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Review

Assessing physiological complexity

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Summary

Physiologists both admire and fear complexity, but we have made relatively few attempts to understand it. Inherently complex systems are more difficult to study and less predictable. However, a deeper understanding of physiological systems can be achieved by modifying experimental design and analysis to account for complexity. We begin this essay with a tour of some mathematical views of complexity. After briefly exploring chaotic systems, information theory and emergent behavior, we reluctantly conclude that, while a mathematical view of complexity provides useful perspectives and some narrowly focused tools, there are too few generally practical take-home messages for physiologists studying complex systems. Consequently, we attempt to provide guidelines as to how complex systems might be best approached by physiologists. After describing complexity based on the sum of a physiological system's structures and processes, we highlight increasingly refined approaches based on the pattern of interactions between structures and processes. We then provide a series of examples illustrating how appreciating physiological complexity can improve physiological research, including choosing experimental models, guiding data collection, improving data interpretations and constructing more rigorous system models. Finally, we conclude with an invitation for physiologists, applied mathematicians and physicists to collaborate on describing, studying and learning from studies of physiological complexity.

Key words: physiology, complexity, experimental design, modeling, information theory, chaos.

Introduction: what is complexity?

Physiological systems are inherently complex, and often far more complex than usually appreciated. This truism, which in many ways comprises a contrary position to Occam's razor, has emerged from repeated observations that physiological systems are best assumed to be *more* complex than at first apparent until we can demonstrate otherwise. For example, the physiological regulation of water fluxes across biological membranes was long assumed to be based on relatively simple osmotic forces and bulk flow – until, that is, we learned the important role of the family of aquaporins in regulating membrane water permeability (e.g. Agre et al., 1995). In fact, Agre et al. (1995) were sufficiently confident to write '*The long-standing biophysical question of how water crosses plasma membranes has been answered by the recent discovery of the aquaporins.*' Aquaporins were then evoked to explain everything we hadn't previously known about water fluxes – until, that is, we learned of 'water pumps' in the form of a K^+/Cl^- cotransporter in the choroids plexus epithelium, the H^+/lactate cotransporter in the retinal pigment epithelium, the intestinal Na^+/glucose cotransporter (SGLT1) in enterocytes and the renal Na^+/dicarboxylate cotransporter in *Xenopus* oocytes, all of which can operate in the absence of both favorable osmotic gradients and aquaporin expression (for reviews see Zeuthen, 2000; Loo, 2002). These water pumps emerged as an alternative mechanism for water transport – until, that is, we learned that the functions of water pumps and aquaporins can be interactive and complementary (e.g. Zeuthen, 2002). Thus, 'until, that is, …' seems to be the most useful paradigm as we await the next installment in the description of transmembrane water transport!

Our historical progression in understanding of water flux in guts, kidneys, eyes and other organs exemplifies the fact that complexity riddles everything we do as physiologists. As physiologists, we may admire complexity, but we also fear it, because inherently complex systems are more difficult to study and often far less predictable. Moreover, few of us can actually offer any definition of this ubiquitous characteristic we call 'complexity'. Just as we observe that Monet's water colours contain great beauty, but are at a complete loss to quantify the metrics we have used in making this observation, we acknowledge that physiological systems contain great complexity, yet we can't clearly express the metric we use to

come to this conclusion. Even if physiologists piece together some definition of complexity involving 'patterns of afferent pathways' or 'numbers of possible target tissues for a hormone' or 'elements of motor responses', we are typically unable to indicate quantitatively, or even qualitatively, how complexity changes as the number of afferents, target tissues or motor responses changes. Nor can we readily point to any applied mathematical or statistical tools to help us deal with this ill-defined complexity. Finally, few physiologists in any precise analytical way actually shape our experimental design specifically based on the perceived degree of complexity of the systems we work on.

We will argue in this essay that an appreciation and awareness of the implications of complexity is essential for a deeper understanding of the physiological systems we study. To achieve this deeper understanding, we feel it is necessary to temporarily stand back and take a modestly philosophical view of the field of physiological complexity. Existing paradigms have tended to serve a relatively narrow physiological audience and are not readily exported to other sub-disciplines. For example, there is considerable interest in complexity as revealed in time series analysis of heart rates, endocrine secretion and other physiological phenomena (e.g. Richman and Moore, 2000; Meyer and Stiedl, 2003; Costa et al., 2005). Yet, such non-linear dynamic analyses and related attempts to define complexity provide little insight and even fewer tools to a physiologist working on, for example, the complex interactions of multiple hemoglobins on *in vivo* blood oxygen transport.

We also strongly advocate that additional interdisciplinary work involving mathematicians, physicists and physiologists (and, by extension, all biologists) is needed to increase our understanding and exploration of complex physiological systems. The first step in such collaboration is understanding each discipline's vocabulary. Indeed, even definitions are highly problematic, because common uses of words such as 'complexity' and 'chaos' are often at variance with the more precise and narrower definitions used by mathematicians. Thus, while the jargon appears the same, the ideas being discussed in disciplines may be quite different. For example, from a mathematical perspective, the behavior of a truly chaotic system cannot be precisely predicted in practice, whereas a system considered chaotic by a biologist may be viewed as predictable if only enough data are collected. A mathematician might then counter by saying that the biological system was not complex but merely complicated. While these semantic issues seem pedantic, they have presented substantial barriers to informed collaboration between disciplines. To assist this bridging process, let us now briefly explore some mathematical views of complex systems before moving on to practical implications for physiologists.

A mathematician's view of complexity

Complex systems science encompasses a wide array of study – from fractals to chaos theory to neural networks. Despite the considerable literature on complex systems in mathematics, physics and, increasingly, biology, there is at present not only no unifying theory of complexity but also no consensus definition of what it means for a system to be complex. There is, however, the general conviction that the various manifestations of complexity are examples of some fundamental paradigm. The study of specific systems, as well as the broader pursuit of an integrated framework, has already produced an essential change in our understanding of how systems operate and interact with one another. This section reviews some of the mathematical approaches to complexity that have led to this change. Of course, in such a short discussion, many topics in complexity will be neglected. The emphasis will be on developing principal themes in complexity and considering their relevance to physiology. An introduction to various mathematical aspects of complexity can be found in Bossomaier and Green (2000), Cambel (1993), Wolfram (2002) and Kauffman (1993).

Chaotic systems

A highly productive area in complex systems science is chaos theory. Definitions of a chaotic system differ across disciplines, but two characteristics occur in most descriptions. First, in chaotic dynamical systems there are trajectories that do not converge to fixed points or become periodic. (A trajectory in this context is the state of a system as a function of time.) Rather, the trajectories exhibit aperiodic, highly irregular behavior. Fig. 1 shows trajectories (in this case, time series) for a set of hypothetical systems. Fig. 1A shows a system that converges to a fixed state; the system in Fig. 1B is periodic; and Fig. 1C gives the trajectory for a chaotic system. Ventricular fibrillation is a commonly cited physiological example of a chaotic trajectory.

The second basic property of chaotic systems is what mathematicians call 'sensitive dependence' to initial conditions. This means that small differences in the initial state of a system can lead to dramatic differences in its long-term dynamics (popularly known as the 'butterfly effect'). Thus, limited precision in measuring the initial state precludes accurate predictions of future states of such systems. The inaccuracy of long-range weather forecasts is a popular example of sensitive dependence in chaotic systems. In physiology, an example would be the pattern of change in blood and lung gases and acid–base balance during diving in a vertebrate. Arterial and alveolar oxygen (P_{O_2}) and carbon dioxide (P_{CO_2}) partial pressures and blood pH are rarely exactly the same at the beginning of consecutive dives and, not surprisingly, physiologists have long appreciated that the precise pattern of change in respiratory gases and blood acid–base balance from dive to dive is very difficult to predict (Burggren and Shelton, 1979; Butler and Jones, 1982, 1997; West et al., 1989; Castellini and Kooyman, 1989).

As early as 1890, Henri Poincare reported sensitive dependence while investigating the classic 'three-body problem'. He observed that small differences in the initial state of the set of equations governing the interactions between the

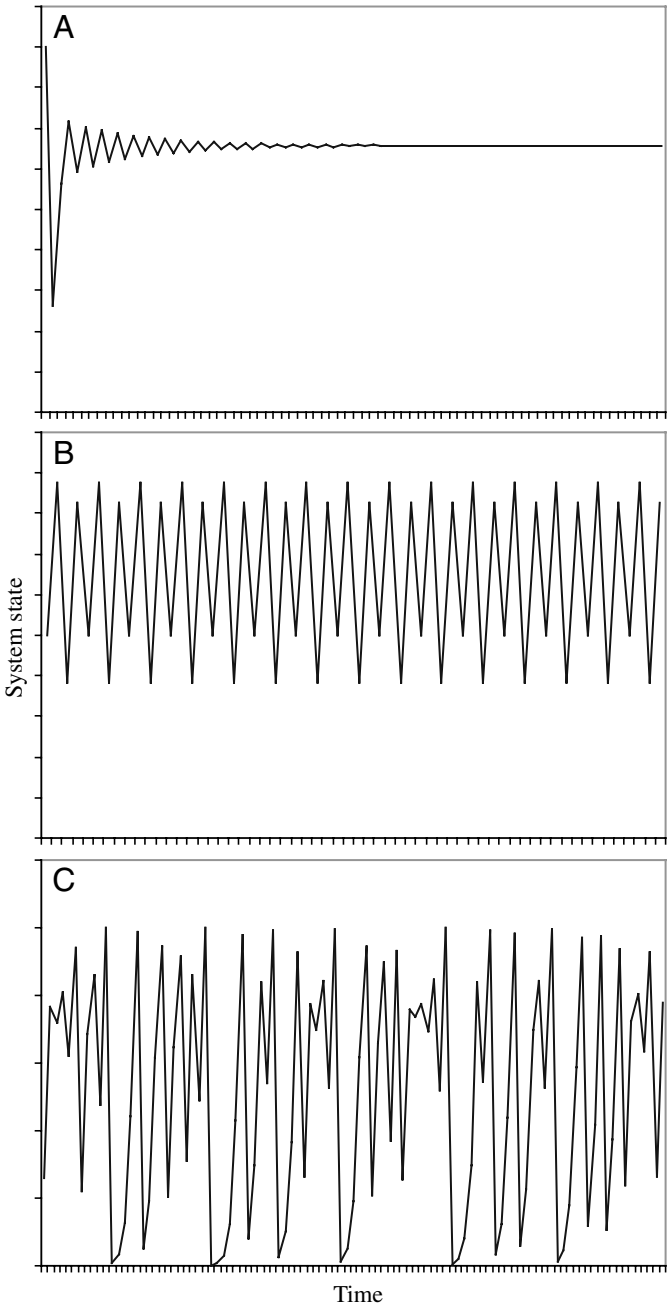

Fig. 1. Time series trajectories. (A) System behavior converges to a fixed state. Such a pattern is commonly seen in the assessment of phase lag and damping in blood pressure recording systems. (B) Periodic system. Such patterns are evident in stable heart rate recordings. (C) Aperiodic trajectory characteristic of a chaotic system. Patterns like these are characteristic of the abnormal beating of hearts in fibrillation.

earth, moon and sun produce large differences in the subsequent time dynamics of this astronomical system. One way to characterize this sensitive dependence is to determine the rate of separation of trajectories starting out adjacent to one another. In chaotic systems, trajectories separate at an exponential rate. Note that a large number of system components is not necessary for sensitive dependence to exist – Poincare (1890) only needed three. Moreover, sensitivity to initial conditions can arise even when the system description is relatively straightforward (although some nonlinearity needs to be present).

The link between sensitive dependence and the ability to predict future states of the system naturally suggests a connection between complexity and information theory.

Information theory

If a system does exhibit sensitivity to initial conditions, then imprecise knowledge of those conditions leads to increased uncertainty in the future states of the system. Another method for characterizing this sensitivity is to quantify the information gained or uncertainty removed from observing the system. To illustrate the basic idea, suppose that you knew a roulette wheel was rigged to always deliver the number 17. No uncertainty on the outcome of a spin of the wheel would be removed by going through the process of spinning it. In this case, the entropy or amount of uncertainty about the outcome of a spin would be a minimum of 0. On the other hand, if the wheel is balanced and gives random numbers, then information is gained – uncertainty is removed – by observing the outcome of a spin. Entropy is maximized when no outcome is any more likely than any other (a fair wheel). Analogously, for a simple (non-chaotic) dynamical system, little information is gained about a trajectory from successive observations. If the initial position is known with a certain degree of accuracy then relatively few observations are needed to maintain approximately the same level of accuracy. To characterize the difference between simple and chaotic systems, mathematicians and physicists use a measure known as the Kolmorgov–Sinai (KS) entropy. Intuitively, the KS entropy measures the average information gained from successive observations of a system. For simple systems, the KS entropy is 0. In chaotic dynamic systems, the KS entropy is positive and is a fundamental property of the system. Information is continually gained from successive observations of chaotic systems. Thus, these systems exhibit behavior more characteristic of random (stochastic) systems, even when the chaotic systems are completely specified and deterministic. For chaotic systems, the challenge of predicting long-term dynamics arises not from lack of knowledge about the system's structure but from sensitive dependence and limited precision in measuring the state of the system at any given time.

Modifications of KS entropy, as well as other metrics, have been used to measure irregularity or lack of predictability of physiologically derived time series (Pincus, 1991; Richman and Moorman, 2000; Costa et al., 2002). When applied to biological data, the objective of these metrics is to quantify the complexity of a time series in order to make inferences about the underlying physiological system producing the series (Pincus, 2001). Some of the literature goes further to equate the complexity of the system with the complexity of the time series, as measured by the time series metric (Costa et al., 2002). However, we argue that the primal definition needed is

just ‘what is a complex system’, and it should be something more than a system that produces a complex time series. Moreover, as mentioned above, solely focusing on a time series view of complexity provides only limited insight to physiologists.

Emergent behavior

Emergent behavior is one of the most compelling but least well-defined concepts in complex systems theory (Morowitz, 2002). Emergent behavior is also immediately relevant to animal physiology. The basic idea of emergence is that, as a whole, a system may exhibit behavior that is unexpected based just on descriptions of its components and their interactions. Assertions about emergent properties of systems range from the fairly innocuous – ‘*the behavior of the system is surprising given the relatively simple description of the components*’ – to the more portentous – ‘*the rules governing the behavior of the system are fundamentally different and independent from the rules governing the components*’. (See, for instance, Mulhauser, 1998.) Both the former (limited) and latter (extensive) interpretations of emergent behavior address questions of level, hierarchy or scale. The limited form takes the approach that emergent behavior at the system level is logically dependent on the rules at the component level, however surprising the higher-level behavior. At the same time, the limited view acknowledges the advantage of adapting descriptions of phenomena according to the level at which they emerge. For example, descriptions of chemical processes generally exclude references, without denying their relevance, to the laws of physics underlying the processes.

The extensive view of emergence has a more fundamental implication: it is not just that the higher-level behaviors were not predicted, rather they could not have been deduced from the lower-level rules. A hierarchy of rules is necessary – not just useful – for describing such systems. The extensive perspective, while not explicitly stated, is often implied in statements about the observance of emergent behaviors. Care must be taken in evaluating such statements in the absence of a clear unambiguous definition of emergence. Further caution should be exercised since what sometimes appears to be an emergent property of a system may merely be an artifact of simulation constraints (cf. Gray, 2003).

A fascinating aspect of emergent behavior is self-organization. Self-organization addresses problems related to governance mechanisms for physiological systems, how these systems develop and how they may have evolved (see Gorshkov and Makar’eva, 2001; Burggren, in press a). Indeed, physiologists and cell and molecular biologists are now using a self-organization construct to look at systems as diverse as protein self-organization in *E. coli* (Howard and Kruse, 2005), neural behavior in cortical minicolumns (Lucke et al., 2004), mesoderm differentiation in the embryo (Green et al., 2004) and signal transduction in cardiac muscle stimulated by epidermal growth factor receptor (Maly et al., 2004). Although an active area of investigation with potentially deep implications to physiology, self-organization is beyond the scope of this short review. However, an introduction into the literature can be acquired from reviews such as Wolfram (2002), Morowitz (2002), Bak (1996) and Jensen (1998).

Working descriptions of complexity

Even with all of the deep, rich theory addressing complexity, mathematics has yet to provide precise definitions that readily map onto the biological world. However, as discussed above, there are central themes that allow us to move beyond the colloquial use of terminology. In particular, complexity is intimately related to the degree by which system dynamics or emergent behavior can be predicted in practice. There must also be significant (e.g. nonlinear) interaction between components. These themes are reflected in the system descriptions we give below. We will consistently use these descriptions when referring to types of systems. When the terms ‘complex’ or ‘complexity’ are used without ‘system’, a more colloquial use of these words may be assumed. Note that our intent is not to provide *the* definition of ‘complex systems’. Our purpose is merely to start the journey down the path towards a common vocabulary between mathematicians, physicists, animal physiologists and other biologists.

Simple systems

A simple system may have few components and little interaction between components. System dynamics and any emergent behavior are straightforward and easy to predict. A physiological example would be a simple nerve synapse in which an action potential in the presynaptic neuron creates an action potential in the postsynaptic neuron.

Complicated systems

A complicated system may have many components, however the interaction between components does not introduce any insurmountable obstacles to predicting the behavior of the system – it may be difficult but it can be done. The nerve network found in the sea slug *Aplysia* appears to be a complicated system as opposed to complex. While the behaviors of *Aplysia* may be manifold, they are understandable and can be predicted.

Complex systems

A complex system is characterized by inherent limitations in the ability to predict the long-term or emergent behavior of the system. It is not that prediction is merely hard or that the system has not been completely specified. Rather, the lack of predictability arises from the nature of the interactions between system components and often from the inability to measure the state of the system at any time with infinite precision. An obvious candidate for such a system is the human brain.

Seeking a complexity definition relevant to physiology

Within the context of the descriptions of simple, complicated and complex systems given above, can more operational definitions for complexity relevant to biology

generally, and physiology specifically, be developed? 'Biocomplexity' is, of course, a burgeoning field of study, and might be expected to offer some practical definitions, but often biocomplexity is used in its colloquial sense to describe '*really, really intricate biological systems*', and biocomplexity studies frequently involve categorizing the components of a system (usually an ecosystem), with less emphasis on specifying the rules that govern their interactions and the predictions of behavior that might subsequently emerge. Fewer studies approach biocomplexity based on mathematical modeling drawn from complexity science (e.g. Anderson, 2003; Ingber, 2004) and fewer still attempt to develop biocomplexity applications relevant to physiology (but see, for example, Freeman et al., 2001; Nicholson et al., 2004). Still, there are several approaches to describing complexity that we, as physiologists, can evaluate and begin to use in our experimental design and data interpretation.

Summing structures and processes

A number of intuitive definitions of physiological complexity can be offered, but each has considerable limitations. Rather straightforward is the 'sum of all parts' interpretation of complexity, described in the 'constructability theorem' of Nehaniv and Rhodes (2000). Essentially, this theorem holds that '*a biological system is the sum of low-complexity, interacting components*' (see Burggren, in press a).

Simply counting parts or structures as a way of categorizing complexity is a time-honored, anatomical approach. Consider nervous systems. From a structural perspective, the simple nerve net of *Aplysia* is considered less complex than the radially arranged nervous system of the echinoderm *Asterias*, which in turn is viewed as less complex than the bilaterally distributed, ganglion-based nervous system of decapod crustaceans or vertebrates. There is an attendant assumption of progressing complexity from sea slug to starfish to snake because of an anatomical progression, as measured by numbers of structures, cell types, tissue types, etc. As physiologists, we often fall into a similar trap. Instead of equating the numbers of structures to complexity, we merely equate the number of processes to complexity. Yet, neither a structure- nor processes-based view adequately defines the true complexity of a nervous system. For example, the neural network of *Aplysia* is relatively simple as defined by the numbers of neurons and its repertoire of behaviors (see review by Croll, 2003). Yet, this 'simple' neural system producing 'simple' behaviors is capable of complex information processing (Brembs, 2003; Croll, 2003) and, as such, has become a contemporary model for investigating the role of neural plasticity in non-associative and associative learning (Cropper et al., 2004; Leonard and Edstrom, 2004). Indeed, Bullock (1993, 1999, 2003) challenges us to expand our view of nervous system complexity beyond structures and processes to include the numbers of transactions, sensory discriminations and behavioral alternatives. Such an approach begins to address the issue of the numbers of possible *interactions* of components in addition to numbers of components in complex systems.

Interactive approaches

If counting structures or processes yields the sum of the parts, then examining the potential interactions between parts and processes describes 'a whole that exceeds the sum of the parts'. Indeed, this particular phrase, although not particularly helpful in a quantitative sense, is appearing more and more frequently in lay literature as symbolic of complexity and emergence thinking (e.g. Morowitz, 2002; Laughlin, 2005). How do we define the whole? Nehaniv and Rhodes (2000) have offered several axioms for describing complexity in biological systems. Their 'non-interaction axiom' can be simply paraphrased as '*complexity only increases if the combined components actually interact*' (Burggren, in press a). Interactions among components are typically governed by a set of rules. Consider again the nervous system, whose rules include one-way information transmission across synapses and a fixed size of an action potential conducted by any given neuron. Bullock (1993, 1999, 2003) has emphasized that the measure of brain complexity is most accurately graded by what he refers to as 'connectivity' between neural components, which in turn leads to memory and larger numbers of more complex behaviors.

If a view of physiological complexity based on interaction is to provide practical guidance in the design of physiological experiments, any definition of complexity used by physiologists has to account for at least three prominent attributes of complex physiological systems: (1) lack of high predictability of output; (2) sensitivity to initial conditions; and (3) non-linear interactions between structural components. Let us consider each in turn.

Even the best-understood physiological systems are not entirely predictable. A tachycardia induced by decreased blood pressure is certainly an anticipated response in most tetrapods, but physiologists don't expect the magnitude of this chronotropic reflex to be the same each time a blood volume or blood pressure drops due to the variability inherent in all cardiovascular control systems (e.g. Ursino and Magosso, 2003). Indeed, as physiologists, we are suspicious of small standard deviations (whereas a physicist, for example, might be suspicious of large ones). The magnitude of uncertainty of output from a regulated physiological system generally equates with the degree of complexity of the system regulating it.

Complex physiological systems are sensitive to what mathematicians would call 'initial conditions' – that is, the values of the system variables at the point at which a series of measurements is made. Returning to the example of the interaction of blood pressure and heart rate in baroreflexes, the change in heart rate that one anticipates in response to a given reduction in blood volume will depend greatly upon the initial blood volume and initial blood pressure as the experiment begins.

Finally, when we consider interaction as a measure of complexity, we must account for non-linear interactions between structural components. Putting it differently, not all components in a complex system will interact equally or identically. Nehaniv and Rhodes' (2000) 'bounded emergence

axiom' addresses this perspective thus: '*interaction between components increases complexity, but one-way interaction sets bounds on the possible increase*' (see Burggren, in press a). For a physiological example, consider respiratory development in developing anuran amphibian larvae. Just prior to metamorphosis, many anuran larvae use a combination of gills, skin and lungs (three components) to breathe air and water (two processes). While respiratory complexity in these intermediate developmental stages could be described as the sum (N=5) of all the components (N=3) and all the processes (N=2), a more meaningful complexity index of anuran respiratory development is compiled from the *product* of all respiratory components (N=6) and all respiratory process (Burggren, in press a, b). However, mindful of the bounded emergence axiom described above, not all respiratory organs are involved in all processes in this example of anuran respiratory development. For example, gills do not interact effectively with air, nor lungs with water! Thus, while complexity certainly increases during development, our description of changes in complexity during metamorphosis must be tempered by the nature of *actual* interactions between the various components. Moreover, complex dynamics can arise in systems with relatively few components and straightforward interaction rules. Thus, a corollary to Occam's razor appears to hold: complex behavior does not necessarily imply a system with complicated sets of components and interactions.

Types of physiological complexity: kinetic vs *potential*

The notion that true complexity depends on the actual pattern of interaction between components leads to another perspective of complexity. Many physiological regulatory systems have present or future *capability* for complex actions. Must they actually be involved in regulatory actions to confer complexity to the system? One might similarly ask '*is an automobile sitting silently in a garage only "potentially" complex until its engine is started and it is driven down the street?*' Answering such a question may have more practical implications than might at first be imagined. Consider how well the concept of kinetic *vs* potential energy has served the physical sciences, dating back beyond Ludwig Boltzman and James Maxwell to Rudolf Clausius and even back to Robert Boyle. School children around the world are taught early on about the potential energy stored in a stretched elastic material, only released as kinetic energy performing work when the elastic material recoils. As an example of applying this concept of potential *vs* kinetic energy to biological complexity, consider physiological development. A fertilized egg has all the 'potential complexity' of the most complex period in that animal's life cycle. The egg's 'kinetic complexity' only becomes evident when it develops physiological systems that are actually regulated (again underscoring the importance of the non-interaction axiom, where complexity only increases if components actually interact). As another example, consider a relaxed muscle fiber loaded with ATP, and with actin and myosin poised for cross bridge formation. As long as the fiber's membrane remains polarized, it exhibits only potential complexity. Of course, with the release of acetylcholine from a motor neuron onto the fiber's post-synaptic receptor, the depolarization of the muscle membrane, and the accompanying Ca^{2+} stimulated actin–myosin cross bridge formation leading to fiber shortening, the muscle fiber's kinetic complexity that was waiting in the wings now becomes amply evident in muscle fiber contraction! To show how potential and kinetic complexity can be nested, consider that while a relaxed muscle might be considered to be in its state of greatest potential complexity, this derives *from the perspective of actin and myosin cross-bridge formation.* Yet, from the perspective of considering regulatory proteins (troponin, tropomyosin), the kinetic complexity of these proteins and their interactions with myosin might be at its greatest during muscle relaxation. Thus, kinetic and potential complexity are highly context dependent.

Do the concepts of potential and kinetic complexity help shape physiological experimentation and the interpretation of those experiments? If we fail to acknowledge the potential complexity of a poorly understood system, we then mistakenly view all physiological observations as reflecting the maximum possible complexity of that system. Not acknowledging the potential complexity of the system leads us to underestimate the complexity of its ultimate emergent behaviors. For example, a kidney processing urine in a human showing water and salt balance does not reveal its potential ability to secrete highly hypertonic urine. Only after the salt load of, for example, a typical fast-food meal does antidiuretic hormone regulation of collecting duct water permeability become evident, revealing a higher level of kinetic complexity of the kidney. Thus, while the complexity of well-understood systems seems obvious, how much potential complexity remains undiscovered until we make observations under new configurations of physiologically relevant conditions?

Complexity and reductionism

The mathematician Georg Polya remarked that if there is a difficult problem that you don't understand, then there is also a simpler problem you don't understand (Polya, 1957). The implication of this statement has been a mainstay of mathematics and science. To understand a system – whether a theoretical mathematical construction or a complicated physiological regulatory system – we are taught to break it down into smaller observable processes and study those first. The reductionist approach has served science in general and physiology in particular remarkably well. Indeed, physiologists (as well as physicists, chemists and mathematicians) have a long tradition of breaking down complex systems into simpler components for individualized study. [In our experience, physiology graduate students are better car mechanics than most other biologists, because they are used to diagnosing systems by breaking them down and swapping out various components until the particular component of interest (e.g. the defective car part) is identified.] For example, the discovery of the regulation of

gastric secretion was simplified through many separate series of experiments focusing on chronological phases of secretion (e.g. cephalic phase, gastric phase), the individual secreted hormones and their pathways for synthesis, the separate chemoreceptive and mechanoreceptive regulation of gastric secretion, etc. This same pattern of 'divide and conquer' is repeated in studies of the cardiovascular, nervous and osmoregulatory systems. As physiologists, we appreciate and admire complexity, but often our proximate goal is to divide a complex system into many simpler components for individualized study – i.e. convert kinetic complexity into potential complexity. Essentially, we are focused on (if not fixated on) the approach of holding all but one variable constant, creating a single dependent variable whose performance we can monitor. The task of assembling the newly emerging pieces of the puzzle into a more sophisticated understanding is viewed by most participants as a task for another day, if not a task for other more patient researchers.

Unfortunately, for reductionist proponents, theoretically 'reassembled' systems often do not perform as predicted by the reductionist-derived models employing the system's components, in part because their interactions are often not fully accounted for. Variation between predicted and actual behavior is typically attributed to system noise. Indeed, system noise is often granted some meta-physical identity of its own, averting the worrisome conclusion that the real system may actually be more than the sum of its parts. However, this very conclusion may be inescapable when the observed variability is large. Interestingly, reductionism without more sophisticated attempts to reassemble the whole appears to be increasingly viewed in retrospect as an important stepping stone – a step that was helpful along the way of understanding the meaning of everything physiological but is no longer the sole pathway (or even a desirable pathway). Advocacy for a balance between reductionism and synthesis is waxing (for reviews, see Rose, 1998; Roenneberg and Merrow, 2001; Moalem and Percy, 2002; Van Regenmortel, 2002; Powell, 2004; Burggren and Warburton, 2005). Indeed, as Neugebauer et al. (2001) comment, '*The part is never the whole, and it is impossible to understand the whole through limited dissections of its parts. The understanding of complex systems requires approaches other than those of explanatory reductionism.*' Importantly, we are not advocating an abandonment of reductionism (as some would) – instead, we seek to stimulate a discussion regarding alternative and/or complimentary approaches that physiologists can use to study complex systems.

The value of understanding physiological complexity

Intensified interactions between mathematicians, physicists and physiologists will help develop concepts of complexity that jointly satisfy mathematical expectations and physiological reality. Until that occurs, how do physiologists interested in complexity actually go about improving experiment design and data interpretation?

Choosing appropriate models

If we appreciate physiological complexity – both potential and kinetic – we may be able to avoid choosing animal systems or animal models that are unnecessarily complex, thus contributing to our near-term understanding rather than confusion. The roundworm *Caenorhabditis elegans*, with just under a thousand cells, is arguably one of the most useful animal models to emerge in decades. Indeed, it is a prime example of this approach of avoiding unnecessary complexity. Consider, for example, the complexities of hypoxic tolerance in metazoans, which involves a huge array of metabolic/biochemical responses, ranging from evolutionary adaptations such as increased O_2–hemoglobin affinity to acute physiological adjustments such as hyperventilation. Physiologists have long been interested in hypoxia tolerance for a variety of reasons, spanning basic research in understanding the evolution of air breathing in fishes (Randall et al., 1981; Little, 1983; Graham, 1997) all the way to treating ischemia and mycocardial infarctions in human patients (James, 1997; Kloner and Rezkalla, 2004; Kolar and Ostadal, 2004). While the study of lungfish or mice, respectively, has certainly provided a level of understanding of hypoxia tolerance in vertebrates, some of our most exciting revelations have emerged from using the relatively simple (at least, physiologically) *C. elegans* to investigate the biochemistry and physiological genomics of hypoxic tolerance (Nystul et al., 2003; Padilla et al., 2003; Treinin et al., 2003).

Given the utility of the less complex *C. elegans*, should we direct all of our resources toward this model? Emphatically not! For all its strengths, *C. elegans* does not exhibit the behaviors of more complex metazoans – it does not generate internal circulatory convection and does not actively ventilate dedicated respiratory organs. From an overarching integrative perspective, recognizing the kinetic physiological complexity of *C. elegans* allows us to predict more accurately the as yet unrevealed potential complexity of more derived animals.

Guiding data collection

Complex systems are often characterized by considerable dependence on initial conditions (e.g. sensitivity of ventilation rate to initial states of metabolism, body temperature, blood pH). Small changes in environmental conditions may produce not only large but also *unexpectedly* large subsequent variations in system performance over time. Thus, complex systems require much more frequent monitoring with multiple observations over time to be able to accurately forecast their near-future emergent behaviors. Whether the system turns out to be complex or 'merely complicated', near term predictions are improved by a higher sampling frequency. As an example, consider the different conclusions that might be drawn from differences in heart rate sampling frequencies when cardiac patterns are very intricate, as in the pupae of the moth *Manduca sexta* (Fig. 2). The more elaborate the observed heart rate pattern (or any other such physiological variable) being measured, the greater is the sampling rate needed to reveal the overall pattern.

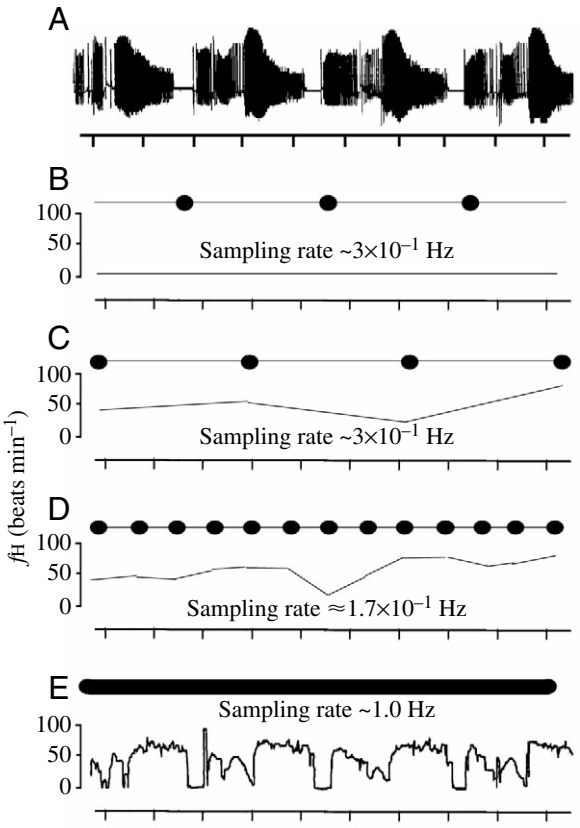

Fig. 2. Effect of sampling frequency on apparent complexity of heart rate (f_H) patterning in the adult moth of the tobacco hornworm, *Manduca sexta*. (A) Heart rate in the resting, intact adult moth at 20°C. Time marker in 10 s intervals (after Smits et al., 2000). (B–E) Effect of sampling frequency on the apparent heart rate pattern observed during a 1.5 min period. Note how in B and C the same low sample frequency can yield a heart rate of zero or alternatively a range from 20 to 70 beats min^{-1}. As sampling frequency increases, the apparent complexity of the observed pattern of heart rate increases. Note that the potential and kinetic complexity remain identical in each case – the change is merely an artifact of sampling frequency.

While this relationship between sampling frequency and pattern delineation may seem obvious, an important but less obvious implication of sampling frequency comes from trying to predict future behaviors of a system based on past, measured ones. If the goal is prediction, then the more complex the system, the shorter the time window over which predictions are likely to remain accurate. Thus, in more complex systems, higher sampling frequencies are necessary to maintain accurate predictions of subsequent system behavior. While also true for complicated systems, the rate at which predictability degrades is much faster for complex systems. Fig. 3 illustrates this notion by comparing predictability (and its inverse, uncertainty) between a simple, complicated and complex system as a function of sampling frequency. The longer the period after a measurement, the more uncertain we are of the state of the system. Just as more frequent sampling provided a better overall picture of heart rate in *Manduca sexta*, more frequent sampling helps maintain a more accurate track of physiological system performance. How might this help experimental design? If a system is simple or merely complicated, then an experimenter needs to factor in less sampling into their protocol than if the system is truly complex. As a physiological example of degradation in predictability, consider the blood chemistry and apnea length of a diving animal such as a freshwater turtle or seal. We know that both falling arterial P_{O_2} and falling arterial blood pH during a dive will stimulate the termination of diving and the onset of lung ventilation (cf. Hochachka, 2000). These straightforward rules nonetheless lead to very complex patterns of intermittent breathing in diving animals (West et al., 1989; Williams et al., 2000). Predicting future dive durations only comes from detailed knowledge of not only the rules but also the blood chemistry and duration of recent dives, which in turn only comes from frequent sampling. In the freshwater turtle *Pseudemys scripta*, for example, dive length can be predicted in part from the pattern of P_{O_2} change in lung gas and arterial blood: an initial slower rate of decline during the early minutes of the dive typically signals a longer dive length (Burggren and Shelton, 1979). Such patterns are only revealed by frequent lung gas and blood sampling, but such frequent sampling would be a waste of time if, for example, the goal was to correlate blood gases at the end of diving to apnea length (as opposed to predicting diving length). Thus, appreciating physiological complexity can lead to more efficient data sampling protocols.

Improving interpretation of data from complex systems

The more we appreciate potential and kinetic complexity as it applies to physiology, the less likely we are to misinterpret simple outputs as coming from what we mistakenly think are simple physiological systems. To illustrate this point of view, a mathematician tends to judge the complexity of a system by the complexity of the system's emergent behavior or output. The more complex the system, the more complex and unpredictable its output. Yet, very complex physiological regulatory systems are often characterized by quite simple and predictable emergent properties, in contrast to a mathematician's expectations. Thus, for example, thermoregulation in a typical mammal results in a simple emergent behavior – a body temperature of ~37°C – despite radiation, convection, diffusion and conduction resulting in a variety of conditions and mechanisms for both heat gain and loss. Strictly on the basis of its simple output then, a thermoregulatory system might be *mis*classified as non-complex. Yet, this very intricate physiological regulatory system (as evident from the number of parts and their interactions) has evolved to be complex *precisely so that its output is highly predictable*. In this respect, the complexity of the external environment must be matched with an equally complex internal regulatory system, and the result is a disarmingly simple behavior. As long as we appreciate physiological complexity, even when masked by simple emergent behaviors, we can guard against overly simple interpretations (recall how, until recently, we thought that aquaporins described all aspects of transmembrane water flux).

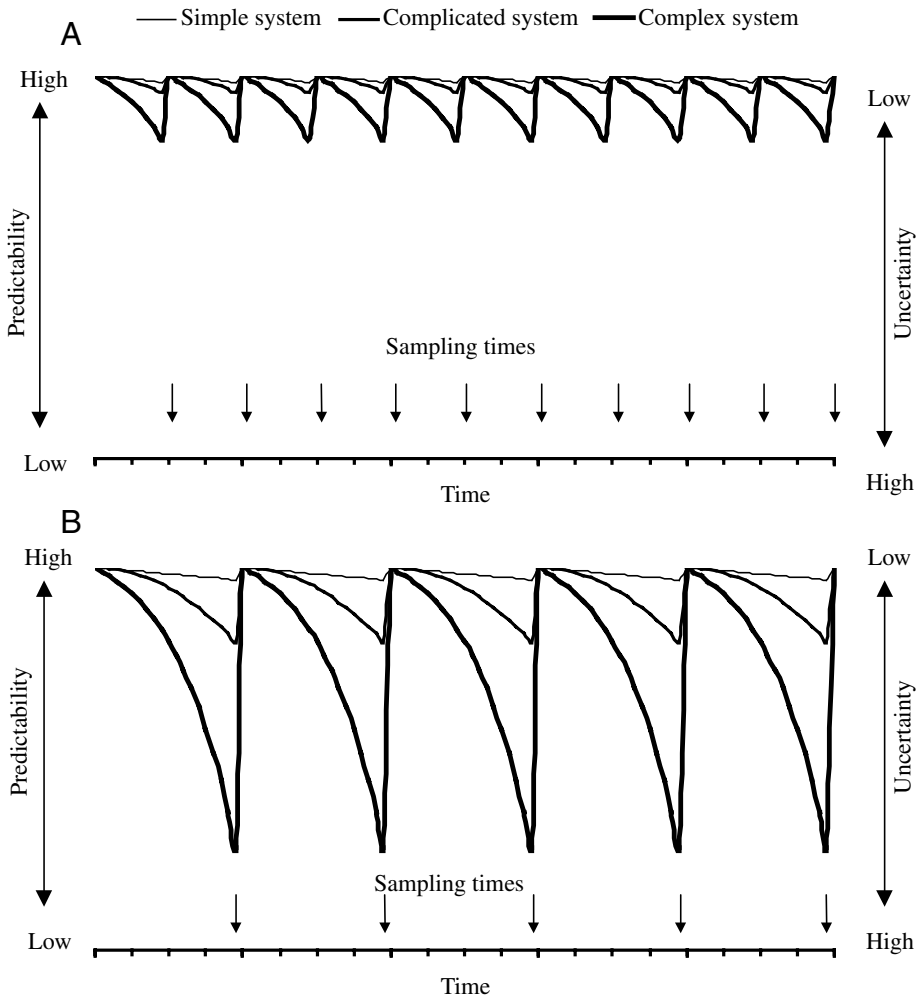

Fig. 3. Changes in predictability and uncertainty as a function of sampling frequency in simple, complicated and complex systems. (A) When sampling times are frequent, the degree of predictability is high (uncertainty low) as time progresses from the last sampling. Note also that the rate of degradation is largest in complex systems and smallest in simple systems. (B). Predictability is degraded (uncertainty increases) rapidly at lower sampling frequencies.

As physiologists, we have not yet defined complexity, but we have nonetheless allowed the concept to influence our perceptions of the progression of everything from evolution to development. Consider, for example, the interpretation of data relating to complexity change during ontogeny. Physiologists typically view complexity as increasing progressively – even linearly – with development. Yet, a view of complexity driven by an integrative view using anatomical components, physiological processes and their interactions reveals prominent examples in vertebrate development where physiological complexity actually peaks at some intermediate point in the life cycle, with terminal, mature stages actually being less complex (Burggren, 2005, in press a, b). In the larvae of a terrestrial amphibian such as a terrestrial toad, for example, early stages are characterized by water breathing with gills and skin. As the larvae develops, however, air breathing with the lungs begins to occur, such that the late larval stage is characterized by two respiratory processes (water breathing, air breathing) and three sites of gas exchange (gills, lungs, skin). Finally, with the advent of metamorphosis, the terrestrial toad 'reverts' to a simpler respiratory situation where it breathes air with lungs (and marginally, with skin). Thus, in this amphibian, respiratory physiological complexity builds during larval development to a peak just before metamorphosis, then declines considerably in the terminal stage.

The progression of complexity during evolution of physiological systems might be viewed similarly to that for development. A progressive increase in complexity is seen as a hallmark of evolution of physiological processes (e.g. Maina, 2002; Morowitz, 2002; Battail, 2004). Because we tend to view the most derived ('highly evolved') forms as having the most complex physiology, we can mistakenly overlook, or at least de-emphasize, some very sophisticated physiology. An excellent example in this regard is the cardiovascular physiology of reptiles. Some physiologists would view the chelonian and squamate heart as a three-chambered heart – essentially a defective mammal heart desperately awaiting 'evolutionary repair'. In fact, the heart of turtles and snakes is a sophisticated blood delivery system capable of responding to waxing and waning levels of oxygen in the lungs and redistributing blood in a highly efficient manner to the oxygen-consuming tissues during intermittent breathing (for reviews, see Burggren et al., 1997; Axelsson, 2001; Hicks, 2002). The heart of the crocodile is even better adapted in this regard, operating as a dual-pressure pump with separate pulmonary

and systemic blood streams during lung ventilation, but then being able to generate a progressively larger pulmonary bypass during extensive periods of breath holding. The latter cardiovascular system, by virtue of its more complex array of physiological responses, is better suited to intermittent breathing and diving than the circulation of diving mammals, which is actually constrained by having permanently divided pulmonary and systemic circuits.

These examples show that developmental or evolutionary stages currently viewed as intermediate can be more, rather than less, complex than more mature or more evolved stages. We are especially likely to overlook complexity in developmentally or evolutionarily intermediate stages if emergent behaviors of physiological systems are simple on first examination. Clearly, we can meaningfully look for signs of complexity in places where it may have been formerly overlooked.

Modeling complicated physiological systems: focus on prediction

Unlike economists or astronomers, physiologists are typically more focused on the 'here and now' than the future. We make physiological measurements (e.g. blood pressure, urine formation, neural discharge) and then interpret what these data mean. If we want to know what happens in the future, we often just wait until the future arrives, and then make the measurement! At the same time, many physiologists are interested in modeling data, particularly in an effort to understand complicated, if not complex, systems. If a model allows prediction of complex behavior a short time into the future, then that model is particularly robust, as it incorporates not only components and their interactions but also how these interactions influence in the near term. As physiologists develop more and more predictive models *and* as we come to learn the impact of complexity on our models, then we can begin to use models not just to affirm our understanding of the system but to predict future behaviors of physiological systems. For example, physiologists may set up experiments in which the desired behavior (e.g. molting, jumping, yawning, feeding, sleeping, etc.) is aperiodic or has low predictability and then spend inordinate amounts of either investigator time or hard disk space collecting extraneous data while awaiting the occurrence of the actual behavior of interest. By assessing the complexity of the behavior's pattern of appearance, it may become possible to predict with a reasonable degree of accuracy both the sampling frequency and the time period in which data collection is actually necessary to capture that phenomenon (Fig. 2).

The future for physiological complexity studies

Physiological complexity abounds. As physiologists, we vaguely recognize it, vaguely respond to it and – at some level – vaguely don't care about it. Yet, as we have indicated in this essay, a full appreciation of complexity has potentially huge implications for a deeper understanding of regulatory systems and the physiological processes they oversee. If we can understand the impacts of complexity on our research, then we can make better decisions about our choices of animal models, design more efficient methodologies for data collection and then interpret the resulting data in more meaningful ways.

Future interdisciplinary collaborations between physiologists, mathematicians and physicists are vitally important on several fronts. Discourse with other quantitative scientists can stimulate physiologists to think about our experimental design in more rigorous ways and also interpret the data we produce with far greater insight. As physiologists, we can also help expand the view of mathematicians away from abstract descriptions of complexity into more applied avenues ripe for exploitation in the physiological sciences.

Collaborative efforts must recognize that semantics are hugely important and that they present a large but surmountable barrier to the interdisciplinary study of complexity. Consider how the very words 'complex' and 'complicated' are taken to mean different things by mathematicians, physicists and physiologists. It may sound disparaging to a physiologist who has been working for decades on an intricate system to hear the focus of their attention described by a mathematician as 'merely complicated', but appreciating these seemingly subtle and technical semantic distinctions is important if we are to communicate effectively and stay engaged with our mathematical colleagues. (In fact, embarrassingly far into the writing of this essay, the physiologist–mathematician author team was still struggling to calibrate their respective use of words that had both common English and technical definitions!) Thus, however tedious the process, stripping away jargon to reveal the common core ideas is of crucial importance for real conceptual advances in complexity studies.

Finally, efforts by physiologists to incorporate elements of complexity science into their research are highly likely to yield tangible results in the near future. To illustrate this point with an example used earlier, is water transport across biological membranes a complicated yet predictable process now known to involve a variety of mechanisms, including aquaporins and water pumps, or does it remain a complex and thus unpredictable mechanism with as yet undiscovered components? If, as physiologists, we learn the characteristics of complicated *vs* complex systems (e.g. greater predictability of the former), then we may be able to concentrate our studies on the interaction of a known, complete list of components of a complicated system, rather than searching for additional unknown components of a complex, unpredictable system.

If the reader of this essay had hoped for precise definitions of complexity, and clear pathways to improved experimental design, they have no doubt realized that they are not yet forthcoming. A great deal of interdisciplinary collaboration between physiologists and other quantitative scientists must first be realized to understand physiological complexity – but *appreciating* physiological complexity is an important first step.

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