



50 years of comparative biochemistry: The legacy of Peter Hochachka

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A B S T R A C T

Peter Hochachka was an early pioneer in the field of comparative biochemistry. He passed away in 2002 after 4 decades of research in the discipline. To celebrate his contributions and to coincide with what would have been his 80th birthday, a group of his former students organized a symposium that ran as a satellite to the 2017 Canadian Society of Zoologists annual meeting in Winnipeg, Manitoba (Canada). This Special Issue of CBP brings together manuscripts from symposium attendees and other authors who recognize the role Peter played in the evolution of the discipline. In this article, the symposium organizers and guest editors look back on his career, celebrating his many contributions to research, acknowledging his role in training of generations of graduate students and post-doctoral fellows in comparative biochemistry and physiology.

1. Introduction

Peter Hochachka (Fig. 1), a giant of both intellect and persona, passed away from cancer prematurely at the age of 65 in 2002. His insights and infectious enthusiasm brought metabolic biochemistry into the broader field of comparative physiology, pushing the frontiers to identify the mechanistic underpinnings of how animals work at the level of enzymes and metabolites. Much has been written about Peter's astounding contributions to diverse disciplines in comparative biochemistry and physiology, particularly his perspectives on the understanding of temperature adaptation of metabolism, facultative anaerobiosis in ectotherms, hypoxia in diving mammals, adaptation to high altitude in humans, and exercise metabolism (Storey, 2004; Somero and Suarez, 2005). In addition to a long career contributing to science through his inspiring books (Hochachka, 1980; Hochachka and Somero, 1984; Hochachka, 1994a; Hochachka and Somero, 2002), synthetic reviews (Hochachka and Mommsen, 1983; Hochachka, 1986a; Hochachka, 1988; Hochachka et al., 1996a; Hochachka, 1998) and his many seminal papers, he also had a remarkable record of training students. Many of Peter's students have gone on to careers in research, mentoring successive generations of researchers of their own. Fifteen

years after his death, and coinciding with what would have been his 80th birthday, a group of former students organized a symposium that ran as a satellite to the 2017 Canadian Society of Zoologists Annual Meeting held at the University of Manitoba. The venue was appropriate because Peter was the recipient of the highest award from the society, the Fry Medal, in 1995. This award honours a researcher who has made an outstanding contribution to knowledge and understanding in zoology. Comparative Biochemistry and Physiology agreed to produce this Special Issue commemorating Peter's career and this symposium. Peter became Editor-in-Chief of CBP in 1994, and along with his co-editor Tom Mommsen, brought the journal into the era of peer review.

Peter's personal research portfolio spanned many areas of science (Fig. 2) but his contributions also inspired many researchers to push the boundaries of what can be considered comparative physiology. With his long-time friend and colleague, Charlotte Mangum, he argued the merits of a broad integrative discipline, where studies might focus on the mechanistic consequences of biochemical differences (independent of their origins) as well as origins of the differences, integrating gene and protein regulation with evolutionary history (Mangum and Hochachka, 1998). The discipline itself has changed over the decades since Peter began his research program, largely because of his efforts to

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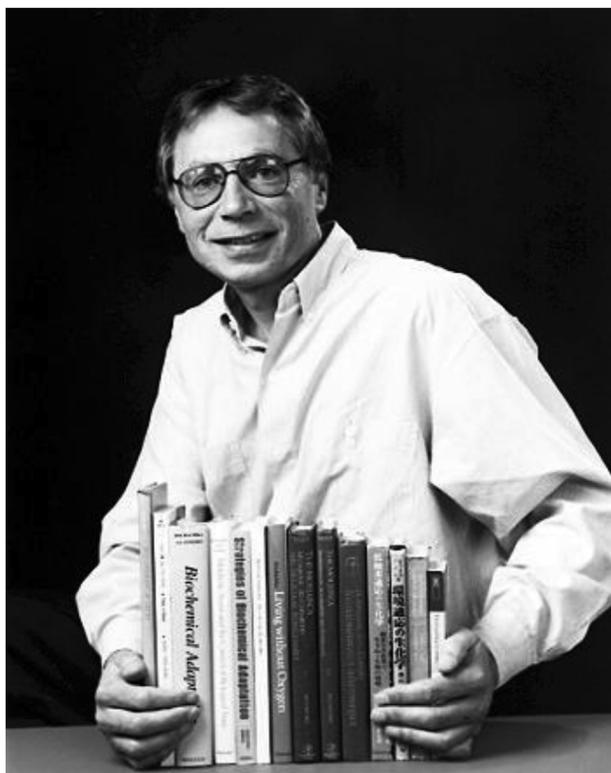


Fig. 1. Peter W Hochachka.

integrate and synthesize from disparate fields. The collection of papers in this special issue reflects this foundation in mechanistic biochemistry, but with an emphasis on integration across levels of biological organization and awareness of the role evolution plays in the origin of diversity of physiological capacities. As a framework for reviewing Peter's contributions to the discipline, we have organized this paper into themes reflecting the organization of the satellite symposium: temperature, hypoxia, bioenergetics, evolution of metabolism, and applied biochemistry. These themes reflect Peter's research interests and are presented in an approximately chronological order although several (hypoxia, bioenergetics and evolution of metabolism) were continuous through his career.

2. Temperature

Peter's predecessors realized that temperature was one of the most important abiotic factors influencing organismal performance. One of Peter's enduring legacies is in uncovering the underlying biochemical mechanisms that link cellular and whole animal temperature sensitivities.

Temperature has pervasive influences on organisms through a combination of physicochemical effects on molecular interactions and emergent effects arising from living systems striving for homeostasis. Changes in temperature of fluids – from external water to internal

compartments – modify solubility and diffusivity of gases and salts, the pH of neutrality, density and viscosity. Changes in temperature also influence weak bonds, leading to changes in the three-dimensional structure of macromolecules, particularly proteins, and assemblages of macromolecules, such as membranes. Since many of these changes affect enzymes and transporters, temperature also influences metabolic pathways and metabolite levels. Extreme increases in temperature can cause proteins to denature, triggering heat shock responses. Cold temperatures cause formation of ice, causing both osmotic stress and mechanical damage from ice crystals. In most ecosystems on our planet, thermal change is a given. Diurnal and seasonal changes are reasonably predictable but stochastic variation in environmental temperatures is the norm, not the exception. How animals adapt to thermal changes on these different time scales is a major focus of scientists in the field of comparative biochemistry and physiology. Peter's vision of biochemical adaptations integrated into organismal physiology is invaluable for current comparative physiologists to understand the potential impacts of climate change, which has given a new urgency to these studies (Somero, 2010; Somero, 2011).

Broadly speaking, the animal world is divided into endotherms and ectotherms. For both categories, changes in environmental temperature can be challenging, though the biochemical solutions to thermal environmental stress differ. Endotherms regulate their biochemistry and physiology to meet the demands for metabolic heat production to achieve homeothermy. Conversely, ectotherms face the brunt of thermal fluctuations in the environment. Changes in body temperature of ectotherms alter the properties of structural and metabolic proteins, biological membranes, and metabolic pathways. If thermal fluctuations are predictable (i.e. diurnal or seasonal), organisms may be able to acclimate to these changes, assuring metabolic integrity despite the fluctuating conditions. If, however, thermal changes are stochastic, the challenge of maintaining metabolic stability increases. When examining how temperature affects biochemical and metabolic function, fish are often the organisms of choice, as their body temperature follows that of the environment, except in select species where specialized circulatory arrangements allow regional heterothermy. However, a great many vertebrate and invertebrate models have been studied in a comparative context to compare and contrast how ectotherms cope with thermal challenges.

Peter's focus on energy metabolism and its regulation was immediately apparent upon entering his laboratory at UBC, in which the classic map of intermediary metabolism was prominently displayed. The spatters on the map attested to its long-term residency on those walls. This metabolic map focused on mammals, but generally was assumed to apply to all animals. Peter encouraged his students to question how well this map reflected metabolism in all animal groups and to reflect upon how flux through these pathways was controlled in different metabolic backgrounds. Mechanisms of metabolic regulation were a major focus of his research, starting with his kinetic studies of isolated enzymes and moving into the evaluations of metabolic changes during shifts from rest to activity, or from normoxia to hypoxia, with his many seminal contributions concerning the nature and regulation of metabolic depression.

Peter recognized that environmental conditions, such as

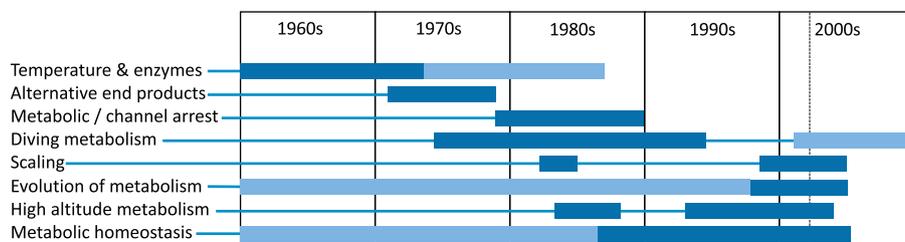


Fig. 2. Timeline of publication areas for Peter Hochachka with his students and colleagues. Darker bars show phases where the theme was a major emphasis of Peter and his lab.



Fig. 3. Researchers and crew of the 1967 Alpha Helix Expedition to the Amazon.

temperature and pressure, profoundly affected enzyme structure and function, and as a result, mechanisms of metabolic regulation. He reasoned that this would lead catalytic and regulatory mechanisms of enzymes from ectotherms to differ from those in mammals. Studies on the impact of temperature on glucose and acetate metabolism (Hochachka, 1968) and kinetic studies with lactate dehydrogenase from a range of fish species began his work as a solo, independent investigator (Hochachka, 1965; Hochachka, 1968; Hochachka, 1974). His enthusiastic and energetic application of a biochemical lens to zoological questions was a breath of fresh air in the discipline. This was also a time in the discipline where researchers started to delve deeply into multidisciplinary studies, exemplified by the Alpha Helix expeditions (Fig. 3). In most of these early studies, enzymes needed to be purified from tissues using tedious chromatography before their kinetic properties were examined as a function of temperature. The abundance of isozymes in salmonid fish - a consequence of gene and genome duplications in this lineage - provided an intriguing mechanism of adaptation to thermal change (Hochachka and Lewis, 1970; Baldwin and Hochachka, 1970; Moon and Hochachka, 1971; Moon and Hochachka, 1972; Somero and Hochachka, 1969). This work illustrated the importance of examining enzyme function using sub-saturating substrate levels, where allosteric regulators have their strongest effects. Out of these classic studies grew the idea that the thermal optimum of thermodynamic and kinetic properties would lie at the temperatures preferred by the organism (Gunderson and Stillman, 2014). Recognizing that the animals themselves experience changes in physiochemical characteristics, it also became clear to Peter that enzyme properties need to be evaluated under conditions that closely mimic those in the cell (pH, ionic composition, buffer type, temperature). Almost all of Peter's enzymology was with soluble, primarily glycolytic enzymes, but he did delve into membrane systems with his examination of temperature effects upon acetylcholinesterase (Baldwin and Hochachka, 1970; Hochachka, 1974).

Many of the earliest threads in Peter's research began via collaboration with his first postdoctoral fellow, George Somero. This work initially focused on temperature. In 1968, they co-authored *The adaptation of enzymes to temperature*, in which they demonstrated the activation of lactate dehydrogenase isozymes by decreasing temperature (Somero and Hochachka, 1968). Their first book, *Strategies of*

Biochemical Adaptation, was the first comprehensive exploration of how biochemistry differed among organisms in relation to environmental challenges (Hochachka and Somero, 1984). The book's successor, *Biochemical Adaptation* (Hochachka and Somero, 2002), brought the understanding of how environmental conditions influence biochemical function to a much wider public than generally attained through scientific papers. George Somero and his own progeny have continued to evaluate the many ways by which temperature affects biochemical function in ectotherms (Somero et al., 2017).

Peter continued building connections between temperature and metabolism by exploring causes and effects of shifts in metabolic rate, particularly the connection between declining temperature and metabolic depression. In many of these studies of metabolic depression, a decrease in metabolic rate accompanies a decline in body temperature. Influential studies from the Hochachka lab explored how hypothermia affects metabolic properties of ground squirrels in hibernation (Staples and Hochachka, 1997; Staples and Hochachka, 1998). In this special issue, the theme of temperature effects on metabolism continues with models exploring the integrative biology of hibernation in marsupials (Luu et al., 2018a; Luu et al., 2018b; Wijenayake et al., 2018a; Wijenayake et al., 2018b) and the naked mole rat, a heterotherm (Houlahan et al., 2018). This special issue also includes papers that examine proximal mechanisms of thermal regulation of metabolism (Callaghan et al., 2018) and the evolution of the ability to tolerate thermal challenges (Kelly et al., 2018). Any evaluation of the effects of temperature on organisms benefits from a broad perspective to generate mechanistic explanations for metabolic patterns and to explore the origins of the mechanisms.

3. Hypoxia

Throughout the 1960's, Peter was mainly interested in the thermal adaptations of the kinetic parameters of enzymes from poikilothermic organisms. By the early 1970's Peter began applying the same concepts of biochemical adaptation to better understand how animals coped with another environmental challenge: oxygen limitations. This transition was probably influenced by Ladd Prosser and Knut Schmidt-Nielsen, who were both interested in physiological adaptation to temperature and hypoxia.

Hypoxia is a condition whereby reductions in ambient or internal oxygen levels impact an organism's physiology and biochemistry. In mammals, hypoxia is typically defined as an oxygen level that triggers increased breathing and heart rates, and an accumulation of blood lactate. However, in many species, including select mammals, something different happens: breathing and heart rates decrease and elevations in blood lactate levels are attenuated. Hypoxic conditions are common in nature and can result from natural phenomena beyond an organism's control (deep ocean and oxygen depleted zones in lakes and oceans, water bodies with high biological oxygen demand, tidal ebb and flow, seasonal ice cover, or even subterranean/high altitude environments). They can also result from behaviour activities, such as prey capture/avoidance or breath-hold diving. Though some humans voluntarily expose themselves to environmental or functional hypoxia (e.g., pearl divers, high altitude explorers), hypoxia is usually associated with a clinical dysfunction, such as cancer, stroke, and cardiac infarction.

Peter's early work in hypoxia focused on invertebrate anaerobes, including gastrointestinal parasites and bivalve molluscs (Hochachka and Mustafa, 1972; Mustafa and Hochachka, 1973a; Mustafa and Hochachka, 1973b). However, there were only a few publications that suggested vertebrates were capable of hypoxia tolerance and these focused on tissue preparations. Peter conducted the first set of experiments that not only demonstrated that select diving vertebrates (sea turtle, seal, sea lion and porpoise; Hochachka et al., 1975b) had the ability to produce multiple anaerobic end products during breath-hold exercise, but showed that humans could also produce succinate and alanine as metabolic end products (Hochachka and Dressendorfer, 1976). Later, his lab would also discover the alternative anaerobic endproducts, alanopine and strombine (Fields et al., 1980). Throughout the 1970's and 80's, he expanded on the biochemical basis of hypoxia tolerance by studying diverse species collected from around the world: cephalopods (Fields et al., 1976; Hochachka et al., 1975a; Hochachka et al., 1977a; Storey et al., 1978), Amazonian fish (Hochachka et al., 1979), African lungfish (Dunn et al., 1981), goldfish (Shoubridge and Hochachka, 1980), deep-sea fish (Baldwin et al., 1975), turtles (Storey and Hochachka, 1974), echidna (Hochachka et al., 1984), seals (Murphy et al., 1980; Hochachka and Mottishaw, 1998), dolphins (Owen and Hochachka, 1974), and high-altitude adapted humans (Hochachka and Storey, 1975; Hochachka et al., 1996b; Hochachka, 1998). The central concepts and recurring themes were synthesized in his influential books (Hochachka, 1980; Hochachka and Somero, 1984; Hochachka and Somero, 2002). Peter's papers from this period involving the detailed analysis and synthesis of carbon flow from amino acids to the anaerobic end products, succinate and alanine, via reversal of succinate dehydrogenase are still commonly referenced by medical researchers (e.g., Chouchani et al., 2014), while the 1980 Science paper on ethanol production by anoxic goldfish (Shoubridge and Hochachka, 1980) is a mainstay of many comparative physiology courses and continues to be cited in works focused on the evolution of this pathway (e.g., Fagernes et al., 2017).

As Peter so clearly outlines in *Defense strategies against hypoxia and hypothermia* (Hochachka, 1986a), severe hypoxia (anoxia) creates a mismatch between ATP supply and ATP demand in most scenarios. The loss of oxidative phosphorylation reduces the efficiency of energy extraction from glucose by ~90%. The Pasteur effect, whereby glycolysis increases ~10-fold to maintain the rate of ATP production, is a means by which cells can produce enough ATP to maintain cellular homeostasis, including the electrochemical gradients across membranes. However, it became clear to Peter and others that those animals able to survive without sufficient oxygen for long periods cannot survive simply by elevating glycolytic rate, and must have a means of reducing total metabolic demands. The importance of ion gradients as a metabolic cost began to emerge as early as 1977, while Peter was on sabbatical with Warren Zapol, an anesthesiologist at the Massachusetts General Hospital. In the 1986 Science paper (Hochachka, 1986a) he

proposed that anoxia-tolerant species ought to have fewer ion channels per unit membrane or a mechanism to regulate ion channel permeability; this became the ion channel arrest hypothesis. He also proposed the complimentary metabolic arrest hypothesis, that stated anoxia-tolerant species ought to have mechanisms to reduce overall metabolic rate to conserve metabolic substrate (glucose) and prevent the accumulation of anaerobic end products (lactate). Peter and his lab continued to investigate the mechanisms of metabolic and ion channel arrest delving deeper into the cellular mechanisms in several model species including turtles (Buck et al., 1993; Land et al., 1993; Doll et al., 1991a; Doll et al., 1991b; Hochachka et al., 1996a; Keiver et al., 1992) and fish (Staples et al., 1995). Additional studies on mammals focused on fuel preferences during exercise following high altitude acclimation (McClelland et al., 1998), or in human populations native to high altitudes (Sherpas, Quechuas) which inevitably led to the synthesis of the field and the development of a novel hypothesis regarding their phylogenetic origins in our lineage (Hochachka, 1998; Hochachka et al., 1999).

The above noted collaboration with Warren Zapol in the 1970's on lactate handling in Weddell Seals (Hochachka et al., 1977b) also ignited a long-term interest in diving physiology (Fig. 4) that expanded upon pioneering studies of the mammalian dive response initiated decades earlier by Scholander, Irving, and colleagues. Peter had the sense that studies conducted on captive divers might not reflect what was happening in nature, where metabolic responses addressed the potential conflicting demands of diving and exercise (Hochachka, 1986b). As an alternative, Peter embraced remote sensing technologies to study free-ranging divers in the field (Guppy et al., 1986; Hill et al., 1987). These studies, and those that followed, not only helped to refine parameters of the dive response but led to a greater understanding of the physiological and behavioral specializations underlying extreme breath-hold endurance (Hochachka, 2000). By the mid-1990's, and again cognisant of the diminishing insights stemming from field studies, Peter's lab began to pursue two very different approaches in his search for the next breakthrough; these two approaches were also evident in his later work on human hypoxia tolerance. The first involved emerging medical diagnostic tools - magnetic resonance imaging (MRI) nuclear magnetic resonance spectroscopy (NMR) - to image and monitor internal structures and metabolites in real time during simulated dive and recovery bouts to provide an unparalleled understanding of several mechanistic and biochemical underpinnings of the dive response. In particular, these studies revealed new insights into blood flow and stroke volume dynamics of elephant seals while submerged, and helped elucidate the role of the hepatic sinus in regulating erythrocyte metering into the circulation (Thornton et al., 2001; Thornton et al., 2005). Up until this time it was commonly accepted that the myriad mechanistic components of the dive response were all "adaptations" (i.e. evolved and were



Fig. 4. Peter getting acquainted with diving mammals.

maintained under selection), though these qualitative assessments were based more on intuition than analytical scrutiny. A second approach thus championed by Peter (and others) was to employ modern quantitative tools of evolutionary biology to examine the evolution of the physiological mechanisms central to the dive response. Interestingly, this work identified a number of conserved non-malleable plesiomorphic reflexes (e.g. maximal bradycardia, peripheral vasoconstriction) that were recruited from capacities present in non-diving ancestors (Hochachka et al., 1999; Mottishaw et al., 1999), and hence did not evolve as a specific response to diving. By contrast, other traits (e.g. body size, spleen size) are strongly correlated with diving capacity, and thus predominantly underlie variations in diving abilities among species. An additional benefit of this approach recognized by Peter and co-workers was that this could “shed light on both the branches and tips of the phylogenetic tree” (Mangum and Hochachka, 1998), thus inspiring a new generation of studies focused on ancestral reconstructions of ancient physiological phenotypes in diving mammals (Mirceta et al., 2013).

This special issue includes papers that continue the tradition of using diverse models to explore the mechanistic basis of hypoxia tolerance. Turtles continue to be important models for studying hypoxia tolerance in terms of the role of ion channel regulation (Buck and Pamerter, 2018) and oxidative metabolism (Gomez and Richards, 2018). The utility of model systems, such as zebrafish (Dhillon and Richards, 2018) and mole rats (Kirby and Pamerter, 2018) reveals how metabolic function is controlled in real time. Hypoxia is an area that is also ripe for cross-over between disciplines, with mechanistic work explaining hypoxic effects on animals in the natural world (e.g., Seibel et al., 2018) and animal research providing insight into more medically oriented questions such as design in cardiopulmonary systems (e.g., Walker and Land, 2018).

4. Bioenergetics

Bioenergetics was at the core of much of Peter's work. His earliest articles studied energy reserves and carbohydrate metabolism in fish and lobsters (Hochachka, 1961a; Hochachka et al., 1962), which led to his career-long interest in bioenergetics and biochemical adaptation. Throughout his career, Peter approached bioenergetics from a broad perspective, combining available technical approaches and integrating across levels of organization. His work on metabolic scaling, exercise metabolism and high altitude adaptation all focused on the intricate details of metabolic regulation.

Comparative physiologists are frequently confronted with the effects of body size on physiological properties of organisms. The study by Emmett and Hochachka (1981) showed that scaling of metabolic rate in mammals trickled down to the cellular level, as had been shown in fish (Somero and Childress, 1980). This paper demonstrated reciprocal relationships of aerobic and anaerobic enzyme activities consistent with the differential effects of body size on sustained versus burst locomotion. His contribution on this classic physiological conundrum was revived in the early 2000s with papers offering mechanistic explanations for the metabolic scaling patterns (Darveau et al., 2002; Hochachka et al., 2003). Peter's broad thinking on the subject even made him plot Gaia's metabolic rate on the mouse to elephant curve (Hochachka and Somero, 2002) but mostly to make the point that these plots can be insensitive to variation at their extremes.

Despite more than a century of investigation, the scaling of metabolic properties of animals remains a major ongoing topic in comparative physiology and biochemistry. Peter's academic progeny continue to integrate the effects of body size on energetics. Subsequent studies by Peter and his students have explored the underlying cellular/genetic basis for establishing the metabolic phenotype in fish (Burness et al., 1999) and mammals (Kocha et al., 2011) in relation to body size. The work of Rodríguez et al. (2015) recently tackled the evolution of phospholipid composition of muscle cellular membranes, proposed to

be a metabolic pacemaker by Hulbert and Else (2000). Rodríguez and colleagues not only showed that muscle membrane composition and flight metabolic rate scale with body mass across species of tropical bees, but also teased apart the correlated evolution of these traits among closely related species. In this special issue Rodríguez et al. (2018) further investigate how species diversity in muscle membrane composition is associated with body mass and thermoregulatory capacity in endothermic poikilothermic insects.

Much of Peter's work examined how tissues and cells respond to perturbations in energetic homeostasis. There are a number of paradigms that can be used to explore the consequences of mismatches in ATP supply and demand. The relationship is critical in skeletal muscle, where the transition from rest to intense exercise can increase metabolic rates by up to 500-fold (Hochachka and McClelland, 1997). Likewise, the metabolic challenges associated with high altitude hypoxia involve a combination of metabolic and evolutionary solutions (Hochachka, 1985). When lowlanders experience increasing altitude, they show a progressive decline in aerobic scope to a point where performance is severely impaired (Hochachka, 1991). Peter articulated key features of a hypoxia defense mechanism that would allow mammals to maintain sufficient levels of aerobic performance at high-altitude: 1) redistribution of cardiac output to working tissues, 2) a switch to substrates with favourable yields of ATP per mole of oxygen or per mole substrate, and 3) an increase in the flux of oxygen from lungs to mitochondria (Hochachka, 1985). These strategies should reflect changes in the structural components of O₂ and substrate supply pathways and/or in the regulation of flux through the various steps as they converge at sufficient rates to support aerobic ATP production by mitochondria. This includes sufficient rates of alveolar ventilation, lung diffusive capacity and cardiac output for convective transport of oxygen. This is coupled with the appropriate mobilization of substrates from storage tissues for delivery to working muscles. Working on humans meant he had to use relatively non-invasive methods to uncover these defense strategies and he was one of the first comparative physiologists to embrace nuclear medicine imaging and diagnostic technologies outside a clinical setting. Peter and his collaborators demonstrated that highland natives from Peru (Quechua) and Nepal (Sherpas) show metabolic rearrangements of cardiac and skeletal muscles beneficial for life at high elevation (Hochachka et al., 1996b; Matheson et al., 1991). These data also helped Peter speculate on the mechanistic underpinnings for the long-standing observations (Dill et al., 1931) that blood lactate accumulation is reduced during exercise in hypoxia in highland natives and acclimatized lowlanders, which he and others termed the lactate paradox (West, 1986; Hochachka, 1988). Peter's influence on the field of high altitude physiology has been long lasting. Advancements in respirometry have allowed a more detailed assessment of mitochondrial phenotypic plasticity and adaptation in lowland and highland natives (Murray and Horscroft, 2016; Mahalingam et al., 2017). Studies continue to build on Peter's theories and discoveries with a new appreciation for adaptive variation in adult acclimatization responses (Storz et al., 2010).

Peter's work on bioenergetics most often focused on cellular metabolism. One of his last contributions (Hochachka, 2003) aimed at generating discussion on the apparent conflict between metabolic homeostasis and metabolic regulation. This topic had preoccupied him for some time (Hochachka, 1994b), leading to many reflections on the subject (Hochachka et al., 1998; Hochachka and Somero, 2002). He was trying to partition how various mechanisms contributed to metabolic regulation of pathways during large changes in energy flux such as the transition from rest to activity. Mechanisms that he considered included: 1) traditional effects of substrate and product concentration on reaction rates, 2) allosteric regulation of enzymes, 3) the effects of phosphorylation, protein interaction, redox state, Ca²⁺ and translocation on protein activity, and 4) metabolic control analysis (Hochachka and Somero, 2002). Peter also recognized that intracellular structure and organization must play a role in channelling substrates for

regulation of different pathways (Hochachka, 2003).

Metabolic regulation remains central to the research of many of Peter's academic progeny. Mathers et al. (2017) recently showed that hibernating ground squirrels reduced electron flux substantially, accompanied by lower enzyme activity but no changes in protein content, implicating post-translational modifications. The central role that mitochondria play in energetics is now being revisited for their contribution as regulators of reactive oxygen species (Treberg et al., 2015; Munro and Treberg, 2017; Treberg et al., 2018). Moreover, mitochondrial bioenergetics research is expanding from strictly mechanistic biochemistry to studies tackling microevolutionary patterns and their underlying basis (Pichaud et al., 2013a; Pichaud et al., 2013b). This special issue also includes papers that explore the mechanisms used by animals to transition into hypometabolism (Giraud-Billoud et al., 2018; Luu et al., 2018a; Luu et al., 2018b; Wijenayake et al., 2018a; Wijenayake et al., 2018b). It is clear that the study of bioenergetics is more integrative than ever. Peter would find it more than just “very interesting, very interesting” and probably “fascinating”.

5. Evolution of metabolism

Energy is the fundamental currency of life, and thus the ability to acquire energy and appropriately allocate it to processes such as growth, maintenance and reproduction is thought to be a key determinant of fitness (Brown et al., 1993). Because of this fundamental importance of energy in biology, it is no surprise that the study of energy metabolism, or the various pathways by which organisms obtain energy from nutrients and use this energy to perform their functions, has been an important theme in Peter's work, and indeed, comparative biochemistry and physiology since the beginnings of the field.

The central importance of energy metabolism to comparative biochemistry can be clearly seen in Peter's work. Although he addressed a wide variety of questions throughout his career, many of these questions were united by their emphasis on the importance of the enzymes and pathways of central energy metabolism, and how these mechanisms allow animals to cope with the challenges of life in a wide variety of environments. This focus on metabolism is evident from his earliest work on glycogen reserves in rainbow trout (*Oncorhynchus mykiss*) (Miller et al., 1959; Hochachka, 1961b) and continued throughout his career even to his work with his last graduate student Charles Darveau and colleagues on energy metabolism in Orchid bees (e.g. Suarez et al., 2005a; Suarez et al., 2005b; Darveau et al., 2005a; Darveau et al., 2005b). This study of energy metabolism was conducted within the broad evolutionary framework that forms the fundamental structure of comparative biochemistry and physiology.

In a seminal review, Charlotte Mangum and Peter Hochachka (Mangum and Hochachka, 1998) clearly articulated the two central goals of comparative biochemistry and physiology as “(1) elucidation of mechanisms and their adaptive significance, and (2) elucidation of the evolution of mechanisms and adaptations”. These two goals outline the complementary ways in which evolutionary biology plays a role in comparative biochemistry and physiology, and both types of studies are represented within the work of Peter and his scientific lineage and can be seen in his studies of the evolution of metabolism.

The research program to achieve the goal of elucidation of mechanism can be termed mechanistic/adaptational physiology and biochemistry (Mangum and Hochachka, 1998). The biological diversity generated through adaptation to particular (often extreme) environments provides experimental material that allows the investigator to gain insight into physiological mechanisms. This approach is a specific application of the Krogh Principle: *for many problems there is an animal on which it can be most conveniently studied* (Krebs, 1975). This mechanistic/adaptational approach has been a major component of comparative biochemistry and physiology, and, as clearly articulated by George Somero, “analyses of these natural curiosities...by means of the comparative method have often led to major new concepts about the

most basic aspects of physiology” (Somero, 2000). One potential criticism of the mechanistic/adaptational approach is that the mechanisms being studied are sometimes simply assumed to be adaptive (see Bennett, 1997 for a discussion of this issue). However, it is not clear that it is necessary to formally demonstrate that a particular trait represents an adaptation for it to be useful in the study of mechanism. On the other hand, mechanistic/adaptational physiologists should always be on guard against uncritical thinking and writing about the putative adaptive significance of the mechanisms that they study.

The research program to achieve the second goal of comparative biochemistry and physiology (the elucidation of the evolution of mechanisms and adaptations) was simply termed “evolutionary physiology and biochemistry” by Mangum and Hochachka (1998). In this research program, the goal is to understand how the diversity of lineage-specific physiological and biochemical mechanisms came to be. Thus, evolutionary physiology has a fundamentally different logical structure from mechanistic/adaptational physiology, as it primarily asks questions about ultimate causation (“Why?” questions), rather than proximate causation (“How?” questions) (Mangum and Hochachka, 1998). They go on to outline three broad categories of approaches to questions in evolutionary physiology, which they term the “gene-to-physiology approach”, the “use of interindividual variation”, and “the interspecies comparative approach”. Each of these approaches can be found within the work of Peter and his students.

Peter's early work on the evolution of metabolism can be clearly classified as belonging to the “mechanistic/adaptational” research programme. In this work he took advantage of the diversity produced by evolution to identify novel pathways of energy metabolism. Using organisms ranging from the chambered nautilus (Hochachka et al., 1977a) and the goldfish (Shoubridge and Hochachka, 1980), he revolutionized our understanding of pathways of anaerobic metabolism in animals, demonstrating that animals could use a variety of other biochemical mechanisms of energy supply as an alternative to the fermentation of glucose to lactate in anoxia (Hochachka and Mustafa, 1972). These seminal discoveries laid the groundwork for ongoing research into the strategies that animals use to survive periods of oxygen lack in a wide diversity of organisms (Pamenter et al., 2011; Park et al., 2017; Stecyk et al., 2017; Warren and Jackson, 2017; Regan et al., 2017; Hand et al., 2016; Vornanen and Haverinen, 2016; Welker et al., 2016).

Although Peter is probably best known for his application of the mechanistic/adaptational approach in comparative biochemistry, he also made a wide variety of contributions that can be classified as falling within the paradigm of evolutionary physiology. He made excellent use of inter-individual and inter-population variation in his studies of high-altitude adaptation in humans (Hochachka, 1992; Hochachka et al., 1999; Rupert and Hochachka, 2001; Hochachka and Rupert, 2003). Peter also applied phylogenetically informed interspecies comparative approaches to his studies of energy metabolism through investigations of diving in marine mammals (Hochachka and Mottishaw, 1998; Mottishaw et al., 1999; Hochachka, 2000) and in understanding the control of flux using an interspecific comparative approach in Orchid bees (Darveau et al., 2005a; Darveau et al., 2005b; Suarez et al., 2005a). These early papers embraced the transition to incorporating more rigorous evolutionary considerations into comparative physiology.

Although mechanistic/adaptational physiology and biochemistry and evolutionary physiology and biochemistry are often thought of as distinct approaches, as can be seen from Peter's work, and as he himself clearly pointed out (Mangum and Hochachka, 1998) “an integration of the two approaches seems to present the most productive trajectory [for comparative biochemistry and physiology] into the next century”. Indeed, it seems appropriate to close this short introduction to the study of the evolution of metabolism with Peter's own words: “the integrated approach advocated here ensures that, in the interplay between mechanistic/adaptational physiology and biochemistry, on the one hand,

and evolutionary physiology, on the other, there is a bidirectional flow of information and ideas" (Mangum and Hochachka, 1998). Indeed many evolutionary biologists today are interested in the mechanisms that underlie adaptation. This marriage of physiology and evolution was promoted by Peter.

Many of the papers in this special issue reflect Hochachka's approach of using variation among closely related groups to explore underlying mechanistic differences in metabolism, including the evolution of aerobic capacity in fishes (Dalziel et al., 2018) and hemoglobin structure and function (Campbell et al., 2018). The special issue also includes papers that explore the broader evolutionary patterns in metabolic enzymes and pathways, including AMP-activated protein kinase (Craig et al., 2018) and cytochrome c oxidase (Little et al., 2018), which are both found in all animals. McDonald et al. (2018) explore the diversity in metabolic fuels used in animal tissues, with a special focus on underappreciated "alternate" fuels. Seebacher (2018) examines the overarching patterns of metabolic pathway regulation, integrating energy sensing, hormones and signal transduction pathways. The role of epigenetics in determining the metabolic phenotype is becoming increasingly important, influencing the links between transcriptional plasticity and biochemical adaptation (Best et al., 2018).

6. Applied biochemistry

While the core of Peter's interests was in the study of temperature and hypoxia, he was keenly aware of the importance of building bridges between disciplines, and he strived to do work that had broader ramifications (e.g. medicine, environmental science). As a result, many of his trainees took from Peter's lab very broad research interests, and used these skills to contribute in disciplines that were peripheral to Peter's main interest; a fact that Peter would have appreciated, if not expected, from his trainees! However, in all his trainees he instilled an appreciation of the power of comparative biochemistry, and the August Krogh principle in particular, at uncovering fundamental biological patterns and processes. In fact, the diversity of research questions and model organisms pursued by his trainees is a testament to his influence, and is exemplified by the papers in this issue.

Peter and his trainees' extensive work on hypoxia tolerance and metabolic depression has had wide-ranging medical applications. For example, mechanisms utilized during hibernation and dormancy have informed development of drugs to arrest the heart during cardiac surgery (Dobson and Letson, 2015), and allowed for the creation of a "pharmacological tourniquet" for reducing internal bleeding (Letson and Dobson, 2017). The latter has obvious applications for trauma in military combat and in remote locations. Peter's seminal paper, *Defense strategies against hypoxia and hypothermia* (Hochachka, 1986a) has had a particularly lasting impact on medicine. This paper has been cited over 800 times, with recent citations including such diverse medical topics as enhancing survival of stem and progenitor cells in cord blood (Vlaski et al., 2014), lung preservation (Machuca et al., 2013), and protection of individuals from cosmic radiation during space exploration (Cerri et al., 2016).

In one of his more unusual medical contributions, Peter recognized the parallels between hypoxia tolerant animals and the metabolic profiles of cancers (Hochachka et al., 2002). It had been known for decades that many cancer cells were tolerant of low oxygen levels, produced large quantities of lactate via anaerobic metabolism (Warburg, 1956), and accumulated fatty acids via de novo fatty acid synthesis (Kuhajda, 2000). Peter drew on his understanding of animal hypoxic defense strategies and hypothesized that the upregulation of the fatty-acid synthase pathway was a mechanism cancer cells use to regulate redox balance under hypoxic conditions (Hochachka et al., 2002). This paper remains frequently cited in relation to cancer metabolism in general and prostate cancer specifically. For example, it was also the inspiration for a study by one of his former students on the metabolic phenotype of prostate cancer cell lines (Higgins et al., 2009).

Thus, as often seemed to be the case, a provocative idea by Peter prompted a subsequent (and often sustained) flurry of research activity.

Environmental degradation, particularly in aquatic environments, is associated with extremes in temperature and oxygen. From his earliest work, Peter recognized the importance of using physiology and biochemistry to promote a deeper understanding of ecological issues. For example, in a now prescient paper published in the ecology/evolutionary biology journal *American Naturalist* (Hochachka, 1964), he argued that to maximize the predictive power in studies of ecological competition, it is necessary to ask both *how* populations differ at a physiological level, and *why* they differ (i.e., what ecological factors are driving the differences in physiology)? These ideas have come full circle and have been incorporated into the emerging discipline of *Conservation Physiology*, contributing to the on-going discussions surrounding climate change, and the 'winners' and 'losers' associated with such changes (Somero, 2010).

Environmental degradation can also be directly associated with human impacts when considering the consequences of xenobiotics within aquatic environments. Although Peter did not directly study these diverse human manufactured chemicals, many of these xenobiotics are known to target the same metabolic pathways and physiological responses affected by physiological effectors (e.g., Fent et al., 2006). Peter's early studies on endocrine effects (Hochachka, 1962) and the impacts of temperature on metabolic branch points (Hochachka, 1968) using tissue slices, advanced the use of tissue preparations for metabolic, but most importantly, for toxicant (e.g., Behrens et al., 2001) and endocrine (e.g., Mommsen and Moon, 1989) studies. Toxicants and tissue preparations are used widely throughout the literature, with many of Peter's trainees making direct contributions (e.g., Al-Habsi et al., 2018).

Molecular tools are now commonly used in all modern research labs, but Peter's career just missed this major innovation. However, Peter's very early work on LDH isozymes lead him to propose an evolutionary phylogeny for these in both salmonid and cyprinid fishes, where patterns are generally extensive (Hochachka, 1966). Though much of Peter's work was conducted prior to the widespread use of molecular biology, he would have been pleased to see how his lineage evolved to incorporate the diverse tools of molecular biology to understand factors that impact both adaptive and disease states.

Peter's research involved choosing species primarily because of their unique biochemical traits but he also recognized the intrinsic appeal of studying models that were also economically important. In setting up a lab on Canada's Pacific Coast, there were a multitude of reasons to study the metabolic biochemistry of migratory salmon. A striking demonstration of the links between metabolic processes and life history can be seen in the 1100 km anorexic migration of sockeye salmon, on the Pacific coast of North America (Mommsen et al., 1980; French et al., 1983; reviewed in, Mommsen, 2004). Peter and his trainees showed that fish lost almost all their lipid and about half their white muscle mass over the 6-week migration, with carbohydrates reserved for brief bursts of exercise, much as the final activity of a marathon runner 'hitting-the-wall' (Rapoport, 2010). Importantly, many of the catabolized fats and proteins became the building blocks required for oogenesis, providing a biochemical mechanism for the classic life-history trade-off between reproduction and survival. Others have expanded upon these early studies to explore the metabolic phenotype of sockeye salmon muscle in relation to migration (Miller et al., 2009; Morash et al., 2013), and the impacts of warming temperatures on aerobic scope (Eliason et al., 2011).

As impressed as Peter was by salmon migration, he would have been astonished by the more recent discovery of non-stop transatlantic migrations by small birds (Gill et al., 2009). Identifying such astonishing feats of endurance has only become possible with the increasing miniaturization of satellite tags, allowing the movement of individuals to be tracked in real time. In fact, remote monitoring was one of Peter's predictions for the future trajectory of comparative physiology and

biochemistry. Recent development of miniaturized telemeters now allows the energetic costs of activity to be estimated using heart rate (Bishop et al., 2015), as well as real-time monitoring of changes in body temperature (McCafferty et al., 2015). Such is the logical extension of the on-board computers pioneered by Peter and his colleagues to study the diving physiology of free-ranging seals (Guppy et al., 1986). The recent measurement of uric acid in vivo during flight in pigeons represents a very exciting development, as it may allow protein catabolism to be studied in vivo (Gumus et al., 2015); “taking the lab to the field,” as Peter would say.

The extensive work by Peter and his offspring on comparative exercise biochemistry allowed for identification of evolutionarily conserved patterns, but also provided a framework against which exceptions could be explored. For example, although protein catabolism is important in both migrating salmon and birds, different functional reasons underlie the use of protein in the two systems. In contrast to salmon, small migrating songbirds risk desiccation, which can be ameliorated by catabolizing their muscles and organs to liberate water (Gerson and Guglielmo, 2011). Additionally, during high intensity flight, birds rely on fat oxidation, contrasting with the reliance on carbohydrates by exercising mammals, as proposed by Peter and his students (McClelland et al., 1999). The adaptive significance of such deviations could be explored through formal multispecies comparative analyses. Although Peter was initially skeptical of incorporating phylogenies into his studies, he later came to realize that such analyses had an ability to reveal unexpected patterns of evolutionary change (Mottishaw et al., 1999).

7. Conclusions

It is not all that uncommon for the principal investigator of a large lab to be a coauthor on dozens of papers a year. Peter's publication record, though impressive, is not unprecedented in that regard. However, he did publish an average of one solo author paper a year between 1983 and 2003. Each of these papers was an enigmatic mixture of scholarly review, novel synthesis and opinion piece. He had a knack for transforming literature reviews for grant applications into Science papers. He also published a remarkable seven books, each of which was a unique synthesis of disparate fields encompassed by comparative physiology and biochemistry (Fig. 2). Those who knew him marvelled at his ability to synthesize information, often on the fly, bringing together ideas from broad fields to provide a perspective that served as a foundation for generating testable hypotheses. This symposium focused on his trainees in recognition of his role in “peeling the reptilian scales from our eyes” to see beyond somewhat tedious experiments to challenge our imagination and put the work into broader relevance within the field.

Peter Hochachka made a great many contributions to the field of comparative physiology, but in addition to the body of research itself, he had a remarkable track record in training students, and a very high proportion of these pursued careers in academia in Canada and around the world. Peter was a wonderful pack leader (Fig. 3). By our count, Peter supervised 16 PhDs, 31 PhD and 11 MSc candidates over his 37 years at the University of British Columbia. Of his graduate students, 27 obtained positions as faculty members at universities, supervising students of their own. From this cohort, 17 held or still hold faculty positions at Canadian Universities. As of June 2017, this first generation of trainees (F1) had themselves supervised more than 750 individuals (Peter's F2 generation). Many of the F1s have years of productivity remaining, so it seems likely that the number of F1 and F2 trainees will surely surpass 1000. In total, 57 F1s and F2s assumed faculty positions in Canada, a country with less than 100 universities. As these researchers go on to train their own students, the number of direct academic descendants of Peter will number in the thousands, which is an astounding imprint on science.

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