39.1

CLONING AND EXPRESSION OF SALMONID CARDIAC TROPONIN C: ROLE IN TEMPERATURE SENSITIVITY OF CARDIAC MYOFIBRILS. C. D. Moyes*, T.J. Borgford, L.LeBlanc, G.F.Tibbits. Simon Fraser Univ., Burnaby, BC, V5A 1S6, Canada

The relationship between Ca^{2+} sensitivity, temperature and pH exhibited by salmonid cardiac myofibrils differs in several aspects from that of mammals. We investigated the role of the myofibrillar Ca^{2+} binding protein, troponin C (TnC), in determining these properties. Cardiac TnC has 3 functional Ca^{2+} binding sites. Steady state occupancy of the 2 high affinity sites promotes binding of TnC into the troponin complex. Beat-to-beat binding to the single regulatory Ca^{2+} binding site is responsible for activation of myofibrillar ATPase. Rainbow trout cardiac cTnC was cloned (lambda Zap), sequenced and expressed in a bacterial system (*E. coli* strain QY13) as a fusion protein (cII-TnC). Comparison of salmonid TnC amino acid sequence with those of mammals reveals complete homology in the single regulatory Ca^{2+} binding site. Several differences in primary structure occur in the inactive site I and throughout the high affinity domain. These regions are not directly involved in Ca^{2+} binding at the regulatory site, however they are implicated in Ca^{2+} dependent interactions with TnI. Recombinant salmonid TnC exhibits spectral properties (circular dichroism, intrinsic fluorescence) resembling those of TnC purified from Atlantic salmon ventricle. To facilitate titration with Ca^{2+}, a mutant TnC was produced (FW27-cTnC) by site-directed mutagenesis (single-stranded PCR). Introduction of a tryptophan residue into the non-functional site I results in a protein which increases in fluorescence several fold in response to Ca^{2+} binding at the functional regulatory site II. Ca^{2+} affinity of isolated TnC differs in several important respects (temperature and pH sensitivity) from that of intact myofibrillar preparations (ATPase, isometric tension). These data suggest that the differences in Ca^{2+} sensitivity between species are not due solely Ca^{2+} binding properties of TnC but also depend upon functional interactions between TnC and other troponin proteins. Funded by NSERC (Canada)

39.2

TEMPERATURE SENSITIVITY OF SALMONID MYOCARDIAL Ca^{2+} SENSITIVITY C.Churcotte, C.D.Moyes*, G.F.Tibbits*. Simon Fraser Univ., Burnaby, V5A 1S6, Canada

Mammalian heart demonstrates marked decreases in Ca^{2+} sensitivity and isometric tension in response to decreasing temperature. Poikilothermic vertebrates must maintain myocardial contractility at temperatures that are cardioplegic for mammals. We postulated that interspecies differences in myofibrillar Ca^{2+} sensitivity ($K_{0.5}CA$) or its temperature dependence ($dK_{0.5}CA/dT$) may prevent de-sensitization in salmonid heart at low temperature. As $K_{0.5}CA$ is also pH dependent (approx. 1pCa/pH), the pHi regulatory strategy influences functional Ca^{2+} sensitivity *in vivo*. We compared the influence of temperature and pH changes in Ca^{2+} sensitivities of ventricular myofibrillar ATPase and skinned fiber force generation from rat and rainbow trout. Salmonid myofibrils are inherently more sensitive to Ca^{2+} than are mammals, when assayed under similar conditions (pH 7.0-7.2: 7-21°C). Salmonids exhibit a greater sensitivity to temperature at fixed pH (0.35 pCa/10°C) than do mammals (0.08-0.16pCa/10°C). Although salmonids demonstrate temperature dependent desensitization, the higher inherent Ca^{2+} sensitivity results in similar *functional* Ca^{2+} sensitivities when assayed at their respective physiological temperatures. There is a potential role for intracellular pH regulation in modifying the effects of temperature on Ca^{2+} sensitivity. An α-stat pattern of intracellular pH regulation (-0.014pH/°C) would negate the effects of temperature in mammals. Although salmonids and mammals have a similar pH sensitivity, salmonids exhibit a greater temperature sensitivity. This property, along with the established "less-than-α-stat" pattern of cardiac pHi changes, suggests that pHi regulation *in vivo* would be insufficient to overcome the effects of temperature on Ca^{2+} sensitivity. We conclude that one mechanism by which salmonid heart is able to maintain contractility at low temperatures is through higher Ca^{2+}-sensitivity of the contractile element, compared to mammalian species. Supported by NSERC (Canada).

39.3

IS ONE PERFORMANCE MEASUREMENT ENOUGH? ALTERNATIVES TO U_{crit} PROCEDURES FOR EVALUATING FISH LOCOMOTOR PERFORMANCE. **Jay A. Nelson, Shannon Reidy, Dale Webber and Steve Kerr. Dalhousie Univ. Halifax, N.S., Canada B3H 4J1**

Ever since J.R. Brett introduced the critical swimming speed protocol (U_{crit}) in 1964, it has been the method of choice for investigators wishing to evaluate locomotor performance in fishes. Unfortunately, while the procedure has the advantage of producing a single performance number for each fish, little information is obtained concerning either the aerobic or anaerobic capabilities of a particular animal. Indeed, recent work from R.G. Boutilier's laboratory demonstrates that Atlantic cod (*Gadus morhua*) with identical U_{crit}'s can use substantially different amounts of anaerobic metabolism during their swimming effort. Furthermore, under certain environmental conditions, the U_{crit} test becomes almost entirely a test of aerobic swimming capacity in cod. For these and other reasons, we developed two alternative swimming performance tests designed to evaluate an animal's anaerobic swimming capacity. The first test, which we term "burst performance protocol", U_{burst}, is nothing more than an accelerated U_{crit} protocol whereupon the animals are forced to swim against water accelerating at a rate of 10 cm/s/min until exhausted (about 7 min). Measurements of U_{burst} in cod are repeatable and are correlated to U_{crit}. The second test involved measuring "fast-start performance" with a computerized "drag strip" utilizing laser light detection. Measurements of acceleration and terminal velocity during the fast-starts were repeatable for Atlantic cod, both on a given day and over several months time. The relative advantages and disadvantages of these methods as well as the methodological details of these tests will be presented.

39.4

Environmental Determinants of White Muscle Performance: Lessons From the Deep-Sea. Russell Vetter, N. Chin Lai[1], and Edward Goolish[1]*. NOAA, La Jolla , CA 92038 and [1]Scripps Institution of Oceanography, Center for Marine Biotechnology and Biomedicine, U.C. San Diego, La Jolla, CA 92093.

Oxygen-delivery properties (hematocrit, p50, hemoglobin, heart size), and metabolic-demand properties (white muscle and heart lactate dehydrogenase, malate dehydrogenase, and citrate synthase activities), were measured in fish species that occupy different, narrowly-defined depth ranges, and in confamilial species that ontogenetically migrate from shallow to deep water. Four Scorpaenid rockfish species and four Pleuronectid flatfishes were collected at 100m depth intervals from the continental shelf (100m) down to (1400m) including the Oxygen Minimum Zone (<.5 ml/l O_2 at 600-800m). By using the shallow-water representatives of each family, we were able to examine the environmental effects ofdeep water (darkness, hypoxia and low-food availability), as they were overlain on accepted patterns of metabolic scaling. The shallow-water rockfish, *Sebastes goodei*, and flatfish,*Paralichthys californicus*, showed size-specific increases in LDH and decreases in CS These were in accordance with cost-of-transport arguments. Deeper-living species had progressively more inverse (negative) size-scaling relationships for LDH, and steeper decreases in CS with increasing body size. The ontogenetically migrating flatfish, *Microstomus pacificus*, took on the pattern of the deep-water species when it migrated to deeper water. Laboratory experiments with *Microstomus pacificus* confirmed that muscle performance is strongly influenced by the deep-sea environment. Fish removed from deep water and maintained in the laboratory took on scaling patterns and oxygen delivery properties typical of shallow-water. Long-term experimental hypoxia revealed remarkable interactions between hypoxia and ration such that fish maintained under hypoxia grew as well or better than normoxic controls, but did so by consuming half the ration. (Supported by NOAA and the National Academy of Sciences)

39.5

REVERSIBLE BINDING OF PHOSPHOFRUCTOKINASE TO MYOFIBRILS DURING EXERCISE AND RECOVERY. Hélène Migault * and Mary Sue Lowery. University of San Diego, San Diego, CA 92110.

Phosphofructokinase (PFK) binds to fast-twitch, glycolytic (white) muscle myofibrils during exhaustive exercise in the barred sand bass, Paralabrax nebulifer. In rested fish, 43.1% of white muscle PFK is bound to myofibrils, with bound PFK increasing to 65.9% in exercised fish. Increased PFK binding corresponded to a decrease in muscle pH from 7.26 to 6.56 and an increase in muscle lactate from 11.4 to 31.2 mmoles/g in exercised fish. During the initial phase of recovery, PFK binding decreased as the intracellular muscle pH and lactate returned to resting values. However, PFK binding increased at 9 hours of recovery indicating that factors other than pH may contribute to binding. Total PFK activity doubled after exercise and continued to increase during the first 3 hours of recovery. Both cytoplasmic and myofibril-bound PFK activity increased, with a larger increase seen in bound enzyme. PFK activity returned to resting levels by 9 hours of recovery. PFK responds to decreased intracellular pH by binding to myofibrils, as seen *in vitro*. Contrary to results of *in vitro* kinetic studies where low pH inhibits PFK, PFK activity in exercised muscle increased under acidic conditions. Reversible binding to myofibrils may serve to enhance glycolysis during exercise or protect PFK activity during extended acidosis.

39.6

AEROBIC AND ANAEROBIC CAPACITIES IN LOCOMOTOR MUSCLE OF TUNAS AND ECTOTHERMIC SCOMBRID FISHES. Michael Hansen* and Kathryn Dickson. California State University, Fullerton, CA 92634

Tunas (Family Scombridae) are unique among teleost fishes in using physiological mechanisms to maintain muscle temperatures elevated significantly above ambient water temperature (endothermy). The maximal activity of key enzymes that limit flux through ATP-generating pathways in fish muscle were used as indices of metabolic capacity, and were compared in six tunas and four ectothermic scombrid species. In red myotomal muscle (RM), the activity of citrate synthase (CS), an index of aerobic capacity, did not differ significantly between the endothermic tunas and the ectothermic scombrid species. In white myotomal muscle (WM), on the other hand, tunas had significantly greater activities of both CS and lactate dehydrogenase (LDH), and index of anaerobic capacity, as well as a greater buffering capacity. Thus, tunas and their closest relatives, ectothermic scombrids, have similar RM aerobic capacities, which may be near maximal for locomotor muscle, but only the tunas maintain elevated RM temperatures, which may be as much as 13°C above water temperature in free-swimming fish. By elevating the temperature of a relatively small amount of tissue (RM is 4-13% of body mass in tunas), tunas can significantly increase red muscle ATP production rate, contraction speed, and power output, and thereby may be able to increase sustainable swimming performance. Tuna WM, on the other hand, lacks extensive heat exchangers and has lower and more variable temperatures than RM; its aerobic and anaerobic capacities are greater than those of ectothermic scombrids. The greater WM aerobic capacities suggest that tuna WM is comprised of more than one muscle fiber type, that it contracts aerobically during sustainable swimming, and/or that it participates in processing the lactate generated during anaerobic bursts. The high WM anaerobic capacity probably results in higher burst speeds and/or duration of bursts in tunas.