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A7–CARDIOVASCULAR RESPONSES TO CHANGES IN METABOLIC STATE

Organised by T. Wang and E.W. Taylor for the Respiration Group

A7.1 The roles of neuroendocrine peptides in cardiovascular and metabolic regulation in reptiles

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The full complement of genes encoding the known neuroendocrine peptides in mammals appear to be expressed in reptiles but evolutionary pressure to conserve the primary structures of the different peptides, both amongst reptiles and between reptiles and mammals, has been very variable. A role for the tachykinins in cardiovascular regulation in snakes is suggested by the observation that python substance P and neuropeptide γ are potent vasodilators in the systemic circulation of the python but have little effect upon the pulmonary circulation. Effects are mediated primarily through interaction with NK1-type receptors but do not involve increased NO synthesis. Evidence from mammals suggests that hypoxia increases sensitivity of pulmonary vessels to NK1 agonists. Both the primary structures and cardiovascular actions of bradykinin (BK) differ markedly among reptiles. Python BK ([Ala¹,Thr⁶]BK) produces a norepinephrine-mediated vasopressor response and increase in heart rate in the python whereas rattlesnake BK ([Val¹,Thr⁶]BK) produces a pronounced vasodepressor response in the rattlesnake systemic circulation but no change in pulmonary vascular resistance. Turtle/alligator BK ([Thr⁶]BK) produces falls in systemic vascular resistance in the respective species of origin. In contrast to the kinins, the structures of the isoforms of endothelin (ET-1) have been strongly conserved during evolution of tetrapods but alligator/human ET-1 and alligator ET-3 ([Tyr⁴]ET-3) produce a pronounced initial vasodepressor response in the alligator that is not seen in mammals. The primary structures of insulin, glucagon and glucagon-like peptide 1 are known for several species of reptiles but their roles in metabolic regulation remain to be established.

A7.2 The length-tension relationship in ventricular myocytes from the rainbow trout (*Oncorhynchus mykiss*)

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Increased cardiac output in fish is achieved largely by an increase in stroke volume. This is due to an increased end-diastolic ventricular volume and a stretch-induced increase in myocardial contractility known as the Frank-Starling mechanism. Despite the physiological importance of this mechanism in regulating cardiac output in fish, relatively few details are understood at the cellular level. We used carbon fibre transducers to apply axial stretch to the longitudinal axis of single trout ventricular myocytes. Cells were stimulated by external electrodes at 0.5 Hz at room temperature to contract auxotonically. Mean end diastolic sarcomere length (SL) was $1.84 \pm 0.002 \mu\text{m}$ ($n=19$) at slack length. When cells were progressively stretched there was an increase in the passive tension (during diastole) and a larger increase in the active tension (additional tension developed during systole). The results predicted that each 1% increase in SL above slack SL caused an approximate 20% increase in active tension (linear regression, $R=0.75$, $P<0.001$). Furthermore, we found that trout myocytes appear to operate over a greater range of sarcomere lengths with maximum force development occurring at longer sarcomere lengths than mammals. This may be a necessary adaptation to allow the trout heart to modulate cardiac output via changes in stroke volume.

A7.3 Cardio-metabolic responses to exercise in ectotherms: the effect of temperature

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During aerobic activity, the rate of oxygen consumption ($\dot{V}O_2$) increases through augmentation of cardiac output

(the product of heart rate [f_H] and stroke volume) and/or extraction of oxygen from the circulating blood. In ectotherms, $\dot{V}O_2$ at rest and during exercise also increases with an increase in temperature. The objective of the studies was to determine if factorial aerobic scope (maximum $\dot{V}O_2$ /pre-exercise $\dot{V}O_2$) is maintained at different temperatures. For Rosenberg's goanna (*Varanus rosenbergi*) during treadmill exercise a factorial aerobic scope of 6.5 was maintained across the temperature range of 14–36°C, and at both 25°C and 36°C similar increases in oxygen extraction (2.2-fold), f_H (2.0-fold) and stroke volume (1.8-fold) were observed. Preliminary experiments on Murray cod (*Maccullochella peelii peelii*) swimming in a flume also indicate that factorial aerobic scope is maintained in the face of changing temperature and that it is primarily the result of increases in f_H as opposed to increases in oxygen extraction or stroke volume. Despite interspecies differences in the contribution of the variables involved in O_2 delivery by the circulatory system, the constancy of the proportional change in each variable across a biologically relevant temperature range ensures that factorial aerobic scope is temperature independent. This means that the maximum gain for each variable is temperature independent and, as a result, an ectotherm at a higher temperature can increase its aerobic activity in absolute terms to a greater extent.

A7.4 The use of heart rate variability as an index of activity patterns in fish

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In mammals there is a positive relationship between heart rate (f_H) and oxygen consumption (MO_2). In fishes this has been shown not to be the case in some species where stroke volume appears to be more important than f_H in determining cardiac output. We examined whether heart rate variability (HRV) may be a better index than f_H for this purpose, estimated using either geometric statistics (standard deviation of successive R–R intervals of the e.c.g., SDRR) or power spectral analysis (PSA). Studying benthic, labriform swimmers meant that the traditional method of varying MO_2 by use of exercise flumes was inappropriate, so we tracked changes due to recovery from surgery and following feeding (specific dynamic action, SDA). From northern temperate, southern temperate and Antarctic species it is clear that SDRR tracks changes in MO_2 in a linear manner, while PSA shows changes in one spectral peak that may be related to activity patterns. However, PSA also demonstrates that erroneous conclusions about the vagal influence on f_H may be made if the animals are not fully recovered from stress due to surgery or disturbance. This work was supported by NERC.

A7.5 Endothelin receptors in teleost fish: cardiovascular effects and branchial distribution

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An ability to regulate the functional respiratory surface area of the gills should be of fundamental importance for the respiratory and ion-regulatory physiology of fishes, especially during challenges such as exercise or hypoxia. By observing gill blood flow using epi-illuminating microscopy in parallel with cardiovascular recordings and immunohistochemistry, we have tried to identify the endothelin-receptor mediating pillar cell contraction, and redistribution of intra-lamellar blood flow in the Atlantic cod (*Gadus morhua*). Intra arterial injection of the specific ET_B -receptor agonist BQ-3020 induced dose dependent increases in ventral aortic blood pressure, gill vascular resistance and pillar cell area (indicating contraction). The specific ET_A antagonist BQ-610 was unable to prevent pillar cell contraction and increased gill vascular resistance induced by ET-1 injection.

The cardiovascular responses were corroborated by the detection of ET_B receptor-like immunoreactivity (IR) associated with pillar cells in the lamellar region and in neuroendocrine cells. ET_B receptor-like IR was also found lining the muscle layer of lamellar arterioles and filamental arteries. In contrast, strong ET_A receptor like IR was located on branchial nerves throughout the filament. In addition, ET like IR was concentrated to neuroendocrine cells in the filament and lamellae. We also present data suggesting that ET mediated pillar cell contraction is widespread among teleost fish, including Atlantic cod, rainbow trout (*Oncorhynchus mykiss*), sculpin (*Scomber scombrus*) and mackerel (*Scomber scombrus*).

Taken together our results suggest that an ET_B -like receptor mediates pillar cell contraction in fishes, while ET_A -like receptors may serve another function in the gill as they are found on branchial nerves.

A7.6 Cardiorespiratory Changes During Sleep; From Lounging Lizards to Somnolent Seals

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Sleep is a state associated with a change in central neural arousal and reductions in activity and metabolic rate. The fall in metabolic rate (MR) is generally greater than would be predicted by the reduction in activity levels

alone. In this study we have explored the changes in oxygen delivery, manifested as reductions in heart rate and ventilation, and the changes in respiratory chemosensitivity, that are associated with this change in state, in a variety of animals. We found that the falls in heart rate, and ventilation associated with the profound reductions in metabolic rate ($\sim 80\%$) during sleep in tegu lizards often preceded the daily reductions in T_b . In species of burrowing mammal capable of hibernation, despite equally impressive falls in MR ($\sim 40\%$) during slow wave sleep (SWS), the relative sensitivity to hypoxia and hypercapnia was increased, decreasing the amount of time spent in SWS. In harbour seal pups there was an insignificant fall in MR in SWS that was associated with a decrease in the air convection requirement and respiratory chemosensitivity was unchanged. Thus, the data reveal tremendous species differences in the changes that occur in different components of the blood gas transport cascade during sleep, some of which can be correlated easily to life style differences, but many of which cannot. Supported by the NSERC of Canada.

A7.7 Loading and limiting factors affecting swimming performance in fish

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The capacity for sustained aerobic exercise is believed to influence the success with which active fish colonise a given ecological niche. Many studies have revealed, however, that swimming ability is profoundly affected by various endogenous and environmental factors. These have been categorised by the manner in which they influence performance, as either loading or limiting. Loading factors are of two general types: those that increase routine metabolic costs and constrain the allocation of energy for exercise, and those that increase the net costs of exercise by impairing the biochemical and physiological processes that define swimming efficiency. Examples of the first include environmental stressors, relative growth rates, nutritional status (in particular specific dynamic action), and prior exercise to exhaustion; examples of the second include toxic pollutants. Limiting factors are also of two types, those that limit the maximal capacity for oxygen delivery, and those that impair the biochemical and physiological processes that define maximal swimming performance. The major example of the first is hypoxia; examples of the second are pH, salinity, and toxic pollutants (e.g. ammonia). Some factors, particularly environmental stressors, are both loading and limiting (e.g. salinity). Sensitivity to loading and limiting factors may be greatest in those species where metabolic costs of exercise are closely matched to the capacity for oxygen delivery. Although the literature indicates that this may be the case in salm-

onid species, evidence will be presented to indicate that it is not the case in other teleosts, such as the common carp and European eel.

A7.8 Effects of hypoxia on the venous circulation in Rainbow trout (*Oncorhynchus mykiss*)

Erik Sandblom and Michael Axelsson, Department of Zoophysiology, Göteborg University, Sweden

Systemic cardiovascular responses to environmental hypoxia are extensively studied in fish. Hypoxia is generally associated with bradycardia, with an unaltered cardiac output due to a compensatory increase in stroke volume. Although changes in venous pressure and flow are important determinants of stroke volume and cardiac performance in teleosts, the role of the venous vasculature during hypoxia has not yet been thoroughly studied. Rainbow trout (*Oncorhynchus mykiss*) were subjected to severe ($P_{wO_2} = 7.3 \pm 0.2$ kPa) or mild ($P_{wO_2} = 11.7 \pm 0.1$ kPa) hypoxia. Venous pressure (P_{ven}), dorsal aortic pressure (P_{da}), heart rate (f_H) and cardiac output (Q) were recorded *in vivo*. Both levels of hypoxia triggered a significant increase in P_{ven} . Severe hypoxia was associated with bradycardia and an unaltered Q , showing that an increase in venous pressure is coupled to a compensatory increase in stroke volume during hypoxic bradycardia. Pharmacological treatment with Prazosin (1 mg kg^{-1}) and (or) Atropine (1.2 mg kg^{-1}) did not fully reveal the underlying mechanisms controlling venous pressure. We speculate that α -adrenergic blockade with Prazosin in combination with local hypoxic effects reduced the total peripheral resistance, thus leading to a passive increase in P_{ven} during hypoxia. The present study emphasizes the influence of venous blood pooling, reduced peripheral resistance and altered venous tone, on the changes in venous pressure observed during hypoxia.

A7.9 Determinants of contractile performance and energy turnover of the anoxic turtle myocardium: A heart that works against all odds

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The freshwater turtles *Trachemys scripta* and *Chrysemys picta* can hibernate for extended periods in anoxic water. While continued propulsion of blood remains essential for survival, these turtles markedly depress cardiac output concomitantly with the metabolic depression associated with cold anoxia. All energy production of the heart is derived from glycolysis during anoxia and cardiac function is maintained despite a substantial acidosis

and increased concentrations of potassium. These negative effects are counteracted by elevated levels of calcium and adrenaline.

Here we report that anoxia (severe hypoxia) markedly improve energy economy of cardiac strip preparations from *Trachemys scripta*, which may contribute to their remarkable hypoxia tolerance. Firstly, cellular energy liberation and energy demand of non-contractile processes decreased 2/3 in the myocardium during hypoxia. Secondly, in comparison to anoxia sensitive species, turtle myocardium maintained a higher cellular energy state and twitch force development at a given rate of energy production. Thus, twitch force increased relative to ATP-hydrolysis during hypoxia.

In a separate study on *Chrysemys picta*, acclimated to either 25°, 5° or 5°C anoxia, we found that twitch force of myocardial preparations was highest at the acclimation temperature of the turtle. Anoxia, acidosis, adrenergic stimulation and elevated Ca^{2+} concentrations each changed twitch force by at most 40%, while hyperkalemia (10 mmol l⁻¹ K⁺) caused irregular contractions and dramatically reduced twitch force. When all treatments were combined, twitch force was higher at 5°C than at 25°C although it was dramatically reduced at both temperatures.

A7.10 Shutting down or remaining active: Contrasting anoxic-survival strategies in anoxia-tolerant species reflected in cardiovascular control

J.A.W. Stecyk and A.P. Farrell, Biological Sciences, Simon Fraser University, Burnaby, Canada; J. Overgaard and T. Wang, Zoophysiology, University of Aarhus, Denmark; K.O. Stensløkken and G.E. Nilsson, Physiology Program, University of Oslo, Norway; C. Bock and H.O. Pörtner, Alfred-Wegener Institute for Polar and Marine Research, Bremerhaven, Germany

Prolonged anoxic survival requires balancing energy supply and demand and coping with anaerobic end products. In ectothermic vertebrates, this is usually accomplished through a reduction in metabolic rate that is mirrored by a decreased cardiovascular function. However, the degree and determinants of anoxic cardiovascular depression vary with anoxia-tolerance and thus, might represent a fundamental difference in anoxic survival between truly anoxia-tolerant and hypoxia-tolerant species. Anoxic freshwater turtles reduce metabolism by 90%, enter a comatose-like state and buffer lactate with their bone and shell. Correspondingly, cardiac power output is reduced by 95%, precluding an up-regulation of glycolysis, and autonomic cardiovascular control is blunted. In contrast, the common carp, which can only survive 24h of anoxia at 5°C, relies upon a partial up-regulation of anaerobiosis to meet its cardiac energy

demands and maintains autonomic cardiovascular control. However, recent study has revealed that the extremely anoxia-tolerant crucian carp maintains cardiovascular function at normoxic levels during a 5-day anoxic exposure at $8 \pm 1^\circ\text{C}$ with sustained autonomic cardiovascular control. By maintaining cardiac activity during anoxia, rapid distribution of glucose from the crucian carp's liver glycogen store, the largest among vertebrates, can support up-regulation of glycolysis. Further, rapid shuttling of lactate to the muscle, the sole site for ethanol production, and ethanol to the gills for excretion is possible, allowing the fish to circumvent anoxic acidosis. Thus, the degree of cardiac depression and suppression of autonomic cardiovascular control during anoxia does not correlate with ability to survive prolonged anoxia, but reflects a more complex anoxia survival strategy.

A7.11 Eat and Run: Prioritization of oxygen delivery during elevated metabolic states

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The principal function of the cardiopulmonary system is the matching of oxygen and carbon dioxide transport to the metabolic requirements of different tissues. Increased oxygen demands ($\dot{V}\text{O}_2$), for example during physical activity, result in a rapid compensatory increase in cardiac output and redistribution of blood flow to the appropriate skeletal muscles, matched by suitable ventilatory increments. This matching of cardiopulmonary performance and metabolism during activity seems universal among vertebrates, and has been demonstrated in a number of different taxa. In some animals, large increments in aerobic metabolism are also associated with physiological states other than activity. In particular, $\dot{V}\text{O}_2$ increases following feeding due to the energy requiring processes associated with prey handling, digestion and the ensuing protein synthesis. This large increase in $\dot{V}\text{O}_2$ is referred to as "specific dynamic action" (SDA). In reptiles, the increase in $\dot{V}\text{O}_2$ during SDA may be 3–40 fold above resting values, peaking at 24–36 hours following ingestion, and remaining elevated for up to 7 days. In addition, to the increased metabolic demands, digestion is associated with secretion of H^+ into the stomach resulting in a large metabolic alkalosis (alkaline tide), with plasma $[\text{HCO}_3^-]$ nearly doubling. Consequently, the cardiopulmonary system must meet the simultaneous challenges of an elevated oxygen demand and a pronounced metabolic alkalosis. This paper will compare and contrast the patterns of cardiopulmonary response to similar metabolic demands during these different physiological states (exercise and/or digestion), and during the simultaneous

challenge of postprandial exercise in a variety of reptiles, including the Burmese python, *Python morulus*, savannah monitor lizard, *Varanus exanthematicus*, and American alligator *Alligator mississippiensis*.

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A7.12 Pressure separation in the undivided snake heart: Correlations with high metabolic rate during digestion or incubation of eggs?

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Vascular pressure separation by virtue of a two-chambered ventricle evolved independently in mammals and birds from a reptilian ancestor with a single ventricle and allowed for high systemic perfusion pressure, while protecting the lungs from oedema. The hearts of varanid lizards are, nevertheless, functionally separated and systemic blood pressure vastly exceeds pulmonary blood pressure. This has been regarded as a unique adaptation to an active predatory life style and high metabolic rate. The Burmese python (*Python molurus*) also exhibits a remarkable flow and pressure separation within the ventricle¹, and we have demonstrated similar abilities in other species of *Python* (*Python regius* and *Python reticulatus*). Pythons have high metabolic rates during digestion, but other large snakes with large metabolic responses to digestion do not possess ventricular pressure separation. Thus, the systolic pressures of the systemic and pulmonary circulations are identical in Boa (*Boa*), anacondas (*Eunectes*), rattlesnakes (*Crotalus*) and vipers (*Bothrops*). *Python molurus*, and some other species of pythons, are unique by being able to increase body temperature through shivering thermogenesis while incubating their eggs. This associated with long-lasting and profound increases in metabolic rate. The link between shivering thermogenesis and ventricular pressure separation is, however, not obvious. Thus, while *Python reticulatus* has been reported to incubate their eggs, *Python regius* does not. It is possible that shivering thermogenesis and ventricular pressure separation co-evolved in the stem group of pythons, but that some species subsequently lost incubation of eggs, while retaining a functionally divided heart.

¹Wang, Altimiras, Klein and Axelsson (2003). *J.Exp.Biol.* 206: 4241–4245.

A7.13 Differential heating and cooling rates in bigeye tuna (*Thunnus obesus*): Options for cardiovascular adjustments

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Temperature and depth data from archival tags retrieved from bigeye tuna (*Thunnus obesus*) at liberty for as long as 57 days were analyzed using a mathematical model of heat exchange. This model takes into account the transfer of heat between the red muscle and the water, as well as between the red muscle and the internal temperature sensor of the archival tag, which was placed intraperitoneally. Our analysis shows that the recorded internal temperature (sensor temperature, T_s) during vertical excursions can be successfully predicted over the entire sampling period of every fish provided that the rates for cooling (k_{low}) and heating (k_{high}) are very dissimilar. Least squares fitting of k_{low} and k_{high} for the entire sampling period of each fish yield values generally in the ranges 0.02 – 0.04 min^{-1} and 0.2 – 0.6 min^{-1} with an average ratio k_{high}/k_{low} of around 12. There is a significant negative correlation between k_{low} and size, whereas there is no correlation between k_{high} and size. This is reflected in a lack of correlation between k_{low} and k_{high} . The maximal swimming speeds during vertical excursions of ca. 2 BL sec^{-1} occur approximately midways which is inconsistent with a passive effect of increased cardiac output on heat exchange.

A7.14 Swimming and recovery: intraspecific differences among salmon

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Studies of critical swimming speed (U_{crit}), when coupled with respirometry, provide valuable information on the metabolic costs of swimming and recovery. To examine for intraspecific differences, swimming energetics were compared over a range of ambient temperatures for two stocks of adult sockeye salmon (*Oncorhynchus nerka*) from different locations in the same watershed. Fish were intercepted on their return spawning migration near their natal stream, and a mobile Brett-type respirometer allowed measurements to be made in field locations. Each fish swam twice to U_{crit} , with a 45 min recovery period between the end of one test and the start of the next. U_{crit} and maximum oxygen consumption (Mo_{2max}) were similar for both tests, and were averaged to examine the effects of ambient temperature. Salmon stocks had temperature optima for Mo_{2max} and U_{crit} . However, the significantly higher values for Mo_{2max} , U_{crit} and cost

of transport for the stock with the higher optimum temperature could not be accounted for entirely by temperature. Excess post-exercise oxygen consumption was also higher for this stock, suggesting either a greater willingness or greater ability to perform non-aerobic swimming. The cardiovascular basis for such intraspecific differences is unknown, but a higher anaerobic effort likely exposes the heart to lower venous oxygen tensions, as well as more severe hyperkalemia and acidosis. Under such conditions, our recent studies with perfused hearts have revealed that a protective adrenergic stimulation is of critical importance. Consequently, future studies of intraspecific differences in cardiac adrenergic sensitivity and coronary artery development may prove informative.

A7.15 Hypometabolism and respiration in mammals: newborns to adults — effects of temperature and hypoxia

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In mammals the rate of oxygen consumption ($\dot{V}O_2$) is roughly equivalent to the production of ATP. If the level of inspired O_2 declines, regulated adjustments in either conduction (structural parameters) and/or functional changes in the partial pressure gradient for all or any step in the oxygen cascade ensures that $\dot{V}O_2$ more or less is maintained constant. When the supply of oxygen can no longer sustain aerobic ATP production the animal must either down-regulate its energy requirements or find alternative sources of energy. In either case, $\dot{V}O_2$ will decline. Alternatively, the decline in $\dot{V}O_2$ could occur before oxygen became limiting if another process that utilized oxygen was attenuated. In small mammals, in general, the hypoxic decrease in $\dot{V}O_2$ is mostly related to a decrease in thermogenesis and hence is associated with a decline in body temperature; the result of a decrease in thermoregulatory operational points rather than hypothermia. Most interestingly is that in situations where supply is not limiting, $\dot{V}O_2$ is appropriately matched to changes in ventilation such that convection requirement per unit of O_2 uptake is maintained appropriate for the level of inspired O_2 regardless of the change in $\dot{V}O_2$ that may or may not have occurred. This suggests that supply is correctly matched to demand and this step in the O_2 cascade is therefore appropriately regulated. Despite appropriate ventilatory responses to hypoxia, the presence or absence of thermoregulatory responses in mammals can complicate the interactions between hypoxia and temperature and these are discussed in terms of respiratory control.

A7.16 Heat Stress in *Pagothenia borchgrevinki*, a Stenothermal Antarctic Fish

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The Antarctic notothenioid fish, *Pagothenia borchgrevinki*, is found immediately below the fast ice in McMurdo Sound, Ross Sea, where it lives at a constant, year-round temperature of -1.86°C . Regarded as an extreme stenotherm, it is regarded as having an upper lethal limit of $+5^\circ\text{C}$, although this has only been demonstrated using acute temperature changes.

Acutely raising the temperature to a level below the lethal limit causes little change to any measures of stress. Both heart rate and ventral aortic pressure increase, though in many cases this is transitory. At 6°C , haematological stress markers are not immediately apparent, but at times greater than 24h they progressively become elevated suggesting a breakdown of systems in the animal. As with the lower temperatures, heart rates and pressures increase but often only for a short time. Catecholamines are not a reliable indicator of stress at this temperature. At the very high temperature of 10°C fish lose equilibrium after 10 min. Catecholamines increase, heart rates and ventral aortic pressures are high. During recovery, catecholamines take almost 2h to return to initial levels.

It is possible to keep *P. borchgrevinki* at 4°C . After a month at this temperature, the fish show distinct changes to their cardiovascular physiology, indicating that they have acclimated to rather than simply tolerated this high, sublethal temperature.

A7.17 Breathless crucian carp remodel its gills

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We have previously shown that crucian carp (*Carassius carassius*) living in normoxic water have gills without protruding lamellae. This is due to a cell mass that fills up the space between adjacent lamellae, effectively burying the lamellae. During exposure to hypoxia ($0.75\text{mg } O_2 \text{ l}^{-1}$) 50% of this cell mass disappears due to cell cycle arrest and transient apoptosis. This morphological alteration is reversible and results in a 7.5 fold increase in the respiratory surface area.

We have investigated if not only oxygen supply but also oxygen demand can trigger the response. We exposed crucian carp to different temperatures (10 , 15 and 20°C) for 1 month. There was a 3 fold difference in oxygen consumption (39 and $118 \text{ mg } O_2 \text{ kg}^{-1} \text{ h}^{-1}$) between fish kept at 10 and 20°C , as measured by closed res-

pirometry. Still, the fish kept at 20 °C had gills without protruding lamellae. However, temperature dramatically increased the speed of the morphological alteration when exposed to hypoxia. At 8°C the morphological alteration takes 3 days while at 15 and 20°C it only takes a few hours, occurring as the fish is exposed to progressive hypoxia in the respirometer. At high metabolic rates, it is probably more important for the crucian carp to have an ability to rapidly increase the respiratory surface area when faced with hypoxia.

A7.18 Chromogranin A-derived peptides in “zero steady-state error” Homeostasis: vasostatsins and the heart

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Recently, we have proposed that vasostatsins (VS), i.e. the Chromogranin A (CGA)-derived peptides corresponding to amino acids 1-76 (VS-1) and 1-113 (VS-2), respectively, may act as cardio-inhibitory agents (Tota et al., 2003).

In an evolutionary perspective, we report here the VS actions on the heart of eel, frog and rat as paradigms of fish, amphibian and mammals, respectively.

On working *Anguilla anguilla* heart, VS and the synthetic CGA7-57 fragment decreased stroke volume and stroke work. This negative inotropism involved Gi/o proteins, muscarinic and adrenergic receptors, calcium channels and required an endocardial endothelium (EE)-NO-cGMP-PKG signaling. Notably, VS-1 counteracted the adrenergic-mediated (i.e. isoproterenol; ISO) positive inotropism.

On ejecting *Rana esculenta* heart, VS induced negative inotropism, counteracting ISO-mediated positive inotropism. VS negative inotropism involved neither Gi/o proteins nor the EE-NO-cGMP system while required K⁺ channel activation.

On *in vitro* Langendorff rat heart, VS-1 while increasing heart rate decreased left ventricular end diastolic pressure, maximal contraction rate (dP/dt Max), and rate pressure product, without changing coronary pressure. In contrast, VS-2 had no effects on both chronotropism and inotropism. Under ISO stimulation, while both VS did not modify the adrenergic-mediated chronotropism, they antagonised ISO-mediated positive inotropism.

These data support an early cardio-suppressive role of VS in vertebrates, emphasizing at heart level the importance of counter-regulation and co-secretion of CGA. Tota et al., (2003) Regul. Pept. 114:123–130

A7.19 A quantitative model of vertebrate resting metabolic rate: Balancing energetics and oxygen transport during activity

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How levels of resting metabolism affect activity metabolism has not been well understood. Bennett and Ruben (1979) proposed that factorial aerobic scope is around 10 in most vertebrates, no matter the physiological state, because of an unspecified constraint that restricts how much resting rate can be increased, with maximal oxygen uptake limited to around an order of magnitude above resting levels. We have developed a mathematical model that argues there is no such restriction. Our results suggest that rates of resting metabolism in vertebrates are maintained at levels that are optimal with respect to two key opposing variables: (1) the energetics of metabolic systems, which favor the lowest possible resting rates to conserve energy expenditure and capacity, and (2) the speed of cardiovascular response to activity, which would select for an increased resting metabolism. The latter point is based on our theoretical finding, obtained from the Fick equation for convective transport, that changes in oxygen consumption require increments of blood flow that vary as a nonlinear function of initial rate. Thus, the size of response required by the cardiovascular system to meet the added oxygen demands of activity decreases hyperbolically as resting rate increases. By maintaining metabolic levels at rest to around 10% of $\dot{V}O_2$ max, therefore, vertebrates strike an optimal balance in the design of their metabolic systems: providing the greatest reduction in the incremental blood convection requirement during activity for the least energetic allocation to resting metabolic rate.

A7.20 *In vivo* myocardial energy status of 5- and 21°C-acclimated anoxic turtles (*Trachemys scripta*) investigated by MRI and ³¹P-MRS

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To determine the putative relationships of energetic status of the heart and cardiac performance *in vivo*, we used ³¹P-nuclear magnetic resonance spectroscopy and magnetic resonance imaging to measure intracellular pH of

cardiac muscle (pH_i), intracellular concentrations of high-energy phosphate, heart rate (f_H) and central vascular blood flows in the freshwater turtle (*Trachemys scripta*) during normoxia and anoxia at 21°C and 5°C (3 h and 11 days, respectively). At 21°C, anoxia caused a reduction in f_H from 14 to 10 min^{-1} , and a reduction of cardiac pH_i by 0.4 units. Concurrently, ATP remained at 80% of control levels through the creatine–kinase reaction, as creatine phosphate (PCr) decreased three-fold, while inorganic phosphate (P_i) rose by 40%. Nevertheless, a continuous increase in the $[\text{P}_i]/[\text{PCr}]$ ratio indicates that cardiac cellular energetic state was partially compromised during anoxia, although f_H remained stable. At 5°C, P_i continuously increased to approximately 400% of control levels by day 11, but reductions in ATP and PCr reached a plateau at 50% of control, and $[\text{P}_i]/[\text{PCr}]$ ratio stabilized by 6 h of anoxia. Despite these plateaus, bradycardia and a reduction in pH_i of approximately 0.1 units persisted. Thus, a simple correlation does not seem to exist between cellular energy state and cardiac performance during anoxia. Consistent with previous studies^{1,2}, pulmonary blood flow was markedly reduced during anoxia, less in the left aorta, and maintained near control normoxic levels in the carotid arteries.

1. Hicks and Wang. 1998. *Physiol. Zool.* 71, 1–14.
2. Stecyk et al. 2004. *J. Exp. Biol.* 207, 269–283.

A7.21 Cardiovascular status and its control in cold, anoxic crucian carp (*Carassius carassius*)

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While anoxic mammals die within minutes, freshwater turtles (genera *Trachemys* and *Chrysemys*) and crucian carp (*Carassius carassius*) survive anoxia for days to months, depending on temperature. To achieve this, anoxic freshwater turtles reduce metabolism by 90%, enter a comatose-like state and buffer lactate with their bone and shell. Correspondingly, cardiac power output (PO) is reduced by 95%, precluding an up-regulation of glycolysis, and autonomic cardiovascular control is blunted. In contrast, anoxic crucian carp remain active, up-regulate glycolysis to meet the brain energy needs, and avoid acidosis by converting lactate into ethanol. Given the activity level and survival strategies of anoxic crucian carp, we hypothesized that their cardiovascular

status and control are maintained during anoxia. Consequently, we measured cardiac output (Q), heart rate (f_H), stroke volume (V_S), ventral aortic blood pressure (P_{VA}) and respiration rate (f_R), and calculated peripheral vascular resistance (R) and PO of crucian carp during five days of anoxia at $8 \pm 1^\circ\text{C}$ and also following intra-arterial injections of cholinergic, β -adrenergic and α -adrenergic antagonists during normoxia and anoxia. After an initial adjustment period, f_H , Q , V_S , PO and f_R all returned to pre-anoxic levels for the remainder of the anoxia exposure. A 40% reduction in R indicated widespread vasodilation, with a resultant 30% decrease in P_{VA} . Unlike turtles, autonomic cardiovascular control was maintained in anoxic crucian carp, probably to sustain circulatory functions. These results suggest that the crucian carp cardiovascular system has similar convection requirements for both normoxic gas transport and anoxic nutrient and metabolite transport.

A7.22 Cardiorespiratory Responses to Changes in Metabolic State. Allometric scaling of the microcirculation in striped bass (*Morone saxatilis*)

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Past studies have demonstrated allometric scaling of enzymes involved in metabolism in fish, and studies on the scaling of the capillary supply on land mammals have been performed. We correlated changes in enzyme activities with morphometric indices of aerobic capacity in striped bass. Red muscle from nine fish ranging in size from 22.9g to 1595g was sampled. Sections were taken for light microscopy to determine mean fibre area, capillary density (CD) and capillary: fibre ratio (C:F). Mean fibre area increased with body mass whilst CD decreased. This suggests that during growth fibre hypertrophy leads to a reduced tissue oxygen supply only partially compensated for by capillary growth, seen as a greater C:F in larger fish. A decrease in mitochondrial volume density with increasing distance from the capillary was found in both the largest and smallest fish, indicating a greater metabolic demand for oxygen close to capillaries. Perivascular pericyte coverage was greater in small fish which also have smaller capillaries. A 27.5g fish had a lower CD and mean fibre area indicative of greater hypertrophy than a 22.9g fish. The transition towards a more mature capillary bed identifies this size range in which capillary growth decreases. This work was supported by NERC.