**What are the major goals of the project?**

This project has had three major goals. The first and main one is the long-term goal of our continuing program: to understand how regulation of physiological processes unfolds during development in vertebrate embryos. Considering almost all prior research has focused on individual systems and their regulation, the second goal in this project emphasized the largely unexplored yet vitally important embryonic interactions between cardiovascular and renal systems.  Our objectives integrate innovative developmental physiology experiments on cardiovascular and renal systems of avian model embryos together with multi-faceted research training from undergraduate through post-doctoral levels.  The third goal focused on integrating an international training and student development (including personal and cultural development) into this experimental program.

**What was accomplished under these goals (you must provide information for at least one of the 4 categories below)?**

Major Activities:

Our major activities occur in two categories: 1) the actual experimental work and 2) the broader impacts activities.

Experimental work: Pharmacological and environmental stressors have been used to modify renal development, while documenting attendant changes in heart structure and performance. Additional work has looked at pulmonary effects, as well.  Experiments to determine the converse - how changes in cardiovascular performance affect kidney development - continue.

Broader Impacts: Many of the University of North Texas' students are Mexican-Americans who, ironically, may have little exposure to Mexican culture, history and traditions, let alone Mexican-based science. Through international workshops with the Autonomous University of the State of Mexico, we are breaking now the borders and establishing deep and lasting international collaborations, in both Anglo and Hispanic UNT student.

Specific Objectives:

Four Specific Objectives were specified in the original proposal:

* Explore the influences of experimentally-modified embryonic renal development on normal embryonic heart structure and function in the second half of incubation.
* Determine the timing of onset of the normal physiological interactions between the cardiovascular and renal systems, as well as the critical windows during which their sensitivity may be adjusted.
* Describe and quantify the phenomenon of fetal programming (embryonic programming in birds) as it relates to cardiovascular and renal development.
* Expand our research training and outreach efforts to include international research experiences for US undergraduates in our research program in Mexico.

Significant Results:

* Sylvia Branum, a PhD student, had determined that changes in environment (humidity, salt-loading) that alter renal performance and structure also influence cardiovascular structure and performance. Additionally, we have determined that modification of heart performance (blood pressure, pulse pressure) leads to modifications of the renal system, as well.
* The "edges" of the critical windows for renal and cardiovascular development are poorly understood.  We have now developed (and published) some new theoretical constructs that relate to the notion of critical windows.  Such windows don't have hard edges, but rather the onset and termination of phenotypic change is dependent upon the "dose" of the environmental stressor.
* Josele Flores, a PhD student, investigated fetal programming by examining both the physiological performance (pressure-volume loops) and anatomical structure of the hearts of quail that have been either normal or undersized as hatchlings, mimicking the effects of very low birth weight (VLBW) in humans.
* Training and outreach have been reported in other areas of this report. Briefly, we have, through our experiments, workshops, and other interactions, generated a student-based network of collaboration between the University of North Texas and the Autonomous University of the State of Mexico.

Key outcomes or Other achievements:

In this, the 5th year of the grant, there have been two "Key Outcomes":

1. Cardiovascular-Renal Interactions: Experiments in our laboratory have revealed that developing renal structures are indeed influenced by cardiovascular perturbations, as we originally hypothesized. This results in morphological modification of the kidneys, as well as significant adjustments in renal performance.
2. Epigenetic Influences Upon Developmental Physiology: The serendipitous component of this reporting period is that we have discovered the potentially profound effect of maternal/paternal environment upon the F1 generation, a form of non-genetic inheritance. This has manifested itself in both zebrafish and *Daphnia* in hypoxia tolerance in offspring as well as morphological changes in body mass and size.

No stated goals were unmet.

### What opportunities for training and professional development has the project provided?

This project continued to center around training and professional development. We highly value the eyes, hands and minds of a talented cohort of young trainees. By the numbers, the PI has in this last period trained 3 post-doc, 4 PhD students, 3 MSc students, and 4 undergraduate students. Additionally, through the international collaborations promoted by this grant, the PI is on the doctoral committee of 2 additional students located in Toluca, Mexico.

### How have the results been disseminated to communities of interest?

We have used our websites (described elsewhere) to disseminate our findings. Additionally, we have hosted a wide range of high school students, ranging from those in formal groups (e.g. Upward Bound) to individuals who are intrigued by a career in science, but have not yet experienced research. In some cases, this simply comprises tours of our facilities.

### What do you plan to do during the next reporting period to accomplish the goals?

Nothing to report

## **Impacts**

### What is the impact on the development of the principal discipline(s) of the project?

The original goal of this project was to begin to unravel the co-development of the cardiovascular and renal systems, using an avian model with which our lab is very familiar.  We have published several papers during the fourth year of the project that have indicated the co-dependence of these two systems during ontogeny. Specifically, we find that renal morphology is highly dependent upon the interaction of environmental conditions and cardiovascular performance, as our hypotheses originally predicted.

### What is the impact on other disciplines?

As reported last year, one of the unpredicted but rewarding aspects of this project is that we have unexpectedly been drawn into the arena of epigenetics. While this field has been growing by leaps and bounds in recent years, surprisingly little is known about the epigenetics of physiological processes. Our research on development of cardiovascular and renal process has led us to discover that epigenetic mechanisms can have a potentially profound effect on our data, and we continue to publish journal articles - both review and original data papers - that address this issue.

### What is the impact on the development of human resources?

The broader impact component of our project, inextricably woven into both the narrative of our proposal but also our activities in the first three years of the grant, involves a strong component of international development with respect to Mexican physiology programs and students (both domestic and foreign). The PI's lab is in the process of training two Mexican PhD students and three undergraduates, and has hosted ~20 Mexican graduate and undergraduate students this past year for shorter stays. Our two universities have established a seed grant program that is supporting biological research projects in both Texas and the Central Mexico Area.

### What is the impact on physical resources that form infrastructure?

At UNT, the grant has provided major new equipment that has enhanced our capacity for physiological and histological measurements.

### What is the impact on institutional resources that form infrastructure?

At the Autonomous University of the State of Mexico, our collaborative research efforts have directly resulted in the establishment of a BioTechnology Program with a strong Animal Physiology component (where none previously existed) and the development of a 30,000 sq. ft. facility to house this program.

### What is the impact on information resources that form infrastructure?

Nothing to report.

### What is the impact on technology transfer?

Nothing to report.

### What is the impact on society beyond science and technology?

Texas and Mexico have an affinity that few areas of the United States experience with another country. In fact, the borders have moved North and South while more than 6 generations of Tejanos have stayed on the same land. Through our collaboration with the Autonomous University of the State of Mexico, we have not only greatly strengthened scientific and engineering collaborations, but now collaborations in engineering, music, art, political science, journalism, economics and psychology, to name a few. Perhaps reflecting the efforts of the UNT in engaging our foreign partner, in September 2012 the PI was named "Rector Honoris Causa" (Honorary President) of the Autonomous University of the State of Mexico.

**Outcomes**

**Humidity:**

Experiments using different humidity incubation environments have shown that eggs incubated in low (20%) humidity lost a significant amount of water compared to control (60%) and high humidity incubated eggs (85%). The lowered incubation humidity decreased wet body mass in later stage embryos (D17,18,19) and hatchlings. The dry body mass was not affected, indicating dry conditions dehydrated the embryos but that the actual growth of the embryo was not affected by the humidity levels during development. High incubation humidity did not show any of these effects on body mass of embryos.

Heart mass was not affected by incubation humidity but it did significantly decrease kidney mass in both the low and high incubation humidity groups. This indicates that the humidity of the environment during development does affect kidney development without affecting the heart. Osmolality, lactate concentration, blood gas variables and hematological variables of the embryos were also recorded. Embryos incubated in low humidity have increased blood osmolality compared the control and high humidity groups. Mean blood lactate concentrations were increased during the latter part of development for both of the treatment groups as well. However, blood gas variables and other hematological measurements, during the last half of incubation were not affected by the humidity treatments.

We found smaller hatchlings from the eggs that were incubated in low humidity but these hatchlings recovered from this decrease in mass by 1-2 days’ post hatch. We have found that lower incubation humidity lowers hatchability and are currently still adding data to support this finding.

**Salt loading:**

Salt loading embryos altered the normal osmoregulation of chicken embryos throughout development. We found that increasing the salt concentration during development and exposing the embryos earlier in development resulted in smaller embryos than the other groups. There is a possible decrease in heart and kidney wet mass in earlier injection days while there was no change in embryos injected later in development.

The CAM showed incomplete and unhealthy development in embryos that were exposed to high salt levels during development. Mortality rates of embryos also increased with the higher salt concentration exposures. Further investigation continues on the glomerular filtration rate and how the high salt levels are affecting the kidneys of the salt loaded embryos.