

7th Integrative Biology Workshop

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ABSTRACTS



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'BET HEDGING' AGAINST CLIMATE CHANGE IN DEVELOPING AND ADULT ANIMALS: ROLES FOR STOCHASTIC GENE EXPRESSION, PHENOTYPIC PLASTICITY, EPIGENETIC INHERITANCE AND ADAPTATION

Warren Burggren and Fernando Mendez-Sanchez

ABSTRACT

Animals from embryos to adults experiencing stress from climate change have a numerous mechanisms available for enhancing their long-term survival. In this review we consider these options, and how viable they are in a world increasingly experiencing extreme weather associated with climate change. A deeply understood mechanism involves natural selection, leading to evolution of new adaptations that help cope with extreme and stochastic weather events associated with climate change. While potentially effective at staving off environmental challenges, such adaptations typically occur very slowly and incrementally over evolutionary time. Consequently, adaptation through natural selection is in most instances regarded as too slow to aid survival in rapidly changing environments, especially when considering the stochastic nature of extreme weather events associated with climate change. Alternative mechanisms operating in a much shorter time frame than adaptation involve the rapid creation of alternate phenotypes within a life cycle or a few generations. Stochastic gene expression creates multiple phenotypes from the same genotype even in the absence of environmental cues. In contrast, other mechanisms for phenotype change that are externally driven by environmental clues include well-understood developmental phenotypic plasticity (variation, flexibility), which can enable rapid, within-generation changes. Increasingly appreciated are epigenetic influences during development leading to rapid phenotypic changes that can also immediately be very widespread throughout a population, rather than confined to a few individuals as in the case of favorable gene mutations. Such epigenetically-induced phenotypic plasticity can arise rapidly in response to stressors within a generation or across a few generations and just as rapidly be 'sunsetting' when the stressor dissipates, providing some capability to withstand environmental stressors emerging from climate change. Importantly, survival mechanisms resulting from adaptations and developmental phenotypic plasticity are not necessarily mutually exclusive, allowing for classic 'bet hedging'. Thus, the appearance of multiple phenotypes within a single population provides for a phenotype potentially optimal for some future environment. This enhances survival during stochastic extreme weather events associated with climate change. Finally, we end with recommendations for future physiological experiments, recommending in particular that experiments investigating phenotypic flexibility adopt more realistic protocols that reflect the stochastic nature of weather.

“MONTHLY VARIATION IN THE LEUKOCYTE PROFILE OF BARISIA IMBRICATA IN AN AGRICULTURAL LANDSCAPE.”

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ABSTRACT

The abundance of each type of white blood cell in the peripheral bloodstream is a useful indicator to understand the health status of an organism. Alterations in leukocyte profiles, (especially changes in the heterophils to lymphocytes ratio) can indicate stress that may be caused by environmental factors, injuries, diseases, and habitat changes. In this study, we determined the abundances of white blood cells (lymphocytes, monocytes, heterophils, eosinophils, and basophils) from a population of the Imbricate Alligator Lizard that inhabits an agricultural environment located at Central México. By examining blood samples under a microscope, we identified each type of white blood cell. We calculated the relative abundances of each cell and determined the leukocyte profile. Additionally, we calculated the heterophil/lymphocyte ratio (H:L) to assess the population's stress levels. Using the obtained data, we determined the variations of each type of leukocyte throughout the year, as well as seasonal changes in the H:L ratio. We compared the data obtained between seasons and sexes. We found no difference in cell proportions between sexes. Monocytes abundances are different between the spring and summer seasons. An increase in H/L ratios was observed during the winter months, indicating high levels of stress.

RESUMEN

La abundancia de glóbulos blancos en el torrente sanguíneo es un indicador útil para comprender el estado de salud de un organismo. Las alteraciones en el perfil leucocitario (especialmente cambios en la proporción de heterófilos / linfocitos) pueden indicar estrés causado por factores ambientales, enfermedades y cambios en el hábitat. En este estudio, determinamos la abundancia de glóbulos blancos (linfocitos, monocitos, heterófilos, eosinófilos y basófilos) de una población del Lagarto Caimán Imbricado que habita un ambiente agrícola ubicado en el Centro de México. Examinamos muestras de sangre bajo el microscopio, identificamos cada tipo de glóbulo blanco. Calculamos las abundancias relativas de cada célula y determinamos el perfil leucocitario. Además, calculamos la relación heterófilos/linfocitos (H:L) para evaluar los niveles de estrés de la población. Con los datos obtenidos se determinaron las fluctuaciones de cada tipo de leucocito a lo largo del año, así como los cambios estacionales de la proporción H:L. Comparamos los datos obtenidos entre temporadas y sexos. No encontramos diferencias en las proporciones celulares entre sexos. La abundancia de monocitos es diferente entre las estaciones de primavera y verano. Se observó un aumento en la proporción H/L durante los meses de invierno, lo que indica altos niveles de estrés.

“SO THAT’S WHY YOU AND I GOT DIFFERENT RESULTS!!”

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ABSTRACT

It has long been perplexing (at least to us) why two different labs making the same cardio-respiratory measurements on the same species get different results!! Researchers (perhaps secretly) think the ‘other guy’ wasn’t careful, didn’t have good equipment, use sick or old animals, etc. etc. But could the answer simply involve genetic differences between animals? More specifically, could variation in cardiorespiratory physiology—even something as straightforward to measure as heart rate—have a genetic basis? We hypothesized that experimental variation in cardiovascular physiology results from genetic variation between individuals. To test this hypothesis, we measured cardiorespiratory variables during development in larval zebrafish (*Danio rerio*) from low heterozygosity NHRGI-1 and AB wild type strains. For all measured cardiorespiratory variables, larvae of low heterozygosity NHRGI-1 larvae typically had a lower Coefficient of Variation during development compared to AB Wild type larvae. We conclude that differences in cardiorespiratory studies on zebrafish conducted in different laboratories could ‘simply’ result from genetic differences within or between populations of what is supposedly the same strain. Thus, studies on “wild type” animals may inevitably lead to variation in physiology as well as morphology. *“There ain’t no ‘good guy’, there ain’t no bad guy—there’s just you and me and we just disagree”* (We Just Disagree, Dave Mason, 1977)

RESUMEN

¡Durante mucho tiempo ha sido desconcertante (al menos para nosotros) por qué dos laboratorios diferentes que realizan las mismas mediciones cardiorrespiratorias en la misma especie obtienen resultados diferentes! Los investigadores (quizás en secreto) piensan que el ‘otro’ no tuvo cuidado, ¿no? tener buen equipo, usar animales enfermos o viejos, etc. etc. Pero, ¿podría la respuesta simplemente implicar diferencias genéticas entre animales? Más específicamente, ¿podría la variación en la fisiología cardiorrespiratoria (incluso algo tan sencillo de medir como la frecuencia cardíaca) tener una base genética? Nuestra hipótesis es que la variación experimental en la fisiología cardiovascular es el resultado de la variación genética entre individuos. Para probar esta hipótesis, medimos variables cardiorrespiratorias durante el desarrollo en larvas de pez cebra (*Danio rerio*) de cepas de tipo salvaje NHRGI-1 y AB de baja heterocigosidad. Para todas las variables cardiorrespiratorias medidas, las larvas de NHRGI-1 de baja heterocigosidad generalmente tenían un coeficiente de variación más bajo durante el desarrollo en comparación con las larvas AB de tipo salvaje. Concluimos que las diferencias en los estudios cardiorrespiratorios en pez cebra realizados en diferentes laboratorios podrían “simplemente” ser el resultado de diferencias genéticas dentro o entre poblaciones de lo que supuestamente es la misma cepa. Por lo tanto, los estudios sobre animales “salvajes” pueden conducir inevitablemente a variaciones tanto en la fisiología como en la morfología. “No hay ningún ‘bueno’, no hay ningún malo; solo estamos tú y yo y simplemente no estamos de acuerdo” (We Just Disagree, Dave Mason, 1977)

ACCLIMATIZATION CAPACITY TO HYPOXIA IN HIGH ALTITUDE AND ITS INFLUENCE ON THE ELEVATIONAL LIMITS OF SPECIES

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ABSTRACT

In montaneous environments, oxygen availability decreases as elevation increases, possibly imposing physiological limits on species migrating from lowlands in search of their optimal habitat. In the context of global warming, this question has conservation importance in the distribution of species and their elevational ranges. Under this scenario, small mammals are particularly vulnerable due to their high metabolic demands. Here we report results from two research projects using the deer mouse (*Peromyscus maniculatus*) and the leaf-eared mouse (*Phyllotis vaccarum*) native highland and lowland. Our focus is to understand: How does the acclimatization capacity of species affect their ability to colonize new environments?. We designed experiments to assess the relative contribution of evolution and phenotypic plasticity on performance and thermogenic aerobic capacity ($\dot{V}O_{2max}$) of mice when challenged to extreme cold and oxygen deficient conditions. We complement the studies with measurements of multiple cardio-respiratory variables involved in the oxygen cascade and also with transcriptomic analyzes to understand the gene expression patterns that underlie the responses under these conditions. Our results expand current knowledge about factors influencing species elevational limits and challenge our knowledge of what is physiologically possible when living in extreme conditions.

RESUMEN

En ambientes montañosos, la disponibilidad de oxígeno disminuye conforme la elevación aumenta, posiblemente imponiendo límites fisiológicos a las especies que migran desde tierras bajas rastreando su hábitat óptimo. En el contexto de calentamiento global, ésta pregunta tiene importancia de conservación en la distribución de las especies y sus rangos elevacionales. Bajo este escenario, los mamíferos pequeños son particularmente vulnerables debido a su alta demanda metabólica. Aquí reportamos resultados de dos proyectos de investigación en los que usamos ratones ciervo *Peromyscus maniculatus* y ratones orejudos *Phyllotis vaccarum* nativos de tierras altas y tierras bajas. Nuestro enfoque es entender: Cómo es que la capacidad de aclimatación de las especies afecta su habilidad para colonizar ambientes nuevos?. Diseñamos experimentos para evaluar la contribución relativa de la evolución y la plasticidad fenotípica en el rendimiento y la capacidad aeróbica termogénica ($\dot{V}O_{2max}$) de los ratones al ser desafiados a condiciones extremas de frío y deficiencia de oxígeno. Complementamos los estudios con medidas de múltiples variables cardio-respiratorias involucradas en la cascada del oxígeno y también con análisis transcriptómicos para entender los patrones de expresión de genes que subyacen las respuestas bajo esas condiciones. Nuestros resultados expanden el conocimiento actual sobre los factores que influyen los límites elevacionales de las especies y desafían nuestro conocimiento de lo que es fisiológicamente posible al vivir en condiciones extremas, propias del fin del mundo.

ACTIN POLYMERIZATION CONTRIBUTES TO CLOSURE OF THE AVIAN DUCTUS ARTERIOSUS (*GALLUS GALLUS*)

Rippamonti, J. and Dzialowski, E.M.

ABSTRACT

The ductus arteriosus (DA) is an oxygen-sensitive embryonic blood vessel present in all developing amniotes. The DA connects the pulmonary artery to the aorta and shunts blood away from nonfunctioning lungs and toward the fetal respiratory organ. Permanent closure of this vessel upon birth or hatch is necessary to establish proper blood circulation in neonates. Smooth muscle contraction, such as in the DA, is governed by myosin light chain kinase stimulating increased cross-bridge cycling and through increases in actin polymerization. The Rac and Cdc42 GTPase pathways increase contractile strength in smooth muscle cells by activating the nucleator Arp2/3, leading to increased actin polymerization. In this study, we investigated the role of the Rac and Cdc42 GTPase pathways on closure of DA in late-term chicken (*Gallus gallus*) embryos. Using a wire myograph, the DA were exposed to inhibitors of the Rac and Cdc42 GTPase pathways, which blocked the ability of vessels to contract in response to 25% oxygen. Similarly, inhibition of the Arp2/3 complex reduced the ability of the vessel to respond to oxygen. These findings suggest that Arp2/3 activation plays a role in active tension of the chicken DA during development by potentially increasing actin polymerization.

ARE THERE CONSTRAINTS ON THE DEVELOPMENTAL PLASTICITY OF METABOLIC PHYSIOLOGY OF AN ENDOTHERMIC INSECT?

Sara Wilmsen and Edward Dzialowski

ABSTRACT

Oxygen levels during development have been found to impact the development of insect respiratory and metabolic systems. For example, adult trachea and mitochondria density in *Drosophila* is dependent on the ambient oxygen levels experienced during larval development. Being ectothermic, these species have a low metabolic demand. Species such as the moth *Manduca sexta* are endothermic having a high metabolic demand for thermoregulation and flight. Here we asked how the higher energy demands of an endothermic insect influence the developmental plasticity of the metabolic system under either hypoxic or hyperoxic developmental conditions. *Manduca sexta* were reared in either 10, 21, or 30% oxygen during their larval and pupal stages. We then examined the tracheal and mitochondrial morphology and physiology of the adult moth. Hypoxia and hyperoxia had significant effects on adult moth size. Hypoxic moths flew at lower oxygen levels compared with hyperoxic moths. Mitochondrial respiration rate in permeabilized flight muscle mitochondria was higher in hypoxic reared moths than in either control or hyperoxic moths. Unlike *Drosophila*, in response to hyperoxia and hypoxia there were no differences in mitochondrial densities or tracheal densities. The developing *Manduca sexta* shows no plasticity in mitochondrial or tracheal densities, potentially constrained by the high metabolic rate required for flight.

BACKNOCK: BACULOVIRUS DELIVERY OF DSRNA TO MOSQUITO OVARIES

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ABSTRACT

Piwi-interacting RNAs (piRNAs) play an essential role in germline development, transposon silencing, and potentially the breakdown of arboviruses. However, key functions of this pathway remain to be elucidated within the developing egg of *Aedes* mosquitoes. This project aims to target Zucchini (Zuc), a key endonuclease involved in piRNA biogenesis, through a baculovirus-mediated knockdown in the mosquito ovary. We will engineer a baculovirus vector to express a nanos 3'UTR gene cassette containing a dsRNA hairpin against Zuc. Baculovirus allows for a systemic infection, while the nanos transcript will direct the dsRNA hairpin to the developing oocyte. Impacts on Zuc transcript levels will be evaluated by qPCR. To assess effects on the piRNA pathway, we will perform small RNA sequencing on ovaries at various stages of embryogenesis. Bioinformatic analysis will evaluate changes in piRNA development and transposon silencing. Off-target activity due to viral infection can be analyzed with modified cassettes. This project will pioneer the use of the baculovirus system for targeted gene knockdowns in *Aedes* mosquito tissues, expanding the toolbox of methods to explore piRNA biogenesis within early egg development. Overall, this project aims to significantly advance understanding of the piRNA pathway in mosquitoes through targeted manipulation of a key nuclease.

RESUMEN

Los ARN piwi-interaccionantes (piARN) juegan un papel esencial en el desarrollo de las células germinales, el silenciamiento de transposones y potencialmente en la ruptura de arbovirus. Sin embargo, aún se deben dilucidar funciones claves de esta vía dentro del desarrollo del huevo de mosquitos *Aedes*. Este proyecto tiene como objetivo apuntar a Zucchini (Zuc), una endonucleasa clave involucrada en la biogénesis de piARN, a través de un silenciamiento mediado por baculovirus en el ovario del mosquito. Ingeniaremos un vector de baculovirus para expresar un casete con la región 3' UTR de nanos que contenga una horquilla de ARN de doble cadena contra Zuc. El baculovirus permite una infección sistémica, mientras que el transcrito de nanos dirigirá el pelo de ARN de doble cadena al ovocito en desarrollo. Se evaluarán los impactos en los niveles de transcripción de Zuc mediante PCR cuantitativa. Para evaluar los efectos en la vía piARN, realizaremos la secuenciación de ARN pequeños en ovarios en diversas etapas de la embriogénesis. El análisis bioinformático evaluará los cambios en el desarrollo de piARN y el silenciamiento de transposones. La actividad fuera de objetivo debido a la infección viral se puede analizar con casetes modificados. Este proyecto sentará las bases para el uso del sistema de baculovirus para silenciamientos génicos dirigidos en tejidos de mosquitos *Aedes*, expandiendo las opciones metodológicas para explorar la biogénesis de piARN dentro del desarrollo temprano del huevo. En general, este proyecto tiene como objetivo avanzar significativamente en la comprensión de la vía piARN en mosquitos a través de la manipulación dirigida de una nucleasa clave.

BAROREFLEX SENSITIVITY IN THE AMERICAN ALLIGATOR (ALLIGATOR MISSISSIPPIENSIS)

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ABSTRACT

The baroreflex is a control mechanism that is a critical component in the maintenance of arterial blood pressure regulation. Baroreflex control has been studied in other crocodilians like the saltwater crocodile (*Crocodylus porosus*) but has yet to be assessed in the American alligator (*Alligator mississippiensis*). However prior studies have not investigated if the reflex exhibits greater sensitivity to increases or decreases in arterial pressure. Additionally, exposure to chronic hypoxia during development has been shown to alter the baroreflex sensitivity and increase the baseline mean arterial blood pressure in adult chickens (*Gallus gallus domesticus*). Prior work has established that hypoxic incubated alligators have a reduced mean arterial pressure, possibly indicating a difference in the set point for the cardiac limb of the baroreflex. Juvenile alligators (7-19 kg), from both hypoxic (10% O₂) incubated (n=12) and normoxic (21% O₂) incubation (n=10) were instrumented for measurements of systemic arterial blood pressure (P_{sys}) and heart rate (fH). Following instrumentation, the animals were injected with the vasoconstrictor phenylephrine (PE) or the vasodilatory sodium nitroprusside. Baroreflex function was estimated using dynamic method consisting of fitting changes in heart rate with changes in arterial pressure to a nonlinear four-variable sigmoidal logistic function. Preliminary results indicate alligators exhibit greater sensitivity to decreases in arterial pressure compared to increases in pressure. Hypoxic incubated alligators also showed a reduced baroreflex response, indicating they are less sensitive to changes in pressure.

BRIDGING ECOPHYSIOLOGY AND DATA SCIENCE: OPTIMIZING BIO-INSPIRED STRUCTURES THROUGH CONSTRUCTAL DESIGN

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ABSTRACT

The harmonious integration of ecophysiology, constructal theory, and data science holds transformative potential for advancing bio-inspired designs in various technological applications. This lecture aims to shed light on the intricate fractal patterns intrinsic to biological systems—ranging from the labyrinthine gills in fishes to the geometries in plant vascular systems—and extrapolate their governing principles for applications such as lab-on-a-chip technologies, thermophoretic nano-engineering and acoustic bio-metamaterials. First, we delve into the constructal theory as a unifying lens to interpret the optimization of flows in biological systems. Using the gill structures in aquatic organisms as a prime example, we elucidate how these natural systems have evolved to optimize the flow of essential elements like oxygen, setting a precedent for bio-inspired microfluidic systems. Second, we explore the concept of acoustic bio-metamaterials, inspired by natural pressure flows, to design phononic crystals with unique acoustic properties. Central to this discussion is the application of data science methods, particularly Fisher information metrics, in optimizing these bio-inspired designs. By bridging the foundational principles of ecophysiology with cutting-edge data analytics, this review posits a framework for the iterative design and optimization of next-generation bio-inspired technologies. The article serves as a nexus for interdisciplinary approaches that leverage the best of biology, physics, and data science to solve complex engineering challenges.

RESUMEN

La integración armoniosa de la ecofisiología, la teoría constructal y la ciencia de datos tiene un potencial transformador para avanzar en diseños bioinspirados en diversas aplicaciones tecnológicas. Este artículo de revisión tiene como objetivo arrojar luz sobre los intrincados patrones fractales intrínsecos a los sistemas biológicos, que van desde las branquias laberínticas en los peces hasta las geometrías en los sistemas vasculares de las plantas, y extrapolar sus principios rectores para aplicaciones como tecnologías de laboratorio en un chip, nanoingeniería termoforética y biomateriales acústicos. En primer lugar, profundizamos en la teoría constructal como una lente unificadora para interpretar la optimización de los flujos en sistemas biológicos. Utilizando las estructuras branquiales en organismos acuáticos como un ejemplo principal, elucidamos cómo estos sistemas naturales han evolucionado para optimizar el flujo de elementos esenciales como el oxígeno, estableciendo un precedente para sistemas microfluídicos bioinspirados. En segundo lugar, exploramos el concepto de biomateriales acústicos, inspirados en flujos de presión naturales, para diseñar cristales fonónicos con propiedades acústicas únicas. Central en esta discusión es la aplicación de métodos de ciencia de datos, particularmente métricas de información de Fisher, en la optimización de estos diseños bioinspirados. Al conectar los principios fundamentales de la ecofisiología con análisis de datos de vanguardia, esta revisión plantea un marco para el diseño iterativo y la optimización de tecnologías bioinspiradas de próxima generación. El artículo sirve como un nexo para enfoques interdisciplinarios que aprovechan lo mejor de la biología, la física y la ciencia de datos para resolver desafíos de ingeniería complejos.

**CARDIAC ACTIVITY DURING THERMOREGULATION IN THE ALLIGATOR LIZARD
BARISIA IMBRICATA (SQUAMATA: ANGUIDAE): IMPORTANCE OF CHOLINERGIC AND
ADRENERGIC MECHANISMS**

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ABSTRACT

The effectiveness of thermoregulation can be increased by changes in internal heat transfer caused by changes in heart rate and blood flow. The heart of some reptiles may have an increase in heart rate during the increase in temperature that will be faster than during the decrease in temperature. The Mountain Alicante, *Barisia imbricata*, is a species of lizard of which there are records at considerable altitude elevations, extreme temperatures (-3 to 35 °C) and relative hypoxic environments. It is proposed that *B. imbricata* possesses cardiovascular adaptive physiological mechanisms regulated by cholinergic and/or adrenergic mechanisms to cope with these stochastic environments. This study suggests that the cardiac hysteresis response in *B. imbricata* will be mediated by a humoral response. To determine the humoral route, we will perform sympathetic and parasympathetic pharmacological stimulation (Propranolol and Atropine). Everyone will undergo a Propranolol treatment prior to instrumentation for the detection of cardiac activity. Subsequently, after 48 hours, the following Atropine treatment will be applied. Adult *B. imbricata* individuals will be instrumented with heart rate sensors (Add Instruments®) and then subjected to a regime of temperature changes (5°C to 35 °C).

RESUMEN

La efectividad de la termorregulación puede incrementarse por cambios en la transferencia interna de calor provocados por modificaciones en la frecuencia cardíaca y el flujo sanguíneo. El corazón de algunos reptiles puede presentar incremento en la frecuencia cardíaca durante el aumento de temperatura que será más rápido que durante la disminución de temperatura. El Alicante de Montaña, *Barisia imbricata*, es una especie de lagartija de la cual se tienen registros a elevaciones de altitud considerable, temperaturas extremas (-3 a 35 °C) y ambientes de hipoxia relativa. Se plantea que *B. imbricata* posee mecanismos fisiológicos adaptativos cardiovasculares regulados por mecanismos colinérgicos y/o adrenérgicos para hacer frente a estos ambientes estocásticos. Este estudio plantea que la respuesta de histéresis cardíaca en *B. imbricata* será mediada por una respuesta humoral. Para determinar la ruta humoral realizaremos estimulación farmacológica simpática y parasimpática (Propranolol y Atropina). Cada individuo será sometido a un tratamiento de Propranolol previo a la instrumentación para la detección de actividad cardíaca. Posteriormente después de 48 horas se aplicará el siguiente tratamiento Atropina. Se instrumentarán individuos adultos de *B. imbricata* con sensores de ritmo cardíaco (Add Instruments®) para luego someterlos a un régimen de cambios de temperatura (5°C hasta 35 °C).

DESIGN OF A BIO-INK FOR CORNEAL 3D-BIOPRINTING

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Abstract.

The cornea is the main optical element of the ocular system. Corneal diseases or physical damage can cause opacity, resulting in corneal blindness; the only accepted treatment is corneal transplantation. Although corneal transplant is the most common type of tissue transplantation worldwide, there is an increasing shortage of cornea donors. Tissue engineering of corneas suitable for transplantation has become one of the most promising solutions to meet the demand of corneas. Given the numerous advantages of 3D-printing in the field of biofabrication, different strategies have emerged aiming the 3D-bioprinting of corneal equivalents. Here, we present preliminary results regarding the formulation, rheological characterization, printability testing and optical assessment, of a bio-ink for 3D-Bioprinting of corneal constructs. Diseño de una biotinta para Biopresión-3D de córnea

Resumen.

La córnea es el principal elemento óptico del sistema ocular. No obstante, es afectada por distintas enfermedades o lesiones físicas que pueden causar opacidad, resultando en ceguera corneal; el único tratamiento disponible es el trasplante de córnea. A pesar de que el trasplante de córnea es el más común a nivel mundial, existe un creciente déficit de donadores. El desarrollo de córneas trasplantables mediante la Ingeniería de Tejidos se ha convertido en una de las alternativas más prometedoras para satisfacer la demanda de tejido. Dadas las numerosas ventajas de la Impresión 3D en el campo de la biofabricación, diferentes estrategias se han reportado con el objetivo de fabricar equivalentes de córnea a través de la Bioimpresión-3D. En este trabajo, presentamos algunos resultados preliminares respecto de la formulación, caracterización reológica, pruebas de impresibilidad y evaluación de la transparencia de una biotinta para la Bioimpresión-3D de constructos corneales.

DEVELOPMENTAL CRITICAL WINDOWS IN THE ZEBRAFISH (*DANIO RERIO*)

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ABSTRACT

Developmental critical windows are specific periods of time in development where the phenotype is sensitive to intrinsic (*e.g.*, hormones) and extrinsic factors (*e.g.*, oxygen availability). Alterations during early development may: 1) increase mortality, 2) promote long-term affectations, or 3) induce self-repair mechanisms to 'return' the animal to its original developmental trajectory. In the current study, NHGRI-1 zebrafish were employed to reveal morphological (total length, body mass) and physiological (cardiac function, oxygen consumption) developmental critical windows as a function of high temperature (33°C) and hypoxia (10 kPaO₂). Incubation was divided into five developmental periods: gastrulation, organogenesis, onset of heart beat, fin bud formation, and hatch. Higher temperature groups showed shorter developmental time compared to controls. Hypoxic fish showed increased developmental time, especially prior hatching. Body mass was decreased in hypoxic fish between fertilization and organogenesis. Total length increased with increased temperature. Cardiac output was decreased in hypoxic fish after fin bud formation, and it increased in high temperature groups. Our results suggest that exposure to environmental factors during specific times in early development may lead to multiple developmental trajectories. However, further research is needed to determine whether alterations are permanent or may be restored ontogenetically or trans generationally.

Las ventanas críticas de desarrollo son periodos de tiempo en el desarrollo en que el fenotipo es sensible a factores intrínsecos (*e.g.*, hormonas) y extrínsecos (*e.g.*, disponibilidad de oxígeno). Las alteraciones durante el desarrollo temprano pueden: 1) incrementar la mortalidad, 2) promover alteraciones, o 3) inducir mecanismos de auto reparación para 'retornar' al animal a su trayectoria original de desarrollo. En este estudio, peces cebras de la línea NHGRI-1 se utilizaron para revelar ventanas críticas morfológicas (longitud total, masa corporal) y fisiológicas (función cardíaca, consumo de oxígeno) de desarrollo en función de alta temperatura (33°C) e hipoxia (10 kPaO₂). El periodo de incubación se dividió en cinco periodos: gastrulación, organogénesis, inicio del ritmo cardíaco, formación del botón de la aleta pélvica y eclosión. Los peces a mayor temperatura mostraron tiempos de desarrollo mas cortos que el control. Los peces en hipoxia mostraron un incremento en tiempo de desarrollo, especialmente en el periodo antes de la eclosión. La masa corporal de los peces en hipoxia disminuyó entre fertilización y organogénesis. La longitud total fue mayor en peces expuestos a alta temperatura. El esfuerzo cardíaco disminuyó en peces en hipoxia después de la formación del botón de la aleta pélvica, e incrementó con mayor temperatura. Los resultados sugieren que la exposición a factores ambientales durante periodos específicos en el desarrollo temprano puede resultar en múltiples trayectorias de desarrollo. Sin embargo, se requiere de más investigación para determinar si estas alteraciones son permanentes o pueden re establecerse en etapas posteriores de la ontogenética o de manera transgeneracional.

DIESEL EXHAUST PARTICULATE EXPOSURE ALTERS HORMONE SIGNALING IN FEMALE MICE

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ABSTRACT

Several studies report a correlation between air pollution and increased risk of cardiovascular disease (CVD). Both epidemiological and animal studies have revealed significant sex differences in both CVD and sex-dependent responses to particulate matter (PM) exposure. However, the underlying mechanisms that drive these sex dependent responses are not fully characterized and most of existing studies focus on outcomes in males. Therefore, it is critical to identify contributing stressors and the underlying mechanisms driving the air pollution-mediated progression of CVD in women. To investigate this, we exposed female C57Bl/6 mice on either a low-fat diet (LF) or high-fat diet (HF) to either 35 µg of suspended DEP or sterile saline for 4 weeks. RT-qPCR and multiplex immunoassays were used to quantify endpoints. Hormone multiplex assays revealed significant alterations in hormone signaling following DEP exposure. These results were exacerbated by the addition of a high-fat diet. Air pollution exposure appeared to have the most significant influence on estradiol and thyroid hormone levels. Further studies will elucidate the possible mechanism of disrupted hormone signaling in driving a CVD phenotype.

RESUMEN

Varios estudios informan una correlación entre la contaminación del aire y un mayor riesgo de enfermedad cardiovascular (ECV). Tanto los estudios epidemiológicos como los realizados en animales han revelado diferencias significativas entre sexos tanto en las enfermedades cardiovasculares como en las respuestas dependientes del sexo a la exposición a partículas (PM). Sin embargo, los mecanismos subyacentes que impulsan estas respuestas dependientes del sexo no están completamente caracterizados y la mayoría de los estudios existentes se centran en los resultados en los hombres. Por lo tanto, es fundamental identificar los factores estresantes que contribuyen y los mecanismos subyacentes que impulsan la progresión de las enfermedades cardiovasculares mediada por la contaminación del aire en las mujeres. Para investigar esto, expusimos ratones hembra C57Bl/6 con una dieta baja en grasas (LF) o una dieta alta en grasas (HF) a 35 µg de DEP suspendida o solución salina estéril durante 4 semanas. Se utilizaron RT-qPCR e inmunoensayos multiplex para cuantificar los criterios de valoración. Los ensayos hormonales múltiples revelaron alteraciones significativas en la señalización hormonal después de la exposición a DEP. Estos resultados se vieron exacerbados por la adición de una dieta alta en grasas. La exposición a la contaminación del aire pareció tener la influencia más significativa sobre los niveles de estradiol y hormona tiroidea. Estudios adicionales dilucidarán el posible mecanismo de alteración de la señalización hormonal que impulsa un fenotipo de ECV.

DISTRIBUCIÓN POTENCIAL ANTE EL CAMBIO CLIMÁTICO DE ESPECIES DEL GÉNERO CROTALUS EN MÉXICO

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One of the main threats to biodiversity worldwide is climate change, climate change is predicted to lead to changes in species distribution and extinctions over the coming decades, Particularly reptiles are vulnerable to this phenomenon, since they are ectothermic organisms and are very susceptible to climatic agents. Modeling the distribution of species at risk is extremely important for their conservation and management. In this study we will use ecological niche models to predict the potential distribution of rattlesnake species living in Mexico (*Crotalus* spp.) different climate change scenarios at present and in projections for the years 2050 and 2070, under contrasting climate change scenarios, using the machine learning technique of maximum entropy in the MAXENT software, this shall be calculated using current database records and explanatory and biophysical variables. Finally, a model will be calculated to calculate the risk of extinction of species of the genus *Crotalus* in Mexico.

Una de las principales amenazas para la biodiversidad en todo el mundo es el cambio climático, se predice que el cambio climático provocará cambios en la distribución de las especies y extinciones durante las próximas décadas, particularmente los reptiles son vulnerables a este fenómeno, puesto que son organismos ectotermos y son muy susceptibles a los agentes climáticos. Modelar la distribución de especies en riesgo es extremadamente importante para su conservación y manejo. En el presente estudio utilizaremos modelos de nicho ecológico para predecir la distribución potencial de especies de serpientes de cascabel que habitan en México (*Crotalus* spp.) ante diferentes escenarios de cambio climático en la actualidad y en proyecciones para los años 2050 y 2070, bajo escenarios de cambio climático contrastantes, mediante la técnica basada en machine learning de máxima entropía en el software MAXENT, esto se calculará utilizando registros de bases de datos actuales y variables explicativas y biofísicas. Finalmente se calculará un modelo para calcular el riesgo de extinción de especies del género *Crotalus* en México.

EFFECTS OF SUBTERRANEAN WATER FROM THE LERMA RIVER ON THE BEHAVIOR OF DANIO REIRO

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ABSTRACT

Fish are highly sensitive to changes in water quality and are known to exhibit a variety of behavioral changes when exposed to contamination. Among these behavioral changes, swimming behavior has been shown to be negatively correlated with high levels of contamination. One of Mexico's most important water sources, the Lerma River, is becoming increasingly polluted at an alarming rate. The Lerma River is home to many endemic fish species and is connected to the Toluca Valley aquifer, a major source of groundwater, supplying drinking water to millions of people. This emphasizes the importance of understanding how this water potentially affects life. Zebrafish, *Danio rerio*, is a frequently used model species due to their physiological similarities to mammals, sensitivity to change, well-documented behavior, and low cost for care and maintenance. Therefore, this project proposes to use *D. rerio* as an animal model to examine how contamination levels from groundwater connected to the Lerma River affect aquatic life, by evaluating the changes in its behavior in response to the mixture of physicochemical characteristics derived from contamination.

RESUMEN

Los peces son sensibles a la variación en la calidad del agua y exhiben una diversidad de cambios de comportamiento cuando se exponen al agua contaminada. Entre los cambios, se ha demostrado que el comportamiento de nado se correlaciona negativamente con altos niveles de contaminación. Una de las fuentes de agua más importantes de México, el río Lerma, se contamina cada vez más a un ritmo alarmante, lo que trae consigo un sinnúmero de problemas, particularmente porque el río Lerma alberga muchas especies endémicas de peces y está conectado con el acuífero del Valle de Toluca, una importante fuente de agua subterránea que abastece de agua potable a millones de personas. Lo anterior enfatiza la importancia de comprender cómo esta agua afecta potencialmente la vida. El pez cebra, *Danio rerio*, es una especie modelo utilizada con frecuencia debido a sus similitudes fisiológicas con los mamíferos, sensibilidad al cambio, comportamiento bien documentado y bajo costo de cuidado y mantenimiento. Por lo anterior, este proyecto propone utilizar *D. rerio* como modelo de animal para examinar el efecto de la contaminación del agua subterránea en la vida acuática, mediante la evaluación de los cambios en su comportamiento.

ENGINEERED LIVING MATERIALS FOR BUILDING INDUSTRY, A BRIEF REVIEW

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Abstract:

During the last decade, the need for more efficient and viable materials has become bigger than ever. Technological progress allows to endow the materials with useful and novel properties. Engineered Living Materials are a viable option to satisfy the need of more environmentally friendly and novel materials due to their less polluting production processes and the potential to be applied to multiple areas. Engineered Living Materials are obtained from living organisms, such as cells and bacteria. In this piece of research, we present a short review of the state of the art regarding Engineered Living materials utilized in the building industry.

Materiales Ingenieriles de origen biológico para la industria de la construcción, una revisión breve

Resumen:

Durante la última década, la necesidad de materiales más eficientes y viables se ha vuelto más grande que nunca. El avance tecnológico hace posible dotar a los materiales con propiedades útiles y novedosas. Los materiales ingenieriles de origen biológico son una opción viable para satisfacer la necesidad de materiales novedosos y ambientalmente amigables, debido a su producción menos contaminante y su potencial para ser aplicados a múltiples áreas. Los materiales ingenieriles de origen biológico son obtenidos a partir de organismos vivos; tales como células y bacterias. En el presente trabajo mostramos una revisión corta sobre el estado del arte de los materiales ingenieriles de origen biológico empleados en la industria de la construcción.

FUNCTIONAL MECHANISM, MOLECULAR BASIS, AND EVOLUTION OF THE UNIQUE CO₂/HCO₃⁻-SENSITIVITY OF CROCODILIAN HEMOGLOBIN

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ABSTRACT

Crocodylians are semiaquatic reptiles that dive to avoid predators or kill prey by drowning them. This extraordinary breath-hold diving capacity has been ascribed to a unique mode of allosterically regulating hemoglobin (Hb)-oxygenation by bicarbonate (HCO₃⁻), which forms in red blood cells upon hydration of CO₂. Although known for decades, the functional mechanisms, the molecular basis, and the evolution of this remarkable mode of allosteric control were unknown until recently. We disentangled and provided direct evidence of this phenomenon through a series of studies: Study 1: We addressed the hypothesis that CO₂ primarily binds to hemoglobin, rather than being accumulated in plasma as in other vertebrates, during diving in caimans. We demonstrate that CO₂ primarily accumulates within the erythrocyte during diving and that most of the accumulated CO₂ is bound to hemoglobin. Furthermore, we show that this HCO₃⁻-binding is tightly associated with the progressive blood deoxygenation during diving. Study 2: We provide the first quantitative analysis of the separate allosteric effects of CO₂ and HCO₃⁻ on purified Hb of the spectacled caiman (*Caiman crocodilus*). Using thin-layer gas diffusion chamber and Tucker chamber techniques, we demonstrate that both CO₂ and bicarbonate bind to Hb with high affinity and strongly decrease O₂ saturation. Study 3: We investigated the origin and mechanistic basis of this novel biochemical phenomenon by performing directed mutagenesis experiments on resurrected ancestral Hbs. The gain of bicarbonate sensitivity in crocodilian Hb involved the direct effect of few amino acid substitutions at key sites in combination with indirect effects of numerous other substitutions at structurally disparate sites.

GENETIC DIVERSITY AND STRUCTURE OF PLESTIODON COPEI, A HIGH-MOUNTAIN LIZARD FROM CENTRAL MEXICO

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ABSTRACT

Plestiodon copei is an endemic lizard of the Trans-Mexican Volcanic Belt (FVT) that is threatened by habitat fragmentation, illegal trade and intentional killing because it is considered venomous. In this study, the structure and genetic diversity of *P. copei* were analyzed. Six microsatellite loci were analyzed, which showed high genetic diversity (H_e : 0.580-0.776) in the six populations studied. Likewise, significant values of F_{ST} showed significant genetic differentiation among populations. Similarly, the presence of barriers between populations associated with genetic discontinuities was detected. Finally, temporal changes in migration rates between *P. copei* populations were detected, with a significant decrease in present-day rates observed relative to estimated historical rates, supported by a model of isolation by distance. The data suggest that the genetic diversity of *P. copei* populations has not been affected despite severe habitat modification by recent anthropogenic activities and the null gene flow between adjacent populations; however, it is necessary to evaluate the mechanisms (i.e., multiple paternity, local adaptation) used by populations to maintain high genetic diversity even in isolation.

RESUMEN

Plestiodon copei es una lagartija endémica de la Faja Volcánica Transmexicana (FVT) que se encuentra amenazada por la fragmentación de su hábitat, el comercio ilegal y la muerte intencional por considerarse venenosa. En este estudio se analizó la estructura y diversidad genética de *P. copei*. Se analizaron seis loci de microsatélites, los cuales mostraron alta diversidad genética (H_e : 0.580-0.776) para las seis poblaciones estudiadas. Asimismo, valores significativos de F_{ST} mostraron una diferenciación genética significativa entre poblaciones. También se detectó la presencia de barreras entre poblaciones asociadas a discontinuidades genéticas. Finalmente, se detectaron cambios temporales en las tasas de migración entre poblaciones de *P. copei*, observándose una disminución significativa en las tasas actuales en relación con las tasas históricas estimadas y respaldado por un modelo de aislamiento por distancia. Los datos sugieren que la diversidad genética de las poblaciones de *P. copei* no se ha visto afectada a pesar de la severa modificación del hábitat por actividades antropogénicas recientes y el nulo flujo genético entre poblaciones adyacentes; sin embargo, es necesario evaluar los mecanismos (i.e., paternidad múltiple, adaptación local) utilizados por las poblaciones para mantener una alta diversidad genética, incluso estando en aislamiento.

HERENCIA EPIGENÉTICA INTRAGENERACIONAL DE FENOTIPOS TOLERANTES A HIPOXIA EN EL PEZ CEBRA (*DANIO RERIO*)

Gil M. Bautista y Warren W. Burggren

Numerosos estudios han asociado las alteraciones en el fenotipo de la descendencia (F_{1+n}) con condiciones estresantes experimentadas por su generación parental (F_0). En esta investigación se evaluó la transferencia epigenética de fenotipos tolerantes a hipoxia de la F_0 a individuos de diferentes puestas dentro de una generación subsecuente (F_1). La generación parental de peces cebras de la línea NHGRI-1 se expusieron a condiciones control de normoxia (21 kPaO₂) y 28°C, y a tres tratamientos de hipoxia a 9, 12 y 15 kPa durante tres semanas. Después, cada dos semanas se reprodujeron los peces para producir cuatro puestas ($F_{1,\#1}$ a $F_{1,\#4}$). Después de la exposición se evaluó el consumo de oxígeno ($\dot{M}O_2$), la tolerancia a la hipoxia (tiempo a la pérdida del equilibrio – $TLoE$) y el factor de condición (K) en la F_0 . En la descendencia se evaluó la masa corporal (BM), la longitud total (L_T), la función cardíaca y $TLoE$ a los 7 días post fertilización. En la F_0 , K no fue afectado por la hipoxia, pero $\dot{M}O_2$ y $TLoE$ incrementaron con la disminución en la PO₂. En la descendencia, se observaron diferencias en morfología solo en la $F_{1,\#1}$. En $F_{1,\#1,\#2}$ disminuyó $TLoE$ en peces de F_0 expuestos a 9 y 12 kPaO₂ y desapareció en $F_{1,\#3,\#4}$. El ritmo cardíaco disminuyó en $F_{1,\#1}$ en los tres tratamientos con hipoxia. El volumen sistólico y el esfuerzo cardíaco se mantuvieron elevados en la descendencia de peces expuestos a 9 kPa a lo largo del experimento. Los resultados sugieren que la severidad de la hipoxia puede modular la transferencia epigenética de fenotipos tolerantes a hipoxia en múltiples puestas dentro de una generación subsecuente a la generación parental. Además, este estudio provee perspectivas sobre la sensibilidad de la modificación epigenética y su estabilidad temporal.

HYPOXIA LEADS TO ALTERED CARDIAC PERFORMANCE IN THE ZEBRAFISH *DANIO RERIO*

Gil M. Bautista, Brandt Smith, Dane Crossley, Warren Burggren

ABSTRACT

Aquatic hypoxia occurs in marine and freshwater environments because of natural and anthropogenic causes. Fish cardiac performance is an important process to maintain homeostasis under hypoxic conditions. This study assessed heart/body mass ratio (HBR), force of contraction (FC), rate of contraction (RC), and 50% rate of relaxation (ROR) in AB and NHGRI-1 (inbred) zebrafish *Danio rerio* under control and hypoxic conditions. Moreover, assessments of variability were carried out. AB fish were divided into 1) controls (~ 21 kPaO₂), 2) exposed to four weeks of hypoxia (4WH; ~ 10 kPaO₂), and 3) life-long hypoxia (LLH; ~ 10 kPaO₂). NHGRI-1 fish were divided only into controls and 4-weeks hypoxia. Temperature was 28°C for all fish. LLH AB fish showed decreased HBR compared to controls and 4WH. No differences in HBR were observed within NHGRI-1 groups. LLH AB fish had increased FC, RC, and ROR compared to controls and 4WH fish; no differences were observed in NHGRI-1 fish. LLH AB fish showed $\sim 40\%$ higher contractility than controls and 4WH fish during hypoxic challenges. No differences were observed in NHGRI-1 fish after hypoxic challenge; however, better-sustained contractility was observed in 4WH NHGRI-1 fish. Decreased variability in FC, RC, and ROR was only observed in 4WH NHGRI-1 fish during hypoxic exposure, compared to control fish. Our results suggest that LLH exposure elicited morphological and physiological changes allowing fish to sustain better cardiac performance in control and hypoxic conditions. Moreover, the use of animal models with decreased heterozygosity may be useful to reduce variation during morphological and physiological assessments.

La Hipoxia Conduce a Alteraciones en el Desempeño Cardíaco en el Pez Cebra *Danio rerio*

RESUMEN

La hipoxia acuática ocurre en ambientes marinos y dulceacuícolas debido a causas naturales y antropogénicas. El desempeño cardíaco de los peces es un proceso importante para mantener la homeostasis durante condiciones hipóxicas. Este estudio evaluó la relación corazón/masa corporal (HBR), la fuerza de contracción (FC), la tasa de contracción (RC), y la tasa al 50% de relajación (ROR) en peces cebra *Danio rerio* de las líneas AB y NHGRI-1 (endogámica) en condiciones control e hipóxicas. Además, se evaluó la variabilidad en los datos. Los peces de la línea AB se dividieron en 1) control (~ 21 kPaO₂), 2) cuatro semanas en hipoxia (4WH, ~ 10 kPaO₂), y 3) criados y mantenidos en hipoxia (LLH, ~ 10 kPaO₂). Los peces de la línea NHGRI-1 solo se

dividieron en control y 4WH. La temperatura fue de 28°C para todos los peces. Los peces AB LLH mostraron menor HBR comparado con los controles y 4WH. No se observaron diferencias en HBR en peces NHGRI-1. Los peces AB LLH mostraron mayor FC, RC y ROR comparados con los controles y 4WH; no se observaron diferencias en peces NHGRI-1. Los peces AB LLH mostraron contracción cardíaca ~40% mayor que los controles y 4WH durante el desafío de hipoxia. No se observaron diferencias en peces NHGRI-1 durante ensayo de hipoxia; sin embargo, los peces 4WH mantuvieron mayor contractibilidad. Solo durante exposición a hipoxia se observó menor variabilidad en FC, RC, y ROR en peces 4WH NHGRI-1. Los resultados sugieren que la exposición LLH promovió cambios morfológicos y fisiológicos permitiendo mantener un mejor desempeño cardíaco en condiciones control y de hipoxia. Además, la utilización de modelos animales con heterocigosidad disminuida pueden ser utilizados para reducir la variación en estudios morfológicos y fisiológicos.

HYPOXIA'S INFLUENCE ON LEAD TOXICITY, CALCIUM, AND TRANSCRIPTION IN ZEBRAFISH

Mckenzie Metzner, Mehrnaz Moghimi, Cameron Emadi, Allie Burdette-Lapuz, Haneen Ahmad, Mohammed-Izhan Lakhani, Martin Grossell And Edward Mager

ABSTRACT

Evidence suggests that the hypoxia signaling pathway influences freshwater fish ionoregulation. A previous study revealed that hypoxia (20% air saturation) leads to hypocalcemia in embryonic/larval zebrafish likely due to a reduction in ionocytes expressing the epithelial calcium channel (ECaC). Given that Pb is believed to enter fish through Ca transport pathways, such as ECaC, we sought to elucidate whether Pb-hypoxia co-exposure would reduce or enhance Pb toxicity. Preliminary results demonstrated that whole body Pb and Ca accumulation at 96-hours was lowest at 40% air saturation compared to 20% and 100% air saturation. We also found that 40% air saturation resulted in the highest mortality of zebrafish embryos when co-exposed to Pb. These results suggest that exacerbated hypocalcemia triggered by competition between Pb and Ca uptake is the likely cause of greater Pb toxicity in hypoxia. To further characterize the role of hypoxia in mediating Pb toxicity, we are conducting additional bioassays of Pb-hypoxia co-exposure at 30% and 50% air saturation to assess acute mortality and Pb/Ca accumulation. A separate time-course experiment will examine the mRNA expression for target genes of interest involved in the hypoxia response and Ca homeostasis: hypoxia inducible factor (HIF1ab), N-myc-downstream regulated genes (NDRG1a), and ECaC.

RESUMEN

Influencia de la Hipoxia en la Toxicidad del Plomo, el Calcio y la Transcripción en el Pez Cebra.

Las evidencias sugieren que la vía de señalización de la hipoxia influye en la ionorregulación de los peces de agua dulce. Un estudio previo reveló que la hipoxia (20% de saturación de aire) conduce a la hipocalcemia en embriones/larvas de pez cebra, probablemente debido a una reducción en los ionocitos que expresan el canal de calcio epitelial (ECaC). Dado que se cree que el Pb (plomo) ingresa a los peces a través de las vías de transporte de Ca, como ECaC, buscamos dilucidar si la coexposición de Pb e hipoxia reduciría o aumentaría la toxicidad del Pb. Resultados preliminares demostraron que la acumulación de Pb y Ca en todo el cuerpo a las 96 horas fue menor al 40% de saturación de aire en comparación con el 20% y el 100% de saturación de aire. También encontramos que el 40% de saturación de aire resultó en la mayor mortalidad de los embriones de pez cebra cuando estuvieron expuestos al Pb simultáneamente. Estos resultados sugieren que la hipocalcemia exacerbada, desencadenada por la competencia entre la captación de Pb y Ca, es probablemente la causa de una mayor toxicidad del Pb en la hipoxia. Para caracterizar aún más el papel de la hipoxia en la mediación de la toxicidad del Pb, estamos llevando a cabo bioensayos adicionales de coexposición de Pb e hipoxia al 30% y al 50% de saturación de aire para evaluar la mortalidad aguda y la acumulación de Pb/Ca. Un experimento de series temporales separado examinará la expresión de ARNm para genes de interés involucrados en la respuesta a la hipoxia y la homeostasis del calcio: el factor inducible de hipoxia (HIF1ab), los genes regulados por las corrientes de N-myc (NDRG1a) y ECaC.

IDENTIFYING PATHWAYS INVOLVED IN AIR POLLUTION-EXPOSURE PROMOTION OF OBESITY.

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ABSTRACT

Exposure to air pollutants, including those generated by traffic-generated sources (e.g., vehicle exhaust), has been associated with promoting metabolic dysfunction and obesity in children and adults; however, the mechanisms involved have not been well characterized. Considering that the worldwide rate of obesity in the population has nearly doubled over the past 30 years, it is imperative to understand the factors and signaling pathways that drive the etiology of abnormal metabolic disease states and obesity and how environmental exposures may contribute. Ongoing studies in our laboratory have revealed that the gut microbiome profile, signaling from the gut, as well as systemic and adipose-tissue specific inflammatory and renin-angiotensin system (RAS) signaling, are altered with inhalation exposure to traffic-generated air pollutants. Each of these factors is known to regulate the homeostasis of adipocyte structure and function. Furthermore, these outcomes are further exacerbated when environmental exposures are coupled with a high-fat diet. Understanding the role of common-source environmental air pollutants in promoting toxicological outcomes across organ systems can provide essential information necessary to determine potential therapeutic targets and also evidence for environmental policy reform.

INTRAGENERATIONAL EPIGENETIC INHERITANCE OF HYPOXIA-TOLERANT PHENOTYPES IN ZEBRAFISH (*DANIO RERIO*)

Gil M. Bautista and Warren W. Burggren

ABSTRACT

Numerous studies have associated alterations in the phenotype of the offspring (F_{1+n}) with stressful conditions experienced by their parental generations (F_0). In the current research, the epigenetic transfer of hypoxia-tolerant phenotypes from F_0 to multiple clutches within an F_1 was assessed. Adult NHGRI-1 zebrafish were exposed to control conditions of normoxia (~21 kPaO₂) and 28°C, and three hypoxic treatments at 9, 12, and 15 kPa (all at 28°C) for three weeks. After every two weeks, fish were bred to produce four clutches ($F_{1,\#1}$ to $F_{1,\#4}$). After the exposure, oxygen consumption ($\dot{M}O_2$), hypoxia tolerance (time to loss of equilibrium – $TLoE$) and condition factor (K) were assessed in the F_0 . Body mass (BM), total length (L_T), cardiac function and $TLoE$ were evaluated in the F_1 when at 7 days post fertilization. In the F_0 , K was unaffected by hypoxia, but $\dot{M}O_2$ and $TLoE$ increased with decreases in PO₂. In the offspring, differences in morphology were observed only in $F_{1,\#1}$. Hypoxia tolerance was increased in $F_{1,\#1,\#2}$ from F_0 exposed to 9 and 12 kPaO₂ and it washed out in $F_{1,\#3,\#4}$. Heart rate was decreased in $F_{1,\#1}$ in the three hypoxic treatments. Increased stroke volume and cardiac output were maintained throughout experimentation in fish from 9 kPa. Our results suggest that the severity of hypoxia may modulate the epigenetic transfer of hypoxia-tolerant phenotypes across multiple clutches within a subsequent generation, after parental exposure to decreased oxygen levels. Additionally, this study provides insights about the environmental sensitivity of epigenetic modification and its stability over time.

RESUMEN

Numerosos estudios han asociado las alteraciones en el fenotipo de la descendencia (F_{1+n}) con condiciones estresantes experimentadas por su generación parental (F_0). En esta investigación se evaluó la transferencia epigenética de fenotipos tolerantes a hipoxia de la F_0 a individuos de diferentes puestas dentro de una generación subsecuente (F_1). La generación parental de peces cebra de la línea NHGRI-1 se expusieron a condiciones control de normoxia (21 kPaO₂) y 28°C, y a tres tratamientos de hipoxia a 9, 12 y 15 kPa durante tres semanas. Después, cada dos semanas se reprodujeron los peces para producir cuatro puestas ($F_{1,\#1}$ a $F_{1,\#4}$). Después de la exposición se evaluó el consumo de oxígeno ($\dot{M}O_2$), la tolerancia a la hipoxia (tiempo a la pérdida del equilibrio – $TLoE$) y el factor de condición (K) en la F_0 . En la descendencia se evaluó la masa corporal (BM), la longitud total (L_T), la función cardíaca y $TLoE$ a los 7 días post fertilización. En

la F_0 , K no fue afectado por la hipoxia, pero $\dot{M}O_2$ y $TLoE$ incrementaron con la disminución en la PO_2 . En la descendencia, se observaron diferencias en morfología solo en la $F_{1,\#1}$. En $F_{1,\#1;\#2}$ disminuyó $TLoE$ en peces de F_0 expuestos a 9 y 12 kPa O_2 y desapareció en $F_{1,\#3,\#4}$. El ritmo cardíaco disminuyó en $F_{1,\#1}$ en los tres tratamientos con hipoxia. El volumen sistólico y el esfuerzo cardíaco se mantuvieron elevados en la descendencia de peces expuestos a 9 kPa a lo largo del experimento. Los resultados sugieren que la severidad de la hipoxia puede modular la transferencia epigenética de fenotipos tolerantes a hipoxia en múltiples puestas dentro de una generación subsecuente a la generación parental. Además, este estudio provee perspectivas sobre la sensibilidad de la modificación epigenética y su estabilidad temporal.

INTRAGENERATIONAL EPIGENETIC INHERITANCE OF HYPOXIA-TOLERANT PHENOTYPES IN ZEBRAFISH (*DANIO RERIO*)

Gil M. Bautista and Warren W. Burggren

ABSTRACT

Numerous studies have associated alterations in the phenotype of the offspring (F_{1+n}) with stressful conditions experienced by their parental generations (F_0). In the current research, the epigenetic transfer of hypoxia-tolerant phenotypes from F_0 to multiple clutches within an F_1 was assessed. Adult NHGRI-1 zebrafish were exposed to control conditions of normoxia (~21 kPaO₂) and 28°C, and three hypoxic treatments at 9, 12, and 15 kPa (all at 28°C) for three weeks. After every two weeks, fish were bred to produce four clutches ($F_{1,\#1}$ to $F_{1,\#4}$). After the exposure, oxygen consumption ($\dot{M}O_2$), hypoxia tolerance (time to loss of equilibrium – $TLoE$) and condition factor (K) were assessed in the F_0 . Body mass (BM), total length (L_T), cardiac function and $TLoE$ were evaluated in the F_1 when at 7 days post fertilization. In the F_0 , K was unaffected by hypoxia, but $\dot{M}O_2$ and $TLoE$ increased with decreases in PO₂. In the offspring, differences in morphology were observed only in $F_{1,\#1}$. Hypoxia tolerance was increased in $F_{1,\#1;\#2}$ from F_0 exposed to 9 and 12 kPaO₂ and it washed out in $F_{1,\#3;\#4}$. Heart rate was decreased in $F_{1,\#1}$ in the three hypoxic treatments. Increased stroke volume and cardiac output were maintained throughout experimentation in fish from 9 kPa. Our results suggest that the severity of hypoxia may modulate the epigenetic transfer of hypoxia-tolerant phenotypes across multiple clutches within a subsequent generation, after parental exposure to decreased oxygen levels. Additionally, this study provides insights about the environmental sensitivity of epigenetic modification and its stability over time.

IS THE MOUNTAIN CRAWFISH CAMBARELLUS MONTEZUMAE AN OXICONFORMER?

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ABSTRACT

El objetivo de este trabajo fue determinar la tasa metabólica indirecta de *Cambarellus montezumae* aclimatados al laboratorio y provenientes de la presa Isidro Fabela, Temoaya, Estado de México a una altitud de 3800 m.s.n.m. Se utilizaron como fuentes de variación el sexo y el estado de desarrollo en experimentos de respirometría cerrada para evaluar el consumo de oxígeno (mmol/kg/hr) y la tolerancia a la hipoxia (PO₂crit). Se presentan los resultados parciales solo para individuos adultos. La tasa metabólica de los machos fue en promedio de 4.66 y la de las hembras de 5.05, la presión parcial crítica de los machos en promedio fue de 1.13 y la de las hembras de 1.2685. Estos organismos presentaron un patrón de oxiregulador contrario a lo esperado para invertebrados que es de oxyconformista.

RESUMEN

The goal of this work was to determine the indirect metabolic rate of *Cambarellus montezumae* acclimated to laboratory conditions and collected from the Isidro Fabela dam, Temoaya, State of Mexico at an altitude of 3800 m.a.s.l. Sex and developmental stage were used as sources of variation in closed-respirometry experiments to evaluate oxygen consumption (mmol/kg/hr) and hypoxia tolerance (PO₂crit). Partial results are presented only for adult individuals. The metabolic rate of males was on average 4.66 and that of females was 5.05, the critical partial pressure of males was on average 1.13 and that of females was 1.26. These organisms presented an oxyregulatory pattern contrary to what is expected for invertebrates, which is oxyconformist.

METHODS FOR SURVEYING THE AMPHIBIAN PATHOGEN *BATRACHOCHYTRIUM DENDROBATIDIS*

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ABSTRACT

Batrachochytrium dendrobatidis (Bd), or the amphibian chytrid fungus is a pathogen that affects many Anurans and other amphibians globally. Surveying this disease can allow us to better understand the dynamics of this pathogen as well as its impact on local wildlife. However, to survey for this disease, methods vary. This study outlines the methods we used to survey Bd. Amphibians were sampled in 30 meter transects for 15 person minutes. Width of the transects varied by accessibility. The amphibians caught were swabbed 10 times on each proximal thigh, 10 times on the ventral abdomen, and 10 times per toe. In addition to swabbing the amphibians, we measured weather data, SVL, mass, and sex. We used the Qiagen DNeasy Blood and Tissue DNA extraction kit to extract and purify the DNA from the swabs. This kit was preferred over PrepMan as our swabs were high retention. Therefore, the swabs absorbed a large volume of PrepMan and would not release it, rendering it an inefficient and costly method. The qPCR protocol followed the methods of Kerby et al 2013. This method was used as it is low volume and fast. This allows more replicates per sample and more qPCR runs per day.

MICROCLIMATE BY SPATIAL AND BEACH PROFILE IN OLIVE RIDLEY TURTLE (LEPIDOCHELYS OLIVACEA) NESTS IN RESERVE LA ESCOBILLA, OAXACA.

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ABSTRACT

The microclimate in sea turtle nests is the result of interactions between biotic and abiotic factors. Since sea turtles are in danger of extinction, it is necessary to evaluate the microclimates according to the beach profile to have a greater certainty of the embryonic development process, and interaction with variables such as: humidity, carbon dioxide and temperature. The knowledge and interpretation of these variables is necessary to understand the process of clutches selection in females and how these climatic change regimes caused by global warming, as well as to propose the improvement of management and conservation plans of the species, to from non-invasive methods. In this study, the climatic variables present in artificial and natural olive ridley turtle nests will be determined according to the spatial profile (upper region, lateral region, lower region and central region) and beach (berm and dunes) during nesting, as well as as the correlation in hatching success and granulometry. Evaluating 10 nests by spatial and beach profile in La Escobilla reserve, Oaxaca and using sensor equipment for measurements at day 1, 10 and 50 of embryonic development.

RESUMEN

El microclima en nidos de tortugas marinas es el resultado de interacciones entre factores bióticos y abióticos. Debido a que las tortugas marinas están en peligro de extinción, es necesario evaluar los microclimas de acuerdo con el perfil de playa para tener una mayor certeza del proceso del desarrollo embrionario, e interacción con las variables como: humedad, dióxido de carbono y temperatura. El conocimiento e interpretación de dichas variables es necesario para comprender el proceso de selección de puesta de las hembras y como estos pueden cambiar ante regímenes climáticos ocasionados por el calentamiento global, así como proponer la mejora de planes de manejo y conservación de la especie, a partir de métodos no invasivos. En este estudio, se determinarán las variables climáticas presentes en nidos artificiales y naturales de tortuga golfina de acuerdo con el perfil espacial (región superior, región lateral, región inferior y región central) y de playa (berma y dunas) durante la anidación, así como la correlación en el éxito de eclosión y granulometría. Evaluándose 10 nidos por perfil espacial y de playa en la reserva La Escobilla, Oaxaca y utilizándose equipo de sensores para las mediciones climáticas al día 1, 10 y 50 de desarrollo embrionario.

MOTOR VEHICLE EMISSION EXPOSURE INCREASES OXIDATIVE STRESS IN MOUSE BRAINS

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ABSTRACT

Studies have examined the effects of air pollutants on generation of reactive oxygen species (ROS) and degradation of the blood brain barrier (BBB), but there is a gap on the interaction of age and diet. Diseases such as Alzheimer's are more prevalent in areas of high air pollution, and there are currently no cures, so identifying mechanisms of toxicity and points of environmental stress is essential.

To investigate this, aged (18 mo.) male C57BL/6 mice on either a low-fat diet (LF) or high-fat diet (HF) (21% fat by content; 42% kcal from fat) were exposed to either MVE (300 µg PM/m³) or filtered air (FA) for 6 hr/d, 7 d/wk, for 50 d. Immunofluorescence and RT-qPCR were used to quantify endpoints.

MVE exposure when combined with a HF diet decreased tight junction (TJ) proteins, suggesting increased BBB permeability. Xenobiotic response elements, AhR and CYP1B1, were upregulated in aged mice exposed to MVE and fed HF diet. NOX subunits and DNA oxidation were increased with MVE exposure and further exacerbated by HF diet. These findings show that MVE exposure has detrimental outcomes on BBB TJ proteins and induces xenobiotic response factors known to contribute to oxidative stress in aged wildtype mice.

RESUMEN

Los estudios han examinado los efectos de los contaminantes del aire en la generación de especies reactivas de oxígeno (ROS) y la degradación de la barrera hematoencefálica (BBB), pero existe una brecha en la interacción de la edad y la dieta. Enfermedades como el Alzheimer son más frecuentes en áreas de alta contaminación del aire, y actualmente no hay curas, por lo que es esencial identificar los mecanismos de toxicidad y los puntos de estrés ambiental.

Para investigar esto, ratones C57BL/6 macho de edad (18 meses) con una dieta baja en grasas (LF) o una dieta alta en grasas (HF) (21 % de grasa por contenido; 42 % de kcal de grasa) fueron expuestos a MVE (300 µg PM/m³) o aire filtrado (FA) durante 6 horas/día, 7 días/semana, durante 50 días. Se utilizaron inmunofluorescencia y RT-qPCR para cuantificar los puntos finales.

La exposición a MVE cuando se combina con una dieta HF disminuyó las proteínas de unión estrecha (TJ), lo que sugiere una mayor permeabilidad de BBB. Los elementos de respuesta xenobióticos, AhR y CYP1B1, aumentaron en ratones envejecidos expuestos a MVE y alimentados con dieta HF. Las subunidades de NOX y la oxidación del ADN aumentaron con la exposición a MVE y se exacerbó aún más con la dieta HF. Estos hallazgos muestran que la exposición a MVE tiene resultados perjudiciales en las proteínas BBB TJ e induce factores de respuesta xenobióticos que se sabe que contribuyen al estrés oxidativo en ratones de tipo salvaje envejecidos.

ONSET OF CARDIOVASCULAR REGULATION BY VAGAL INNERVATION OF THE HEART IN GULF KILLIFISH EMBRYOS (*FUNDULUS GRANDIS*)

Steven Williams, Ben Dubansky, Gil Martinez Bautista and Warren Burggren

ABSTRACT

Elevated water temperature significantly influences cardiac performance in both embryonic and adult zebrafish (*Danio rerio*). However, decreased water temperatures affecting zebrafish larvae during critical developmental windows after hypoxia-exposure is less explored. Specifically, how the interaction between fluctuating temperature and temperature-dependent oxygen solubility in water influences cardiac recovery in response to injury. Here, we evaluate how 21 and 28 °C water temperatures influence heart rate, stroke volume, and cardiac output in normoxic control and hypoxia-exposed larval zebrafish. Preliminary results show a lower heart rate for the hypoxia-exposed larvae compared to normoxic controls from 48 to 72 hours post-injury (hpi) at 21 °C, yet a higher stroke volume for the hypoxia-exposed larvae during this period culminating in similar cardiac output values for both groups at 96 hpi. At 28 °C, increased heart rates for both groups compared to 21 °C but decreased cardiac output for hypoxia-exposed larvae attributed to lower stroke volumes, prolonging recovery time. These data may suggest that cooler water may enhance cardiac recovery in response to injury, possibly due to increased oxygen solubility, opening to future studies evaluating how hypoxia-exposure and water temperature modulates cardiac injury response during critical developmental windows of larval zebrafish.

OXYGEN PARTIAL PRESSURE EFFECT ON GESTATION AND EMBRYO DEVELOPMENT OF THE VIVIPAROUS FISH GIRARDINICHTHYS MULTIRADIATUS.

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ABSTRACT

Girardinichthys multiradiatus (Meek, 1904), is a freshwater fish endemic to the Upper Lerma River Basin, it is one of the few species that survives contamination and the rapid deterioration of its habitat. It is a viviparous fish, classified as tolerant to hypoxia (low partial pressure of oxygen "PO₂" in its habitat) threatened with extinction, unlike the viviparous ones that are extinct or extirpated from the same ichthyogeographic region. Apparently, viviparity plays an important role in their tolerance to low atmospheric, ecological and systemic oxygen availability, which can be particularly severe during the gestation period and embryonic development.

Derived from the above, the effect of hypoxia on reproduction variables will be determined: gestation period, litter number and size of *G. multiradiatus* offspring. Similarly, the HIF-1 α protein will be quantified in the tissues of the mother and the tissues of the embryo at different stages of development as a molecular marker of chronic exposure to severe hypoxia.

RESUMEN

Girardinichthys multiradiatus (Meek, 1904), es un pez dulceacuícola endémico de la Cuenca Alta del Río Lerma, es una de las pocas especies que sobrevive a la contaminación y al rápido deterioro de su hábitat. Es un pez de vivíparo, catalogado como tolerante a la hipoxia (baja presión parcial de oxígeno "PO₂" en su hábitat) amenazado de extinción, a diferencia de los vivíparos que están extintos o extirpados de la misma región ictiogeográfica. Al parecer la viviparidad juega un papel importante en su tolerancia a la baja disponibilidad de oxígeno atmosférico, ecológico y sistémico, que particularmente puede ser severa durante el periodo de gestación y el desarrollo embrionario. Derivado de lo anterior, se determinará el efecto que tiene la hipoxia en variables de reproducción: duración del periodo de gestación, tamaño de la camada y tamaño de las crías de *G. multiradiatus*. De igual manera, se cuantificará la proteína HIF-1 α en los tejidos de la madre y los tejidos del embrión en diferentes etapas de desarrollo como marcador molecular de la exposición crónica a hipoxia severa.

P38-INHIBITORS ENHANCE CARDIAC FUNCTION IN LARVAL ZEBRAFISH AFTER MYOCARDIAL INFARCTION.

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Myocardial infarction (MI), also known as heart attack, is one of the major causes of death worldwide. It is caused by the shortage of oxygen in the myocardial tissue (ischemia), leading to cardiac remodeling, consisted of cardiomyocyte death and replacement by scar tissue. Myocardial geometry is compromised after MI, with a significant decrease in cardiac output. Novel therapies, such as p38 inhibitors, are promising in increasing cardiac function after MI. Zebrafish larvae in severe acute hypoxia (1 kPa during ~18 - 20 min) are a good model to mimic and study the effects of MI. We investigated p38 inhibitors in seven different concentrations (0.03 – 100 μmol) administered in four different times of exposure (6, 12, 18, and 24 h). Videos were recorded at three different time points: before hypoxic exposure, 48 h, and 72 h after treatment. Low concentrations (0.1-1 μmol) during intermediate times showed a significant increase versus control group. The concentration 0.3 μmol showed an increase of 144% in cardiac output ($P<0.001$), increased by 134% stroke volume improvement, with no changes in heart rate. This positive inotropic effect in fish larvae treated with p38 inhibitors may help to further elucidate the treatments of MI in humans.

Inhibidores de p38 aumentan la función cardíaca en larvas de pez cebra después de infarto al miocardio.

El infarto al miocardio (IM), también conocido como ataque al corazón, es una de las principales causas de muerte a nivel mundial. Es causado por la escasez de oxígeno en el tejido miocárdico (isquemia), lo que lleva a la remodelación cardíaca, con la muerte de cardiomiocitos y su reemplazo por tejido cicatricial. La geometría miocárdica es afectada después de un infarto al miocardio, con una disminución significativa del gasto cardíaco. Algunos nuevos fármacos, como los inhibidores de p38, han sido prometedores para aumentar la función cardíaca después de un infarto al miocardio. Las larvas de pez cebra en hipoxia aguda severa (1 kPa durante ~ 18 - 20 min) son un buen modelo para imitar y estudiar los efectos de IM. Se investigaron los inhibidores de p38 en siete concentraciones (0,03 - 100 μmol) administradas en cuatro tiempos diferentes de exposición (6, 12, 18 y 24 h). Los videos se grabaron en tres puntos de tiempo diferentes: antes de la exposición hipóxica, 48 h y 72 h después del tratamiento. Las concentraciones bajas (0,1 - 1 μmol) durante tiempos intermedios, mostraron un aumento significativo frente al grupo control. La concentración (0,3 μmol) mostró el mayor aumento, con 144% incremento en el gasto cardíaco ($P<0.001$), con un 134% en incremento del volumen sistólico, sin cambios en el ritmo cardíaco. Este efecto inotrópico positivo en larvas de pez cebra tratadas con inhibidores de p38 puede contribuir a dilucidar en mayor medida los tratamientos de IM en humanos.

PERTURBATION OF AGO3 AND ZUCCHINI IN YELLOW FEVER MOSQUITOS ABSTRACT

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ABSTRACT

Genes expressed in the ovarian tissues are challenging to study; mutations in such genes often result in ablated viability or sterility. Transposons are mobile genetic parasites, and their regulation is crucial to maintaining genomic integrity. Using the ovary-specific exuperentia promoter, we will express Cas-9 such that mutations only occur in the ovarian tissues. This study will use this technology to understand better the germline defense against transposons within yellow fever mosquito (*Aedes Aegypti*) ovaries. The experiment aims to understand the interplay between Ago3 and Zuc gene knockout and transposon activity within the adult organism. Leveraging deep mRNA sequencing and High-Resolution Melt Analysis (HRMA), we dissect the impact of gene perturbations on transposon dynamics and further understand how to influence germline fitness with minimal effect on the parental generation. Knockout of both Ago3 and Zuc in the egg stage arrest development before the adult stage, and we aim to understand better the role of the piRNA pathway germline regulation in adult mosquitos. We expect to find increased transposon activity and abnormalities within the egg cells of the ovaries 24 hours post-blood meal. We hope this will lead to a decrease in viability and quantity of eggs laid for each female. The CRISPR/Cas-9 system has ushered in a revolutionary era of precision genome editing, paving the way for genetic manipulation across diverse organisms. This research holds the promise of developing novel strategies for combating mosquito-borne diseases through precision gene editing not possible through traditional means.

PREDICTING FUTURE HABITAT SUITABILITY OF TWO ELASMOBRANCHS WITH DIFFERING ENVIRONMENTAL REQUIREMENTS IN A CHANGING OCEAN ENVIRONMENT

Imogen Brown, Andrew Carr and David Wells

ABSTRACT

Understanding how environmental variables drive shark distribution is essential to being able to accurately predict populations and conserve them. Sharks play key roles in ocean ecosystems and a loss has the potential to cause trophic cascades. This paper used scalloped hammerhead shark (*Sphyrna lewini*) and lemon shark (*Negaprion brevirostris*) catch data, alongside biologically relevant environmental variables, to assess the environmental drivers of presence and to predict future distribution along the Texas coast under Representative Concentration Pathways (RCP) 4.5 and 8.5 for the years 2040-2050. Models were generated using the Maximum Entropy algorithm (Maxent). The resulting environmental variable graphs determined temperature as the most important driver of distribution for *S. lewini* and pH as the most important driver for *N. brevirostris*. The suitability distribution models showed a decline, with losses over 50% from present distribution to RCP 4.5 for both species. There was little difference between scenarios 4.5 and 8.5, likely as the full impact of 8.5 was not captured within the time-frame or the damage done in RCP 4.5 between present and 2040 is catastrophic to the species distribution. The loss of suitable habitat highlights a need for conservation efforts on the Texas coast for the two species presented, particularly between now and 2040. This paper can be influential in developing marine protection zones for these species within the Texas gulf and furthers the knowledge on ecological drivers.

PROMOTING A STANDARD METHODOLOGY FOR PHYSIOLOGICAL PHENOTYPING OF SQUAMATE REPTILES

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ABSTRACT

Current methodologies for measuring relevant physiological variables across temperatures in squamates are likely adequate for comparison within studies, but the lack of standardization limits broader comparative application. This is important given the need for information relating to rapidly changing global temperatures, temperature related phenomena across regions and taxa, and comparisons across time. Here, I propose a set of measurements and experimental temperatures that will allow such comparisons to be easily made across space, time, and taxa. Such measurements can be routinely collected in physiological ecology laboratories across the globe, published in relevant journals, and submitted to a common repository for ease of comparison. Compiling a global data set of physiological phenotypes gathered with standardized methodologies will ultimately allow us to make comparisons and predictions that could result in better informed conservation actions and a better general understanding of how physiology, particularly metabolism, affects biodiversity and the distribution of life on earth.

RESUMEN

Las metodologías actuales para medir variables fisiológicas relevantes a través de las temperaturas en los escamosos probablemente sean adecuadas para la comparación dentro de los estudios, pero la falta de estandarización limita una aplicación comparativa más amplia. Esta estandarización es importante dada la necesidad de información relacionada con las temperaturas globales que cambian rápidamente, los fenómenos relacionados con la temperatura entre regiones y taxones, y las comparaciones a lo largo del tiempo. Propongo un conjunto de medidas y temperaturas experimentales que permitirán que tales comparaciones se realicen fácilmente a través del espacio, el tiempo y los taxones. Dichas medidas se pueden recopilar de forma rutinaria en laboratorios de ecología fisiológica de todo el mundo, publicarse en revistas relevantes y enviarse a un depósito común para facilitar la comparación. La compilación de un conjunto de datos globales de fenotipos fisiológicos reunidos con metodologías estandarizadas nos permitirá por última instancia hacer comparaciones y predicciones que podrían resultar en acciones de conservación mejor informadas y una mejor comprensión general de cómo la fisiología, particularmente el metabolismo, afecta la biodiversidad y la distribución de la vida en la tierra.

REDUCING MALACHITE GREEN'S TOXICITY IN DAPHNIA PULICARIA WITH CORONAPLASMA

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ABSTRACT

Malachite green is a non-biodegradable dye that contaminates drinking water and fertile soil, threatening the ecosystem's balance. However, it has numerous applications in aquaculture, industry, serving as an antiseptic, bactericide, pH indicator, and for parasite and fungus control. Hence, it's crucial to find wastewater treatment methods. *Daphnia pulicaria*, known for its high environmental sensitivity, is widely used as a bioindicator. Its survival was measured in four different treatments (N = 40; n1 = 10; n2 = 10; n3 = 10 and n4 = 10) to determine how crown plasma, with or without malachite green, affects its life history. The mean effective concentration EC50(24-48) = 0.1250 mg/L (N=60; n4 m/L; n2 m/L; n1 m/L; n0.5 m/L; n0.250 m/L and n0 m/L), ranking it in the top 10 of the most sensitive species to malachite green. The plasma treatment broke down the malachite green molecule in approximately 15 minutes, resulting in a 9.1% decrease in

RESUMEN

El verde malaquita es un colorante no biodegradable que reduce el agua potable y la tierra fértil, llegando a amenazar el equilibrio del ecosistema, sin embargo, tiene una gran variedad de aplicaciones en la acuicultura, industria, como antiséptico, bactericida, indicador de pH, para combatir parásitos y hongos. Es por ello, que es de suma importancia encontrar métodos para tratar las aguas residuales. La especie *Daphnia pulicaria* tiene una alta sensibilidad a los cambios ambientales y es ampliamente utilizada como bioindicador, por lo cual se midió su sobrevivencia en 4 tratamientos diferentes (N=40; n1=10; n2=10; n3=10 y n4=10) para X X 2 determinar cómo afecta el plasma tipo corona con y sin verde malaquita a su historia de vida. Se determinó la concentración efectiva media EC50(24-48) = 0.1250 mg/L (N=60; n4 m/L; n2 m/L; n1 m/L; n0.5 m/L; n0.250 m/L y n0 m/L) y la colocho en el top 10 de las especies más sensibles al verde malaquita. El tratamiento de plasma en tan solo 15 min aproximadamente rompió la molécula del verde malaquita llegando a afectar el 9.1% de la supervivencia de *Daphnia pulicaria*. En cambio, cuando contiene verde malaquita en concentraciones letales sobrevive el 23.4% de la población.

**RESPIRATION PHYSIOLOGY AND COMBINED TEMPERATURE AND HYPOXIA
TOLERANCE OF THE AIR-BREATHING LOACH *MISGURNUS ANGUILLICAUDATUS***

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ABSTRACT

Air breathing has evolved in multiple fish species as an adaptation to hypoxia and other extreme conditions. The air-breathing weather loach, *Misgurnus anguillicaudatus*, possesses adaptations that facilitate atmospheric oxygen uptake through the intestinal walls. To investigate the aerial and aquatic contributions to gas exchange during acute temperature changes, we developed a closed respirometry system in which PO_2 and temperature could be experimentally manipulated. We independently measured aquatic and aerial oxygen consumption ($\dot{M}O_2$), gill ventilation frequency and air breathing frequency in unrestrained, undisturbed adult (10-12 months old) loaches acclimated to 23°C and a PO_2 of ~150 mmHg and measured acutely at 23°C and 26°C. Mean body mass was 6.69g (n=10) for tests at 23°C and 10.84 (n=5) at 25°C. Weather loaches increasingly supplemented gill ventilation with air breathing as temperatures increased. Mean gill ventilation frequency increased sharply from 34 /min at 23°C to 137/min at 26°C ($Q_{10} > 100$ for gill ventilation). Concurrently, mean air breathing frequency increased from 9/hour at 23°C to 39/h at 26°C ($Q_{10} > 130$ for air breathing). Thus, air breathing increased ~ proportionately with the increase in gill ventilation. Aquatic $\dot{M}O_2$ increased from 0.58 $\mu M/g/h$ at 23°C to 1.50 $\mu M/g/h$ at 26°C ($Q_{10} \sim 24$). Larger loaches (typically females) had smaller rates of $\dot{M}O_2$ than smaller loaches (typically males). In summary, the data shows correlation between temperature and $\dot{M}O_2$ as well as body mass and $\dot{M}O_2$. This study provides an insight of the respiratory response of tropical air-breathing fish under a seasonal change scenario in natural populations. Moreover, these results contribute to understanding the implications of severe and permanent climate change on those populations in the future should global temperature trends continue.

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SEX DIFFERENCES IN MOLECULAR RESPONSE TO AIR POLLUTION IN MICE

Victoria L. Youngblood¹, Amie K. Lund¹

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ABSTRACT

Several studies report a correlation between air pollution and increased risk of cardiovascular disease (CVD). Both epidemiological and animal studies have revealed significant sex differences in both CVD and sex-dependent responses to particulate matter (PM) exposure. However, the underlying mechanisms that drive these sex dependent responses are not fully characterized and most of existing studies focus on outcomes in males. Therefore, it is critical to identify contributing stressors and the underlying mechanisms driving the air pollution-mediated progression of CVD in women.

To investigate this, male and female C57Bl/6 mice on either a low-fat diet (LF) or high-fat diet (HF) were exposed to either 35 µg of suspended DEP or sterile saline for 4 weeks RT-qPCR and multiplex immunoassays were used to quantify endpoints.

Hormone multiplex assays revealed sex-dependent differences in hormone signaling following DEP exposure. Results of the multiplex assays also revealed significant alterations in hormone signaling in HF diet-fed mice of both sexes. Thyroid hormone signaling appeared to have the most significant sex-dependent differences. Further studies will elucidate the possible mechanism of disrupted hormone signaling in driving a CVD phenotype.

RESUMEN

Varios estudios informan una correlación entre la contaminación del aire y un mayor riesgo de enfermedad cardiovascular. Tanto los estudios epidemiológicos como los realizados en animales han revelado diferencias significativas entre sexos tanto en las enfermedades cardiovasculares como en las respuestas dependientes del sexo a la exposición a partículas (PM). Sin embargo, los mecanismos subyacentes que impulsan estas respuestas dependientes del sexo no están completamente caracterizados y la mayoría de los estudios existentes se centran en los resultados en los hombres. Por lo tanto, es fundamental identificar los factores estresantes que contribuyen y los mecanismos subyacentes que impulsan la progresión de las enfermedades cardiovasculares mediada por la contaminación del aire en las mujeres.

Para investigar esto, se expusieron ratones C57Bl/6 machos y hembras con una dieta baja en grasas (LF) o una dieta alta en grasas (HF) a 35 µg de DEP suspendida o solución salina estéril durante 4 semanas. Se utilizaron RT-qPCR e inmunoensayos multiplex para cuantificar los criterios de valoración.

Los ensayos hormonales múltiples revelaron diferencias dependientes del sexo en la señalización hormonal después de la exposición a DEP. Los resultados de los ensayos multiplex también revelaron alteraciones significativas en la señalización hormonal en ratones de ambos sexos alimentados con una dieta rica en grasas. La señalización de la hormona tiroidea pareció tener las diferencias más significativas según el sexo. Estudios adicionales dilucidarán el posible mecanismo de alteración de la señalización hormonal que impulsa un fenotipo de ECV.

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SPATIAL AND TEMPORAL VARIATION OF ZOOPLANKTON FROM CHIMALIAPAN MARSH

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ABSTRACT

In the present work, partial results of the spatiotemporal variation of the zooplankton of the Ciénega Chimaliapan at Ciénegas de Lerma National Reserve are presented. Samples were collected from 5 points in the Ejidos of San Pedro Tultepec and Capulhuac. At each point, a monthly sample was carried out during a year with a low mesh opening net, collecting zooplankton. Partial results are showing existing organisms from the families of Copepods: Diaptomidae and Cyclopidae and 3 species of Cladocerans have been identified: *Daphnia pulicaria*, *Daphnia pileata* and *Moina wierzejskii*. Subsequently, the zooplankton biomass (gr/m³) and the relative abundance of each species will be quantified per sampling point and per month. These results will be integrated with the water quality results to determine the trophic state of the swamp.

RESUMEN

En el presente trabajo se presentan resultados parciales de la variación espacio temporal del zooplancton de la Ciénega Chimaliapan en el ANP Ciénegas de Lerma. Se colectaron muestras de 5 puntos en los Ejidos de San Pedro Tultepec y de Capulhuac. En cada punto se realizó un arrastre mensual durante un año con una red de baja apertura de malla, realizando una colecta de zooplancton, hasta el momento se han identificado organismos existentes de las familias de Copepodos Diaptomidae y Cyclopidae y 3 especies de Cladoceros: *Daphnia pulicaria*, *Daphnia*

pileata y *Moina wierzejskii*. Posteriormente se cuantificará la biomasa zooplanctónica (gr/m³) y la abundancia relativa de cada especie por punto de muestreo y por mes. Estos resultados se integrarán a los de calidad del agua para determinar el estado trófico de la ciénega.

THE ROLE OF RAC GTPASES IN CLOSURE OF THE AVIAN DUCTUS ARTERIOSUS (*GALLUS GALLUS*)

Rippamonti, J. and Dzialowski, E.M.

RESUMEN

The ductus arteriosus (DA) is an oxygen-sensitive embryonic blood vessel present in all developing amniotes, connecting the pulmonary artery to the aorta to shunt blood away from nonfunctioning lungs and toward the fetal respiratory organ. Closure of this vessel upon birth or hatch is necessary to establish proper blood circulation in the neonate. Smooth muscle contraction, such as in the DA, is governed by myosin light chain kinase (MLCK) stimulating increased cross-bridge cycling and through increases in actin polymerization. The Rac GTPase pathway increases contractile strength in smooth muscle cells by activating Arp2/3 leading to increased actin polymerization. In this study, we investigated the role of the Rac GTPase pathway on closure of DA in late-term chicken (*Gallus gallus*) embryos. Using a wire myograph, late-term chicken DA were evaluated for vessel responsiveness, changes in baseline tension, and exposed to inhibitors of the Rac GTPase pathway. Exposure to the Rac GTPase inhibitor EHT 1864 (10 μ M) blocked the ability of vessels to contract. Similarly, inhibition of the Arp2/3 complex with CK666 (100 μ M) reduced the ability of the vessel to respond. However, inhibition of the p-21 activated kinase pathway with IPA3 (10 μ M) did not alter vessel contraction. These findings suggest that the Rac GTPase-Arp2/3 pathway plays a role in closure of the chicken DA during development by potentially increasing actin polymerization.

THYROID HORMONES INFLUENCE DEVELOPMENT OF SKELETAL MUSCLE MITOCHONDRIA PHENOTYPE AND THERMOREGULATION IN NESTLING RED-WINGED BLACKBIRDS

Ed Dzialowski, Tushar Sirsat, Sarah Goy Sirsat, Megan Pineda, Edwin Price and Janna Crossley

University of North Texas

ABSTRACT

Thyroid hormones are key regulators of development and metabolism in vertebrates. Red-winged blackbirds are an altricial species that exhibit increases in plasma 3,3', 5-triiodo-L-thyronine (T3) levels during the first 5 days post-hatch (dph), begin to develop endothermic metabolic responses by 7 dph, and fledge within 10 days of hatching. To better understand the role thyroid hormones play in endothermic metabolic development in an altricial species, we treated nestling red-winged blackbirds on 2, 3, and 5 dph with either methimazole (MMI) to induce hypothyroidism or supplemental T3 to induce hyperthyroidism. We examined mitochondria function in breast and thigh skeletal muscle at 5, 7 and 9 dph in permeabilized muscle fibers. Hypothyroid conditions prior to day 5 post-hatching induced lower oxygen flux during oxidative phosphorylation through complexes I and II than controls in both breast and thigh skeletal muscles of 7 and 9 dph nestlings. Elevating thyroid hormones during the first 5 dph resulted in no change in oxidative phosphorylation respiration during the nestling period. These data suggest that thyroid hormone levels play a role in development of skeletal muscle mitochondrial function and may be a foundational mechanism by which endothermic capacity is achieved by neonate altricial birds. Supported by NSF Grant IOS-1146758

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TOXICITY ASSESSMENT AND METABOLIC TRAIT RESPONSES OF JUVENILE GIANT FRESHWATER PRAWN (MACROBRACHIUM ROSENBERGII) TO AMMONIA EXPOSURE AT DIFFERENT SALINITIES

Emadi, Cameron, Fabio Dos Santos Neto, Breana Smithers, Miguel Acevedo and Edward Mage

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ABSTRACT

Macrobrachium rosenbergii, commonly known as the giant freshwater prawn, has gained significant attention in global aquaculture initiatives. As a catadromous species, *M. rosenbergii* relies on brackish water for early life stage development, making it an ideal candidate for cultivation in desalination projects that produce concentrated brine as a byproduct. The culturing of *M. rosenbergii* is characterized by high concentrations of individuals, resulting in the potential for harmful nitrogenous waste accumulation in the form of ammonia. Ammonia is a critical environmental factor in aquaculture systems, affecting the survival, growth, and overall health of aquatic animals. However, the interactive effects of ammonia and salinity on *M. rosenbergii* remain poorly understood, particularly during the juvenile stages that coincide with the transition to brackish water. This study aims to determine the 24 and 48-hour ammonia LC50 values across three different salinities (0, 10, and 20 ppt). Informed by these LC50 concentrations, the acute real-time effects of ammonia exposure on the routine metabolic rate of juvenile *M. rosenbergii* will be investigated using static intermittent respirometry. This research will assist in developing a sentinel respirometry system that can detect changes in water quality in real-time by monitoring the metabolic rate of the prawns.

UNRAVELING THE MORPHOLOGICAL DEVELOPMENT AND ONTOGENY OF ABOS IN BRISTLENOSE PLECOS

HALEY VALDEZ AND ED DZIALOWSKI

ABSTRACT

Bristlenose plecostomus (*Ancistrus cirrhosus*) are a freshwater species of armored catfish found in the Paraná River basin of Argentina and Uruguay. Their subfamily Loricariidae are commonly studied for their air-breathing capabilities and unique jaw and musculature structures present in Loricariidae's evolutionary history. *A. cirrhosus*' stomach evolved to act as air-breathing organ (ABO), which is an adaptation that allows these fish when living in hypoxic environments to breathe with their stomach. Previous studies in our lab observed a critical developmental window of 1.8 to 3.0 cm for the onset of air breathing. This study aims to identify morphological changes occurring in the Bristlenose plecos' ABO during the development of air breathing. Whole animals were euthanized using buffered MS-222. Fish lengths and masses were recorded, and animals were preserved in 4% paraformaldehyde. Following preservation, animals were embedded in paraffin and sectioned for histological study. Tissues were stained using the hematoxylin and eosin (H&E) technique to visualize the ABO and the gills to characterize the ontogeny and morphology of the organs during larval development. We expect to develop a new detailed understanding of the morphological and ontological threshold for the incurrence of air breathing in juvenile bristlenose plecos.

USING ENVIRONMENTAL PHYSIOLOGY TO IMPROVE ENVIRONMENTAL REGULATIONS

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ABSTRACT

We study the impact of changes in the environment on the physiology of aquatic organisms, with a focus on the mechanisms involved in gill function and internal homeostasis. When these changes involve toxicants, understanding gained from experimental physiology may provide powerful approaches to improve environmental regulations. I will illustrate this point with examples drawn from the research of my group and that of my collaborators. An understanding of the effect of low water pH on gill ion transport led to the realization that environmental acidification was a multifactorial problem dependent on water chemistry and the mobilization of metals, particularly aluminum, as specific gill toxicants. A simple model presented to the US Congress was influential in the passage of modifications of the US Clean Air Act in 1990 that substantially reduced transborder atmospheric fluxes of acidifying gases, thereby greatly reducing acid precipitation, allowing the progressive recovery of acidified lakes and rivers in the US and Canada, and providing important human health benefits. Subsequent research on the effects of different metals and water chemistry on gill ion transporters led to the Biotic Ligand Model which has been adopted in various formats for deriving site-specific Ambient Water Quality Criteria for metals throughout the world. At present, we are working on a model based on gill electrophysiology to regulate major ion toxicity ("salt pollution") in freshwaters.

VAGAL INNERVATION TOPOGRAPHY IN THE ALLIGATOR LIZARD *BARISIA IMBRICATA*

Sofía Aimeé Quiroz-Adrián, Josele Flores-Santin, Mónica Vanessa Garduño-Paz and Oswaldo Hernández Gallegos

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ABSTRACT

Ectothermic animals have certain physiological adaptations. Depending on the height at which they are found, these adaptations correspond to the cardiovascular system and are observed in response to thermoregulation with the phenomenon of cardiac hysteresis. The function of the ANS is to maintain homeostasis and make the body react to changes in the internal and external environment. Within the ANS we find the parasympathetic system, in charge of restorative responses and originates in the cranial and sacral region, where it innervates the cranial nerves III, VII, IX and X. Within the vagus nerve (X), we find that it has the most extensive distribution of the entire peripheral nervous system and plays an important role in the regulation of cardiorespiratory function in reptiles. *B. imbricata* will be used as an object of study since it is known that this species presents cardiac hysteresis and is found at elevations of 2600 masl. A nerve staining technique will be performed, which makes other soft tissues transparent while staining the nerves. It allows mapping complete patterns of innervation of organs, skeletal muscle, mucosa, skin, and other structures; having the advantage that the nerves within the sample can be visualized in their three-dimensional positions.

RESUMEN

Los animales ectotermos presentan ciertas adaptaciones fisiológicas. Dependiendo de la altura a la que se encuentran, estas adaptaciones corresponden al sistema cardiovascular y se observan en respuesta a la termorregulación con el fenómeno de histéresis cardíaca. El SNA tiene como función el de mantener la homeóstasis y hacer reaccionar al cuerpo a los cambios del medio interno y externos, dentro del SNA encontramos el sistema parasimpático, encargado de las respuestas restauradoras y tiene su origen en la región craneal y sacra, donde inerva los nervios craneales III, VII, IX y X. Dentro del nervio vago (X), encontramos que tiene la distribución más extensa de todo el sistema nervioso periférico y juega un papel importante en la regulación de la función cardiorrespiratoria en reptiles. Se usará a *B. imbricata*, como objeto de estudio ya que es sabido que esta especie presenta histéresis cardíaca y se encuentra a elevaciones de 2600 msnm. Se realizará una técnica de tinción de nervios, la cual, transparenta otros tejidos blandos mientras tiñe los nervios. Permite mapear patrones completos de inervación de órganos, músculo esquelético, mucosas, piel y otras estructuras; teniendo como ventaja que los nervios dentro de la muestra se pueden visualizar en sus posiciones tridimensionales.

VOCALIZACIONES EN UNA POBLACIÓN SILVESTRE DE *ASPIDOSCELIS COSTATUS* *COSTATUS* EN TONATICO, ESTADO DE MÉXICO, MÉXICO

Edgar De La Rosa-Silva and Hernández-Gallegos Oswaldo, and Labra Lillo Antonieta

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ABSTRACT

The study of vocal production in lizards has started to receive more attention in recent years, however, in México there are still few studies in this regard, considering the great diversity which it possesses. Previously, within a population in Ixtapan de la Sal, the emission of complex calls was identified in the Balsas Basin Whiptail (*Aspidoscelis costatus costatus*) when the individuals were restrained. The following study analyzed a population of this species in Tonatico, Estado de México, to determine if vocalizations would be typical of the species, rather than of the studied population. Seven organisms were captured (five males, two females), from which, the 71% (four males, one female) vocalized. The 16 vocalizations examined presented characteristics very similar to those of the population of Ixtapan de la Sal, being short, low-pitched and with multiple harmonics, so it is proposed that vocalizations may be potentially produced by vocal cords, or analogous organ, which to this date has not been described in the genus. The vocalizations would be a characteristic of the species, and we discuss the possible defensive functions of these vocalizations against predators.

RESUMEN

El estudio de la producción vocal en lagartijas ha empezado a recibir más atención en años recientes, sin embargo, en México aún existen comparativamente muy pocos estudios al respecto, considerando la gran diversidad con la que cuenta. Previamente, en una población en Ixtapan de la Sal, se identificó la emisión de llamadas complejas en el Huico Correlón (*Aspidoscelis costatus costatus*) al ser los individuos apresados. El presente estudio analizó una población de esta especie en Tonatico, Estado de México, para saber si las vocalizaciones serían propias de la especie, más que de la población estudiada. Se capturaron siete individuos (cinco machos, dos hembras), de los cuales el 71% vocalizó (cuatro machos, una hembra). Las 16 vocalizaciones examinadas presentaron características muy similares a las de la población de Ixtapan de la Sal, siendo sonidos cortos, agudos y con múltiples armónicos, por lo que se propone que las vocalizaciones son potencialmente producidas por cuerdas vocales, o un órgano equivalente, el cual a la fecha no han sido descrito en el género. Las vocalizaciones serían una característica de la especie, y se discuten las posibles funciones de defensa de estas vocalizaciones ante los depredadores.