Thyroid hormone plays a key role in proper skeletal development in vertebrates. Previous data using thyroid-ablated zebrafish has shown that a great number of bones are affected in size, shape, and mineralization. As such, zebrafish are an excellent model system with which to investigate the developmental consequences of thyroid disruption. Disruption of thyroid hormone in zebrafish leads to several morphological changes in the adult skeleton. Some of the most affected elements include the lower jaw, hyomandibula, pharyngeal jaws, and tripus. In addition to being significantly impacted by thyroid disruption, these bones are all functionally important. The lower jaw plays a role in food capture, the hyomandibula supports the jaw and connects it to the neurocranium, the pharyngeal jaws process food, and the tripus is part of the Weberian apparatus which connects the swim bladder to the inner ear for hearing. Given that these bones are significantly impacted by thyroid disruption, an ontogenetic sequence of zebrafish was cleared and stained and investigated to document changes to timing, ossification, and shape of these bones from larval to adult stages. Overall thyroid disruption led to delayed growth and ossification in hypothyroid zebrafish, and accelerated growth and ossification in the hyperthyroid condition. Furthermore, thyroid disruption led to changes in the shape of bones in both hypo- and hyperthyroid zebrafish. By determining ontogenetic changes in morphogenesis and ossification of these bones we can better direct future studies which will investigate both the molecular pathways that thyroid disruption affects as well as the functional impact of altering the morphology of these bones.

The Role of Thyroid Hormone in Phenotypic Effects on Cypriniform Cranial Morphology

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With over 4000 described species Cypriniformes is a diverse group of freshwater fishes showing nearly unparalleled diversity in trophic anatomy. Specifically, this group is characterized by several feeding novelties which include pharyngeal jaws comprised of hypertrophied fifth ceratobranchials as well as kinethmoid-mediated premaxillary protrusion. The hypertrophied 5th ceratobranchials have teeth ankylosed to them and since upper pharyngeal jaws have been lost, these fishes process food against the basioccipital pad at the base of the skull. Here, we show that thyroid disruption has profound effects on the morphology of the pharyngeal jaws, phenocopying some of the natural variation seen within cypriniform pharyngeal jaws. In contrast to the euthyroid condition, the pharyngeal jaws of hypothyroid zebrafish have significantly more teeth, the posterior and anterior arms are the same length, and the supporting struts are thinner and more disorganized. Alternatively, the pharyngeal jaws of hyperthyroid zebrafish have significantly fewer teeth, and pronounced differences associated with skeletal architecture of these jaws. Kinethmoid-mediated premaxillary protrusion was also strongly affected by thyroid hormone. The kinethmoid, a sesamoid bone that sits ventral to the ascending process of the premaxilla, has been shown to play a vital role in cypriniform jaw protrusion. In the hypothyroid zebrafish, the kinethmoid failed to ossify properly and lacked the characteristic lateral wings and dorsal process. In the hyperthyroid zebrafish, the kinethmoid is hyperossified and misshapen. Thyroid hormone therefore appeared to regulate the proper development of these feeding novelties, and evolutionary modulation of thyroid hormone may give rise to some of the trophic diversity observed within Cypriniformes. Understanding how the modulation of thyroid hormone changes these bones, and thus their ability to function, may lend insight into how they may have evolved.

Bats to Belugas: Functional Anatomy of Biosonar in Air and Water

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During the explosive period of mammalian radiation, two groups, Microchiroptera and Odontoceti, emerged with highly evolved adaptations for ultrasonic hearing and echolocation in radically different media - air vs water. In the last 50 million years, these groups proliferated into diverse species, all of which possess highly sophisticated biosonar capabilities. This paper addresses media related functional commonalities and differences of biosonar receptors of bats vs dolphins, comparing the auditory peripheries of these groups; i.e., structural variations in the outer, middle, and inner ears, highlighting convergences in the cytoarchitecture and morphometry of their inner, middle, and outer ear structures. Data presented are taken from behavioral, electrophysiological, microscopical, ultrahigh resolution imaging, and finite element simulation studies. Inner ear anatomy is fundamentally similar across the groups although differences exist in both neural densities and distributions as well as basilar membrane dimensions and cochlear spiral configurations. Specialist ears are present in both groups, suggesting at least one odontocete has cochlear specializations consistent with those found in some CF-FM bats (bats producing constant frequency (CF) and frequency modulated (FM) vocalizations), including specialized basilar membrane regions and high frequency neural foci. Cochlear specializations in both groups are primarily linked to peak spectra of their echolocation signals, expanded frequency representation, and in some cases, possibly enhanced tuning in adjacent ear segments derived from standing wave phenomena. Differences that are consistent with processing of aerial vs aquatic borne sound, such as the fatty tissue pinna analogues in odontocetes, are found primarily in the outer and middle ear elements. Other differences among species within each group are correlated with signal type, prey, and/or habitat complexity. Funding sources: Hanse Wissenschaftskollegg, ICBM Fellowship; Helmholtz International Fellow research program; Joint Industry Programme on Sound and Marine Life.

Allometric Variation of Skull and Mandibula Shape in Nyctalus noctula (Schreber, 1774) (Chiroptera: Vespertilionidae)

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Intraspecific variation of skeletal structures can be a result of genetic or environmental factors. It may reflect either phylogenetic divergence or phenotypic plasticity. *Nyctalus noctula* is one of the most widespread species of bats in Europe that prefers forest habitats for breeding and hibernates in urban areas. It is a long-distance migratory species, capable for long, up to 1600 km migrations. *N. noctula* is thought to be genetically uniform (Petit et al. 1999, Evolution 53: 1247-1258). The aim of our study was to estimate variation in the shape and size of *N. noctula* skull and mandible. We used the geometric morphometrics approach with several projections of each skull and mandible to study intraspecific allometric variation. In the results we describe the cranial morphometric differences that are associated with the size of the *N. noctula* skull and mandible and demonstrate which cranial structures change most of all due to the size increase. We also estimate between-sex differences and describe associated shapes. Estimating *N. noctula* intraspecific and between-groups cranial variation with the methods of geometric morphometry complements the few data available about cranial variation of this widespread species. This study provides evidence for microevolution processes in *N. noctula* populations.

**PORCN Inhibition Stimulates Chondrocyte Differentiation**

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Porcupine (PORCN) is an endoplasmic reticulum protein belonging to the membrane bound O-acyl transferase superfamily. This molecule is necessary for the attachment of long chain fatty acids to WNT proteins. PORCN is expressed in most tissues of the body and animals with loss of function in PORCN exhibit embryonic lethality at early developmental stages with extensive gastrulation defects. Several dominant mutations in PORCN were described in human patients, who demonstrated significant skeletal abnormalities. Recently, a powerful PORCN inhibitor C59 has become commercially available and has been successfully used to inhibit WNT pathway in several cancer lines. C59 negatively affects WNT signaling by inhibition of WNT palmitoylation, which is necessary to acetylate WNT ligands before their secretion and binding to a carrier protein. Here, we analyzed the possible effect of this inhibitor on skeletogenesis with focus on endochondral bone formation. We observed, enhanced growth of mesenchymal condensations and also increased number of cartilage nodules in primary micromass cultures established from the early limb bud, which were treated by increasing concentrations of C59. This trend was also confirmed by analysis of peanut agglutinin expression visualizing early mesenchymal condensations. Also the amount of extracellular matrix produced by differentiated chondrocytes was enhanced as was shown by Alcian Blue staining on six day cultures. Moreover, *in vitro* cultures of embryonic tibias treated by C59 displayed a massive increase of cartilaginous mass. Histological analysis revealed enlargement of the hypertrophic cartilage zone. The direct effect of C59 on chondrogenesis has not been determined yet. As this inhibitor can influence both canonical and non-canonical WNT signaling, we next plan to analyze changes in activation of WNT members during chondrogenesis using molecular approaches. This study was supported by the MEYS CR (CZ.02.1.01/0.0/0.0/15_003/0000460).

**Osteoderms of Heloderma suspectum – A New Nano-Micro Hierarchical Biomimernalized Structure in Vertebrates**

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Helodermatidae is a family of venomous lizards that has evolved osteoderms in the skin. Since they were first reported, osteoderms have generally been described as bone-like structures, and this concept has never been thoroughly challenged. Here, the nano/micro characteristics of the osteoderms of *Heloderma suspectum* are evaluated and compared to bone and tooth samples from the same animal. This characterization study was done using advanced physical-chemical analyses, such as electron microscopy with focused ion beam (FIB). At the micro scale, SEM results show that the osteoderms can be divided into three regions: a highly mineralized dense material present in the upper region of the osteoderm; a bone-like material that runs through the osteoderm, apparently surrounding vasculature; and a mineralized region, with collagen fibers organized in a three-dimensional mesh. At the nano scale, TEM of samples prepared by FIB demonstrated that each of the three different regions is unique, with different nanostructures and crystallinity, as demonstrated by x-ray diffractometry. Comparison with samples of bone and teeth taken from the same animal reveal that the osteoderm is indeed a unique mineralized tissue and not a bone-like tissue. These results are an indication that in vertebrates, natural non-pathological hard tissues are more diverse than suspected, suggesting the existence of completely novel cellular and biochemical biomimeralization systems. Further preliminary results indicate that the three structures described here are the fundamental components of osteoderms in lizards, with variations in the shape and proportion of these three structures between species. Finally, beyond the biological, evolutionary and ecological significance of these hard tissues in vertebrates, the hitherto unknown nano/micro structures described here might prove valuable in future translational applications, including the creation of bioinspired materials with special properties.