50 Years of Comparative Biochemistry: The Legacy of Peter W. Hochachka

A satellite symposium of the
Canadian Society of Zoologists
May 13 & 14, 2017

Professor Peter W. Hochachka, O.C., Ph.D., D.Sc., F.R.S.C. – 1937-2002

Peter Hochachka, a pioneer in the field of Comparative Biochemistry passed away in 2002 at the age of 65. Much has been written about Peter's intellectual contributions to his discipline in areas such as temperature adaptation, facultative anaerobiosis in ectotherms, diving in marine mammals, human high altitude adaptation, and exercise metabolism. It is well recognized that his work transformed the field of comparative biochemistry (G. Somero and R.K. Suarez, Annu. Rev. Physiol. 67:25–37, 2005; K.B. Storey, Comp Biochem Physiol B Biochem Mol Biol. 39:359-69, 2004). But Peter's legacy as a mentor of students is less well documented, especially his impact on science across Canada and internationally. Peter supervised approximately 16 PDFs, 31 PhD and 11 MSc candidates over his career. His trainees have to this date supervised more than 750 individuals (i.e. the F2s) and given that many of the F1s have years of productivity remaining, the number of Hochachka F2s will surely surpass 1000! A tabular summary of this information will be linked to the Hochachka Memorial Lecture page (http://www.zoology.ubc.ca/seminar-series/hochachka). Peter's PDFs include George Somero who fathered an intellectual family of his own but it was really Peter's supervision of PhD candidates that has made an outstanding impact. Of Peter's 32 PhD students (Trish Schulte counted as a PhD for purpose of this document), 27 went on to supervise students of their own. Of these individuals, 17 held or still hold faculty positions at Canadian Universities. Of the current F2's, approximately 40 hold faculty positions at Canadian universities and again this number will increase. In total, 57 F1s and F2s assumed faculty positions in Canada in a country with less than 100 degree granting institutions. What more needs to be said!

On what would have been the occasion of his 80th birthday, a Special Symposium was held at the University of Manitoba on May 13 and 14, 2017 to celebrate Peter's contribution in this domain. The symposium was conceived by a group of Hochachka students over dinner one evening in San Diego in Oct. 2014. In attendance were four of Peter's PhD students Jim Staples, Les Buck, Chris Moyes, Bill Driedzic, and Johanne Lewis (PhD student of Driedzic). This group formulated the idea of having a meeting in which students of Peter's students would be showcased. Thereafter, other members of the Hochachka lineage were brought into the loop – Trish Schulte, Helga Guderley, Tom Moon, Grant McClelland, Charles Darveau, Gary Burness, and Kevin Campbell. The decision was made to hold the meeting as a satellite symposium to the CSZ 56th Annual Meeting to be held in Winnipeg, May 2017. Kevin Campbell, who had been a postdoc with Peter, was also conscripted for the local organization of the event, and was our champion on the ground.

The symposium was organized around 5 major themes that ran throughout the Hochachka laboratory: hypoxia, temperature, bioenergetics, evolution of metabolism, and ecophysiology and adaptive change. Each session was organized by two of Peter's first generation trainees and each session was to include 5 speakers who were further down the Hochachka lineage. This was a formidable challenge since 25 individuals were to be selected from a F2 generation population of more than 750 and a F3 generation that is already in the many hundreds.

By all accounts, the meeting (the abstracts are attached) was a great success. A generation of students now better appreciate their lineage. Peter was most certainly celebrated and his important contributions of mentoring his students so that they would become mentors was recognized.

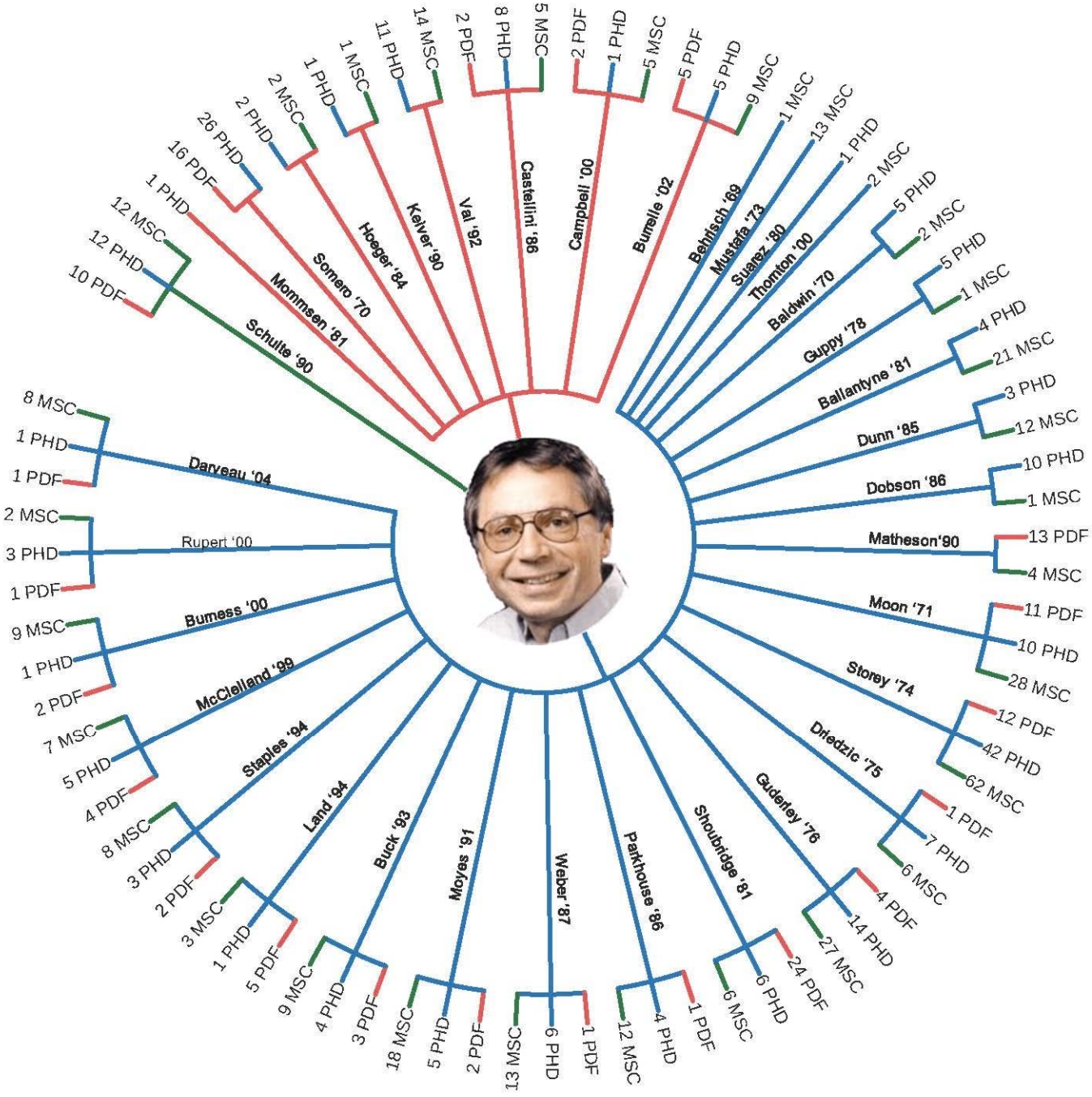
Peter Hochachka, un pionnier du domaine de la biochimie comparée, nous a quitté en 2002 à l'âge de 65 ans. Plusieurs ont rapporté par écrit la contribution intellectuelle de Peter tels que l'adaptation à la température, l'anaérobiose facultative des ectothermes, la plongée chez les mammifères marins, l'adaptation à la haute altitude chez l'humain, ainsi que le métabolisme lors de l'exercice. Il est bien établi que son travail a transformé le domaine de la biochimie comparée (G. Somero and R.K. Suarez, Annu. Rev. Physiol. 67:25–37, 2005; K.B. Storey, Comp Biochem Physiol B Biochem Mol Biol. 39:359-69, 2004). L'héritage de Peter en tant que mentor pour ses étudiants n'a pas été aussi bien documenté, surtout son impact en science au Canada et internationalement. Peter a dirigé environ 16 postdocs, 31 PhD et 11 MSc tout au long de sa carrière. Ses étudiants ont jusqu'à présent dirigé plus de

750 individus (la génération F2 de Peter), et puisque plusieurs de la génération F1 ont encore bien des années de productivité à venir le nombre de F2 Hochachka devrait dépasser 1000! Un tableau résumant ces informations sera joint à la page web de la série de conférences commémorative Hochachka (http://www.zoology.ubc.ca/seminar-series/hochachka). Les postdocs de Peter inclus George Somero, ayant lui-même généré une famille intellectuelle nombreuse, mais ce sont vraiment ses PhD qui ont eu un impact remarquable. Des 32 PhD de Peter (Trish Schulte est comptée comme une PhD pour les fins de ce document), 27 ont dirigé des étudiants eux-mêmes. De ceux-ci, 17 ont été ou demeurent professeurs dans des universités canadiennes. De la génération F2, environ 40 ont des postes dans des universités canadiennes et ce nombre augmentera certainement. Un total de 57 individus de la génération F1 et F2 ont un poste de professeur dans une université au Canada, un pays qui compte moins de 100 institutions conférant des diplômes. Que dire de plus!

À l'année qui célèbrerait son 80e anniversaire, un symposium spécial a eu lieu le 13 et 14 mai 2017 à l'Université du Manitoba afin de célébrer la contribution de Peter à son domaine. Ce symposium a été conçu par un groupe d'étudiants d'Hochachka lors d'un souper à San Diego une soirée d'octobre 2014. Quatre étudiants PhD de Peter y étaient, Jim Staples, Les Buck, Chris Moyes, Bill Driedzic, ainsi que Johanne Lewis (étudiante PhD de Driedzic). Ce groupe développa l'idée d'une rencontre où les étudiants des étudiants de Peter seraient mis de l'avant. Par la suite, d'autres membres de la lignée Hochachka ont été inclus dans l'organisation : Trish Schulte, Helga Guderley, Tom Moon, Grant McClelland, Charles Darveau, Gary Burness et Kevin Campbell. La décision a été prise de tenir la rencontre comme symposium satellite lors de la 56e rencontre annuelle de la SCZ ayant lieu à Winnipeg en mai 2017. Kevin Campbell, ayant été un postdoc avec Peter, a été recruté pour l'organisation locale de l'événement et notre champion sur le terrain.

Le symposium a été organisé autour de 5 thèmes majeurs étant centraux au laboratoire Hochachka : hypoxie, température, bioénergétique, évolution du métabolisme, et écophysiologie et changement adaptatif. Chaque session fût organisée par deux étudiants de première génération de Peter et chaque session comptait 5 orateurs étant de générations subséquentes de la lignée Hochachka. Ce fut tout un défi puisque seulement 25 individus étaient sélectionnés de la génération F2 comptant plus de 750 membres et une génération F3 comptant déjà plusieurs centaines de personnes.

De toutes évidences la rencontre (les résumés sont joints) fût un grand succès. Des générations d'étudiants ont maintenant une plus grande appréciation de leur lignée. Peter a certainement été célébré et son importante contribution au mentorat de ses étudiants pour qu'ils puissent à leur tour être mentor e été reconnue.



Peter W. Hochachka academic phylogeny
—the legacy lives on—

Program

50 years of Comparative Biochemistry: The Legacy of Peter W. Hochachka

On what would have been the occasion of his 80th birthday, a Special Symposium will be held to celebrate the contributions of Peter Hochachka, a pioneer in the field of Comparative Biochemistry. Although he passed away in 2002, his legacy thrives through his many research contributions and mentoring of the students and post-doctoral fellows that passed through his lab over nearly three decades of research.

When and where: This meeting will be held May 13 and 14, 2017, as a satellite symposium preceding the 56th annual meeting of the Canadian Society of Zoologists at the University of Manitoba in Winnipeg.

Oral Presentations:

Hypoxia: Organized by Les Buck, University of Toronto & Kevin Campbell, University of Manitoba

Temperature: Jim Staples, Western University & Chris Moyes, Queen's University

Bioenergetics: Grant McClelland, McMaster Univ. & Charles Darveau, Univ. of Ottawa

Evolution of Metabolism: Trish Schulte, UBC & Helga Guderley, Université Laval

Ecophysiology & Adaptive Change: Tom Moon, Univ. Ottawa & Gary Burness, Trent University

Poster presentations: In addition to invited oral presentations, attendees are encouraged to contribute to a Saturday evening poster session.

Comparative Biochemistry and Physiology: A special issue of CBP-B: Biochemistry and Molecular Biology will be published in association with the meeting. The Special Issue will be a combination of primary publications and reviews, each subject to the regular peer-review process. Tentative deadline for submissions: May 1, 2017.

For information on the meeting, contact the organizers: Bill Driedzic, Memorial University of Newfoundland, Helga Guderley, Université Laval, Kevin Campbell, University of Manitoba, Chris Moyes, Queen's University

For information on the Special Issue of CBP, contact Chris Moyes, Editor in Chief CBP-B

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Day 1: Saturday, May 13, 2017

8:00 Breakfast @ Daily Bread Café

8:45 Welcome and Perspectives (***Driedzic***) – 118 St. John's College

1.0 Hypoxia (Buck and Campbell) – 118 St. John's College

9:00 Opening Comments (***Les Buck***)
9:10 **1.1** Matt Pamenter: *Keeping cool in hottest Africa: mitochondrial adaptations to hypoxia in a fossorial mesotherm*
9:35 **1.2** Neal Dawson: *The effects of high-altitude ancestry and hypoxia acclimation on diaphragm function of the deer mouse, Peromyscus maniculatus.*
10:00 **1.3** Anthony Signore: *Parallel elevations in the oxygen affinity of adult and prenatal hemoglobins during the aquatic transition of Sirenia*
10:25 Coffee Break @ Daily Bread Café
10:40 **1.4** Sajeni Mahalingham: *Mitochondrial adaptations to high altitudes in deer mice*
11:05 **1.5** Jeff Richards: *Suppress and Survive: mechanisms of hypoxia and anoxia tolerance in ectotherms*
11:30 Lunch @ Daily Bread Café

2.0 Temperature (Staples and Moyes) – 118 St. John's College

12:50 Opening Comments (***Jim Staples***)
1:00 **2.1** Anne Todgham: *When two plus two doesn't equal four: understanding responses to multiple stressors through investigations of energy metabolism.*
1:25 **2.2** Dillon Chung: *Mitochondrial function in local adaptation and thermal acclimation to temperature.*
1:50 Break
2:00 **2.3** Alexander Little: *Thyroid hormone, cold acclimation and the evolution of endothermy.*
2:25 **2.4** Simon Lamarre: *Understanding the effects of environmental parameters on protein metabolism in ectothermic animals*
2:50 **2.5** Jason Brown: *Changing How We Teach Our Students About Thermal Physiology*
3:15 Coffee Break @ Daily Bread Café

3.0 Ecophysiology and adaptive change (Burness and Moon) – 118 St. John's College

3:30 Opening Comments (***Tom Moon***)
3:40 **3.1** Paul Craig: *Micro problems in a macro world: Environmental influences on microRNA in teleost*
4:05 **3.2** Chris LeMoine: *Chasing waterfalls: Ecophysiology and metabolism of the climbing gobies*
4:30 Break
4:40 **3.3** Jan Mennigen: *Micromanaging metabolism: The role of miRNAs in salmonid energy metabolism*

5:05 **3.4** Tyson MacCormack: *Fishing for mechanisms of toxicity: energy metabolism and physiology at the nano-scale*

5:30 **3.5** Alex Gerson: *Phenotypic flexibility of migratory birds: Causes and Consequences*

7:00-late Poster Session and buffet @ The Hub (3rd floor University Centre) – catered

Day 2: Sunday, May 14, 2017

8:00 Breakfast @ Daily Bread Café

4.0 Bioenergetics (McClelland and Darveau) – 118 St. John's College

9:00 Opening Comments (***Charles Darveau***)

9:10 **4.1** Cayleih Robertson: *Baby its cold outside: Developmental adaptations to thermoregulating at high altitude*

9:40 **4.2** Enrique Rodriguez: *From bees to bivalves: exploring the mechanisms and processes linked to metabolic rate and longevity*

10:10 **4.3** Kate Mathers: *Mitochondrial bioenergetics in stressful environments: A journey from lake to prairie*

10:40 Coffee Break @ Daily Bread Café

11:00 **4.4** Jason Treberg: *Things I've learned about mitochondria that they didn't teach me in school*

11:30 **4.5** Speaker Nicolas Pichaud: *Mitochondrial respiration in Drosophila: what's new from an old model?*

12:00 Lunch @ Daily Bread Café

5.0 Evolution of Metabolism (Guderley and Schulte) – 118 St. John's College

1:20 Opening Comments (***Trish Schulte***)

1:30 **5.1** Anne Dalziel: *Do convergent mechanisms underlie the evolution of aerobic capacity in fishes?*

2:00 **5.2** Pierre Blier: *How much disruption of mito-nuclear co-adaptation can be tolerated?*

2:30 **5.3** Ben Speers-Roesch*: Metabolic organization of sharks and squids: convergences and consequences*

3:00 Coffee Break @ Daily Bread Café

3:20 **5.4** Charles Darveau: *Evolution of metabolic scaling in active animals*.

3:50 **5.5** Allison McDonald*: Diversity of mitochondrial electron transport systems in animals*

4:20 Concluding Remarks (***Helga Guderley***)

Banquet (Driedzic-Master of Ceremonies)

7:00 Banquet @ Daily Bread Café

8:30 Keynote introduction (***Driedzic***) – Schultz Theatre (St. John's College)

8:40 Keynote address (***Storey***) – Schultz Theatre (St. John's College)

Acknowledgements

The organizers of the Hochachka Legacy Symposium would like to thank the following sponsors for their generous financial contributions to the meeting:

Funding for the best poster presentations kindly provided by:

Zoological Education Trust

List of Delegates

Raafay Ali
Brittney Borowiec
Jason Brown
Neal Callaghan
Felix Christen
Paul Craig
Neal Dawson
Bill Driedzic
Eva Enders
Garth Fletcher
Sara Good
Leah Hayward
Anne Kim
Lucy Lee
Alexander Little
Tyson MacCormack
Tábata Martins de Lima
Olivia McMillan
Tom Moon
Gabriele Nandal
Nicolas Pichaud
Jeffrey Richards
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Ken Storey
Anne Todgham
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Alex Zimmer

Michael Berenbrink
Colin Brauner
Heather Bryant
Kevin Campbell
Dillon Chung
Anne Dalziel
Rush Dhillon
Yvonne Dzal
Nann Fangue
Alex Gerson
Helga Guderley
Brenda Hochachka
Simon Lamarre
Chris LeMoine
Sully Lyons
Sajeni Mahalingam
Kate Mathers
Jan Mennigen
Chris Moyes
Matthew Pamenter
Varshinie Pillai
Cayleih Robertson
Graham Scott
Anthony Signore
Jim Staples
Jan Storey
Jay Treberg
Christine Verhille

Pierre Blier
Taylor Brooks
Les Buck
Alicia Cassidy
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Matt Regan
Enrique Rodriguez
Brad Seibel
Ben Speers-Roesch
Nadia Stec
Loïc Teulier
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Photo: Jan Storey

1.0 Hypoxia (Buck and Campbell)

1.1 Keeping cool in hottest Africa: mitochondrial adaptations to hypoxia in a fossorial Mesotherm

Matthew E. Pamenter[1,2]
[1]*Department of Biology, University of Ottawa, Ottawa, Ontario, K1N 9A7*
[2]*University of Ottawa Brain and Mind Research Institute, Ottawa, Ontario, K1H 8M5*

Naked mole rats (NMRs) are among the most hypoxia-tolerant mammals and their brain cells are particularly tolerant to oxygen deprivation. However, the cellular mechanisms that enable this tolerance are poorly understood. Mitochondria are signalling lynchpins that coordinate cellular responses to hypoxia and we hypothesized that brain mitochondria from NMRs differ functionally from those of hypoxia-intolerant mice. High-resolution respirometry was utilized to assess electron transport system (ETS) function in saponin-permeabilized brain from NMRs and mice at their respective physiological temperatures (28 and 37ºC). Relative to mouse brain, NMR permeabilized brain respiratory flux in non-phosphorylating mitochondria was lower throughout the ETS at physiological temperatures and the mitochondrial H+-gradient was more tightly coupled to respiration. Conversely, at 28ºC, mouse ETS flux was markedly lower than in NMRs but mitochondria were less leaky to H+, indicating that mitochondrial respiration is more efficient at physiological temperature in NMRs than in mice. Our data suggest that NMR brain mitochondria are not more adapted to hypoxia *per se*, but rather that NMRs benefit from enhanced mitochondrial respiration efficiency due to their lower body temperature. This may represent an evolutionary trade-off between the conflicting energetic goals of maintaining mitochondrial ATP production during hypoxia while simultaneously reducing metabolic demand.

1.2 The effects of high-altitude ancestry and hypoxia acclimation on diaphragm function of the deer mouse, *Peromyscus maniculatus*

Neal J. Dawson, Sulayman A. Lyons, Danielle A. Henry and Graham R. Scott
Department of Biology, McMaster University, Hamilton, Ontario, L8S 4K1

High-altitude hypoxia increases breathing and diaphragm muscle activity, but it is unclear whether diaphragm function is altered in high-altitude natives to improve respiratory function at high altitudes. To better understand the basis for this physiological feat, we compared oxidative capacity and muscle mechanics in the diaphragm from high- and low-altitude deer mice (*Peromyscus maniculatus*) exposed to hypoxia simulating 4300 m and 7000 m. Acclimation to hypoxia elicited changes in mitochondrial respiratory capacities in both highland and lowland mice; however, respiratory capacities of highland mice rose only when exposed to more severe hypoxia. Hypoxia acclimation also exhibited increases in citrate synthase activity in both populations in concert with increasing severity of hypoxia, while cytochrome c oxidase activity rose only in hypoxia-acclimated lowlanders. Similarly, reactive oxygen species produced via mitochondrial respiration rose significantly in hypoxia-acclimated lowlanders but remained unchanged in highlanders. Curiously, high-altitude natives had lower force production, shorter relaxation times and greater diaphragm capillarity, regardless of acclimation environment. Overall, acclimation to hypoxia seems to increase oxidative capacity, while most differences unique to high-altitude natives are consistent with patterns of counter-gradient variation that may act to oppose the effects of plasticity and preserve the phenotype found in low-altitude mice in normoxia.

1.3 Parallel elevations in the oxygen affinity of adult and prenatal hemoglobins during the aquatic trasition of Sirenia

Anthony V. Signore[1], Phillip R. Morrison[2], Colin J. Brauner[2] and Kevin L. Campbell[1]
[1]*University of Manitoba, Department of Biological Sciences, Winnipeg, Manitoba*
[2]*University of British Columbia, Department of Zoology, Vancouver, BC*

Evolved changes to adult mammalian hemoglobins (Hbs) that optimize O_2 management in unfavorable environments (such as high altitude, underground, aquatic, and the Arctic) have been repeatedly demonstrated. However, little attention has been directed to the impact these changes may have on maternal-prenatal O_2 transfer. Recently, the adult-expressed Hbs of the Order Sirenia were shown to have high O_2 affinity to maximize O_2 extraction from the lungs and limit premature tissue O_2 unloading during a dive. However, this trait may hinder maternal-prenatal O_2 transfer in the absence of parallel changes to Hb isoforms in the developing circulation. As a first step in characterizing the prenatal Hb isoforms of sirenians, we expressed a recombinant embryonic Hb (Gower I) and a putative fetal Hb protein (HbF) from Steller's sea cow (*Hydrodamalis gigas*), and HbF from the dugong (*Dugong dugon*). Functional tests reveal these Hbs to have extremely high O_2 affinities, reduced cooperativity and attenuated Bohr effects. Notably, Steller's sea cow HbF has the highest reported O_2 affinity of any mammalian Hb tested to date. Our results suggest that the Hb-O_2 affinity of prenatal sirenian Hbs increased in parallel to their adult counterparts in order to maintain maternal-prenatal O_2 transfer.

1.4 Physiology of mitochondria from skeletal and cardiac muscles in high-altitude deer mice

Sajeni Mahalingam, Grant B. McClelland and Graham R. Scott
Department of Biology, McMaster University, Hamilton, Ontario, L8S 4K1

Deer mice native to high altitudes have higher aerobic capacities in hypoxia than low-altitude deer mice during exercise and thermogenesis. We tested the hypothesis that changes in the physiology of mitochondria from skeletal and cardiac muscles help enhance aerobic capacity in high-altitude deer mice. Mice from wild populations at high and low altitudes were bred in captivity, and their lab-raised progeny were acclimated to normoxia and hypoxia. The gastrocnemius muscle of highland mice had higher respiratory capacities, total and subsarcolemmal mitochondrial volume densities, and citrate synthase activities than their lowland counterparts. This was not true of the left ventricle of the heart, which had similar activities of citrate synthase in highlanders and lowlanders. However, hypoxia acclimation increased the capacity of heart mitochondria to oxidize carbohydrates relative to fatty acids in highland mice, suggesting that an increased reliance on carbohydrates in hypoxia might help produce more ATP per mole of oxygen compared to lipids. Furthermore, heart mitochondria from highland mice had higher capacities to oxidize lactate as a metabolic fuel. We conclude that mitochondrial function is improved in high-altitude mice in a tissue specific manner, and likely improves performance at high altitudes.

1.5 Suppress and Survive: mechanisms of hypoxia and anoxia tolerance in ectotherms

Jeffrey G. Richards and Crisostomo R. Gomez
Department of Zoology, The University of British Columbia, Vancouver, British Columbia, V6T 1Z4

The freshwater turtle, *Trachemys scripta* is one of the most anoxia-tolerant vertebrates due, in part, to its impressive ability to suppress ATP consuming processes. This ability to suppress ATP consumption extends to the anoxic mitochondria. In anoxia intolerant organisms, anoxia exposure transforms the mitochondria from the major site of ATP production to one of the largest cellular energy consumers

because of the reverse functioning of the F_1F_O-ATPase (complex V), which hydrolyzes ATP to pump protons out of the mitochondrial matrix. Previous work showed however that *T. scripta* can inhibit complex V in heart and brain in response to anoxia exposure, but the regulatory mechanisms are unknown. In an attempt to elucidate the regulatory mechanisms responsible for this inhibition we assessed the role of nitrosylation as a regulatory mechanism and performed mitochondrial proteomics. Our results clearly indicate that the addition of a nitric oxide donor to isolated mitochondria does not affect complex V activity as observed previously in mice. Our proteomics analysis suggests that three subunits of complex V (ATP5A1, ATP5F1, and MT-ATP5J), all associated with the peripheral stalk, decreased in protein expression in response to anoxia. These proteomic changes may point to a potential regulatory mechanism.

2.0 Temperature (Staples and Moyes)

2.1 When two plus two doesn't equal four: understanding responses to multiple stressors through investigations of energy metabolism / Quand deux et deux ne font pas quatre: étude du métabolisme énergétique pour comprendre les interactions entre plusieurs facteurs de stress

Anne E. Todgham
Department of Animal Science, University of California Davis, Davis, California, 95616

High latitude seas are predicted to experience the impacts of CO_2-acidified seawater (ocean acidification, OA) within the next 40 years and are already experiencing ocean warming (OW). Organisms within these oceans may be some of the most vulnerable to environmental change, having evolved under stable conditions for millions of years. Early life stages are of particular concern as they are thought to be more sensitive to changes in climate-related variables than adults. Our research investigated the combined impacts of OA and OW on emerald rockcod (*Trematomus bernacchii*) juveniles and naked dragonfish (*Gymnodraco acuticeps*) embryos. Taking an integrative, multi-stressor approach combining metabolism, growth and development, and cardiorespiratory physiology, this research provides insight into the biochemical and physiological plasticity of early life history stages of polar fishes to changing ocean conditions and how co-occurring stressors can interact synergistically to impact performance during early development. Mechanisms and implications of non-linear interactions between multiple stressors will be discussed, with a focus on energy metabolism.

2.2 Lipid remodeling as a predictor of mitochondrial respiratory capacity in thermally acclimated Atlantic killifish (*Fundulus heteroclitus*) subspecies

Dillon J. Chung and Patricia M. Schulte
Department of Zoology, University of British Columbia, Vancouver, British Columbia, V6T 1Z4

The mitochondrion plays a critical role in maintaining energetic balance in response to environmental change. However, our understanding of the mitochondrial mechanisms used to offset the effects of changes in environmental variables such as temperature is incomplete. The objective of this study was to investigate the role of mitochondrial lipid remodeling as a mechanism for altering mitochondrial function in response to thermal acclimation (5, 15, and 33°C) in locally adapted subspecies of the Atlantic killifish (*Fundulus heteroclitus*). We observed greater mitochondrial respiratory capacity in the northern subspecies, which may underlie previously observed subspecies differences in whole-organism aerobic metabolic rate. Acclimation to 5 and 33°C was associated with a marginal increase and a large decrease, respectively, in mitochondrial respiratory capacity. We characterized mitochondrial lipid remodeling using HPLC-ESI-MS which allows for the quantification of individual phospholipid species. Thermal acclimation altered many phospholipid classes and the direction of these effects differed between the

subspecies. These shifts were pronounced in monolysocardiolipin species, a precursor to cardiolipin which is critical to mitochondrial function. Our results strongly support the involvement of IMM lipid remodeling in the differentiation of mitochondrial function between *F. heteroclitus* subspecies and as part of the thermal acclimation response.

2.3 Thyroid hormone regulates metabolism, muscle function and cardiac performance during cold acclimation in Zebrafish (*Danio rerio*)

Alexander Little[1] and Frank Seebacher[2]
[1]*Rosenstiel School of Marine and Atmospheric Science, Miami, Florida, USA 33149*
[2]*School of Life and Environmental Sciences, University of Sydney, Australia 2006*

Thermal acclimation is often cited as the most important defense ecotherms have against climate change, but the overarching mechanisms that coordinate this process are not known. Thyroid hormone (TH) mediates thermal responses in mammals, and its physiological effects appear similar across vertebrates. We therefore hypothesized that TH regulates thermal acclimation in ectotherms to optimize metabolism, muscle function and cardiac performance to prevailing ambient temperatures. We pharmacologically induced hypothyroidism in zebrafish (Danio rerio) during acclimation to cold (18˚C) and warm (28˚C) conditions. We measured an array of whole-animal and tissue-specific response parameters to assess metabolic, cardiac and skeletal muscle performance. We then verified the role of TH by supplementing hypothyroid fish with TH. We found that TH enhanced swimming performance during cold acclimation, but had little to no effect in warm acclimated fish. This general trend, where fish were more sensitive to TH during cold acclimation, was mirrored in response measures for metabolism, cardiac function and skeletal muscle performance. Our findings suggest the properties that underlie the role of TH in thermal acclimation (temperature sensitivity and metabolic control) may have predisposed this endocrine pathways for a regulatory role in the evolution of endothermy.

2.4 Hot topics in protein metabolism

Simon G. Lamarre
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Protein metabolism refers to the interrelated processes of protein synthesis and protein degradation. Temperature has profound effects on protein metabolism, and it is relatively well established that protein synthesis is close to its maximum rate at the optimal temperature of ectothermic animals. However, far less is known on the relationship between temperature and protein degradation. Until about two decades ago, protein degradation was looked upon as an unregulated and random process, however, it is now clear that it is a highly regulated process. The kinetics of protein synthesis and protein degradation are generally regarded as complicated to measure and are seldom measured in ectothermic animals. In this presentation, I will discuss some of the work I did on the effects of temperature on protein metabolism in a stenothermal cold-adapted fish species, the spotted wolffish (*Anarhichas minor*). The recent developments in techniques to study protein metabolism in ectothermic animals will then be presented. Finally, the relevance of studying the acclimation of ectothermic animals to fluctuating temperatures rather than to fixed and laboratory controlled temperatures will be emphasized.

2.5 Changing the way we teach our students about thermal physiology / Changer la pédogogie de la régulation thermique

Jason C. L. Brown
Dept. of Biological Sciences, University of Toronto Scarborough, Toronto, Ontario, MIC 1A4

Many students have misconceptions about how different animals regulate their body temperature. For example, many students believe that reptiles cannot employ evaporative cooling, or fail to realize that many mammals and birds do employ behavioural thermoregulation. These misconceptions largely arise because of our emphasis on the endotherm-ectotherm divide. In the first part of this talk, I will propose that the heat balance equation should serve as our framework for teaching thermal physiology, and that the similarities in thermoregulation among animals should be highlighted rather than the differences. In the second part of this talk, I will discuss how Picasso has inspired my teaching philosophy in a way that helps my students to develop the kind of thinking and reasoning competency that will greatly benefit them in graduate and medical schools, as well as experience a sense of scientific discovery for themselves as they employ course concepts to explain novel observations and solve problems. While I do not have any quantitative research to present on the effectiveness of my pedagogical approach, I can attest that many of my students have described my exams as "fun" and are excited to debate with me about my exam questions when the timer expires.

3.0 Ecophysiology and adaptive change (Burness and Moon)

3.1 Micro problems in a macro world: Environmental influences on microRNA in teleosts

Paul M. Craig
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My first introduction to Peter Hochachka was through his JEB obituary that Nick Bernier posted in the lab during the first month of my MSc degree. His unfortunate passing prompted both Nick and Pat Wright to change the theme of our graduate physiology class to a tribute of Peter Hochachka's work, legacy, and, of course, his physiological tome, "Biochemical Adaptations". It was through Peter's research that I became fascinated with how organisms 'work' under a variety of extreme environments, and the molecular events that drive adaptive responses. Having had the privilege of working with two of Peter's former graduates, Grant McClelland and Tom Moon, I'm part of a unique family tree of physiologists. This has helped develop my passion for understanding the genetic and enzymatic responses to adverse environments that drive phenotypic plasticity in teleosts. Being unsatisfied with the occasional disconnect between transcript and function, I started investigating epigenetic mechanisms. This path has led to my current research program; understanding the epigenetic mechanisms that drive functional, phenotypic responses following multi-stressor exposures in fish. While the field of epigenetics is relatively new, it still retains the essence of Peter's work in biochemical and genetic adaptation to extreme environments.

3.2 Chasing waterfalls: Ecophysiology and metabolism of the climbing gobies / A la poursuite des cascades: écophysiologie et métabolisme des gobies grimpeurs

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Several freshwater gobiidae inhabiting tropical islands are amphidromous: after being washed out to the ocean as fry, the juveniles return to freshwater to mature, reside and reproduce. During this upstream journey, juveniles face multiple challenges including extreme metabolic demands imposed by extensive

muscle recruitment. To complicate matters, the topography of tropical islands is challenging, as migrating fish have to pass vertical waterfalls and swim against strong currents to reach their upstream destination. To overcome these obstacles, migrating gobies use their pelvic disk to adhere to substrate and climb their way up. Recent collaborative work on closely sympatric species in Guadeloupe (Caribbean) and La Réunion (South West Indian Ocean) Islands indicate that while most Sicydiinae are able to climb, closely related goby species exhibit marked differences in climbing performance and overall locomotory strategy. These differences are also evident within a species as in both natural and artificial conditions, juveniles typically outperform older fish. Interestingly, several metabolic markers also show both intra- and inter-specific differences, which overall parallels individual performance. Ultimately, these physiological traits seem to play an important role in the success and distribution of sympatric gobies species, and may be important determinants of the resilience of these charismatic fishes globally.

3.3 Diet-induced hyperglycemia alters the hepatic microRNA profile in rainbow trout, *O. mykiss*

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MicroRNAs are small, non-protein coding RNAs, which have emerged as potent regulators of glucose metabolism in mammalian model species through destabilization of specific mRNAs and inhibition of protein translation. While many miRNAs are highly conserved in evolution, teleost fish possess additional lineage- and species-specific miRNAs, targeting a potentially vastly different set of mRNA targets. To gain insight into the role of miRNA networks in the 'glucose-intolerant' phenotype in rainbow trout, we profiled hepatic miRNA abundance in the liver of rainbow trout exhibiting diet-induced normo- and hyperglycemia. Our study identified a total of 26 differentially regulated miRNAs, among them several previously identified as being regulated in a hyperglycemic environment in mammalian models. While this suggests at least some regulatory similarities between miRNA regulation in hyperglycemic rainbow trout and mammalian models, predicted mRNA targets suggest a large degree of divergence. Using our identified miRNA targets, future studies will focus on possible causative roles for select hepatic miRNAs towards the glucose-intolerant phenotype in rainbow trout.

3.4 Fishing for mechanisms of toxicity: energy metabolism and physiology at the nano-scale

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Engineered nanomaterials (ENMs) straddle the atomic and molecular scale and exhibit unique physical, chemical, and electronic properties. Their high surface area to volume ratio in combination with these unique properties, make ENMs desirable in a multitude of industrial, clinical, and consumer applications. Unfortunately, these same characteristics also make it difficult to predict the toxicity of novel ENM formulations and their potential risks to human and environmental health. We use a range of physiological, biochemical, and surface chemistry techniques to examine these questions at scales ranging from purified proteins, to isolated cells, to *in vivo* studies on organ physiology and energetics. We exploit the tunable nature of ENMs to address how their physicochemical properties (e.g. size, shape, surface charge) influence interactions with proteins, membranes, and metabolites *in vitro*. This data is then applied to higher-level studies on the biological and ecological relevance of 'nanotoxicity', largely using fish as models. We specifically focus on the cardiorespiratory system, as the pathology of chronic

metabolic and/or physiological stress often manifests as cardiovascular dysfunction. The end goal of this research is to facilitate the responsible development of nanotechnology applications aimed at addressing critical issues in human and environmental health.

3.5 Extreme endurance athletes: How do environmental conditions experienced in flight affect the physiology and ecology of songbirds?

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Migratory songbirds fuel long flights almost entirely with fat, but it has become apparent that protein is also used as a metabolic fuel during flight, resulting in dramatic reductions in organ and muscle mass after flights, that seem to impose a physiological constraint on stopover refueling. Recently we have shown that the rate of protein catabolism is modulated in response to high rates of water loss during flight – directly linking climate with fuel mixture used during endurance exercise. We are now further investigating the functional consequences of tissue losses at multiple levels of organization. By flying migratory songbirds in a wind tunnel for up to 10 hours, we have mimicked the dramatic reductions pectoralis muscle, intestine and liver mass commonly seen in wild birds, and we are investigating structural and functional changes in these tissues after flight and through recovery, while also evaluating long-standing hypotheses about the adaptive role of protein catabolism in flight. Simultaneously, using automated radio telemetry we are taking our findings to the field to investigate the role of climate on fuel use in flight, and how this may influence the stopover ecology and migration speed of these birds.

4.0 Bioenergetics (McClelland and Darveau)

4.1 Baby its cold outside: Developmental adaptions to thermoregulating at high altitude

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Low oxygen and temperatures at altitude are particularly challenging for small mammals due to the high energetic costs of aerobic thermogenesis. In altricial rodents the onset of independent endothermy occurs in the first month of life, a period of high mortality and a potential selective window. We tested the hypothesis that selection has accelerated the ontogeny of thermoregulation at high-altitude. Using lab-reared descendants of deer mice native to low (LA; 400 m a.s.l) and high (HA; 3500 m a.s.l.) altitude environments we characterized the growth and maturation of two thermo-effector organs; skeletal muscle and brown adipose tissue (BAT), in pups aged 0-30 days. We also determined the age of onset of non-shivering and shivering thermogenesis in response to acute cold using video thermography and indirect calorimetry. Contrary to predictions, we found that thermogenesis in HA mice matured slower than in LA mice. This difference may be driven by a greater reliance by HA mice on muscle-based shivering, which increases later in development as muscle assumes a more aerobic phenotype. In contrast, recruitment of BAT occurs earlier after birth and is more apparent in LA pups. Reliance on BAT-based thermogenesis during early development may be too costly in an O_2-limited environment.

4.2 From bees to bivalves: exploring the mechanisms and processes linked to metabolic rate and longevity / Des abeilles aux bivalves: mécanismes et processus en lien avec le taux métabolique et la longévité

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Biochemical mechanisms determining species metabolic rate or maximal lifespan have been subject to much investigation, but remain elusive. We take a look at two prominent theories linking metabolism and longevity to membrane lipid composition, using intra- and inter-specific approaches. The membrane pacemaker theory of metabolism proposes that the relative abundance of polyunsaturated fatty acids in membrane phospholipids sets the metabolic rate of organisms. Using species of tropical orchid bees spanning a 16-fold range in body size, we show that the flight metabolic rate (FlightMR) varies with the relative abundance of linolenate (%18:3 n-3) in flight muscles, according to the predictions of the theory. The membrane pacemaker theory of aging is a component mitochondrial "oxidative stress" theory of aging, and posits that peroxidation susceptibility of mitochondrial membranes determines species lifespan. Recent results from the comparison of the longest-living non-colonial organism known to science, the ocean quahog *Arctica islandica* (maximum lifespan: 507 years), with other marine bivalves of shorter lifespan provide support to the theory. We expand the analysis to populations of ocean quahogs with wide differences in maximum lifespan

4.3 Mitochondrial bioenergetics and environmental stress: from lake to prairie

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Various environmental factors can constrain energy metabolism by limiting the rate of ATP production by animal cells. My research explores how two such environmental stressors - environmental hypoxia and limited fuel availability - affect mitochondrial bioenergetics. Mitochondrial ATP production can be limited at the "end" of the electron transport system by hypoxia. My MSc research focussed on closely related sunfish species that differ in hypoxia tolerance, and naturally produce viable hybrids where the parental species co-exist. I found that hybrids had reduced activity of ETS complexes III and IV in muscle mitochondria which corresponded with reduced hypoxia tolerance. Cold winters with little food can constrain mitochondrial ATP production by limiting the availability of substrate that can "enter" the ETS. In my PhD research I study how suppression of metabolism in hibernating ground squirrels conserves energy throughout the winter. I investigated the mechanisms underlying reversible suppression of liver mitochondria that occurs as squirrels enter torpor. I found suppression of complexes I and II, with activity of these enzymes corresponding with their phosphorylation state. These studies have advanced our understanding of how mitochondrial bioenergetics influences stress tolerance, revealing new mechanistic links between mitochondrial and whole–animal metabolism.

4.5 Mitochondrial reactive oxygen species metabolism: Considerations for comparative studies, or, things I've learned about biochemistry that they didn't teach me in school

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Mitochondria are often blamed for being the major source of reactive oxygen species (ROS) in animal cells thus linking mitochondria to oxidative stress and disruption of redox homeostasis and signalling. The view that mitochondrial ROS are an amorphous 'inevitable consequence of aerobic metabolism' with complex I and complex III being the 'major' sites of ROS production is commonly expressed but overly simplistic. Mitochondrial ROS metabolism involves an intricate network of electron fluxes interacting via mobile electron carriers with the protonmotive force and the reduction state in dozens of redox centres.

These redox centres include >10 sites of ROS production and multiple enzyme-based antioxidant pathways. This network is intimately linked via mitochondrial energetics, adding further complexity by differential substrate-dependent responses, which can be species and tissue-dependent. Here, I elaborate on strategies to evaluate specific components of mitochondrial ROS metabolism with emphasis on extending applications to comparative studies. Finally, by integrating ROS production and antioxidant capacities, I discuss how mitochondria may be better viewed as having an intrinsic capacity to regulate steady-state concentrations of a key redox signalling-link ROS (hydrogen peroxide) in a manner consistent with communicating the current state of mitochondrial energetics to extramitochondrial compartments.

5.0 Evolution of Metabolism (Guderley and Schulte)

5.1 Do convergent mechanisms underlie the evolution of aerobic capacity in fishes?

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The capacity for oxygen uptake, transport and use can influence and animal's ability to perform many of the activities required for survival and reproduction and is predicted to be a common target of natural selection. While many of the physiological mechanism underlying adaptive diversification of aerobic capacity among species have been uncovered, less is known about the initial steps of adaptive divergence, possible constraints, and the genetic basis for differences in aerobic capacity. To address these questions, we have studied the repeated evolution of aerobic capacity among populations of migratory and non-migratory Threespine Stickleback (*Gasterosteus aculeatus*) and limnetic and benthic ecotypes of Lake Whitefish (*Coregonus clupeaformis*). We have found that aerobic capacity can evolve rapidly (≤ 12,000 years), is encoded by multiple genes with relatively small effect sizes, and often occurs by different genetic, molecular, and biochemical mechanisms among populations within a species. Despite extensive non-convergence at the genetic, molecular and biochemical levels, we do see convergence in heart size and skeletal muscle mass and/or fiber type composition. Overall, this work suggests that aerobic capacity can rapidly evolve by a variety of mechanisms in closely related populations of fishes.

5.2 How much disruption of mito-nuclear co-adaptation can be tolerated?

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The co-evolution of mitochondrial and nuclear genomes, and therefore coadaptation of mitochondrial peptides, has been considered as a major constraint in the evolution of organisms. For example, viability of inter-species or even inter-population hybrids is strongly limited by disruption of these coadaptations. A clear manifestation of these disruptions is the exacerbation of oxidative stress. It has therefore been suggested that oxidative stress drives selection of genome compatibility. In order to evaluate to which extent populations can support increase in oxidative stress, we generated four mitonuclear genotypes of *Drosophila* harboring different combinations of a mutation in $tRNA^{tyr}$ in the mitochondrial genome and a mutation in the corresponding nuclear-encoded mitochondrial tyrosine synthetase. We observed

that cells attempt to restore mitochondrial homeostasis in mis-adaptated mitonuclear genotypes but fail to rescue mitochondrial functions.

5.3 Ecophysiological consequences of taxon-dependent metabolic organization: lessons from sharks and squids

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Peter Hochachka was a pioneer in revealing the significance of the metabolic diversity among animals. He also embraced the notion that the 'phylogenetic baggage' of animals can have important consequences for their physiology and ecology. His broad thinking in these areas influenced my work on the distinctive metabolic organizations of cephalopod molluscs (squids and relatives) and chondrichthyan fishes (sharks and relatives), about which Hochachka also made key discoveries. We have found that the energy metabolism of cephalopods and chondrichthyans has converged in certain ways. Specifically, both groups store lipids centrally in the liver (digestive gland in cephalopods), while having a tissue-specific de-emphasis on fatty acids as metabolic fuel that appears to be compensated by a greater reliance on ketone bodies (mobilized from liver lipids) and/or amino acids. The lipid-rich liver is an energy store, particularly during starvation, but also can have a competing role as a buoyancy organ, especially in chondrichthyans and pelagic squid. While the factors that led to the convergence probably differ between cephalopods and chondrichthyans, our work suggests that their characteristic metabolic organization has had important ecophysiological consequences for both groups, constraining the invasion of the oligotrophic deep-sea by chondrichthyans and constraining starvation survival strategies in cephalopods.

5.4 Evolution of metabolic scaling in active animals / Évolution des effets d'échelle sur le métabolisme des animaux actifs

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The effects of body size on metabolism integrate various levels of organization. Among the many challenges metabolic scaling presents, bridging the gap between macroevolutionary patterns and microevolutionary mechanisms remain elusive. Flying insects offer an attractive model as their mode of locomotion imposes high energy demand for muscle function, the dominating contributor to this metabolic state. The close associations between the scaling of morphological traits and biomechanical properties dictate flight metabolic rate scaling. I will show how intraspecific variation meets the conditions for selection to act on traits and ultimately yielding interspecific scaling. The consequences of flight metabolic rate scaling on muscle tissue supporting flight are observed on multiple metabolic phenotypes involved in energy production or associated with cell metabolic activity. Interspecific scaling of flight muscle metabolic enzyme activity follows from flight metabolic rate scaling, and intraspecific variation shows that the same functional steps are linked to metabolic rate variation. Flight muscle membrane phospholipid composition scales with body mass and according to the proposed membrane pacemaker hypothesis, indicating how cellular properties change with tissue metabolic intensity. This presentation will summarize a series of studies highlighting how *mechanism and process in physiological evolution* shed light into metabolic scaling.

5.5 The Diversity of Mitochondrial Electron Transport Systems in Animals

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The electron transport systems in the mitochondria of many organisms contain alternative respiratory enzymes distinct from those of the canonical respiratory system depicted in textbooks. Two of these enzymes, the alternative NADH dehydrogenase and the alternative oxidase, were of interest to a limited circle of researchers until they were envisioned as gene therapy tools for mitochondrial disease treatment. Recently, these enzymes were discovered in several animals. Here, we analyse the functioning of alternative NADH dehydrogenases and oxidases in different organisms and examine their taxonomic distribution in animals. We hypothesize that both enzymes ensure bioenergetic and metabolic flexibility during environmental transitions, particularly during changes in oxygen levels.

6.0 Poster Abstracts

6.1 Bioenergetics flexibility of zebra fish muscle facing environmental constraints: chronic hypoxia *vs*. ion poor water acclimation / Flexibilité de la bioénergétique musculaire du poisson zèbre en réponse à des contraintes environnementales : hypoxie chronique *vs.* eau pauvre en ions

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Hypoxic and ion poor water are two conditions commonly encountered in the wild freshwater environments. Both of them are known as modulating energy budget, leading to a metabolic depression for sparing energy during hypoxia or at the contrary to an increase of energy expenditure due to the additional cost of osmoregulation. In order to investigate consequences of these environmental constraints on muscle bioenergetics, we maintained zebrafish (*Danio rerio*) under chronic hypoxia (20% O_2) or acclimatized to ion poor water (60 µS/cm) during 10 days. Fish muscle is the main organ in terms of relative mass and therefore likely plays a major role into buffering energetic variations under these challenging conditions. We studied red and white muscle, to discriminate the effect of treatments on the two different muscle types that are fueled by contrasted metabolic pathways. Taking account of the different muscle types, we hypothesized that fish red muscle may have been negatively impacted by these environmental constraints compared to white muscle. Using high-resolution respirometers (Oxygraph-2k, Oroboros® Instruments, Innsbruck, Austria), we measured basal and phosphorylating states of both type of muscles fibers. Our results show a decrease of phosphorylating and basal respiration rate (between 30% and 40% for both states) in hypoxic and ion-poor water acclimatized fish in red muscle. In contrast, white muscle bioenergetics was only affected by hypoxic condition (-40%). Therefore, red muscle seems more sensitive to environmental conditions than white muscle and our results are in line with the whole-organism metabolic depression under hypoxia and the hypothesis of an energy reallocation toward other osmoregulation organs, such as gills in ion-poor water condition.

6.2 Ventilatory acclimatization to hypoxia in deer mice native to high altitudes

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Hypoxia at high altitudes constrains O2 supply to support metabolism and thermoregulation in the cold. Many highland taxa exhibit physiological specializations for overcoming these challenges, but the importance of hypoxia acclimation responses in high-altitude natives is not known. Highland taxa often possess a high haemoglobin-O2 binding affinity, which safeguards arterial O2 saturation (SaO2) and tissue O2 delivery in hypoxia, but could influence hypoxia acclimation responses. We examined this issue by comparing captive populations of deer mice (Peromyscus maniculatus) from high and low altitudes. Mice from each population were acclimated to normoxia and moderate hypoxia (12 kPa, the PO2 at 4300m) for 6-8 weeks. Highland mice were also acclimated to more severe hypoxia (9 kPa, the PO2 at ~7000m), which induces the same SaO2 in highlanders that lowlanders experience at 12 kPa. In lowlanders, hypoxia acclimation augmented the hypoxic ventilatory response (HVR), increased SaO2 in hypoxia, and induced right ventricle hypertrophy. Hypoxia acclimation had little effect on the HVR in highlanders, such that the hypoxic control of breathing appeared to be fixed. Right ventricle hypertrophy did not occur in either moderate or severe hypoxia in highlanders, suggesting blunted hypoxic pulmonary vasoconstriction and hypertension. Therefore, high-altitude adaptation appears to have blunted several hypoxia acclimation responses in deer mice.

6.3 How do cuttlefish (*Sepia officinalis*) respond to a moderate hypoxia exposure? Insights from whole animals and isolated tissue

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Naturally occurring hypoxic conditions in warm coastal waters may increase in frequency and severity in the near future. Understanding how marine organisms respond to ecologically relevant hypoxia is therefore important. For sepioids like the common cuttlefish (*Sepia officinalis*) suboptimal dissolved oxygen content (DO_2) is thought to range from 65% to 35%. We challenged cuttlefish for one hour to 50% DO_2 and observed a decrease in oxygen consumption of 37% associated with an 85% increase in ventilation rate. Octopine levels, a marker of anaerobic metabolism, increased by a small but significant level in mantle, whereas there was no change in gill or heart levels. Similarly, the hypoxic period did not result in changes in HSP70 or polyubiquinated protein levels in mantle, gill, or heart. It seems that although metabolic rate decreases there is only a minor increase in anaerobic metabolism and no biochemical changes that are hallmarks of alterations in protein trafficking. Experiments with isolated preparations of mantle, gill, and heart suggest that pharmacological inhibition of protein synthesis could decrease oxygen consumption by 32% to 42% or Na^+/K^+ ATPase activity by 24% to 54% dependent upon tissue type. The decrease in whole animal oxygen consumption is potentially the result of a controlled decrease in the energy demanding processes of both protein synthesis and Na+/K+ ATPase activity.

6.4 Metabolic suppression in the pelagic red crab, *Pleuroncodes plannipes*, in oxygen minimum zones/ Suppression métabolique du crabe rouge pélagique, Pleuroncodes plannipes, dans les zones minimales d'oxygène

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The pelagic red crab, *Pleuroncodes planipes*, is abundant throughout the Eastern Tropical Pacific. Juveniles of the species are among the most prevalent components in the diets of major oceanic predators, including tunas, whales and squids. Crabs migrate daily from the surface to mesopelagic depths where oxygen is a fraction of air saturation. These crabs regulate aerobic metabolism to a critical PO_2 of ~0.8 kPa but encounter much lower PO_2 at depth. In hypoxia, oxygen consumption suppressed and we show a modest increase in lactate levels indicating elevated anaerobic metabolic ATP production. Metabolic suppression is achieved via posttranslational modifications on histone H3, which are associated with a condensed chromatin state (and hence decreased transcription). Under hypoxia, p-H3 S10, Ac-H3 K9, Ac-H3 K14 were 39, 68, and 36% of control values, respectively. Several translation factors also decreased under hypoxia. Transcription and translation are major sinks for metabolic energy. Global suppression of these processes is a common strategy for temporary survival of extreme environmental conditions. Elevated heat-shock proteins suggest that the cellular stress response is triggered during hypoxia in *P. plannipes*. These results are important for the ecology of zooplankton and their predators, as well as the biogeochemical cycles to which they contribute.

6.5 Physiological and mitochondrial responses following an exposure to a high fat diet in *Drosophila melanogaster* / Réponses physiologiques et mitochondriales lors d'une exposition à une alimentation riche en lipides chez *Drosophila melanogaster*

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Mitochondria are central to metabolism as they produce the majority of ATP needed by the cell via the process of oxidative phosphorylation which is coupled to the electron transport system (ETS). The reducing equivalents NADH and $FADH_2$ used by the ETS are produced through different metabolic pathways such as the tricarboxylic acid cycle and the beta-oxidation directly inside the mitochondrial matrix. The cytosolic NADH produced during glycolysis can also reach the mitochondria via the glycerol-3-phosphate dehydrogenase (G3PDH). An exposure to a high fat diet (HFD) is thought to alter metabolic homeostasis, but the underlying mechanisms are still poorly understood. In this study, we investigated several metabolic parameters in order to highlight the modulation of the metabolic pathways involved in the short- and long-term responses after an exposure to a HFD diet in *Drosophila Melanogaster*. Specifically, we measured longevity, as well as key metabolite levels, mitochondrial respiration, and geotaxis at 0, 1, 2, 4 and 10 days following the exposure. Our results showed that short-term exposure caused an increased mitochondrial respiration. However, long-term exposure resulted in decreased longevity and geotaxis, as well as alterations of mitochondrial respiration at the level of complex I, which was partially compensated by increased G3PDH.

6.6 What underlying mechanisms are responsible for the increased lipid oxidation rates observed in thermoregulating highland deer mice (*Peromyscus maniculatus*)? / Quels mécanismes sont responsables de l'augmentation des taux d'oxydation des lipides observés chez la souris sylvestre d'altitude?

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Concurrent hypoxic-cold stress in high-altitude environments is extremely challenging for small endotherms. Deer mice need to sustain the oxygen-demanding process of thermogenesis, despite the low availability of oxygen, to maintain a constant body temperature in the cold. Our research has revealed that both highland and lowland native deer mice fuel thermogenesis primarily by oxidizing lipids rather than carbohydrates; however, highlanders can support higher rates of this lipid-fuelled thermogenesis. The underlying mechanisms responsible for increased lipid oxidation rates in highland deer mice remain unknown. The purpose of this study is to characterize the mobilization, transport, uptake and oxidation of lipids in both highland and lowland deer mice. Lipid storage will be determined in white adipose tissue and muscle using triglyceride assays. Circulating plasma fatty acids will be identified using gas chromatography to assess mobilization. Capacity for uptake will be quantified via Western Blot for fatty acid binding protein and fatty acid transporter CD36 abundance. Soleus lipid oxidation will be measured using the Pulse-Chase technique. The findings from this research will begin to elucidate the mechanistic underpinnings of selection for greater lipid oxidation rates to fuel thermogenesis at high-altitude.

6.7 Endogenous rhythms of thorax brown adipose tissue in the 13-lined ground squirrel

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Hibernating mammals, such as the 13-lined ground squirrel, spend winter in a state of torpor, with reduced body temperature (~5°C). Torpor is interrupted by spontaneous arousals where body temperature rapidly increases to ~37°C, with thermogenesis facilitated primarily by brown adipose tissue (BAT). In non-hibernating eutherians, BAT proliferation requires extensive cold acclimation. In non-food-caching hibernators an endogenous rhythm regulates white adipose tissue proliferation, allowing animals to store sufficient food energy for the hibernation season. In BAT thermogenesis-related genes are also upregulated in autumn, even when animals are housed at warm temperatures, suggesting an endogenous rhythm, but data about BAT growth in hibernators is scarce. Using water-fat MRI we showed that thorax BAT volume increases in ground squirrels as hibernation approaches, even while housed at warm (22°C) temperatures. To further dissect the potential of an endogenous rhythm, we housed two groups of ground squirrels at 12L/12D photoperiod: one at thermoneutral (25°C) and the other at cold (5°C) temperatures. Thorax BAT volume increased in both groups at the same rate initially, but decreased in the thermoneutral group at the time when hibernation would begin in the wild. These results challenge the role of endogenous rhythms in BAT proliferation in this hibernator.

6.8 Dynamic chromatin modification during hibernation

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Hibernation is characterized by a dramatic reduction in energy demand that profoundly alters metabolic state. Changes in metabolic rate during torpor-arousal cycles affect levels of circulating metabolites, which in turn have the potential to induce dynamic alterations in histone acetylation and methylation status. In this study, we investigate how different metabolic and nutritional phases across the annual hibernation cycle affects histone modifications in multiple tissues of 13-lined ground squirrels. Liver, brain, and brown adipose tissue were analyzed in squirrels sampled in summer, winter torpor, winter interbout arousal (IBA) and spring phases. Using an in-house LC-MS/MS workflow, we identified > 50 distinct chromatin signatures that segregate by tissues and metabolic/nutritional state. Hierarchical clustering shows that histone post translational modification (PTM) states of brain and brown adipose are more similar to one another than either are to liver. Histone acetylation, which has a significantly shorter half-life than histone methylation, generally associates with more rapid switches in metabolic state (i.e., torpor *vs.* IBA). In contrast, switches between torpor and either of the two fed states (spring, summer) associate with changes in histone methylation. Collectively, our results suggest that the robust metabolic and nutritional shifts associated with hibernation drive changes in chromatin state.

6.9 The effects of water temperature and oxygen availability on the aerobic metabolic scope of a southern population of Bluegill Sunfish (*Lepomis macrochirus)*

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With the predicted increase in global water temperature and acute hypoxic episodes, knowledge of the effects these stressors can have on local aquatic life is extremely valuable. This study quantified the change in metabolic rate of Bluegill (*Lepomis macrochirus*), both maximum metabolic rate (MMR) and resting metabolic rate (RMR), in Bluegill sunfish in response to varying temperature (20, 25 and 30°C) and dissolved oxygen (> 95% O2 and 40% O_2 saturation). This data was then used to calculate aerobic metabolic scope (AMS) and extrapolate the effects these stressors have on fish performance. Decreases in dissolved oxygen content were determined to result in a decrease in AMS, due to the limiting of MMR. Additionally, increases in acclimated temperature, were shown to lead to an increase in AMS, until the optimum temperature for the species was attained, after which AMS decreased. Through this, it was concluded southern populations of bluegill are at or near their optimum temperature range in the summer months. With the expected increases in temperature due to global climate change, these fish may be pushed past their optimum temperature and into the pejus range, resulting in ecological decline.

6.10 Alternative splicing as a mechanism of thermal acclimation

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Alternative splicing has long been suggested as a potential mechanism involved in thermal acclimation, and evidence for such a role has been detected in plants and insects. However, little is known about these responses in vertebrates. Whole-transcriptome studies of the response of fish tissues to cold acclimation indicate that genes involved in RNA processing are strongly upregulated by cold acclimation, which is potentially consistent with a role for alternative splicing in thermal acclimation. To determine

whether alternative splicing is important in the response of fish muscle to cold, we performed and RNA-seq experiment in fish acclimated to 15°C and 5°C, and identified >7,000 genes that changed in expression in response to cold acclimation. We also identified 574 genes with evidence of differential exon usage in response to cold acclimation. Among these genes were those involved in RNA splicing and RNA stability, the regulation of mitochondrial morphology and abundance, and multiple subunits of electron transport chain Complex I, which we have shown is as a key target of regulation during thermal acclimation in killifish. We also re-analyzed several RNA-seq datasets from other species, and found similar extents of differential exon usage, suggesting that this is a conserved mechanism of thermal acclimation.

6.11 The effects of hypoxia on protein metabolism in the Amazonian cichlid, *Astronotus ocellatus* / Les effets de l'hypoxie sur le métabolisme des protéines chez le cichlide amazonien, *Astronotus ocellatus*

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In the aquatic environment, fish are often exposed to large variations in dissolved oxygen (DO). Some fish are able to alter their behaviour and certain physiological processes when faced with hypoxic conditions in order to prolong survival. The Amazonian cichlid, *Astronotus ocellatus*, is highly tolerant to hypoxia. Some fish, including *A. ocellatus,* are able to reduce their metabolic rate by reducing the activity of energetically expensive metabolic processes, including protein synthesis when oxygen is lacking in their environment. The objectives of this research were to determine how protein metabolism is regulated in *A. ocellatus* during hypoxia. Fish were exposed to a stepwise decrease in DO (100%, 20%, 10% and 5%) for 2 hours at each level, and sampled throughout the experiment. This allowed us to determine for the first time in fish that a decrease in protein synthesis during hypoxia is controlled by signaling pathways (mTOR and eIF2-α), and not simply due to a lack of substrate. We also observed no effect on the capacity of tissues to degrade proteins. There is limited information on the effects of hypoxia on protein metabolism in fish. This relatively unexplored angle could help in our understanding of hypoxia tolerance, which has important implications in conservation.

6.12 Changes in histone methyltransferases during freezing stress in the wood frog

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The wood frog (*Rana sylvatica*) survives freezing of ~65% of body fluids over days or weeks at a time while overwintering supported by adaptations including synthesis of cryoprotectants and entry into a hypometabolic state. Here we report that changes in histone methylation may contribute to hypometabolism by suppressing gene transcription. Relative protein levels of seven histone methyltransferases (HMTs) (SMYD2, SETD7, ASH2L, RBBP5, SUV39H1, EHMT2, SET8), four methylated histone H3 lysine residues (H3K4me1, H3K9me3, H3K27me1, H3K36me2), HMT enzymatic activity on H3K4, and methylation of a non-histone protein (p53, p53K370me2, p53K372me1) were measured in skeletal muscle and liver during freeze-thaw of wood frogs. Results showed tissue-specific changes in HMTs across experimental conditions which correlated with changes in the methyl-histone residues they act on. In skeletal muscle, methyl-histone residues associated with active transcription (H3K4me1, H3K27me1) decreased during freezing, whereas those associated with gene silencing (H3K9me3, H3K36me2) were maintained. During freezing in liver, H3K4me1 decreased, H3K9me3 remained

constant, H3K27me1 increased during thawing, and H3K36me2 rose during freezing. Total p53 did not change but differential methylation of p53 in muscle occurred. The data indicate that histone and non-histone protein methylation plays a role in facilitating natural freeze tolerance.

6.13 Turn down gene expression for WAT: Anti-apoptotic signaling protects white adipose tissue in hibernating 13-lined ground squirrels

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During hibernation, the metabolic rate of 13-lined ground squirrels (*Ictidomys tridecemlineatus*) can drop to just 1-5% of normal resting rate at 37°C, body temperature can plummet to 1-5°C and heart/breathing rates fall to low values. Energy saved by using hibernation allows squirrels to survive the winter without eating, living off lipids mobilized from white adipose tissue (WAT). Defense against conditions that would normally be damaging (e.g. low temperatures, hypoxia, ischemia) is needed for long term survival and to prevent cell death via apoptosis. Stress signals alter the relative amounts and activities of pro- and anti-apoptotic Bcl-2 family members. This study analyzed responses by anti-apoptotic proteins using immunoblotting to assess six time points of the torpor-arousal cycle. These included anti-apoptotic Bcl-2 family members functioning at the mitochondrial level: Bcl-2, p-Bcl-2 (S70), p-Bcl-2 (T56), Bcl-xL, p-Bcl-xL (S62), Mcl-1, p-Mcl-1 (S159) and caspase inhibitors downstream of the mitochondria: x-IAP and c-IAP. Bcl-xL and c-IAP exhibited no changes over torpor-arousal, but significant increases in Mcl-1 and x-IAP proteins occurred during torpor, compared to euthermia, and significant decreases in p-Mcl-1 (S159), Bcl-2, p-Bcl-2 (T56), p-Bcl-2 (S70), and p-Bcl-xL (S62) occurred. The data suggest an overall increase in WAT cell survival efforts during hibernation.

6.14 Regulation of liver glutamate dehydrogenase activity in response to freezing in the wood frog (*Rana sylvatica*)

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Glutamate dehydrogenase is crucial to regulating nitrogen metabolism in liver by mediating conversion of glutamate to α-ketoglutarate with the release of ammonia which can then be utilized to generate urea. Wood frogs can survive prolonged bouts of freezing during the winter, and accumulate substantial quantities of urea to supplement the elevated levels of glucose cryoprotectant. Purification of GDH to homogeneity was performed in two-steps: chromatography on carboxymethyl Sepharose with elution by increasing pH followed by GTP agarose affinity chromatography with elution by increasing salt concentrations. Purified liver GDH from frozen frogs showed higher affinity for substrates than the enzyme from control liver (K_M glutamate decreased 41%, K_M NAD^+ decreased 40%). A higher amount of GTP was needed to inhibit GDH from frozen frogs relative to controls (I_{50}: 260% increase) and GDH maximal activity was higher for freeze-exposed GDH in both directions. Western blotting assessed the relative abundance of various post-translational modifications on the two GDH forms. Results showed a significant increase in the relative amount of acetylation associated with the frozen enzyme compared to controls (1.2 fold). Overall, these results suggest that GDH is modified by deacetylation in response to freezing causing increased enzyme activity.

6.15 No need to diet – just control your metabolism! Regulation of pyruvate dehydrogenase (PDH) in hibernating ground squirrels (*Ictidomys tridecemlineatus*)

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Pyruvate dehydrogenase (PDH) is a vital regulatory enzyme that links glycolysis and the tricarboxylic acid cycle. Inhibition of PDH activity via three serine phosphorylation sites (p-S293, p-S300, and p-S232) regulates metabolic flux through the TCA cycle, decreases glucose utilization, and facilitates lipid metabolism. This study explores the post-translational regulation of pyruvate dehydrogenase mediated by PDH kinases (PDK1-4) over the torpor-arousal cycle in liver, skeletal muscle, and cardiac tissues of hibernating 13-lined ground squirrels (*Ictidomys tridecemlineatus*). Luminex multiplex assays and western immunoblotting were used to measure relative protein expression levels. Skeletal muscle showed little inhibitory regulation of PDH during hibernation, whereas liver and heart exhibited strong inhibitory PDH regulation during late torpor and interbout arousal.

6.16 The hibernating South American marsupial, *Dromiciops gliroides*, displays torpor-sensitive microRNA expression patterns

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The marsupial monito del monte, *Dromiciops gliroides*, is the only known hibernator in South America; its capacity for strong metabolic rate depression allows the species to endure seasonal cold and food scarcity. Hibernation is a complex energy-saving strategy, involving metabolic controls that include changes in gene expression that are elicited in part by regulatory control from the differential expression of microRNAs. To better elucidate the role of microRNAs in orchestrating hypometabolism, a modified stem-loop technique and quantitative PCR were used to characterize the relative expression levels of 85 microRNA species in liver and skeletal muscle of control and torpid *D. gliroides*. Thirty-nine microRNAs were differentially regulated during torpor; of these, 35 were downregulated in liver and 11 were differentially expressed in skeletal muscle. Data reveal an important tissue-specific involvement of differential microRNA expression in torpor facilitation and bioinformatic analysis indicated that these microRNAs contribute to the regulation of selected signal transduction pathways (MAPK, PI3K-Akt, mTOR), thermoregulation, and prevention of muscle disuse atrophy.

6.17 Evolution of the Cytochrome c Oxidase Subunit 4 Paralogs

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The largest subunit of cytochrome c oxidase exists as two paralogs in most vertebrates: a constitutive COX4-1 and hypoxia-inducible COX4-2. Though all vertebrates (except birds) possess two COX4 paralogs, the structural and functional subfunctionalization differs between fish and mammals. In primates and rodents, COX4-2 is a hypoxia-responsive gene encoding a protein with its ATP-binding site disrupted by a disulfide bridge, precluding COX from the allosteric regulation that is exerted through this site in COX4-1. However, in fish, COX4-2 is not hypoxia responsive at either the mRNA or protein level and the fish

COX4-2 protein lacks the paired CYS residues seen in mammalian COX4-2. Thus, the functional discrimination between paralogs in relation to hypoxia differs between these lineages. In fish, COX4-2 shows higher constitutive expression in all tissues and shows intriguing patterns in relation to distribution within cells, between cells of a tissue, and changes with development. The distinctions in COX4-2 between mammalian models (rodents, humans) and other vertebrates (other mammalian lineages, reptiles, fish) bring into question the genetic origins of the paralog pair and its functional evolution during vertebrate diversification (Supported by NSERC Canada).

6.18 High-altitude Adaptation and Hypoxia Signalling in Deer Mice

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The hypoxic and cold environment at high altitudes requires that endothermic animals sustain high rates of O_2 consumption for both locomotion and thermogenesis while facing a diminished O_2 supply. Recent evidence suggests that genes in the hypoxia inducible factor (HIF) pathway have been targeted by natural selection and have contributed to evolutionary adaptation to high altitudes in several species. Here, we examine the role of hypoxia signaling in high-altitude adaptation in deer mice (*Peromyscus maniculatus*). High- and low-altitude populations exhibit extreme allele frequency variation in a non-synonymous single nucleotide polymorphism in *Epas1*, the gene encoding HIF-2α. Transcriptome scans indicate that these differences in *Epas1* allele frequency stem from a history of spatially varying selection between high and low altitudes. Comparisons of the hypoxia response between mice with different *Epas1* genotypes (captured from an admixed population on the summit Mount Evans CO) show that the high-altitude *Epas1* allele is associated with higher heart rates in deep hypoxia, but that it has no association with variation in breathing, pulmonary gas exchange, or blood haemoglobin content. Our results therefore suggest that changes in hypoxia signaling contribute to high-altitude adaptation in deer mice. (Supported by NSERC of Canada and the USA National Science Foundation)

6.19 How to make a hypoxia intolerant fish tolerant: The effect of diel cycling hypoxia on the physiology and biochemistry of rainbow trout (*Oncorhynchus mykiss)*

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Many fish naturally encounter daily cycles of hypoxia but it is unclear if these harden them to subsequent episodes of hypoxia or if they lead to accumulated stress. Rainbow trout were exposed to 1 of 4 different treatments in an intermittent flow respirometer: 1) One group were held for 110 h at 100% DO_2 prior to a prolonged hypoxia (4 h, 20% DO_2); 2) Another was exposed to 110h of diel DO_2 cycling (100-20% over 24h), followed by prolonged hypoxia; 3) A control group was exposed to air-saturated water (100% DO_2); 4) A second control group was exposed to a constant 60% DO_2, the mean DO_2 experienced by fish in group 2. Blood and liver samples were collected to assess physiological and metabolic responses to each treatment. Some responses were similar in cycled and mean fish but overall, we found strong evidence that diel cycling hypoxia improves hypoxia tolerance in trout. Liver HSP70 expression increased and plasma cortisol decreased following multiple cycles of hypoxia, as the activation of protective responses reduced whole animal stress. Metabolic rate was lower during hypoxia in cycled fish and metabolite and signal transduction pathway responses indicated animals transitioned from a catabolic to an anabolic state.

6.20 HIF-1 plays a key signalling role in physiological responses to acute hypoxia in the African naked mole rat, Heterocephalus glaber

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Naked mole rats are among the most hypoxia-tolerant mammals identified. This tolerance is partially achieved by coordinated reductions of metabolic and ventilatory rates by > 85% in acute hypoxia; however, the cellular mechanisms that mediate these responses remain poorly understood. Hypoxia-inducible factor-1α (HIF-1α) is a transcription factor that is central to hypoxic signalling at the cellular level and we hypothesized that endogenous activation of HIF-1α-mediated signalling contributes to the remarkable hypoxia-tolerance of naked mole rats by modulating their metabolic rate. We used pharmacological tools to manipulate HIF-1α expression in naked mole rats and mice and measured metabolic and ventilatory responses to an acute hypoxic challenge (7% O_2 for 1hr) in awake and unrestrained subjects using plethysmography and respirometry. Pharmacological manipulation of HIF-1α expression was confirmed by measuring changes in the expression of several downstream gene targets of HIF-1α. In naked mole rats, blocking HIF-1α reversed the hypoxic ventilatory response and blunted the hypoxic metabolic response. As a result, the naked mole rat air convection requirement (ACR) increased markedly in hypoxia, indicating that naked mole rats were hyperventilating and thus less tolerant of hypoxic stress. Conversely, activating HIF-1α in mice increased ventilation in normoxia and reduced the hypoxic increase in the murine ACR, indicating that HIF-1α-agonist-treated mice were more tolerant of hypoxic stress. We conclude that endogenous upregulation of HIF-1α signalling contributes to the hypoxia-tolerance of naked mole rats, and we demonstrate that this mechanism can be translated to hypoxia-intolerant mice to induce a hypoxia-tolerant phenotype.

6.21 Correlation between thermal tolerance, ROS production and omega-3 content in fish hearts

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In the context of climate change, it is of paramount importance to investigate the thermal sensitivity of aquatic ectotherms. Heat challenge tests have recently been advanced as a good way to assess an individual's robustness and general fitness. In this context, the objective of this study is to assess individual heat challenge performance of fish and shed light on the underlying physiological mechanisms. Previous results demonstrated that mitochondrial activity is impaired and reactive oxygen species production rates level off in the same temperature ranges as the whole organism loss equilibrium. Four strains of charr were used to determine individual heat stress performance. In addition to that, the individual reactive oxygen species (ROS) production of permeabilized heart fibres were measured and the fatty acid profiles of heart tissue were determined. Our results show; 1) that less heat tolerant individuals have higher rates of ROS production, 2) good performers have a higher omega 3 content and less omega 6 in heart tissue. This demonstrates that an organism's performance in heat challenge tests seems to be correlated with its heart's ability to cope with increased ROS production. Moreover, individuals with higher omega3/omega6 ratios seem to be more robust and show better overall performance.

6.22 Mitochondrial adaptations to high altitude in brown adipose tissue of highland deer mice

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High altitude endotherms such as deer mice (*Peromyscus maniculatus*) must support high metabolic rates necessary to guard their body temperature against low ambient temperatures, despite a reduced O_2 availability for mitochondrial respiration. Non-shivering thermogenesis (NST) in deer mice primarily takes place in brown adipose tissue (BAT), an organ specialized to produce heat using uncoupled mitochondrial respiration following activation of uncoupling protein 1 (UCP1) by free fatty acids. Past work has shown that highland deer mice have a greater NST capacity than acclimated lowlanders but no difference in BAT size with acclimation to cold and hypoxia. This suggests that highland deer mice modify BAT mitochondrial function, to increase NST capacity in cold and hypoxia. No population differences in leak or coupled mitochondrial respiration were found before or after cold and hypoxia acclimation. When UCP1 uncoupled respiration was stimulated with palmitoyl-carnitine, both populations had equivalent rates of O_2-consumption before acclimation. After acclimation to cold and hypoxia, highland mice significantly increased uncoupled respiration to rates significantly higher than in acclimated lowland mice. These findings suggest that the enhanced NST capacity in highland deer mice acclimated to cold and hypoxia is partially the result of a greater fatty acid-stimulated uncoupled respiration per BAT mitochondrion.

6.23 Comparative analysis of metabolic rate measures in Westslope Cutthroat Trout (*Oncorhynchus clarki lewsi*) and Rainbow Trout (*Oncorhynchus mykiss*) in relation to environmental variables

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Under the Canadian Species-at-Risk Act, the Alberta populations of Westslope Cutthroat Trout are listed as “Threatened”. The major threats to the species are the alteration in water flow, climate change, and competition from invasive fish species, specifically Rainbow Trout. However, little is known about the physiology of Westslope Cutthroat Trout, particularly the information available on the species’ metabolic rate and ability to adapt to different environmental conditions is sparse. Consequently, it was essential to investigate the species’ metabolic rate in comparison to its main competitor, the introduced Rainbow Trout. Our results showed a higher standard metabolic rate but lower maximum metabolic rate for Westslope Cutthroat Trout in comparison to Rainbow Trout. Subsequently, Westslope Cutthroat Trout have a lower aerobic metabolic scope than Rainbow Trout. This information will allow developing predictive comparative habitat models of how Westslope Cutthroat Trout and Rainbow Trout populations will react to natural and anthropogenic flow and climate when managing Westslope Cutthroat Trout populations.

6.24 Reductions in mitochondrial reactive oxygen species mimic the anoxic response in goldfish neurons

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Although the anoxia intolerant mammalian brain undergoes neuronal death within minutes, the common goldfish *Carassius auratus*, is able to avoid cellular damage in anoxic environments. Due to the suppression of action potentials in excitatory glutamatergic neurons and the increased action potential frequency in inhibitory GABAergic neurons, the goldfish is able to decrease its overall energy expenditure and survive weeks under low oxygen conditions. In conjunction with the decreased oxygen availability complication, these animals also have to overcome the homeostatic imbalance of reactive oxygen

species (ROS). ROS in low concentrations has been proposed to function as part of signal transduction pathways. Given that the cellular mechanism of anoxia tolerance in goldfish remains elusive, we proposed that low ROS contribute to the anoxic response. Using the whole-cell patch-clamp technique, we measured changes in electrophysiological parameters of cortical pyramidal goldfish neurons in response to ROS scavengers. We found that 125 uM NAC was sufficient to depolarize the membrane by 7.8mV, similar to the anoxic response. In addition, firing frequency decreased in pyramidal neurons by approximately 60% and whole cell conductance increased by 60%- mimicking anoxia. MitoTEMPO, a mitochondrial scavenger, exhibited similar results. Together, this implies that ROS plays a signaling role in anoxia.

6.25 Evidence of positive selection in members of the insulin superfamily of peptides and receptors in marine versus freshwater populations of stickleback: possible roles in euryhaline adaptation?

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Three-spine stickleback is a primarily anadromous species of the northern hemisphere but recurrent adaptation from marine to freshwater (FW) streams and lakes occurred ~10,000 years ago. Well known for the dramatic parallel morphological changes associated with FW adaptation, G. aculeatus has become the target of global genome-wide sequencing efforts. These studies have unveiled that the recurrent evolution of similar phenotypic and physiological responses of stickleback populations to FW are mirrored by parallel adaptations at the genomic level either via recurrent sweeps of the same allele (hard sweep) or by independent parallel evolution ('soft sweep'). Since there are well described trade-offs between growth and reproduction for fish in salt water (SW) vs fresh water (FW), we investigated whether any of the 23 members of the insulin superfamily of ligands and receptors, several of which play known roles in osmotic regulation, reproduction and/or growth, exhibit evidence of selection in marine vs freshwater accessions of stickleback. To this end, we performed population genomic analyses on a publically available databases of 21 stickleback genomes, 11 derived from FW and 10 from SW habitats. We observe evidence for positive selection at several ligand and receptor genes, some of which occurs in region of high divergence between SW/FW ecotypes suggesting evidence of genomic hitchhiking.

Campus map

Daily Bread Café is in the basement of St. Johns College