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Commentary

Cardiac plasticity in fishes: environmental influences and intraspecific differences

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Summary

Fish cardiac physiology and anatomy show a multiplicity of intraspecific modifications when exposed to prolonged changes in environmentally relevant parameters such as temperature, hypoxia and food availability, and when meeting the increased demands associated with training/increased activity and sexual maturation. Further, there is evidence that rearing fish under intensive aquaculture conditions significantly alters some, but not all, aspects of cardiac anatomy and physiology. This review focuses on the responses of cardiac physiology and anatomy to these challenges, highlighting where applicable, the importance of hyperplastic (i.e. the production of new cells) *vs* hypertrophic (the enlargement of existing cells) growth to the adaptive response of the heart. In addition, we summarize recent studies that have explored the relationship between the myocardial protection afforded by preconditioning and myocardial hypoxia tolerance. This latter research clearly demonstrates the capacity of the fish heart to adjust to short-term perturbations, and shows that it can be difficult to predict how short-term and long-term alterations in cardiac physiology will interact.

Key words: intraspecific cardiac plasticity, fish, environment, heart, myocardium, hyperplasia, hypertrophy, preconditioning, hypoxia, temperature, maturation, food deprivation.

Introduction

With over 20 000 species of teleost fish, considerable interspecific diversity in cardiac anatomy and physiology is expected. This is the outcome of evolutionary adaptation to different habits, modes of life and activity levels. For example, athletic species have a more powerful heart than sedentary species, and fish such as hagfish, carp and eel normally show a much higher degree of myocardial hypoxia tolerance than species such as salmonids (Farrell, 1991; Farrell and Jones, 1992). Plasticity in cardiac form and function has also been demonstrated during ontogeny, and the cardiovascular flexibility exhibited during embryonic and larval development, is nicely reviewed by Pelster (2003). What is less well appreciated, however, is the high degree of intraspecific cardiac plasticity displayed by post-larval fishes. Accordingly, this review explores what is known about intraspecific cardiac plasticity among juvenile and adult fishes. This intraspecific plasticity, like that exhibited during development, may well reflect individual variability on which natural selection could act.

In this review, we focus primarily on temperature effects, which are relatively well studied, and on the effects of other environmental and biological factors that modify cardiac anatomy and physiology, including food deprivation, sexual maturation, exercise training and rearing under aquaculture conditions. Further, we summarize recent work on cardiac preconditioning and myocardial hypoxia tolerance in fishes, and discuss the potential implications of this work. Preconditioning is a short-term form of cardiac plasticity that has the potential to protect the heart from insults that might normally lead to cardiac damage, dysfunction or death. Preconditioning has been the focus of several thousand mammalian studies (e.g. see review by Yellon and Downey, 2003), and so the handful of recent studies in fish, which already point to important intraspecific differences, may find application outside the piscine world. Similarly, researchers who wish to stimulate cardiac growth to replace damaged myocardial tissue in mammals, may be heartened to discover that fish cardiac tissue, unlike the mammalian heart, does not lose its ability for hyperplastic growth with age. In fact, we suspect that the high degree of intraspecific plasticity that we

describe below is partly related to the fact that fish hearts grow through hyperplasia as well as hypertrophy.

The heart powers an internal convection system for the whole animal, and in this context, global comparisons of cardiac function (e.g. cardiac output, stroke volume) are best represented in units of ml min^{-1} kg^{-1} body mass. However, relative ventricular mass (RVM) can vary considerably (e.g. by 50% intraspecifically, see below), and thus of units of ml min^{-1} g^{-1} ventricular mass or cardiac power output (mW g^{-1} ventricular mass) allow us to interpret whether differences in cardiac function are due to changes in heart size, and/or plasticity in cellular physiology. We utilize both measurements of cardiac function in this review, because as a more mechanistic understanding of cellular plasticity emerges, elucidating the roles of these cellular changes will require increasingly refined comparators of cardiac performance.

Temperature

Temperature has quite rightly been termed the 'ecological master factor' (Brett, 1971) as it has a profound effect on the physiology of all ectothermic animals. Nevertheless, it is well known that fish, like other ectotherms, can compensate for the direct effects of temperature on physical processes and enzymatic reaction kinetics through temperature acclimation or acclimatization. Here we focus primarily on the rainbow trout *Oncorhynchus mykiss* to illustrate cardiac plasticity in response to temperature change.

Salmonids have adapted to exploit the cold water habitats created by retreating glaciers, despite the fact that optimum temperatures for maximum cardiac performance, aerobic scope and swimming ability are commonly around 15–18°C and preferred temperatures typically range from 12 to 18°C (McCauley and Huggins, 1979; Jobling, 1981). Thus, it is perhaps not surprising to find that cardiac function in rainbow trout has a rather low sensitivity to temperature change. Indeed, temperature acclimation between 5 and 18°C results in Q_{10} values in the range 1.2–1.4 for maximum cardiac output ($\dot{Q}_{max}$) and maximum power output (PO_{max}) for rainbow trout (Graham and Farrell, 1989; Keen and Farrell, 1994), rather than Q_{10} values of around 2 if there had been no compensation.

The ability to almost maintain $\dot{Q}_{max}$ and PO_{max} across a broad temperature range clearly involves cardiac plasticity that provides advantages to fish that inhabit an environment with fluctuating temperatures. The mechanisms behind this cardiac plasticity in response to temperature are partially understood. For example, exposure to a 10°C decrease in temperature for 3–4 weeks increases relative ventricular mass by 20–40%, but decreases the proportion of compact myocardium by 15–30% (Farrell et al., 1988; Graham and Farrell, 1989). A larger ventricular muscle mass compensates for a cold-temperature-induced decrease in contractility, thereby helping maintain stroke volume (V_S), $\dot{Q}$ and pressure development. Implicit in this argument is that a decrease in contractility negatively affects end-systolic volume of the ventricle, which in trout is normally very small. At warmer temperatures, ventricular mass could be relatively smaller while maintaining the same $\dot{Q}$ because, in addition to improved force of contraction, rates of ventricular contraction and relaxation are faster. These latter effects would increase the time available for cardiac filling, and may compensate for the effect of increased heart rate on end-diastolic volume. Cardiac enlargement, however, is apparently dependent on other factors beside temperature, because rainbow trout held on a 12 h:12 h light:dark photoperiod show either no or a smaller degree of cardiac enlargement (<15%) when acclimated to different temperatures (Keen et al., 1993; Keen and Farrell, 1994; Sephton and Driedzic, 1995; Aho and Vornanen, 2001). Although we know little about what these environmental and physiological factors might be, recent work by Tiitu and Vornanen (2003) suggests that cold/seasonal cardiac enlargement may be partially related to thyroid state. Thyroid hormones affect many physiological functions in fishes (e.g. osmoregulation, nitrogen excretion, morphological changes associated with smoltification, muscle growth etc.), and these authors found that hypothyroidism was associated with increases in heart size and heart rate in rainbow trout. The involvement of hypertrophic or hyperplastic myocardial growth in cold/seasonal cardiac enlargement is presently unresolved (Driedzic et al., 1996), although hypertrophy is a well-documented compensatory response to cold temperature in tissues such as the liver (Kent and Prosser, 1985).

The intrinsic cardiac pacemaker rate is also reset with cold acclimation, with heart rate (f_H) being higher than it would be following an acute decrease in temperature. This elevation in f_H, which is obviously important in maintaining $\dot{Q}$, involves alterations to membrane ion channel function and density, the details of which have been recently discovered and reviewed (Vornanen et al., 2002a,b). For example, the repolarizing K^+ currents (I_k), which affect the shape and duration of the action potential (AP), are altered in cold-acclimated rainbow trout and this partially compensates for a cold-induced prolongation of the AP. Specifically, the density of the inward rectifier potassium current, I_{ki}, is depressed in the ventricle, while that of the delayed rectifier current, I_{kr}, is strongly increased: the net effect is that AP duration and presumably the refractoriness of the heart are shortened.

Similarly, the delivery of calcium to troponin C, which initiates the contractile event and regulates the strength of cardiac contraction, is clearly plastic in fish and responds to temperature. Calcium entry into cardiomyocytes *via* the L-type Ca^{2+} channel (I_{Ca}) plays an important role in cardiac contractility, including triggering the release of intracellular Ca^{2+} from the sarcoplasmic reticulum (SR) and directly activating the myofilaments. Ion flow through cardiac L-type Ca^{2+} channels in mammals, and surprisingly also rainbow trout, is extremely temperature sensitive, with peak current having a Q_{10} of 1.8–2.1 for acute temperature changes (Kim et al., 2000; Shiels et al., 2000). However, in rainbow trout, a slowing of channel inactivation and a prolongation of the AP counteracts the depressive effect of cold temperature on peak I_{Ca} such that the net calcium charge transfer is essentially independent of an acute temperature change (Shiels et al.,

2000). With cold-acclimation the AP is shortened through the plasticity of the sarcolemmal K^+ channels (noted above), and although the density of I_{Ca} when measured at room temperature is the same for cold- and warm-acclimated rainbow trout and carp, the rate of I_{Ca} inactivation is greater for the cold-acclimated fish (Vornanen, 1998). Given the temperature dependent decrease in myofilament Ca^{2+} sensitivity, it seems likely that a compensatory increase in Ca^{2+} from another source is needed to maintain the same force of contraction at low temperature (see Vornanen et al., 2002a,b). In this regard, cold-induced proliferation of SR (another source of activator Ca^{2+} for contraction) has been observed and cold-acclimated fish respond more robustly to ryanodine (an SR Ca^{2+} release agonist), especially when the tissue is acutely warmed (see Shiels et al., 2002). Thus, to activate muscle contraction, a larger SR capacity could compensate for a smaller SL Ca^{2+} trigger. However, the possibility that cold-induced hyperplastic cardiac growth could enhance the myocyte to surface area to volume ratio, and thus augment sarcolemmal-dependent processes, has not been thoroughly explored.

Extrinsic modulation of the heart is also altered by temperature acclimation, and in this regard certain cellular transduction mechanisms are known to show temperature-dependent plasticity. Wood et al. (1979) showed that cholinergic inhibitory tonus in rainbow trout is more important in setting routine heart rate at cold temperatures, while adrenergic excitatory tonus is relatively more important at high temperature. However, temperature effects on the adrenergic signal transduction pathway that controls ventricular contractility appear to be opposite to those seen for heart rate. In particular, the rainbow trout myocardium becomes more responsive to β-adrenergic stimulation with cold acclimation. This is due to an increase in the density of SL β-adrenoceptors (Keen et al., 1993) and an upregulation of the secondary messenger cascade (Keen, 1992), and the former response clearly needs further study to determine whether receptors are being sequestered and cycled to the membrane, or whether genes are being turned on to make more receptors. β-adrenergic stimulation shortens the AP and stimulates I_{Ca} (Shiels et al., 2002). In fact, the possibility exists that tonic adrenergic stimulation may be critical for adequate L-type Ca^{2+} channel function at cold temperatures in rainbow trout (Shiels et al., 2004), as well as proper atrio–ventricular coordination (Graham and Farrell, 1989).

While much has been learned about the mechanistic basis for cardiac plasticity in rainbow trout, limited studies with other fish species clearly point to alternative patterns of cardiac plasticity. For example, the hearts of Arctic charr *Salvelinus alpinus* reared at 15°C are 15–30% larger, not smaller, than the hearts of fish reared at 5°C (Ruiz and Thorarensen, 2001). Carp are an extremely eurythermal family, and winter dormancy in *Cyprinus carpio* is associated with a suppression of routine cardiac power output (Q_{10} ~4) through intrinsic mechanisms rather than cholinergic suppression of cardiac activity (J. A. W. Stecyk and A. P. Farrell, unpublished data). Conversely, *Carassius carassius*, which survives winter anoxic conditions by fermenting glucose to alcohol, maintains cardiac activity (J. A. W. Stecyk et al., unpublished data) despite increased cardiac refractoriness (Tiitu and Vornanen, 2001). The ability of the Pacific bluefin tuna *Thunnus orientalis* heart to maintain cardiac pumping at cold temperatures that are refractory to hearts from other tuna species appears to be directly related to a high SR Ca^{2+} ATPase activity, and this cardiac feature may be a primary adaptation that allows this species to forage to deeper and colder depths (Blank et al., 2004). Similarly, the burbot *Lota lota*, which also remains active in deep lakes during winter, has an unusually high SR Ca^{2+}-release at 1°C, which is reduced at warmer acclimation temperatures (Tiitu and Vornanen, 2002). The idea that the pattern of cardiac plasticity for cold-active fishes differs from cold-inactive fishes is also supported by data on thermal compensation of heart rate and twitch kinetics in yellow perch *Perca flavescens vs* sea raven *Hemitripterus americanus* (Driedzic et al., 1996), and by data on the cardiac responses of sympatric bass species with differences in winter activity (Cooke et al., 2003).

Sexual maturation

Cardiac enlargement that occurs in salmonids and some other species is associated with reproductive maturation in male, but not female, fish. This sexual dichotomy results in hearts from mature males being 20–90% larger than those of mature females (Luk'yanenko and Raspopov, 1972, Franklin and Davie, 1992; Graham and Farrell, 1992; West and Driedzic, 1999). Functionally, this increases maximum cardiac stroke volume (Vs_{max}) and power output (PO_{max}) (Franklin and Davie, 1992), and is hypothesized to support the increased functional demands placed on the hearts of male fish during spawning. Elevated levels of androgens (testosterone, 11-ketotestosterone) stimulate this cardiac growth (Thorarensen et al., 1996; Davie and Thorarensen, 1997), and increase the proportion of compact myocardium without compromising coronary capillary density (Clark and Rodnick, 1998; R. V. Clark and K. J. Rodnick, unpublished data). However, whether androgen-induced myocardial growth is hypertrophic or hyperplastic has not been resolved. Bailey et al. (1997) concluded that cardiac enlargement in maturing rainbow trout is mainly due to hyperplasia, whereas Clark and Rodnick (1998) indicate that cardiac growth in male trout results from hypertrophy and not hyperplasia. The use of different methodologies (DNA/protein ratios *vs* cardiocyte morphometics) may have contributed to these opposing views, and so further study is required to resolve the relative contributions of cardiomyocyte hyperplasia and hypertrophy to heart growth in maturing male trout.

The trout heart has a significant population of androgen receptors (Pottinger, 1988; Fitzpatrick et al., 1994), which probably mediate the increased protein synthesis needed for maturation-induced cardiac enlargement in response to elevated levels of circulating androgens. However, Clark and Rodnick (1999) provide evidence for two scenarios where changes in haemodynamics with maturation may also promote

Table 1. *Morphometrics and ventricular energy status of fed and food deprived (8–10 weeks) Atlantic cod* Gadus morhua

Fish category	Fish body mass (g)	Fish body length (cm)	Relative ventricular mass (%)	Ventricular triglycerides (mg g^{-1})	Ventricular glycogen (mg g^{-1})	Ventricular protein (mg g^{-1})
Fed (*N*=16)	1039±51	50.7±1.0	0.88±0.03	2.4±0.9	3.6±0.9	82±0.9
Food deprived (*N*=15)	780±47*	51.9±1	0.94±0.03	0.2±0.02*	2.4±0.8*	86±0.2

Values are means ± S.E.M.
*Significant differences (P<0.05) detected using one-way ANOVA (A. K. Gamperl et al., unpublished).

ventricular hypertrophy. For example, an androgen-dependent expansion of blood volume could increase both venous pressure and *V*S (through the Starling response), and cause stretch-induced remodeling. Similarly, work-induced remodeling could occur if androgens increase blood pressure through alterations in vascular tone and resistance.

Feeding, exercise and inactivity

Food deprivation

Long periods of starvation, which occur naturally (Holdway and Beamish, 1984), and may produce mortality, e.g. in Atlantic cod (Dutil and Lambert, 2000), roach (Griffiths and Kirkwood, 1995) and smallmouth bass (Adams et al., 1982), can significantly decrease swimming endurance (e.g. Atlantic cod; Martinez et al., 2003). Given the importance of cardiac function to aerobic swimming performance (Hughes et al., 1988; Kolok and Farrell, 1994; Keen and Farrell, 1994), one might expect that cardiac alterations after extended periods of food deprivation could seriously compromise heart function. This hypothesis was recently tested by depriving Atlantic cod *Gadus morhua* of food for 10 weeks at 8°C (A. K. Gamperl et al., unpublished), and measuring cardiac morphometrics, biochemistry, and *in situ* cardiac performance. Cod deprived of food for 10 weeks were in poor condition (25% lighter, with a 85% decrease in hepatosomatic index), and had smaller hearts that contained dramatically reduced levels of energy substrates (Table 1). However, relative ventricular mass, ventricular protein levels and mass-specific maximum cardiac output ($\dot{Q}_{max}$ expressed in ml min^{-1} g^{-1} ventricle, rather than ml min^{-1} kg^{-1} body mass) were unchanged (Table 2). These results show that although the heart was not spared during prolonged negative energy balance, the relative performance of the cod heart, and thus its capacity to support swimming capacity, was unaffected. Additional evidence of cardiac remodeling with food deprivation was provided by the 15% reduction in intrinsic f_H in food-deprived cod (Table 2). Agnisola et al. (1996) earlier reported a 30% lower heart rate in sturgeon *Acipenser naccarii* Bonaparte fed diets enriched with either omega-3 polyunsaturated fatty acids or saturated fatty acids. Thus, it is possible that starvation altered the membrane lipid composition of the cardiac pacemaker cells, and effected the change in heart rate.

Table 2. *Maximum* in situ *cardiac performance in fed and food deprived Atlantic cod* Gadus morhua

Parameters	Fed	Food deprived
Heart rate (beats min^{-1})	59±1.8	50±2.7*
Cardiac output (ml min^{-1} g^{-1} ventricular mass)	64±5.7	58±3.6
Stroke volume (ml $beat^{-1}$)	1.1±0.09	1.2±0.11

Values are means ± S.E.M. (*N*=8 or 9)
*Significant difference (P<0.05) detected using one-way ANOVA (A. K. Gamperl et al., unpublished).

Exercise training

Aerobic training alters various components of the salmonid cardiovascular system, inducing cardiac growth (Hochachka, 1961; Farrell et al., 1990), and increasing $\dot{Q}_{max}$, certain cardiac enzymes, haematocrit, arterial O_2 content, skeletal muscle capillarity and tissue O_2 extraction (Hochahcka, 1961; Davie et al., 1986; Farrell et al., 1991; Gallaugher et al., 2001). These exercise-induced changes, however, are often small and variable (Davison, 1989), and even the 25% increase in $\dot{M}_{O_2max}$

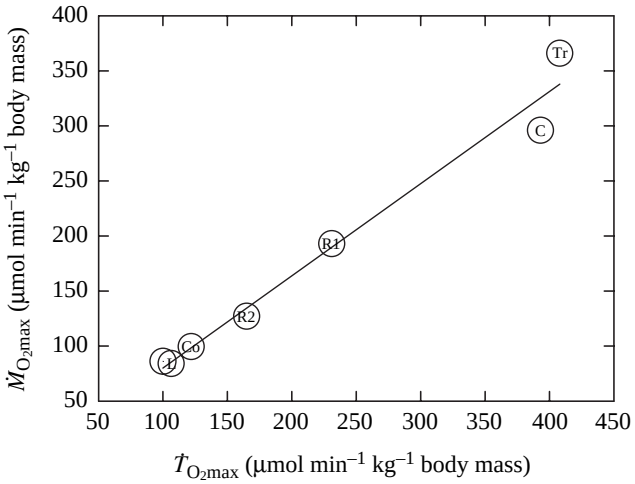

Fig. 1. For swimming studies, a tight relationship exists between maximum oxygen uptake ($\dot{M}_{O_2max}$) and maximum arterial oxygen transport ($\dot{T}_{O_2max}$; the product of maximum cardiac output and arterial oxygen carrying capacity) among a variety of fish species ($\dot{M}_{O_2max}$=0.84$\dot{T}_{O_2max}$–4.03; r^2=0.99). These data also illustrate the modest increase in both $\dot{M}_{O_2max}$ and $\dot{T}_{O_2max}$ produced with intense exercise training in chinook salmon. C, control; Tr, exercise-trained; Co, Atlantic cod, *Gadus morhua*; L, leopard shark; D, dogfish; R1+R2, rainbow trout). Graph taken with permission from Gallaugher et al. (2001).

brought about by a 3 month intense training regime (Fig. 1) is small relative to the twofold variability in $\dot{M}_{O_2max}$ that often exists among individual fish. Thus, although many individual components responsible for internal arterial O_2 convection show plasticity, the sum of the changes in individual components produce, at best, about a 25% improvement in metabolic capacity.

Because tissue O_2 extraction can increase with training, and the O_2 supply to the heart's spongy myocardium comes from oxygen-depleted venous blood (Davie and Farrell, 1991; Farrell and Clutterham, 2003), the possibility exists that training-induced cardiac growth occurs predominantly in the compact myocardium, which receives oxygen-rich coronary arterial blood. This pattern of cardiac growth would be consistent with that seen in sexually maturing male trout (see above); however, this possibility remains to be studied.

Aquaculture

Aquaculture conditions contrast with food deprivation and exercise-training studies in that fish become less active and are often overfed, and cardiac morphology certainly changes in salmonids raised for aquaculture. The normally distinct pyramidal structure of the ventricle (Fig. 2A) becomes more rounded (Fig. 2B,D), resembling the morphology of sedentary fish species (see Santer et al., 1983). Fat deposition can increase around the heart (Fig. 2B,C) and cardiac deformities may develop (Fig. 2E *vs* F). Further, studies show that the enhanced growth rates associated with aquaculture increase the rate of development of coronary arteriosclerosis (Saunders et al., 1992; Farrell, 2002), and that cultured salmonids have a decreased swimming capacity compared to wild fish (Duthie, 1987; Brauner, 1994; MacDonald et al., 1998). While these observations all point to diminished cardiac performance, direct measurements of cardiac performance in fish displaying the above morphological changes have not been performed. Moreover, two recent studies indicate that maximum cardiac function may not be different between wild and hatchery-reared salmonids. Dunmall and Schreer (2003) examined whether there is a genetic component to domestication by measuring swimming performance and *in vivo* maximum cardiac function in genetically distinct adult farmed and wild Atlantic salmon raised in identical conditions, and found no difference between the two groups. Further, maximum *in situ* cardiac function for two groups of pond-reared (domesticated) rainbow trout was found to be no different from either wild or sea-ranched (fish from wild stock, raised in hatcheries until smolts and then released into the wild) steelhead trout (Table 3; A. K. Gamperl et al., unpublished data).

While a definitive answer as to whether aquaculture/domestication affects maximum cardiac function requires more refined/controlled studies, aquaculture practices such as triploidy certainly alter cardiac physiology. Cardiomyocytes

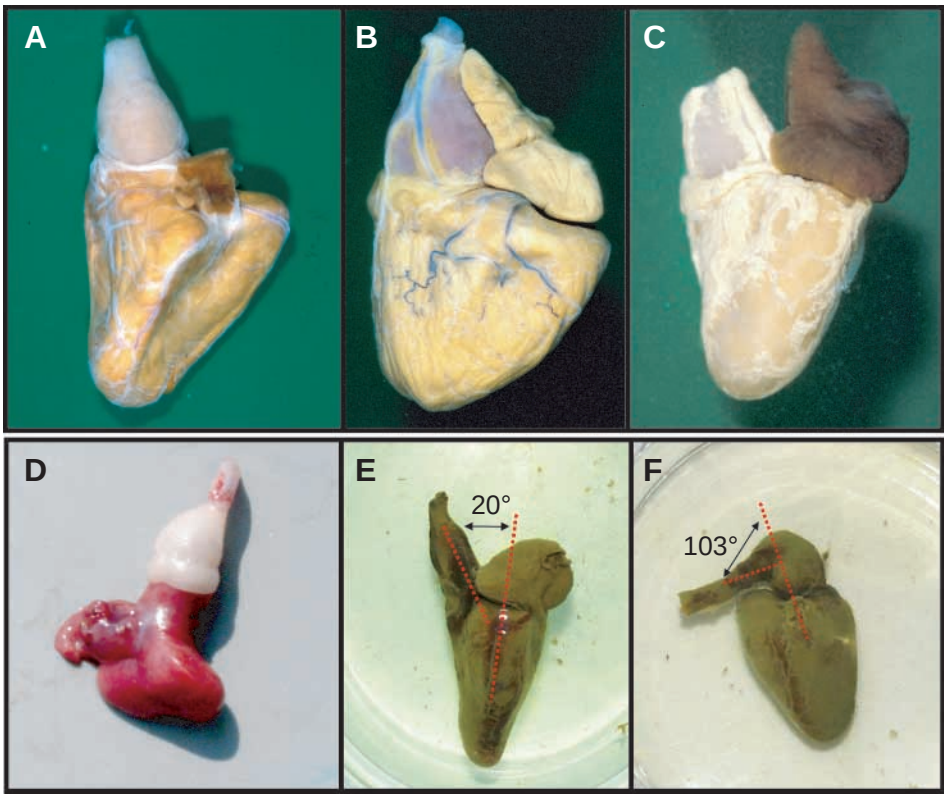

Fig. 2. Photographs of teleost hearts to illustrate normal and abnormal morphology. (A) Normal heart from a wild steelhead trout *Oncorhynchus mykiss* (~5 kg) from Idaho. Note the typical sharp edges to the pyramidal ventricle, and that the coronary arteries are not obvious. (B) An abnormal heart from a farmed rainbow trout *Oncorhynchus mykiss* (~3 kg), which died suddenly in an aquaculture pen in Norway. Note the more rounded shape to the ventricle, and the more superficial (prominant) coronary arteries. (C) An abnormal heart from a farmed Atlantic salmon *Salmo salar* (~4 kg), which died suddenly in an aquaculture pen in Norway. Note the excess fat deposits on the surface of the bulbus arteriosus and ventricle. Photographs A–C were provided courtesy of Dr Trygve Poppe. (D) An abnormal heart from a farmed sea bass *Dicentrachus labrax* (1.4 kg), which died suddenly in an aquaculture pen in France. Note the deformed shapes of the bulbus arteriosus and ventricle. (E) A normal heart from a farmed triploid brown trout *Salmo trutta* (~400 g) taken from an aquaculture pen in France. Note the acute angle subtended by the bulbus arteriosus to the ventricle. (F) An abnormal heart from a farmed triploid brown trout *Salmo trutta* (~500 g). Note the extreme angle subtended by the bulbus arteriosus to the ventricle. Photographs D–F were provided courtesy of Dr Guy Claireaux.

Table 3. *Maximum cardiovascular parameters in hearts of 'wild' and hatchery-reared* Oncorhynchus mykiss

Group	Relative ventricular mass (%)	$\dot{Q}$ (ml min^{-1} kg^{-1} fish)	$\dot{Q}$ (ml min^{-1} g^{-1} ventricle)	*Vs* (ml min^{-1} g^{-1} ventricle)	Power output (mW g^{-1} ventricle)
Wild steelhead (*N*=8)	0.094±0.003	68.6±3.4^{a}	73.2±3.2	1.02±0.05	7.34±0.19
Sea-ranched steelhead (*N*=8)	0.102±0.005	69.3±3.3^{a}	68.0±3.1	0.97±0.04	7.16±0.21
Oregon Hatchery rainbows (*N*=6)	0.101±0.005	77.7±2.8^{ab}	76.6±3.4	1.08±0.06	7.00±0.27
Washington Hatchery rainbows (*N*=8)	0.107±0.006	81.3±3.9^{b}	76.9±5.0	1.08±0.05	7.43±0.45

$\dot{Q}$, cardiac output; *Vs* stroke volume.

No significant differences were found between groups, with the exception of $\dot{Q}$ (ml min^{-1} kg^{-1}) for Washington Hatchery-reared rainbows. This difference was probably related to the number of mature males in this group (5 of 8) (A. K. Gamperl et al., unpublished). Dissimilar letters indicate a significant difference as determined by one-way ANOVA.

are 60% larger in triploid brown trout than in diploid rainbow trout, and they have an increased sensitivity to ryanodine (a blocker of SR Ca^{2+} release; Mercier et al., 2002). Perhaps the enhanced role for SR calcium release in the contraction of triploid cardiac muscle reflects the decrease in cellular surface to volume ratio associated with cell enlargement and a concomitant limitation to I_{Ca} *via* L-type Ca^{2+} channels.

When growth rate is further enhanced using growth hormone (GH) transgenic fish, swimming performance and $\dot{M}_{O_2max}$ can be either reduced (Farrell et al., 1997; Lee et al., 2003a) or no different (Stevens et al., 1998; McKenzie et al., 2000). With respect to the potential for cardiac changes in GH transgenic fish, we are only aware of one study. Pitkänen et al. (2001) found that the relative ventricular mass (RVM) of GH transgenic animals was enhanced by 60% *vs* size-matched controls, and suggested, based on non-significant differences in myocardial DNA contents (2.54 mg g^{-1} in transgenics *vs* 2.69 mg g^{-1} in size-matched controls), that this difference was due to hypertrophy alone.

Hypoxia

Hypoxic acclimation

Low water oxygen content (hypoxia) is a feature common to shallow waters that are highly eutrophic or ice-covered for prolonged periods in the winter, to continental slopes, and to deep basins found in the Baltic, the North Sea, and the East and West Coasts of North America. Surprisingly, however, we are aware of only three studies that have directly examined the effects of hypoxic acclimation on the fish heart, and all have focused on species that routinely experience hypoxic conditions. Paajanen and Vornanen (2003) acclimated crucian carp *Carassius carassius* to hypoxia (P_{O_2}<3 mmHg) for 3 weeks, and reported that the Na^+/K^+ ATPase activity of cardiac homogenates was reduced by 33%. Lennard and Huddart (1992) found that 3 weeks of exposure to hypoxia (P_{O_2} ~40 mmHg) caused numerous morphological changes in flounder *Platichthys flesus* cardiac mitochondria (decreased size, increased budding and cristae density) that would increase the area/volume ratio for oxygen diffusion, and may have led to changes in the concentration of oxidative enzymes. Finally, Driedzic et al. (1985) exposed the pout *Zoarces viviparous* to 4–6 weeks of hypoxia (P_{O_2} approx. 75 mmHg) and found that ventricular strips from hypoxia-acclimated animals were better able to sustain tension development during anoxia in the presence of high levels of external Ca^{2+}, even though no alterations in key enzymes of energy metabolism were detected. Thus, these studies suggest that hearts of species that normally experience aquatic hypoxia, undergo morphological and physiological adjustments that enhance function when exposed to environmental hypoxia.

Whether such adaptations are found in more active, hypoxia-sensitive, species is unclear. Bushnell et al. (1984) reported

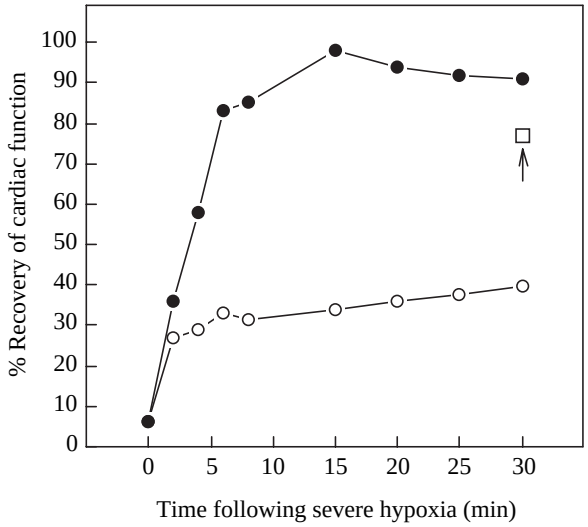

Fig. 3. Recovery of cardiac function following 30 min of severe hypoxia, measured as the percentage recovery of maximum force by ventricular strips [Gesser, 1977; open circles, rainbow trout (*N*=5) and closed circles, carp (*N*=5)], or as the % recovery of $\dot{Q}_{MAX}$ in *in situ* trout hearts (Faust et al., 1994; open square, *N*=8). Workload during the hypoxic period was similar between studies. The ventricular strips used by Gesser (1977) were developing maximum force, but at a contraction rate (0.2 Hz, 12 contractions min^{-1}) much lower than measured *in vivo* (~50–60 beats min^{-1}). By contrast, the power output of the hearts used in Faust et al. (2004) was approx. 1/6th of maximum. Reprinted with permission from the *Journal of Experimental Biology*.

that 3 weeks of hypoxic acclimation (P_{O_2}~40 mmHg) failed to enhance the swimming performance or oxygen consumption of rainbow trout when swum at this O_2 level, which argues against significant hypoxia-induced compensation in trout heart function. In contrast, recent experiments (Faust et al., 2004; *vs* Gamperl et al., 2001; Gesser, 1977; Fig. 3) show cardiac differences among rainbow trout obtained from different hatcheries, and report an unusual degree of myocardial hypoxia tolerance for fish reared at a facility where oxygen and other water quality parameters are sub-optimal. Clearly, further experiments are required to determine whether these differences in myocardial hypoxia tolerance are a result of acclimation to poor water quality (e.g. low O_2 saturation) or of genetic selection by hatchery operators.

Preconditioning

So far, this review has focused on cardiac alterations following long-term environmental change. However, recent research shows that fish can also respond rapidly to acute hypoxic exposure. Zebrafish *Danio rerio* exposed to just 48 h of non-lethal hypoxia (P_{O_2}=15 mmHg) have a significantly increased survival time (by 9× in males and 3× in females) when subsequently exposed to more severe hypoxia (P_{O_2}=8 mm Hg) (Rees et al., 2001). Further, Gamperl et al. (2001) demonstrated a cardioprotective response, in that pre-exposure to only 5 min of hypoxia (P_{O_2}=5–10 mmHg) completely eliminated the loss of *in situ* maximum cardiac function that normally follows 15 min of exposure to hypoxia in rainbow trout (Fig. 4A). This cardioprotective response, termed preconditioning, is broadly defined as the ability of brief periods of stress (e.g. hypoxia, ischaemia, stretch, heat shock) or biochemical/pharmacological substances to make tissues resistant to damage caused by a subsequent period of ischaemia or hypoxia. Gamperl et al. (2001) provided the first evidence (using hypoxia-sensitive trout) that preconditioning exists in fishes, and thus that preconditioning is a mechanism of cardioprotection that appeared early in the evolution of vertebrates. In mammals, numerous cellular pathways and end-effectors are involved in preconditioning (Okubo et al., 1999; Nakano et al., 2000; Yellon and Downey, 2003). No experiments have directly investigated the cellular mechanisms that mediate myocardial preconditioning in fishes, although recent studies suggest that sarcolemmal (Cameron et al., 2003) and mitochondrial (MacCormack and Driedzic, 2002) ATP-sensitive K^+ channels, and MAPK signaling pathways (ERK, JNKs and p38-MAPK; Gaitanaki et al., 2003) may be involved.

The importance and indeed existence of preconditioning in hypoxia-tolerant vertebrate hearts has been questioned in recent years. For example, ischaemic preconditioning failed to improve contractile function following 40 min of global ischemia in hypoxia-tolerant neonatal rat hearts (1 or 4 days post partum), only slightly (by 7%) improved contractile function in relatively hypoxia-sensitive rat hearts tested 7 days post partum (Ostadalova et al., 1998), and Baker et al. (1999) showed that hearts from 7–10 day old rats that were reared in a hypoxic environment (12% oxygen) no longer experienced increased functional recovery in response to preconditioning. In contrast, both Tajima et al. (1994) and Nechář et al. (2002) demonstrated that although hearts from chronically hypoxic adult rats had increased resistance to ischaemia-related damage, preconditioning conferred an additional amount of protection. Thus, to examine whether hearts from hypoxia-tolerant trout can be preconditioned, we recently conducted *in*

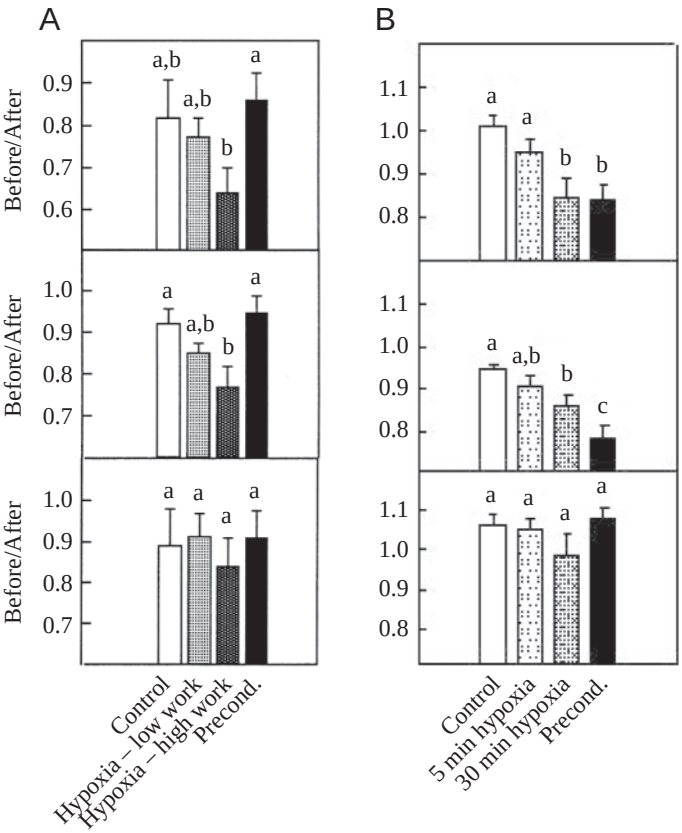

Fig. 4. Comparison of the ability of preconditioning (5 min of hypoxic pre-exposure) to protect (A) hypoxia-sensitive (Gamperl et al., 2001) and (B) hypoxia-tolerant (Gamperl et al., 2004) trout hearts from the myocardial dysfunction that follows more prolonged exposure to hypoxia. In A, 5 min of hypoxic pre-exposure completely eliminated the loss of myocardial function that normally followed the 'Hypoxia-high workload' protocol. In B, preconditioning with 5 min of hypoxia either did not affect, or increased, the amount of myocardial dysfunction following exposure to '30 min of hypoxia'. Top panels, maximum cardiac output; middle panels, maximum stroke volume; bottom panels, heart rate. Note that the hypoxia-tolerant trout hearts in B required twice the duration of hypoxia (15 *vs* 30 min), and 6 times the workload, as compared with hypoxia-sensitive hearts (A) to achieve a comparable (15–20%) decrease in post-hypoxic myocardial function. Values were obtained by comparing maximum *in situ* cardiac function before and after the treatment protocols. All values are means ± S.E.M. (N=7–9). Dissimilar letters indicate a significant difference at P<0.05, as determined by one-way ANOVA. Hypoxia in these experiments was defined as perfusate P_{O_2}=5–10 mmHg. Control hearts were only exposed to oxygenated saline.

situ studies on two different populations of rainbow trout that display an unusual degree of myocardial hypoxia tolerance. Gamperl et al. (2004) performed *in situ* experiments using trout with hearts that Faust et al. (2004; Fig. 3) previously identified as hypoxia-tolerant (again using 5 min of hypoxia as the preconditioning stimulus), while Overgaard et al. (2004) used a population of trout from British Columbia (Canada) and 2× 5 min cycles of hypoxia or exposure to high adrenaline (250 nmol l^{-1}) as preconditioning stimuli. Both studies (e.g. Fig. 4B) showed that hypoxia-tolerant trout hearts could not be preconditioned, and thus that the protection afforded by inherent myocardial hypoxia tolerance and preconditioning was not additive. These data suggest that the relationship between hypoxic adaptation and preconditioning in the trout heart resembles that of the neonatal/immature, not adult, mammalian heart.

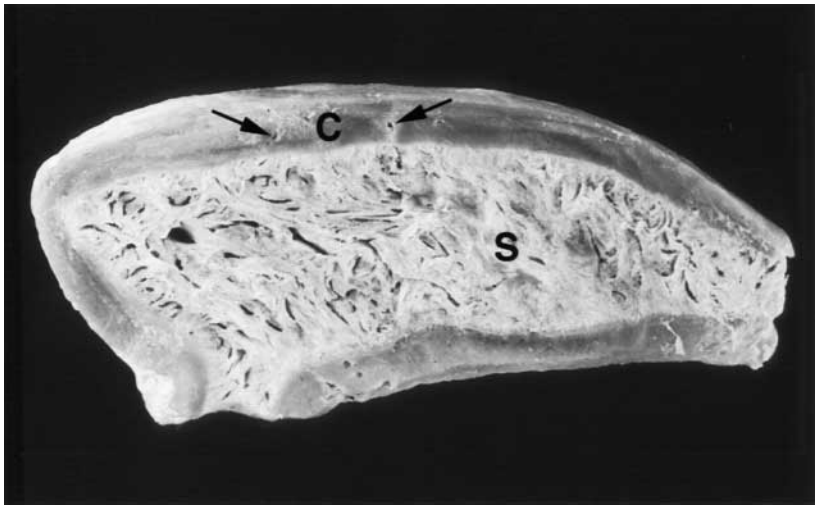

Fig. 5. Medial section through the alcohol-preserved ventricle of a chinook salmon *Oncorhynchus tshawytscha* (10 kg). Note location of, and the proportion of, ventricle occupied by the compact (C) and spongy (S) myocardium. Arrows indicate coronary arteries. Original magnification, ×5. Reprinted with permission from the *American Journal of Physiology* (Gamperl et al., 1998).

It is tempting to associate myocardial preconditioning with myocardium that is supplied with blood from the coronary circulation because the rat heart becomes increasingly dependent on its coronary circulation as it ages, and rainbow trout possess a coronary circulation (Tota et al., 1983) that supplies blood to the compact myocardium, which comprises the outer one-third of the heart (Fig. 5). However, the hypoxia-sensitive heart of Atlantic cod *Gadus morhua*, which lacks a coronary circulation and is composed entirely of spongy myocardium, can be preconditioned (A. G. Genge and A. K. Gamperl, unpublished; Fig. 6) in much the same way as rainbow trout (Gamperl et al., 2001). Why cod hearts that have only spongy myocardium and display a moderate degree of hypoxia tolerance, but not trout hearts that have developed a high degree of hypoxia tolerance (Gamperl et al., 2004; Overgaard et al., 2004), can be preconditioned is not known. However, investigations into the cellular mechanisms that mediate these differences are likely to provide valuable information on how the hearts of fish and other lower vertebrates deal with oxygen deprivation.

Cardiac variability among fish stocks

Perhaps the greatest source of intraspecific cardiac plasticity has yet to be discovered because comparisons among fish stocks are limited. Nonetheless, the few studies that exist often show important differences in cardiac design and function. As described above, certain stocks of domesticated rainbow trout have hearts with a greater hypoxia tolerance than others. Graham and Farrell (1992) showed that lake-dwelling rainbow trout have large ventricles and moderate amounts of compact myocardium, whereas anadromous fish had smaller ventricles (by 10–25%) and higher levels (by 30–40%) of compact myocardium. Likewise, the Pacific salmon have appreciable stock-specific differences in both swimming performance and the optimum temperature for aerobic activity (Tsuyuki and Williscroft, 1977; Taylor and McPhail, 1985; Taylor and Foote, 1991; Lee et al., 2003b), that appear to reflect the large differences in river migratory distances, which can be as little as 100 km and as large as 1000 km. In Iceland, numerous Arctic charr morphs exist that differ substantially in morphology, behaviour and life-history characteristics, and the relative ventricular mass of an anadromous population was 10–20% greater than that of a landlocked population (M. A M. Ruiz and H. Thorarensen, unpublished data). This relationship

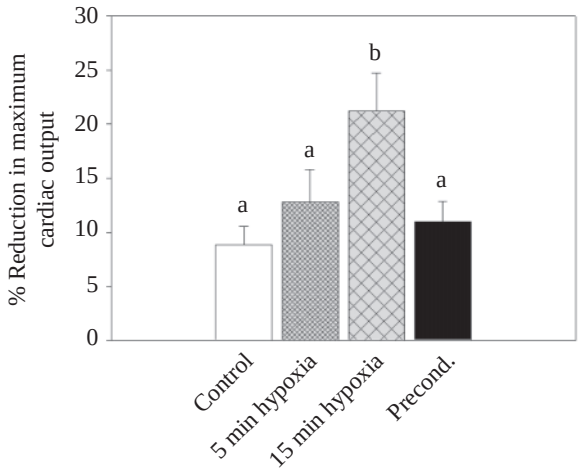

Fig. 6. Recovery of maximum cardiac output in 10°C *in situ* cod (*Gadus morhua*) hearts exposed to oxygenated perfusate only (control), 5 min or 15 min of hypoxia (P_{O_2}=5–10 mmHg), or 5 min hypoxia 20 min prior to 15 min of hypoxia (preconditioning). Values are means ± S.E.M. (N=8–9). Dissimilar letters indicate a significant difference at P<0.05 as determined by one-way ANOVA (A. G. Genge and A. K. Gamperl, unpublished).

between life history and heart size is opposite to that reported for Pacific salmon (Graham and Farrell, 1992), suggesting that natural selection has provided two contrasting model systems to examine intraspecific plasticity at the stock level.

Summary and perspective

The mammalian heart is characterized by its relative intolerance to injury or the lack of oxygen. This is in part related to its high metabolic demand relative to its glycolytic ability, and in part to the fact that post-neonatal cardiac growth is primarily, but not exclusively (Anversa, 2000), through hypertrophy. Fish hearts differ in the manner in which they grow, and it appears that the rainbow trout heart grows through both hyperplasia and hypertrophy during ontogeny (Farrell et al., 1988), and by hyperplasia, hypertrophy or both, depending on the environmental or physiological challenge. While mammalian researchers continue to search for ways to stimulate cardiac growth to replace damaged myocardial tissue, it seems that fish never lose this ability (perhaps these researchers should be looking at fish models too!), and it is likely that the high degree of intraspecific plasticity that we describe may well be related to the fact that fish retain hyperplastic as well as hypertrophic myocardial growth. Further, it appears that cardiac remodeling is not restricted to myocytes, but may be a general characteristic of cells that comprise the fish heart. Egginton and Cordiner (1997) showed that myocardial capillary density is maintained in fish acclimated to 4 *vs* 11°C despite cardiac hypertrophy, and is increased by ~75% in fish acclimated to 18°C. Clark and Rodnick (1998) showed that capillary growth matches cardiac growth associated with sexual maturation in males. Research on rainbow trout indicates that new vessel growth can re-establish coronary blood flow following coronary artery ablation (Daxboeck, 1982; Farrell et al., 1990). Finally, we have observed major remodeling of the bulbus and ventral aorta of Atlantic cod, to allow for the maintenance of cardiac output past the feeding appendages of the haematophagus parasite *Lernaeocera branchialis* (e.g. see Fig. 7).

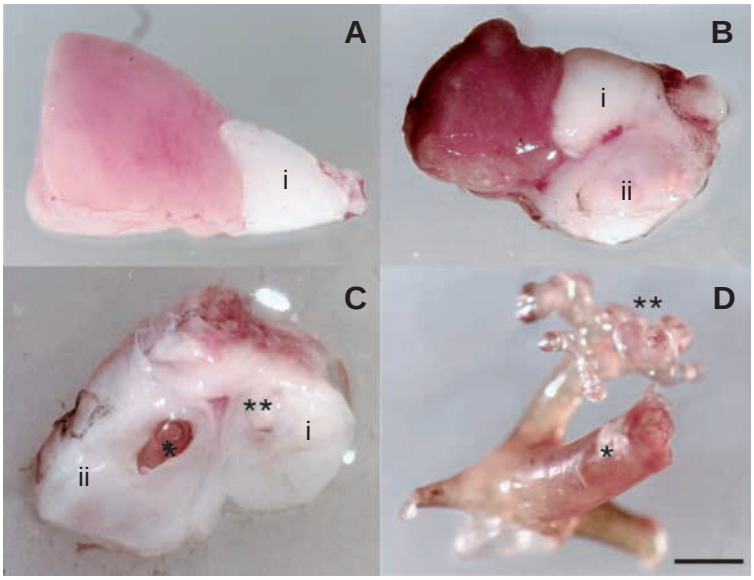

Fig. 7. Photographs of hearts from (A) an uninfected cod (*Gadus morhua*) and (B) one that was infected with an X-stage *Lernaeocera branchialis*. (C) Cross section though the bulbus (i) and connective tissue 'capsule' (ii) that had grown around the parasite and allowed for additional blood flow. * and ** indicate the locations of the labeled portions of the parasite's cephalic anchors. (D) Chitinous exoskeleton of the cephalic anchors that were imbedded in the bulbus and capsule. The anchor in the bulbus (**) occluded a significant portion of the bulbus' lumen. Scale bar in D, 1 mm.

Recently, we have shown that a preconditioning-like phenomenon exists in fishes, that there is a significant degree of intraspecific variation in myocardial hypoxia tolerance and the ability to be preconditioned among rainbow trout, and that the spongy myocardium of cod can be preconditioned. These findings strongly suggest that protective pathways can still be stimulated in myocardium that is normally perfused by blood of low oxygen partial pressure, and that preconditioning and acquired hypoxia tolerance in trout are mediated by the same or similar cellular mechanisms. Further, we provide substantial indirect evidence that trout myocytes are not permanently damaged by exposure to prolonged periods (15 min to 4 h) of severe hypoxia, even though contractile function is diminished (Gamperl et al., 2004; Overgaard et al., 2004; J. Overgaard and J. A. W. Stecyk, unpublished). While this enhanced ability of rainbow trout hearts to tolerate long periods of severe hypoxia as compared with mammals is likely to be related in part to temperatures (10–15°C *vs* 37°C) and absolute workload, we suspect that there are also mechanistic reasons for this difference.

In this review we have demonstrated that the fish heart has tremendous capacity to respond to both short-term and long-term perturbations, and hint at mechanistic explanations of how this is accomplished. However, it is apparent that we have little understanding of the molecular and biochemical signaling pathways that mediate much of this plasticity. Important and obvious questions include: What cellular events are responsible for stimulating hyperplastic *vs* hypertrophic growth of the fish heart? Which signal transduction pathways and end-effectors mediate preconditioning and inherent hypoxia tolerance of the fish myocardium, and how do they compare with those in mammals? Why does the trout heart not experience permanent damage (necrosis) when exposed to severe hypoxia or anoxia for periods up to 4 h? Our challenge, therefore, is to design experiments that will provide insights into the novel control mechanisms that mediate myocardial plasticity and adaptation in fish.

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