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REVIEW

Developmental physiology, animal models, and the August Krogh Principle*

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Summary

The field of development physiology is growing rapidly, and central to its success has been the judicious use of animal models. This essay considers the concept of the "animal model" and how a sharp focus on such models is usually helpful, but in some cases can blind us to alternative models that could accelerate advancement of the field. The rationale is presented for the intense use of popular animal models in studying vertebrate developmental physiology such as the early developmental stages of the zebrafish, mouse, and chicken embryo. The essay reminds readers of the August Krogh principle – *"For many problems there is an animal on which it can be most conveniently studied"* – and suggests that there are undiscovered animal models that could be used profitably in future studies of developmental physiology. Four specific areas of animal characteristics are explored – "macroembryony", transparency, ecological relevance, and life cycles – showing how consideration of each characteristic in experimental animals can help advance the study of physiological ontogeny. Potential new animal models based on the above characteristics are presented (e.g. emu, direct developing frog, armadillo), and examples of data collected from them are presented. The essay concludes by discussing the "universality" of physiological data from developing animals, indicating that the earlier in development, the more similar are both qualitative and quantitative characteristics from a wide variety of vertebrate embryos and larvae. Thus, with caution, development physiologists can meaningfully discuss the common physiological characteristics of the "vertebrate embryo".

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Introduction

The field of modern developmental biology is burgeoning. From its early roots and firm establishment, primarily due to the influence of German embryologists in the 19th century, through to the current global emphasis on molecular and cellular aspects of development, the field of developmental biology now influences almost every aspect of the biological sciences. Though relatively late to get underway in this biological gold rush, the field of *developmental physiology* is now flourishing, and the number of papers published in the main zoological and comparative physiological journals grows sharply each year.

Particularly promising is the amalgamation of techniques, approaches and – perhaps most importantly – ultimate goals of the various scientific participants interested in developmental physiology. From an admittedly biased position that the most interesting biological questions are often the more global ones, it is heartening to see scientific teams combining expertise in genetics, cellular and molecular biology, organ system biology, and even ecology (though still too little of this) to determine comprehensive answers to some of the more pressing questions in developmental physiology.

The influx of new investigators and even newer approaches is highly welcomed, but the overall integration and reconciliation of all emerging data (which surely should be one of our major goals?) is sometimes less efficient than it could be. Often, the

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very same investigators who bring different perspectives to developmental physiology are not immediately able either to share that perspective, or appreciate the perspective of others. This is often evident when biologists from diverse backgrounds speak of "animal models" and their utility to developmental physiology. As will be explored below, the same animals viewed by some investigators as integrated, critical parts of whole ecosystems are viewed by others as simply convenient platforms for reductionistic studies.

The goals of this paper are to:

- explore the concept of the "animal model" as it applies to developmental physiology;
- revisit that guiding principle for much of the last several decades of comparative physiology – the August Krogh principle – and determine how its judicious application can help further advance developmental physiology;
- discuss how some common (and some uncommon) animal models are contributing to the field of developmental physiology;
- evaluate the validity of the concept of "universality" of animal models employed in developmental physiology.

The concept of animal models in developmental physiology

Comparative physiologists and especially physiological ecologists are sometimes attributed with expressing disdain for those who use "animal models" with purportedly little understanding of the biology of their chosen model. On the other hand, molecular biologists and medical physiologists are sometimes attributed with the view of comparative physiologists as a "fringe" group that are more interested in seemingly endless exploration of diversity in lieu of supplying fundamental answers to major questions. Fortunately, these harsh characterisations are rarely true. Unfortunately, both groups have perhaps not done enough to persuade the other of the advantages of their own approaches in advancing the field of developmental physiology. This issue frequently comes to a head over the utility of so-called "animal models".

So, what is an animal model, how are they chosen, and how are they justified? Simply put, an animal model is an animal that is studied frequently and preferentially to tell us more about systems, tissues, cells and biochemical and physiological process in which we are interested. An animal quickly becomes a "model" when it is recognized as easier to study than another animal while attempting to answer a basic biological question (see Exploiting the "August Krogh" Principle, below). When the motivation is to understand human physiology, then an animal model may be the only acceptable approach for reasons of ethics or suitability of current experimental approaches. A prime example here is the use of embryos of the mouse, chicken, zebrafish and even the fruit fly to model those processes that we believe to be occurring during human development, but cannot reasonably investigate in humans (see Chen and Fishman 1997). More pragmatically, animals also become models when their popularity builds momentum to the point that they "corner the market". Often a point is reached when so much begins to be known about a particular animal model – that is, they becomes so popular – that even more compelling potential models are no longer explored[1].

The zebrafish, *Danio rerio*, epitomizes the "animal model". The formerly obscure aquarium fish has risen rather meteorically as a model for studying a variety of developmental questions, now including those with a physiological basis (Roush 1996). The zebrafish has all the hallmarks of a successful animal model in developmental physiology. It exhibits parthenogenesis, lends itself to mutagenic screens, is relatively transparent as an embryo and early larva, has a relatively rapid generation time for a vertebrate, and can be maintained and bred easily in a limited amount of space. The zebrafish also exhibits that most important property of a successful model in having "cornered the market". So much is known about the genetics and, increasingly, the physiology of embryonic and larval zebrafish (for recent reviews see Chen and Fishman 1997) that it is difficult even to contemplate rejecting so much supporting background data and beginning the study of a new model species. Yet, one could indeed argue that models more suitable than the zebrafish must surely exist. Bony fishes represent the greatest evolutionary explosion in the history of vertebrates. There are more than 20,000 living species, and one can predict that there probably exists a species of fish that has larger embryos, grows more rapidly, is more prolific, and can be kept and bred more easily than currently popular zebrafish. (The relatively well-known tilapia *Oreochromis* or the fathead minnow *Pimephales promelas* come close but do not achieve all of these characteristics). However, since superior piscine models are not actively being sought, and since the data on all aspects of zebrafish biology (but especially their genetics) grows almost exponentially, it is quite unlikely that even a demonstrably more superior vertebrate model could displace the zebrafish anytime soon.

[1] There are excellent examples of superior products failing to displace more popular inferior products, or at least arguably equivalent ones, in the field of consumer electronics. Consider the complex history over the last thirty years of the evolution of mass storage devices for music and movies.

Does the increasing prominence of zebrafish in developmental biology – especially in trying to determine the genetic underpinnings of physiology – mean that the emerging field of developmental physiology must make do with an "inferior" model? Most certainly not. As indicated above, when a sufficient data base exists for an animal model, this becomes one of its most compelling features, even when there might be other limitations. Most importantly, however, the beauty of a model is in the eye of the beholder. If one is interested in, for example, the changing ability of developing aquatic vertebrates to withstand chronic hypoxic effects (as is our laboratory), then there is certainly utility to continuing to consider popular models like the zebrafish. Indeed, Figure 1 shows developmental change in P_{crit} (the PO_2 at which an animal can no longer maintain normoxic levels of oxygen consumption) in a developmental series of embryonic, larval and juvenile zebrafish. These data show that the ability to oxyregulate is largely in place by Day 10, when zebrafish have made the full transition from yolk sac to free feeding larvae. A similar rapid onset of oxyregulation has been reported for larval *Xenopus laevis*, though the precise pattern proves more complex than that of the zebrafish (Hastings and Burggren 1995). If, however, one is interested in more ecologically oriented questions such as "how does naturally occurring chronic hypoxia affect early development and how does the animal correct for it?", then the zebrafish may be a less suitable model than some other species that lays its eggs in more hypoxic habitats for the simple fact that *Danio rerio* is unlikely to experience hypoxia in its native habitat of well-oxygenated, flowing streams. However, if one is interested in knowing how chronic swimming affects physiological development, then the zebrafish is a superior piscine model simply because swimming is an early natural behavior as an adaptation to life in the streams of Burma to which it is native. Indeed, our laboratory is currently investigating the metabolic effects (Bagatto B, Pelster B, Burggren, unpublished) and cardiovascular effects (Bagatto B, Schwerte T, Pelster B, Burggren W, unpubl) of chronic swimming at different speeds on zebrafish larvae and fry.

In summary, the concept of animal models is a venerable one. Popular animal models are popular for many appropriate reasons, but even the most widely employed models may not be appropriate for all research questions. A prudent approach in developmental physiology, especially when considering ecophysiological aspects, would appear to be to define clearly the problem and then choose (possibly having had to search for) the appropriate model, rather then assume that currently popular animal models will be suitable.

Exploiting the "August Krogh" principle – potential new animal models for developmental physiology

Suppose an investigator in developmental physiology is contemplating a new set of experiments on embryos – but a set that is not easily carried out with that investigator's currently employed animal model. That investigator, faced with the dilemma of either abandoning the new experimental set or struggling forward under adverse conditions, would do well to learn of, if not simply recall, the *August Krogh Principle*. August Krogh was a remarkable Danish animal physiologist noted for many achievements in the early 1900s, culminating in his receiving the Noble Prize in 1920 for elucidating the motor mechanism of capillaries. August Krogh espoused the view that "For many problems there is an animal on which it can be most conveniently studied" (see Krebs 1975). August Krogh lent his full endorsement to the "animal model" concept, but with a twist. To paraphrase the August Krogh principle, many experiments that are difficult or unachievable with current models can be completed satisfactorily by seeking out more appropriate animal models.

My purpose in (re)acquainting the reader with the August Krogh Principle is to indicate that, in the field of developmental physiology in particular, the opportunity to exploit profitably new models is immense, and largely unexploited. Let us consider several experimental approaches in which an expansion of animal models and experimental paradigms is occurring. For the time being, assume that the data acquired from these alternative models is relevant and transferable to other systems, though this critical issue is addressed in the final section of this paper.

"Macroembryony"

Almost by definition, developmental physiologists work on small, it not microscopic, animals. Despite a proliferation of "micro-techniques" enabling experiments on smaller and smaller animals (see Burggren and Fritsche 1995, Paul *et al* 1997), we still face the challenge of measuring metabolic rate, cardiac output, or renal filtration in an embryo that could weigh only a few milligrams. While experimentalists continue to push hard on the technological envelope, a complimentary (not alternative) approach is to exploit the August Krogh principle more fully, and look for animal models with larger embryos. Our laboratory has taken this approach on numerous instances, to great advantage. For example, in the early 90s, following more than a decade of research on the developmental physiology of amphibian larvae (see Burggren and Just 1992), we were frustrated by our inability to measure chroni-

cally blood pressure along with heart rate in free-swimming bullfrog tadpoles. Though typically reaching 30–40 g, the larvae of *Rana catesbeiana* just did not lend themselves to conventional pressure measurement, and micropressure measurements could only be made on restrained animals with an opened body wall. At this point in the saga, enter *Pseudis paradoxsus*, the paradoxical frog. Native to the sub-tropical regions of Brazil, and occupying a similar ecological niche to the North American bullfrog, *Pseudis* is remarkable for one reason – at >150 g it comprises the world's largest known tadpole. The paradox implied in its Latin name comes from the fact that at metamorphosis it rapidly diminishes in size to produce a froglet of only 4–5 g. (Students of apoptosis could do well to study this animal!). For our purposes, the extraordinary size of the tadpole of *Pseudis* allowed the insertion of an indwelling catheter into the femoral artery of the tadpole (Burggren *et al* 1992). Thus, for the first time we were able to measure vital parameters such as blood pressure in a free-swimming amphibian tadpole. Moreover, we could also inject sympathetic and parasympathetic agonists and antagonists, and observe the natural cardiac reflexes associated with activity, intermittent breathing, etc. (Figure 2). Such measurements and procedures would have been very difficult, if not impossible, in *Rana catesbeiana* or any other common anuran amphibian "model".

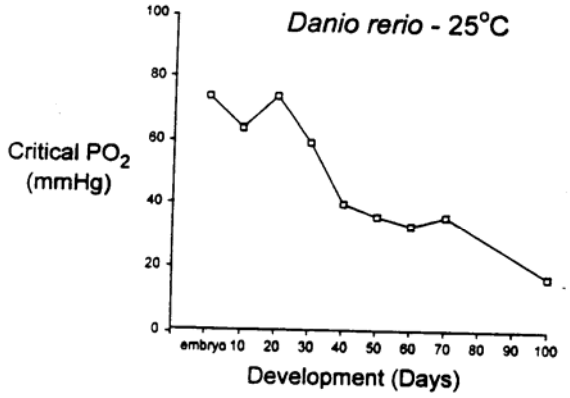

Fig. 1. Critical value of PO_2 (P_{crit}) as a function of development in the zebrafish *Danio rerio*. P_{crit} drops markedly during the first 20 days of development, indicating the rapid onset of the ability to regulate oxygen consumption in the face of acute hypoxic challenge. (After Barrionuevo and Burggren 1999).

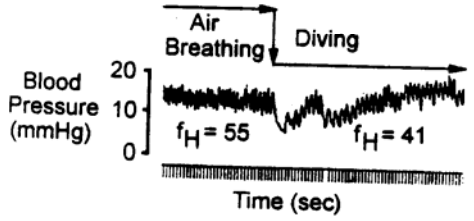

Fig. 2. Arterial blood pressure measured from the cervical artery of a freely swimming unanesthetized Stage 37 larval *Pseudis paradoxsus*. Note that the onset of diving is accompanied by a bradycardia, as evident in adult anuran amphibian. (After Burggren *et al* 1992).

A second, very recent example of the employment of the August Krogh principle – and specifically following the route of "macroembryony" – stems from our laboratory's investigations of cardiovascular regulation in the avian embryo. Almost invariably, the phrase "avian embryos" is taken to mean to embryo of the chicken, *Gallus gallus*. Relative to the embryos/larvae of zebrafish, bullfrogs or other vertebrates, the chicken embryo is relatively large, relatively accessible, and very extensively studied (see numerous chapters in Burggren and Keller 1997). Yet, there are many aspects of the physiology of the avian embryos that are not easily explored because of the size of the chicken embryo. The embryos of the large ratites – emus, ostriches and rheas – are obviously much, much larger than chicken embryos, but until very recently have commanded a price that was simply not compatible with the budgets of developmental physiologists. However, in the U.S. the cost of emu eggs has plummeted, especially in Texas where emu ranches are now common. This new availability of emu embyros in eggs that tip the scales at about 10–15 times the mass of typical chicken eggs is now permitting easier and more convenient study of the onset of cardiovascular regulation in avian embryos by allowing indwelling catheterization of multiple vessels in a single embryo, etc. It also allows the contemplation of experiments that would be extremely difficult with chicken eggs (e.g. longitudinal studies in individual catheterized embryos; tracking of physiological changes during hatching).

Transparency

One if the decade's major technological advancements assisting developmental physiology is the popularization of methodologies that depend upon optical data gathering. Monochromatic illumination and image collection is allowing *in vivo* measurement of hemoglobin oxygen saturation and other variables in microscopic animals (Paul *et al* 1997), while microvideotaping of the intact beating heart of vertebrate embryos has permitted the increasingly reliable calculation of cardiac output and other cardiovascular parameters (see Hou and Burggren 1995b, Burggren and Fritsche 1995,

Fritsche and Burggren 1996, Schwerte and Pelster 2000). The success of these and other optical techniques is very dependent upon the transparency of the embryos. Conventional embryonic models may not lend themselves to such examination because of their opacity. Again, we turn to the August Krogh principle to provide us with new opportunities. Though not in the strict context of development, Steffenson *et al* (1986) showed the power of looking for alternative animal models. These authors were interested in the specialized microvasculature of fishes, and ultilized the nearly transparent glass catfish, *Kryptopterus bicirrhis*, for *in vivo* microscopical visualization of microvasculature perfusion patterns.

The embryos and early larvae of many fishes are sufficiently transparent to allow many types of optical measurements. The early stages of the zebrafish are relatively transparent up to about day 10, and several laboratories are now exploiting this characteristic to measure cardiac output by videotaping the beating heart directly through the clear body wall. Many physiological events of interest occur after 10 days of development, but accumulation of the pigments (primarily melanin) in the pericardium prevent further use of this technique. To circumvent this limitation, Schwerte and Pelster (2000) have used mutant zebrafish with a point deletion that prevents melanin pigment accumulation in the pericardium, thus allowing free optical access to the beating heart for several days longer than the normal, pigmented wild type.

A final example of using the August Krogh principle vis a vis transparency involves circumventing the limitations of an opaque eggshell by finding an animal with a transparent, or translucent, egg shell. Our laboratory has twice ventured to Puerto Rico to study a new world frog, the coqui *Eleutherodactylus*. The coqui is a direct-developing frog that develops over a 20–25 day period in a terrestrial egg about the size of a small pea. Because the egg shell is virtually transparent and the body wall translucent, heart rate in the intact embryo can be recorded visually (Burggren *et al* 1990, Burggren *et al* 1997). Figure 3 indicates how heart rate, observed directly through the transparent egg case and body wall, changes before and around hatching in the cave couqui, *Eleutherodactylus cooki*. These embryos should also prove to be highly amenable to optical measurement of cardiac output and other physiological parameter (perhaps arterial oxygenation) through the transparent egg case.

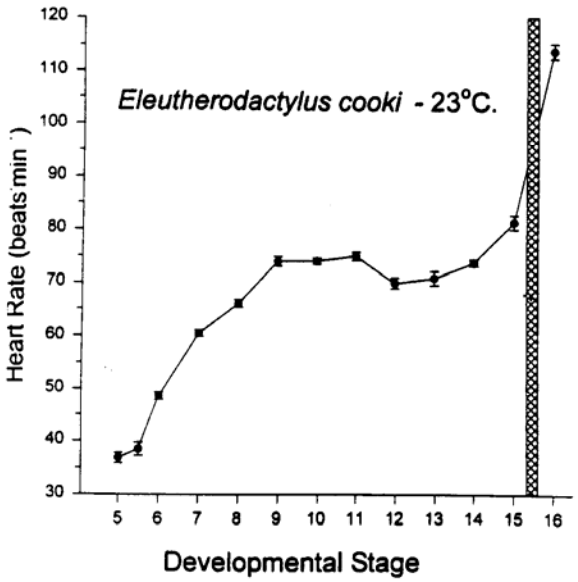

Fig. 3. Mean heart rate measured in the cave coqui, *Eleutherodactylus cooki*. The vertical bar indicates hatching. These data on heart rate were recorded visually by observing the beating heart of the embryo through the transparent egg case and translucent body wall, making the coqui a useful animal model for documenting developmental changes in cardiovascular physiology. (Burggren W, Crossley D, Rogowitz G unpubl).

Ecological Relevance

For many investigators and many investigations, the "ecological relevance" of the experimental conditions is perhaps less important than the ability to make the desired physiological measurements. However, as the basic understanding of physiological development grows, our approach should mature as well, and we should increasingly strive to put our experiments in a meaningful ecophysiological context. (For a dated, but likely still valid discussion of the ecological relevance in physiological studies, see Feder *et al* 1987). A pivotal question to ask is: "Does the animal actually experience the experimental condition anytime in its life cycle?" To answer this question one should know where the animal lives and the characteristics of its natural habitat. The author is more than slightly bemused by the opening statement in *The Zebrafish Book* (Westerfield 1995), an otherwise highly useful and informative source on many aspects of zebrafish biology. The offending statement implies that the zebrafish is found "... in pet stores throughout the world". In fact, as noted above, zebrafish are located in relatively well oxygenated streams of Burma. Thus, studies investigating the chronic effects of hypoxia, temperature fluctuations, high acidity, etc., are more profitably carried out on animals other than the zebrafish that might in fact actually experience such conditions. To give another example, if one wants to study chronic hypoxic effects on avian embryos, then the most ecologically relevant studies are

those carried out on the embryos of fossorial or high altitude birds (Boggs *et al* 1984, see Lutz and Storey 1997 for review) rather than chickens. Certainly, one can use non-ecologically relevant environments to probe both the physiology of embryos and/or their eggs particularly if one is looking at mechanistic questions, but the question of the relevance of the observations frequently lingers.

Another example of using the August Krogh principle in developmental physiology involves the development of renal function, volume regulation and reninangiotensin/baroreceptor reflexes. Certainly, the hydration state of the chicken embryo can be altered by changing the relative humidity in which the eggs are incubated (though this has marked effects on mortality). One could even open the eggshell, cannulate a chorioallantoic artery or vein, and then modify blood volume by either adding saline or hemorrhage while measuring heart rate, cardiac output, blood pressure, etc. However, environmentally-induced volume changes are not likely to occur during natural chick incubation. If the intent is to study the responses to changes in hydric state shown by vertebrate embryos developing in hard-shelled eggs, then the August Krogh principle sends us off in search of shelled vertebrate embryos that naturally experience changes in hydric state. In this respect, the embryos of many oviparous reptiles are highly amenable to such studies. By varying the hydration state of the soil or other incubation medium surrounding the eggs, the actual water content of the eggs and embryo themselves can be modified (see Packard 1991). For example, Dane Crossley, working in our laboratory with the African house snake, *Lamprophis fuliginosis*, has shown that heart rate in intact embryos can be chronically modified by changes in water potential of the incubating medium (Crossley 1997).

Life Cycles

A final example of integrating the August Krogh principle into one's choice of experimental animals involves a consideration of what might be loosely called "life cycles". One of the joys of studying developmental physiology is discovering the evolution of remarkable variations in the patterns of development exhibited by animals. (For a very recent and comprehensive discussion of larval forms, see Hall and Wake 1999). Our own laboratory has considered exploiting various developmental life cycles as we investigate the "nature vs. nurture" argument as it applies to physiology. We were intrigued by the subtle but significant variations in physiological parameters (e.g., heart rate, metabolic rate on a specific day of development) that seem to occur repeatedly in vertebrate embryos (Burggren 1999). Were these fluctuations in physiological performance "noise", reflecting subtle environmental cues, or genetically regulated? That is, is it the genetic makeup of an embryo or the environment in which it grows up more likely to influence developmental patterns of the cardiovascular system (Burggren 1999). Using both avian and amphibian models, we have looked at the "sibling effect" on heart rate development (Burggren, *et al* 1994, Burggren *et al* 1997). Developmental patterns in heart rate are statistically far more similar in siblings than non-siblings when raised under identical environmental conditions. While these data are suggestive of the dominant effect of genes, the arguments remained based on circumstantial (though relatively compelling) arguments.

To test the hypothesis that the common genetic "instructions" for development among siblings leads to the sibling effect, we used the August Krogh principle to find an animal model more tractable than avian or amphibian embryos. Enter an unlikely

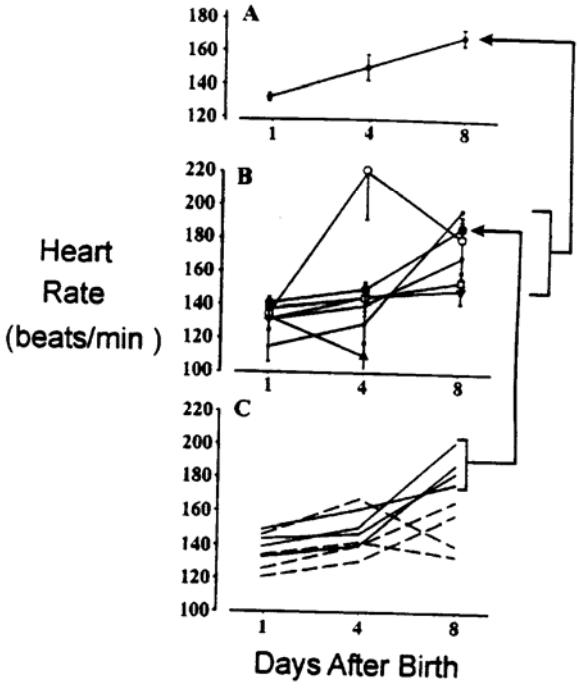

Fig. 4. Within and between litter variation in heart rate in neonates of the nine-banded armadillo, *Dasypus novemcinctus*. The top panel shows mean heart rate for 8 litters of four individuals each (n = 32). The middle panel shows the mean heart rate for each of eight litters (n = 8), and the bottom panel shows individual heart rates from four neonates from two litters (dashed and solid lines). Note how siblings show developmental patterns of resting heart rate that are far more similar to each other than to non-siblings. (Bagatto B, Crossley D, Burggren W unpubl).

paradigm in the form of the nine-banded armadillo, *Dasypus novemcinctus*. Armadillos are unique among mammals in showing a developmental phenomenon called "polymbryony" (Loughry *et al* 1998). Invariable, the blastocyst buds off into four separate embryos, eventually yielding at birth a litter of quadruplets that are genetic "clones" of each other. We have been examining metabolic rate, heart rate and ventilation rate in intact, undisturbed neonates (Figure 4). Fundamental to the study is the comparison of variation within and between litters in the neonatal period. Measured physiological parameters are statistically far more similar in siblings than in non-siblings at birth, Day 4 and Day 8 of neonatal development, suggesting a strong genetic component to their neonatal physiological patterns. Drawing the armadillo into our "nature/nurture" studies has thus allowed us to speak more confidently of the relative impact of genes and environment on developmental variation in physiologial patterns.

As another example of an unusual life cycle that is conducive to answering physiological developmental questions, let us return to the coqui. A considerable amount of both thought and experimentation has gone into the development and evolution of urea excretion but many questions remain unresolved (for reviews see Walsh and Wright 1995). Amphibians are useful as models for studying the evolution of nitrogen excretion, since they typically begin life as aquatic, ammonia-excreting larvae and develop into more terrestrial, urea-excreting adults (see Burggren and Just 1992). The developmental transition from excreting ammonia to excreting urea is of particular interest, in part because it mimics the putative physiological transition in the evolution of terrestriality (Randall *et al* 1981, Little 1983, Graham 1997). Problematic with the conventional amphibian model is that one cannot separate the development of air breathing/terrestriality (i.e. change in habitat) with the development of the adult morph (i.e. changes in developmental stage per se). In this respect, the coqui provides an opportunity to tease apart a change in developmental stage from a change in habitat, since the coqui goes through its entire development to the adult morph within the aquatic environment of the egg capsule, and only then hatches. Thus, the development from tadpole to adult morph is distinct from, and occurs earlier than, the change in habitat from the fluid-filled egg capsule to a terrestrial, air-breathing existence. We have only gathered information on the pH of egg capsule fluid during development, and important indicators such as egg capsule ammonia levels and biochemical analysis of enzymes key to urea formation are missing. However, following the direction to the coqui pointed out by the August Krogh principle, the potential exists to probe the evolution of urea excretion more deeply than previously possible.

On the "universality" of physiological data from developing animals

As evident from the above discussion of how the August Krogh principle leads us to consider "macroembryony", transparency, ecological relevance, and life cycle, there are a host of new experimental paradigms that are just beginning to be tapped. Many more remain to be discovered. However, there remains an important unanswered question: "Are data from non-traditional animal models broadly applicable?". To state this question another way, does an experimental finding from an embryo of a emu, snake or armadillo advance the field of developmental physiology as much as a finding from a chick or mouse embryo? The answer is yes ... and no.

One of the most surprising findings to come from our own studies of developmental physiology – and in particular the ontogeny of the cardiovascular system and its regulation – is the extraordinary similarity of how cardiovascular function unfolds in a wide variety of vertebrate embryos. These similarities are often not just qualitative, but also quantitative. For

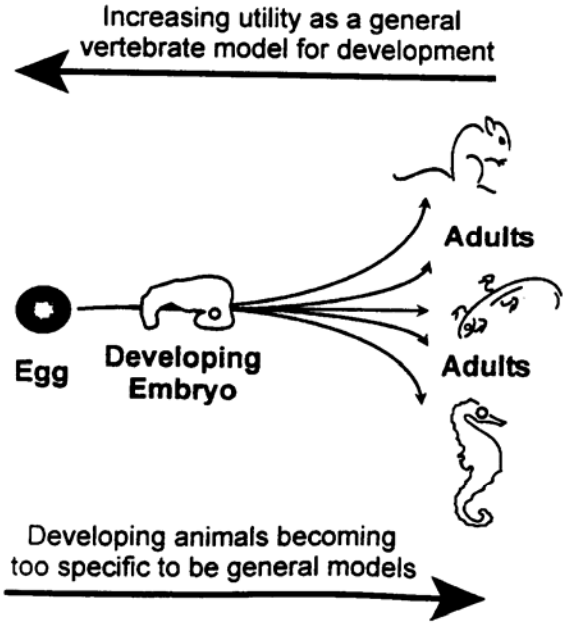

Fig. 5. The "universality" (the broad transferability or applicability of data from various vertebrate embryos) is greatest early in development, allowing one to talk, with a few caveats, about the condition of "the vertebrate" embryo. However, as development progresses, distinctive species-specific physiological and morphological traits render observations less broadly applicable. The transition from "universality" to "specificity" depends both on the species, and the parameters being monitored. (After Burggren 1998).

example, the actual appearance and rate of rise of measurable blood pressure, the dramatic increase in cardiac output, and the fall in peripheral resistance all follow more or less the same pattern in embryos of the zebrafish *Danio rerio* (Pelster and Burggren 1996), the clawed frog *Xenopus laevis* (Hou and Burggren 1995a, 1995b, Fritsche and Burggren 1996, Fritsche 1997), and the chick embryo (see Tazawa and Hou 1997, Keller 1997, Crossley 1999). Because of this similarity in physiological performance, we have found that data gathered from early embryos of one species provides concrete and very useful insights into those of another species. That is, one can enjoy and exploit a certain physiological embryonic universality, and can think of "the vertebrate embryo" and how it develops physiologically (Figure 5).

As embryonic development proceeds, however, the cardiovascular performance of any given species becomes increasingly idiosyncratic, reflecting the appearance of the dual chambered fish heart, the three-chambered amphibian heart, and the four chambered avian heart (among other prominent anatomical and physiological developments). Thus, the "universality" of the embryo and the data derived from it is progressively eroded, and increasingly less common and more distinctive features are seen (Figure 5). Importantly, the point of transition from early universality to later distinctiveness will vary from species to species. Thus, each investigator, using each model, and carrying out each set of investigations, should compare their data with that from other species to assess the extent to which the experimental findings can be generalized.

Conclusions

At no time in its history has developmental physiology been at a more exciting juncture. While we have long recognized critically important questions to ask, never before has there been such an array of appropriate experimental tools combined with robust animal models (and emerging models, guided by the August Krogh principle). There is an emergence of new interest in the (re)amalgamation of evolution and development – witness the launch of Blackwell's new journal *Evolutionary and Developmental Biology*. Thus, the study of developmental physiology should gain additional prominence as a cornerstone of understanding not only how animals develop, but how they have evolved, as well.

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