming in both resting and exercising sockeye salmon (*Oncorhynchus nerka* (Walbaum in Artedi, 1792)) (Steinhausen et al. 2008). In resting fish, this maximum rate was reached at 24 °C; in exercising fish, the maximum rate was reached at only 19 °C and remained unchanged as temperature was further increased.

Maximum heart rate in fishes was suggested to be about 2 Hz (120 beats \cdot min $^{-1}$), with the exception of some tunas (Farrell 1991; Clark et al. 2008b). However, higher heart rates have now been reported for larval fish. For example, embryonic zebrafish (*Danio rerio* (Hamilton, 1822)) (<10 mg body mass) have a heart rate of 3.5–4.0 Hz (Barriónuevo and Burggren 1999; Schwerte et al. 2006). Even so, heart rate decreased to 2.0–2.5 Hz by the time zebrafish approached full development. Furthermore, a heart rate of 4 Hz for a 10 mg zebrafish at 28 °C falls well below that of 15 Hz for a 2 g hummingbird (family Trochilidae), especially when scaling and Q_{10} effects are considered. Why maximum heart rate is limited to such low rates in most

fishes compared with similar-sized mammals remains a mystery.

Extrinsic regulation of heart rate

Vagal inhibition of heart rate via a muscarinic mechanism apparently appeared first in elasmobranch fishes and autonomic adrenergic control followed in the teleosts (Nilsson 1983). Such extrinsic controls have tonic influences on the heart, setting routine heart rate at a level that is rarely the same as the intrinsic pacemaker rate. The degree and nature of cardiac tonus varies widely among species and with temperature, as revealed by measuring heart rate following injections of atropine and a β -adrenergic antagonist (Axelsson et al. 1987; Altimiras et al. 1997; Mendonça and Gamperl 2009). Values vary between 7% and 131% for cholinergic tonus and between 4% and 40% for adrenergic tonus (Axelsson 2005). With cold acclimation, cholinergic tone increases in rainbow trout (Wood et al. 1979). In addition, stress tends to increase the adrenergic tonus and decrease the cholinergic tonus, resulting in elevated heart rates for fish that are either insufficiently rested after surgery or not properly habituated to their holding conditions (Altimiras and Larsen 2000; Campbell et al. 2004).

Oxygen delivery to the heart

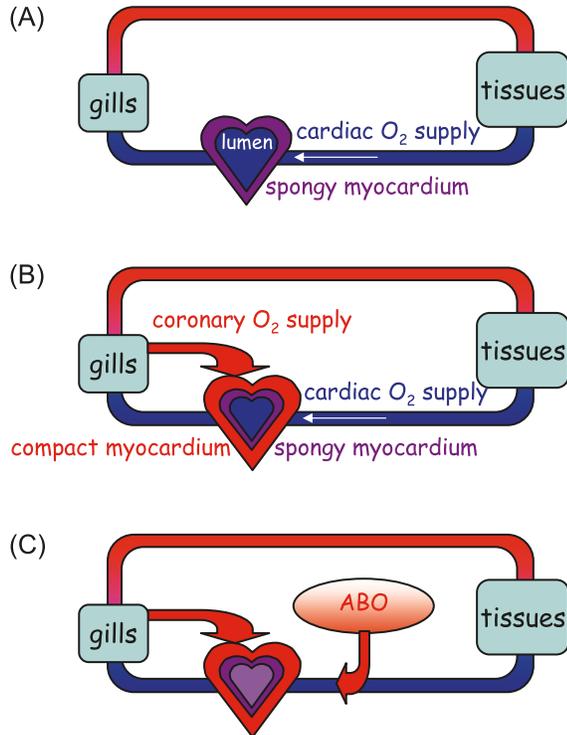
Among fishes, the oxygen supply for the myocardium comes from one of three routes. Consideration of the myocardial oxygen supply of fishes is simplified by separating the ventricle into its two major component muscle types, the spongy myocardium and the compact myocardium. All fish species have spongy myocardium, accounting for as little as 40% or as much as 100% of the ventricular muscle. The spongy myocardium is a mesh-like network of cardiomyocytes arranged in bundles (trabeculae) that crisscross the inner wall of the heart (Pieperhoff et al. 2009). The trabeculae create lacunae filled with venous blood (the venous oxygen supply route for the spongy myocardium). Because end-systolic volume is minimal, these lacunae are largely emptied during each heartbeat when the trabeculae contract. The compact myocardium encases the spongy myocardium and does not have direct access to the venous blood in the lacunae. Instead, it has its own dedicated coronary circulation.

The venous oxygen supply route

Lacking capillaries, the spongy myocardium must extract oxygen from venous blood (termed the venous or luminal oxygen supply, or the cardiac circulation in this regard) (Fig. 1A). The trabecular meshwork (Fig. 2A) greatly increases the surface area for contact between myocytes and venous blood (see also Pieperhoff et al. 2009), presumably facilitating rates of gas diffusion. But as noted above, this diffuse arrangement also exposes the spongy myocardium to deleterious alterations in venous blood composition.

With the ventricle representing just 0.05%–0.15% of body mass in most fish and total myocardial oxygen demand estimated at no more than 4% of metabolic rate (Farrell and Jones 1992; Steffensen and Farrell 1998), oxygen content of venous blood is unlikely to compromise the trabecular oxygen supply. Exceptions include severely hypoxic conditions

Fig. 1. Schematic representation of the circulatory systems, blood oxygenation status (fully oxygenated blood appears in red; deoxygenated venous blood appears in blue), and composition of the ventricle for (A) fishes with only spongy myocardium, a cardiac circulation, and no coronary circulation, (B) fishes with a coronary circulation to an outer compact myocardium, and (C) air-breathing fishes. ABO, air-breathing organ.



(Table 1) and Antarctic icefishes that lack Hb and consequently have a C_{vO_2} that might be one-tenth that of red-blood fishes.

Given the heart's continuous need for oxygen, all fish have a minimum venous PO_2 below which trabeculae become hypoxic. In the first instance, the threshold venous PO_2 will depend on the oxygen demand of the heart (which is proportional to myocardial power output and is approximated by the product of \dot{V}_b and ventral aortic blood pressure), the thickness of the trabeculae, the residence time of blood in the lumen of the heart (set by the inverse of heart rate), the shape and position of the oxygen-Hb dissociation curve, and the environmental conditions. Since all these parameters vary considerably among fishes, the exact venous PO_2 threshold for trabecular function has not been detailed. In salmonids, venous PO_2 can decrease to as low as 0.8 kPa during environmental hypoxia but remains around 2.0 kPa during swimming when cardiac ATP demand is augmented (Davie and Farrell 1991). Whether these venous PO_2 values represent threshold PO_2 levels for these two cardiac work conditions is unclear. A lower ventral aortic blood pressure will reduce myocardial power output and potentially the venous PO_2 threshold. Interestingly, hypoxia-tolerant fishes appear to have relatively low ventral aortic blood pressures (Farrell and Stecyk 2007), as do Antarctic icefishes (Axelsson 2005).

Recent studies with salmonids at rest and during pro-

longed exercise have shown that changes in venous PO_2 , \dot{V}_b , T_{aO_2} and C_{a-vO_2} all cease with acute warming when systemic tissues begin to make the transition to glycolytic metabolism (Farrell and Clutterham 2003; Clark et al. 2008a; Steinhausen et al. 2008). Why tissue oxygen extraction ceases with acute warming is unclear. The low capillarity of white muscle (Mosse 1978; Egginton and Sidell 1989) could be important because it easily accounts for 50% of a fish's body mass and undoubtedly has a diffusion limit for oxygen uptake. Thus, a white muscle diffusion limitation for oxygen might act as a potential "governor" for systemic tissue utilization of oxygen, ensuring that under most conditions venous PO_2 exceeds the venous PO_2 threshold oxygen supply to cardiac trabeculae. The cardiac trabeculae, after all, have the lowest PO_2 gradient driving oxygen diffusion to any of a fish's muscles. However, when fish swim at high water temperature, this protective role may break down and venous myocardial oxygen supply may become the weak link in the entire oxygen cascade system (Olson and Farrell 2006).

Clearly, under conditions of aerobic exercise, hypoxia and warming, the fish heart would benefit from additional oxygen supply routes; there are two others.

The coronary oxygen supply route

Some fishes have the benefit of a dual myocardial oxygen supply: a coronary circulation and a luminal supply (Fig. 1B). The coronary circulation delivers fully oxygenated blood supply to the outer, compact myocardium that wraps the spongy myocardium (Figs. 2B, 2C). Therefore, the coronary circulation is more reliable than the cardiac circulation in supplying oxygen during aerobic exercise because arterial PO_2 is maintained, whereas venous PO_2 decreases. Thus, in terms of oxygen delivery, the degree of capillarisation of cardiomyocytes is of primary importance for the compact myocardium, unlike the spongy myocardium, where venous PO_2 and trabecular dimensions appear to be critical factors. Fish cardiomyocyte diameters are much narrower than adult mammalian cardiomyocytes, possibly no wider than 10 μm (Farrell et al. 1988a; Vornanen et al. 2002), which may be a design feature that helps to minimize the oxygen diffusion distance between blood and mitochondria, which are centrally located in fish cardiomyocytes.

Phylogeny, physiology and environment clearly played prominent roles in the evolution of the coronary circulation in fishes. Coronary capillaries were probably absent in the chambered heart of the first vertebrates because hagfishes and lampreys (family Petromyzontidae) lack a coronary circulation. However, since all elasmobranchs and other primitive fishes (including sturgeon (family Acipenseridae), holosteans, and lungfishes (subclass Dipnoi)) examined to date possess a coronary circulation, the coronary circulation is clearly an old morphological adaptation that was subsequently both lost and enhanced among the teleosts (Farrell 2007c). Across teleosts, those species that are either highly athletic (e.g., salmonids and tunas) or hypoxia tolerant (e.g., freshwater eels (family Anguillidae)) typically have a coronary circulation, while the most athletic fishes have the greatest dependence on the coronary circulation (Farrell 1996).

The proportion of compact myocardium, expressed as a percentage of ventricular mass, is an index of the heart's de-

Fig. 2. (A) A longitudinally sectioned heart of tilapia (genus *Tilapia* Smith, 1840) to reveal the wall architecture (courtesy of Dr. S. Pieperhoff); (B) a section through the outer compact and inner spongy myocardia of a sockeye salmon (*Oncorhynchus nerka*), with the compact layer partially peeled away from the spongy layer; and (C) a vascular cast of the coronary vessels of a sockeye salmon with all tissue digested away leaving only the vessels visible. The upper opening is the ventricular-bulbar canal and the lower opening is the atrio-ventricular canal (courtesy of Dr. M. Axelsson).

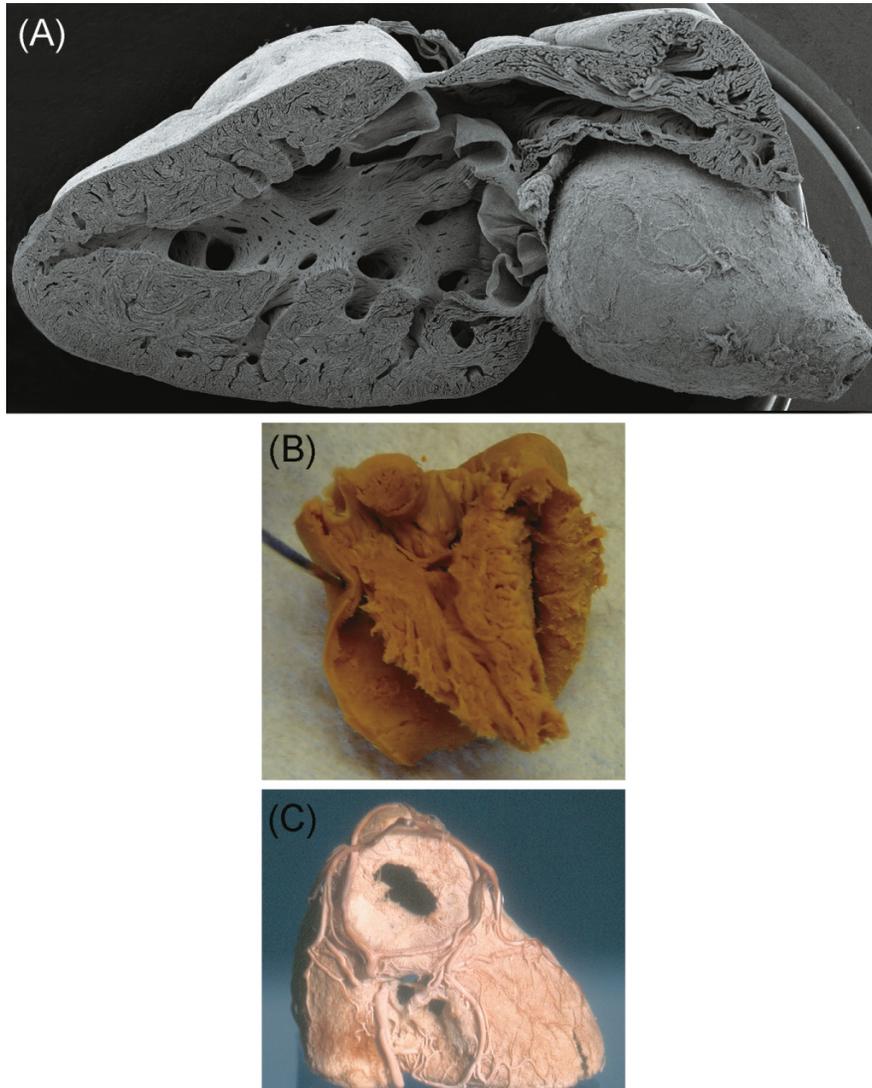


Table 1. A simplified estimate of the cardiac oxygen need and supply of a rainbow trout (*Oncorhynchus mykiss*) under resting conditions, maximally exercising, and under hypoxic conditions.

	Rest ($1.5 \text{ mW}\cdot\text{g}^{-1}$)	Exercise ($7.5 \text{ mW}\cdot\text{g}^{-1}$)	Hypoxia ($1.5 \text{ mW}\cdot\text{g}^{-1}$)
Cardiac \dot{V}_{O_2} , $\text{mL}\cdot\text{s}^{-1}$	2 + 2	10 + 10	2 + 2
Coronary O_2 supply, $\text{mL}\cdot\text{s}^{-1}$ ($q_{\text{cor}} \times C_{\text{aO}_2}$)	1.9 (0.3×6.24)	6.9 (0.9×6.24)	1.8 (0.9×2.0)
Cardiac O_2 supply, $\text{mL}\cdot\text{s}^{-1}$ ($\dot{V}_{\text{b}} \times C_{\text{vO}_2}$)	56.2 (15×3.75)	131.1 (60×2.18)	14.0 (15×0.94)
Venous PO_2 , kPa	4	1.5	0.8

Note: Cardiac \dot{V}_{O_2} is based on the power output (in parentheses) and assumes that this demand is equally divided between the compact and spongy myocardia. Coronary O_2 supply to the compact myocardium is based on measurements of coronary blood flow (q_{cor}) and arterial oxygen content (C_{aO_2}) for the three conditions. Similarly, the cardiac O_2 supply to the spongy myocardium is based on measurements of cardiac output (\dot{V}_{b}) and venous oxygen content (C_{vO_2}) for the three conditions.

pendence on its coronary circulation. It has been estimated that one-third of fish species possess compact myocardium (Santer 1985), but this estimate requires verification. The thickness of the compact myocardium can be estimated ei-

ther from histological analysis, or more simply by peeling off and weighing the compact myocardium (Fig. 2B; Farrell et al. 2007). The proportion of compact myocardium varies among species and also increases with cardiac growth dur-

ing development, but rarely beyond 60% even in the most athletic fishes (Fig. 3). Therefore, for the majority of fishes, the coronary circulation must be described as a supplemental myocardial oxygen supply route. Why a proportion of spongy myocardium is retained in all fishes is unexplained. Perhaps a spongy myocardium permits an end-systolic volume that approaches zero, which in turn ensures that almost all of the venous blood (and the oxygen it contains) is replaced with each heartbeat.

Routine coronary blood flow has been variously estimated as 0.2–0.4 mL·min⁻¹·g⁻¹ ventricular mass (0.6%–3.0% of \dot{V}_b ; Axelsson and Farrell 1993; Gamperl et al. 1994; Gamperl et al. 1995), which is an order of magnitude lower than estimates for endotherms (e.g., 3.5 mL·min⁻¹·g⁻¹ cardiac mass for birds; Smith et al. 2000). The heart works harder and uses more oxygen during exercise (Table 1), pumping a greater quantity of blood at a faster rate and at a higher blood pressure. Myocardial oxygen consumption in fishes is directly proportional to myocardial power output (Farrell and Jones 1992). Coronary blood flow increases by almost threefold when salmon swim aerobically, as compared with a fourfold increase when birds exercise. However, the increase in coronary blood flow in salmon does not quite quantitatively match the increase in cardiac oxygen demand, as measured by the increase in myocardial power output (Gamperl et al. 1995). Therefore, either oxygen extraction from the coronary blood supply increases or the heart becomes mechanically more efficient (Graham and Farrell 1990), or some combination. These increases in coronary blood flow involve appreciable decreases in coronary vascular resistance, but the mechanisms controlling these changes are poorly understood. Given a powerful α -adrenergic vasoconstriction in the coronary circulation, there is the potential for a large coronary vasodilatory reserve (Farrell 1987).

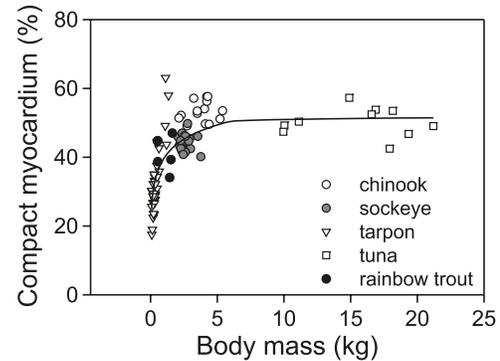
The oxygen supply route from the air-breathing organ

Unique to air-breathing fishes is a third myocardial oxygen supply route. This is because oxygenated blood leaving the air-breathing organ (ABO) is added to the systemic venous blood returning to the heart, boosting C_{vO_2} and P_{vO_2} (Fig. 1C). Thus, air breathing can clearly benefit the spongy myocardium. A simple extension of this benefit is the hypothesis that air breathing evolved in fishes primarily as a means to enhance myocardial oxygen supply during exercise in normoxic conditions (Farmer 1997). However, the universality of this hypothesis must be challenged because the air-breathing Pacific tarpon (*Megalops cyprinoides* (Broussonet, 1782)) does not take air breaths nearly as often while swimming in normoxia as it does when resting in aquatic hypoxia (Clark et al. 2007; Seymour et al. 2007). The fact that many primitive air-breathing fishes also have a coronary circulation, which has yet to be examined in any detailed fashion, means that they possess three oxygen supply routes for the heart (Farrell 2007c).

Fish cardiorespiratory physiology in an era of climate change

Climate change represents a challenge for all zoologists because resource managers are seeking sage advice. The critical information needed includes the resistance, resilience

Fig. 3. The percentage of compact myocardium in a variety of fish species to illustrate the increase in compact myocardium with fish growth and the upper limit for the amount of compact myocardium even in large tunas. Species are Chinook salmon (*Oncorhynchus tshawytscha*), sockeye salmon (*Oncorhynchus nerka*), Pacific tarpon (*Megalops cyprinoides*), southern bluefin tuna (*Thunnus maccoyii* (Castelnau, 1872)), and rainbow trout (*Oncorhynchus mykiss*).

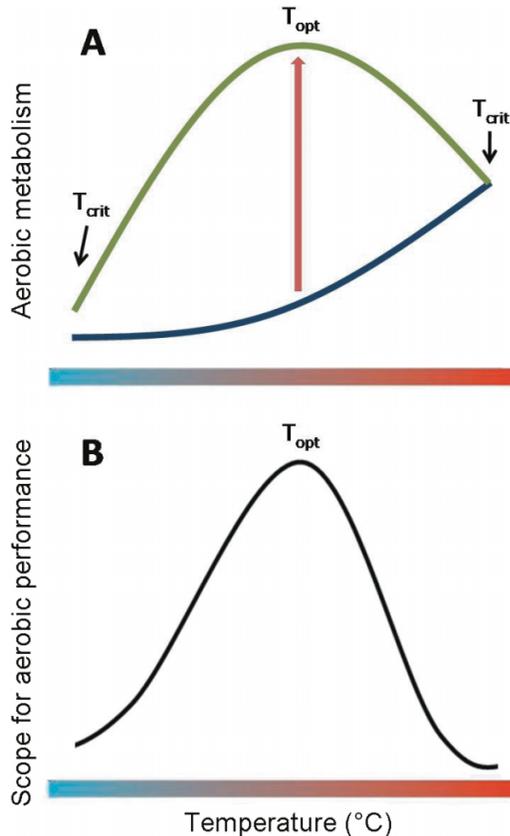


and adaptability of a population to environmental change, especially the global warming trends. Presently, advice is necessarily simplistic because mechanistic explanations, even for temperature effects, are in short supply and certainly do not encompass a wide range of species. In addition to warming, aquatic organisms will be challenged with multiple, interactive environmental changes, such as salinity, pH, ammonia and oxygen.

Environmental temperature is most certainly a primary determinant of biotic distributions and abundances, but finding mechanisms that link physiological effects of temperature to lifetime fitness remains a challenge. A promising conceptual framework is that of aerobic scope (Pörtner 2002; Pörtner and Knust 2007), which builds from ideas originally advanced by a Canadian giant in fish physiology (Fry 1947; Fry and Hart 1948; Fry 1971), who conceptualized the effects of environmental changes such as temperature on fish metabolism and activity as lethal, controlling, limiting, masking and directive. Given the central role \dot{V}_b plays in determining $\dot{V}O_2$ (see above), Brett (1971) then showed a similar temperature optimum (T_{opt}) for maximum swimming activity, $\dot{V}O_2$ and \dot{V}_b in salmon, and paved a way to consider cardiac insufficiency as a potential mechanistic explanation for the effects of extreme temperature on aerobic scope and whole-animal tolerance. This idea is expanded on below, along with an illustration of the clear linkage that can exist between aerobic scope and lifetime fitness of sockeye salmon (Farrell et al. 2008).

Aerobic scope, defined as the difference between basal and maximal metabolic rates (Fig. 4A), is the amount of aerobic energy beyond that required for basic existence that can be allocated to activities that contribute to lifetime fitness (e.g., for growth, activity and reproduction). There is solid evidence that extreme temperatures limit this energy allocation (Brett 1971; Claireaux et al. 2006; Claireaux and Lefrançois 2007). Indeed, the bell-shaped curve for aerobic scope versus temperature in Fig. 4B clearly illustrates how aerobic scope declines above T_{opt} . The shape and position of these aerobic scope curves vary considerably among fishes (Fry 1971). The decline in aerobic scope results from

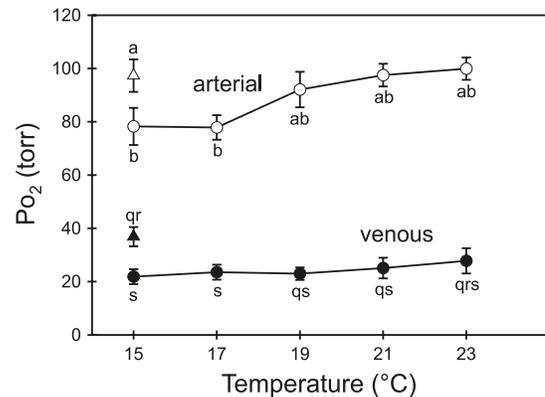
Fig. 4. (A) Changes in minimum and maximum aerobic metabolism as a function of temperature, and (B) the corresponding change in scope for aerobic activity, defined as the difference between minimum and maximum aerobic metabolic rates. Aerobic scope is optimal typically over a small temperature range (T_{opt}), and progressively decreases to zero at critical temperatures (T_{crit}) either side of the T_{opt} . Figure modified from Pörtner and Farrell (2008).



a combination of a continual Q_{10} -related increase in basal metabolism and a corresponding inability to similarly increase maximal metabolic rate with increasing temperature. Extrapolating such curves to zero aerobic scope yields a prediction of the critical temperature (T_{crit}) when anaerobic metabolism must take over to temporarily support activities (Pörtner and Knust 2007). Furthermore, the difference between the estimated values for T_{opt} and T_{crit} represents just 6–7 °C of separation for salmonids (Farrell et al. 2008), which is not a large buffer given some of the global warming scenarios. But mechanistically how does this oxygen limitation come about?

While the mismatch between an animal's demand for oxygen (a Q_{10} effect) and the capacity of the circulatory and ventilatory systems to deliver this oxygen clearly gets progressively worse at temperatures beyond T_{opt} , the mechanistic basis for the decline in aerobic scope is still being debated. Potential mechanisms can be simplified to gill-related, cardiac-related and tissue-related limitations (Brett 1971; Farrell 1997, 2002; Taylor et al. 1997; Pörtner 2002; Farrell 2007b; Clark et al. 2008a). Brett (1971) suggested that the salmonid ventilatory system did not have enough capacity to sufficiently compensate for the $\sim 2\% \cdot ^\circ\text{C}^{-1}$ decrease in water oxygen content (Dejours 1975). If there was

Fig. 5. Oxygen partial pressure (PO_2 ; 1 torr = 133.3 Pa) of arterial (open symbols) and venous blood (closed symbols) from swimming sockeye salmon (*Oncorhynchus nerka*) during acute temperature increase. Triangles represent resting values prior to commencement of the swimming protocol. All values are means \pm SE. Different letters indicate significant differences between temperatures within a group.



a gill limitation (either oxygen delivery to the gills or oxygen diffusion across the gills into the blood), then arterial PO_2 would progressively decrease with increasing temperature, as was observed in resting trout (Heath and Hughes 1973). However, this result has not been replicated with recent experiments. For example, adult sockeye swimming near their maximum $\dot{V}O_2$ did not decrease arterial PO_2 when the water was progressively warmed over several hours (Fig. 5; Steinhausen et al. 2008). This was also true for similar-sized, resting Chinook salmon (*Oncorhynchus tshawytscha* (Walbaum in Artedi, 1792)), although in larger fish arterial PO_2 was reduced with increasing temperature (Clark et al. 2008a). Therefore, there is a good degree of uncertainty as to whether or not a gill-related limitation exists at high temperature in salmonids.

In contrast, a cardiac limitation was clearly identified in the same experiments with adult sockeye swimming near maximum $\dot{V}O_2$. Heart rate, \dot{V}_b and T_{aO_2} all failed to continue to increase with temperature (Fig. 6). As a result, tissues became glycolytic, releasing lactate and causing hyperkalemia and acidemia (Steinhausen et al. 2008). Given the interactive and detrimental effects of hypoxemia, hyperkalemia and acidemia on maximum cardiac performance that are noted above, as well as the possible absence of adrenergic protection at elevated temperature, it seems reasonable to link the changes in venous blood composition to the decline in \dot{V}_b in sockeye salmon and perhaps to the cardiac arrhythmias observed at extreme temperatures in Chinook salmon (Clark et al. 2008a).

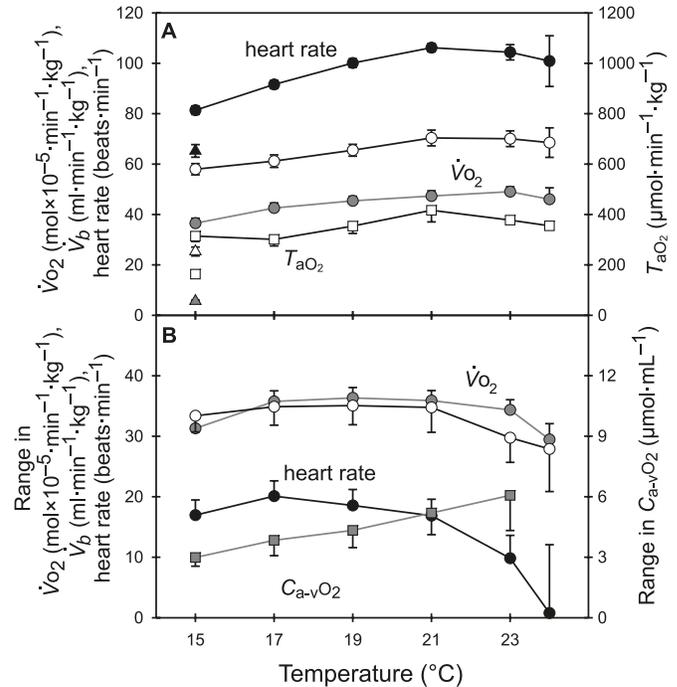
$C_{a-v}O_2$ did increase modestly with increasing temperature in swimming sockeye salmon (Fig. 6), but this was not a result of decreasing venous PO_2 (Fig. 5), which is an important observation when examining for a tissue-related diffusion limitation. Theoretically, venous PO_2 should decrease with increasing oxygen demand in the absence of a tissue-related diffusion limitation. Therefore, this result suggests that a limitation on oxygen diffusion in skeletal muscle does develop (Taylor et al. 1997), and that the modest increase in oxygen unloading (to account for the increase in $C_{a-v}O_2$)

likely occurs as a result of a temperature- and pH-induced right shift in the Hb–oxygen dissociation curve (Steinhausen et al. 2008). Consequently, differences in muscle capillarisation (relative to their oxygen needs) as a result of thermal acclimation and among species (Egginton and Sidell 1989; Taylor et al. 1996; Taylor et al. 1997) may be important in setting thermal limits in fishes.

By combining these observations, we propose the following death spiral for salmon swimming at temperatures well above T_{opt} . With increasing temperature, the heart becomes rate-limited when heart rate reaches its maximum and can no longer increase maximum \dot{V}_b . This places a perfusion limitation on swimming muscles, which then forces venous PO_2 in skeletal muscle down to a point where a diffusion limitation develops. This may be particularly problematic for white muscle because of the anatomical arrangement of the capillaries. Muscles are then forced to work glycolytically to maintain swimming performance, releasing H^+ and K^+ into venous blood. Venous acidemia and hyperkalemia (combined with venous PO_2 being near to the threshold PO_2 and the absence of adequate adrenergic protection) then impair the spongy myocardium, causing a decline in maximum cardiac performance. As maximum \dot{V}_b declines, a positive feedback ensues, making matters progressively worse with increasing temperature and perhaps ultimately leading to cardiac arrhythmias. If arterial PO_2 is also compromised at high temperature, matters become even worse.

It has been proposed that the concept of aerobic scope may apply broadly to aquatic organisms, even beyond fishes, and to environmental interactions (Pörtner and Farrell 2008). These environmental interactions would presumably either load basal metabolic rate or limit maximum metabolic rate, as envisioned by Fry (Fry 1971). Whether such a paradigm applies broadly to other species and situations needs to be tested. Hypoxia tolerance certainly varies among fishes and so the quantitative aspects related above for sockeye salmon are unlikely to apply broadly outside of athletic fishes. The broader issue of fish hearts being able to function under severe hypoxia, and even in anoxia, has been considered elsewhere (Farrell and Stecyk 2007; Stecyk et al. 2008). In addition, when fish are swum in a laboratory respirometer, they have the benefit of ram ventilation. Therefore, resting fish may be taxed somewhat differently if excessive effort is spent on gill ventilation, perhaps increasing the cost of ventilation or respiratory dead space. Nevertheless, cardiac adaptations, such as increased expression of the cardiac SERCA2 protein involved in excitation–contraction coupling, have been implicated in niche expansion for salmon sharks (*Lamna ditropis* Hubbs and Follett, 1947) into the subarctic seas (Weng et al. 2005) and the *Thunnus* lineage into deeper, colder ocean waters (Block and Finnerty 1994; Blank et al. 2004; Dickson and Graham 2004). A fascinating study species might be the Antarctic Hb-free icefish. These fish have extreme cardiovascular adaptations and oxygen supply to the heart may be at the extreme limit of design (large, entirely spongy myocardium and no hemoglobin to boost CO_2 of venous blood). Furthermore, recent work with red-blooded Antarctic fishes has shown their ability to acclimate to temperatures previously thought lethal and perhaps not experienced for tens of thousands of years (Franklin et al. 2007). Whether the aerobic scope and car-

Fig. 6. (A) Changes in oxygen consumption rate ($\dot{V}O_2$), arterial oxygen delivery (T_{aO_2}), cardiac output (\dot{V}_b), and heart rate in swimming sockeye salmon (*Oncorhynchus nerka*) during an acute temperature increase of $2^\circ C \cdot h^{-1}$, and (B) the range in $\dot{V}O_2$, \dot{V}_b , heart rate, and arterio-venous oxygen content difference ($C_{a-v}O_2$), calculated as the difference between routine and active swimming values at a given temperature. All values are means \pm SE.

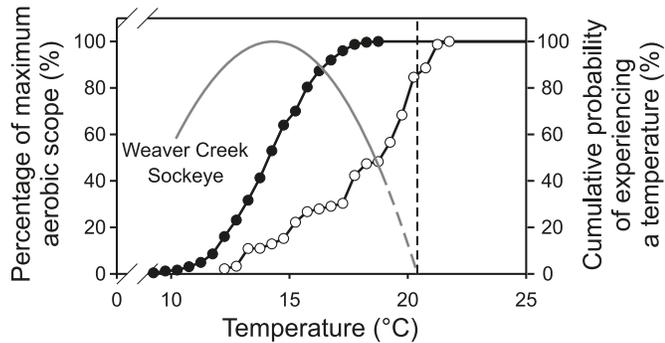


diac capabilities of these Antarctic fishes limit them to the stable frigid temperatures currently found in Antarctica is an intriguing question.

Temperature optima for metabolism and swimming performance obviously vary considerably among species and with thermal acclimation (Brett 1971; Beamish 1978; Randall and Brauner 1991; Taylor et al. 1996, 1997; Clark et al. 2005; Claireaux et al. 2006). The above concept leads to two predictions: (1) the T_{opt} for aerobic scope should match the historic environmental conditions and (2) large deviations from these conditions should have fitness consequences. Both predictions appear to hold true for adult Fraser River salmon, as shown below. Pacific salmon spawn once, and so it is easy to equate an inability to migrate back to natal spawning grounds with zero lifetime fitness (Farrell et al. 2008). Furthermore, thermal acclimation has limited effects on the upper thermal tolerance of stenothermal salmon, unlike the eurythermal goldfish (*Carassius auratus* (L., 1758)) (Brett 1956).

Adult Gates Creek sockeye make a long (400 km) inland migration at peak summer temperatures, and have a maximum aerobic scope of $10.7 \text{ mg O}_2 \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ at a $T_{opt} = 16.3^\circ \text{C}$ and a $T_{crit} = 24.4^\circ \text{C}$. Weaver Creek sockeye have a lower maximum aerobic scope of $7.9 \text{ mg O}_2 \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ and a lower $T_{opt} = 14.3^\circ \text{C}$ and $T_{crit} = 20.4^\circ \text{C}$, which corresponds to a shorter (100 km) coastal migration in early autumn when the river is cooler and slower. The coastal Chehalis River coho salmon that migrate in early winter have an even lower T_{opt} (8.1°C) and T_{crit} (17.0°C) (max-

Fig. 7. Aerobic scope for Weaver Creek sockeye salmon (*Oncorhynchus nerka*), expressed as a percentage of the maximum, and the cumulative probability of experiencing certain river temperatures historically (●) and in 2004 (○). The curve for aerobic scope has been extrapolated (broken line) to derive the critical temperature when aerobic scope is zero. Vertical broken line indicates the upper critical temperature (T_{crit}) of 20.4 °C for this population of sockeye salmon.



imum aerobic scope is $7.5 \text{ mg O}_2\text{-kg}^{-1}\text{-min}^{-1}$) (Lee et al. 2003b; Farrell et al. 2008). Thus, T_{opt} for aerobic scope appears adaptive for river migration at the population level. Indeed, when the historical river temperatures experienced by Weaver Creek sockeye during the past 50 years are compared with the aerobic scope, there is a remarkable correspondence between the mode for temperature experiences and the measured T_{opt} (Farrell et al. 2008). Consequently, while Pacific salmon that migrate farther up river clearly experience harsher hydraulic conditions and warmer river temperatures than coastal salmon, they correspondingly have a greater aerobic scope, critical swimming speed and T_{opt} (Tsuyuki and Williscroft 1977; Lee et al. 2003a, 2003b), as well as a smaller body size, a more fusiform shape and a proportionally smaller total egg mass (Crossin et al. 2004). Our current studies suggest that intraspecific differences in rM_v and ventricular composition for adult sockeye salmon can be related to the relative difficulty of river migration back to natal streams (E.J. Eliason, unpublished data), which may give clues to how a fish might adapt to avoid the abovementioned death spiral at warmer temperatures and harsher swimming conditions.

An acid test for a laboratory-generated idea is whether it is consistent with field observations. Again this appears to be the case for the sockeye salmon. In summer 2004, Fraser River water temperatures reached record highs and Weaver Creek sockeye that entered the Fraser River earlier than their historic norm encountered water temperatures that exceeded their T_{crit} ($T_{crit} = 20.4 \text{ °C}$; Fig. 7). In fact, the 2004 Weaver Creek population had a 50% probability of experiencing water temperatures in excess of 18.5 °C , whereas historically the probability of experiencing such a high temperature was $<20\%$ (Fig. 7). Tens of thousands of these fish did not reach the spawning grounds. Furthermore, when Weaver Creek sockeye were intercepted to implant a biotelemetry device to follow subsequent river migration, just 10% of fish reached the spawning area when the river temperature was around T_{crit} versus 78% when river temperature had seasonally decreased to T_{opt} (14.3 °C) (Farrell et al. 2008). Consequently, collapse of aerobic scope could have

been an important mechanism to explain the high salmon mortality observed during river migration. Concern that excessive temperature acts as a barrier to salmon migration extends to the Columbia River in the USA (Quinn et al. 1997), where there have been reports of salmon temporarily ceasing their migration when river temperature increased to $>21 \text{ °C}$ after migration had begun (Hyatt et al. 2003; Goniea et al. 2006; Keefer et al. 2008). However, while aerobic scope appears to be a promising predictive tool for such migratory behaviour, until we know exactly what proportion of aerobic scope is needed for successful migration, precise modeling of upstream migration will be limited. With the advent of new biotelemetry and biologging technologies (Wilson et al. 2006; Clark et al. 2008b; Green et al. 2009), it may be possible to further strengthen aerobic scope models with real-time recordings of cardiovascular status and activity levels of free-swimming fish in the natural environment.

Acknowledgements

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