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Primary Cell Culture of Adult Zebrafish Spinal Neurons for Electrophysiological Studies

Zebrafish (*Danio rerio*) have increasingly become a popular organism in biological research fields, especially in neuroscience, as a paradigmatic vertebrate model. The mature adult zebrafish model can be used to study neural circuits and the physiological processes within the spinal cord. Building upon previous zebrafish research regarding the spinal cord, we developed a novel *in vitro* primary cell culture for spinal neurons from adult zebrafish. After dissecting and isolating neurons from an intact spinal cord, we cultured robust cells displaying distinct neuronal morphology and behavior with large cell somas, long axons and dendrites, and highly active growth cones. To characterize cells in culture as neurons, immunofluorescence labeling, calcium imaging, and patch clamping were performed. The staining of the cells with NeuN antibody, neuronal nuclear marker, and axonal marker acetyltubulin confirmed neuronal characteristics. Morphologic analysis was supported by calcium imaging which confirmed an excitable cell type. Lastly, analysis from the recordings of whole cell patch clamping revealed strong voltage-dependent currents, both inward and outward, as well as tetrodotoxin-blocked sodium conductance, consistent with a neuronal phenotype. Altogether, these tests successfully and effectively differentiated the spinal neurons from adult zebrafish from other surrounding cells in culture confirming successful isolation. The cultured neurons represent a powerful new system to investigate modulators of endogenous ion channel targets in the zebrafish model system.

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Body mass in female and male fox squirrels (*Sciurus niger*) at Saint Mary's College

Urbanization can affect native species by altering their food availability, habitat structure, and other factors of survival, such as predation. An urban environment provides species with increased anthropogenic food sources that can affect body condition. The Saint Mary's College campus in Notre Dame, IN is an urban environment that supports a robust fox squirrel population (*Sciurus niger*). This arboreal squirrel relies heavily on campus resources for food, breeding grounds, and shelter. Our study focuses on the differences in scaled body mass between female and male fox squirrels (*Sciurus niger*) captured on campus during the months of June and July 2019. Previous work on sexual dimorphism in small mammals has found that males usually weigh more than females for purposes of mate competition and defense. We collected measurements of body mass, body length, tail length, ear length, and hindfoot length. These values were used to generate scaled mass indices for male and female fox squirrels. Each individual was sexed and tagged with a stainless steel ear tag for identification purposes. Scaled body mass is indicative of body condition, which is a more accurate way to assess the health of an individual compared to mass alone. Our results indicate that there is no significant difference in female and male scaled body mass and body condition. Failure to detect differences in scaled body mass between female and male fox squirrels may be attributed to the timing of data collection and small sample size. Future work will include additional year-round data collection over the course of several years to generate a long-term profile of body mass trends within the Saint Mary's College squirrel population.

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Effects of gamete age on development in broadcast spawning marine invertebrates

For broadcast spawning organisms like echinoderms, the timing of gamete release is essential for successful fertilization. However, synchronous release of gametes does not always occur. Delayed fertilization due to asynchronous gamete release or delayed contact between sperm and eggs could potentially have a large impact on the successful development of broadcast spawning organisms, but few studies have tested the effects of egg age on development beyond fertilization. Prior studies have focused on the presence of a fertilization envelope as a marker of successful development, however, the presence of a fertilization envelope alone does not necessarily indicate whether the fertilized zygote will develop into a normal larva and therefore may be a misleading indicator of successful development. We tested the effects of egg age on fertilization and early development in two species of asteroid echinoderms: *Asterias forbesi* and *Acanthaster cf. solaris*. We found that both species exhibited similar developmental responses to delayed fertilization. Fertilization was consistently high (90-100%) within 30 minutes of gamete release and/or egg maturation, but declined variably after that among different females. Importantly, we found that the frequency of normal development drops dramatically at each stage of development (blastula, gastrula, bipinnaria), with fewer than 30% of offspring developing to normal bipinnaria if fertilized more than an hour after gametes have been released. Our work suggests that researchers should carefully monitor gamete maturation and release in asteroid echinoderms, and likely other marine invertebrates, to avoid artifacts of gamete age on experiments with marine invertebrate eggs, embryos and larvae.

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Further studies of axolotl and tiger salamander kinematics

Axolotls (*Ambystoma mexicanum*) are neotenic salamanders that do not typically complete metamorphosis and remain aquatic when they reach sexual maturity; however, some axolotls may undergo spontaneous metamorphosis. Tiger salamanders (*Ambystoma tigrinum*) are non-neotenic terrestrial salamanders that normally complete metamorphosis and are within the same genus as axolotls. These studies investigate kinematics in order to compare movement of the metamorphosed axolotl to the tiger salamander during walking. By digitizing the movements of these salamanders with slow-motion video, the motion and position of specific body parts were tracked, measured and compared.