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Society for Experimental Biology Annual Main Meeting 28th June — 1st July 2009, Glasgow, UK

A1 — BIOLOGY OF ELASMOBRANCHS: FROM GENES TO ECOPHYSIOLOGY AND BEHAVIOUR

A1.1

08:30 Wednesday 1st July 2009

Gas transfer in elasmobranchs: A unique model of CO_2 excretion

Kathleen M. Gilmour (University of Ottawa), Steve F. Perry (University of Ottawa)

Carbonic anhydrase (CA) is a zinc metalloenzyme that catalyzes the reversible hydration–dehydration reactions of CO_2. It is present in high abundance in the cytoplasm of vertebrate red blood cells, where it contributes to CO_2 excretion. A membrane-bound CA isoform (CA IV) is also present in the lungs of mammals and reptiles, but plays little role in CO_2 excretion. The gills of teleost fish appear to lack plasma-accessible CA activity. In elasmobranchs, however, evidence gathered using a variety of physiological, biochemical and molecular approaches suggests that CA IV is present in the gills, and that this CA IV makes a significant contribution to CO_2 excretion by catalyzing the dehydration of plasma HCO_3^-. The contribution of CA IV to CO_2 excretion is favoured by unusually high plasma buffering that aids in the provision of protons for HCO_3^- dehydration. Moreover, reduced emphasis on HCO_3^- flux through the red blood cell may reflect the occurrence of a slower turnover cytosolic CA in elasmobranchs. This model of CO_2 excretion, in which HCO_3^- dehydration in the red blood cell catalyzed by cytosolic CA and HCO_3^- dehydration in the plasma catalyzed by membrane-bound CA IV are of near equal importance, appears to be unique to elasmobranchs. (Funded by operating and equipment grants from the Natural Sciences and Engineering Research Council of Canada.)

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A1.2

09:00 Wednesday 1st July 2009

Osmoregulation in elasmobranch fish

Neil Hazon (University of St Andrews)

In seawater the elasmobranch fish typically maintain body fluids slightly hyperosmotic to the surrounding environment. This is achieved by regulating the concentrations of organic and inorganic osmolytes, principally sodium, chloride, urea, and trimethylamine oxide. The concentrations of these osmolytes are altered, independently of each other, in response to changes in environmental salinity. These alterations are necessarily coupled with changes in the direction and magnitude of osmotic water exchange with the environment.

The elasmobranch osmotic strategy depends on the integration of:

1. hepatic urea production and branchial and renal urea excretion and
2. the balance of salt input with salt excretion at the gills, gut, kidney and via the unique elasmobranch rectal gland.

The mechanisms that control this integrated response depend upon a series of osmoregulatory hormones that can affect the activity of the key osmoregulatory organs. In recent years our understanding of the control of ion and urea metabolism in elasmobranch fish has increased with many more species being investigated and the role of key osmoregulatory hormones being described, In this presentation the osmoregulatory strategies of partially euryhaline and fully euryhaline elasmobranch species will be compared.

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A1.3

09:30 Wednesday 1st July 2009

sAC mediated translocation of V–H^+–ATPase to the basolateral membrane in the Pacific spiny dogfish gill ionocytes

Agnieszka K. Dymowska (University of Alberta), Greg G. Goss (University of Alberta)

In the Pacific spiny dogfish (*Squalus acanthias*), post-feeding metabolic alkalosis is associated with translocation of V–H^+–ATPase from the cytoplasmic vesicles to the basolateral membrane of the gill ionocytes. This mechanism counters the increased metabolic base concentration by pumping hydrogen ions into the blood, which decreases blood pH and energizes an apical Cl^-/HCO_3^- transporter enabling effective base secretion. In insects, translocation of V–H^+–ATPase to the cell membrane is known to be mediated by a cAMP dependent signaling cascade. To examine the signaling pathways responsible for basolateral translocation

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of V–H^+–ATPase in spiny dogfish we incubated dissected gills in IBMX, 8-Br-cAMP, H89 and, KH7 in the presence or absence of high bicarbonate levels in the incubating medium. Preliminary results revealed the possible involvement of soluble adenyl cyclase in the signaling pathway during V–H^+–ATP translocation.

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A1.4

09:50 Wednesday 1st July 2009

Questioning maternal resource allocation in sharks

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Life history theory predicts that organisms will provide an optimal level of parental investment to increase offspring survival balanced against the effects on parent survival and future reproductive potential. Optimal models also predict an increase in reproductive effort with age, as expected future reproduction decreases. To date, maternal investment in sharks has received limited attention. We found that neonatal dusky sharks (*Carcharhinus obscurus*) are not independent from maternal resource allocation at the point of parturition as previously thought. Newborn sharks are provisioned with reserves in the form of an enlarged or *super liver* which constitutes approximately 20% of total body mass. Analysis of long-term archived data sets showed that a large proportion of this *super liver* is utilised during the first weeks of life and therefore indicates that the reported weight loss of newborn sharks signifies a natural orientation process and is not necessarily related to prey abundance and/or indicative of high mortality rates. Interrogation of the condition of near-term pups of pregnant individuals of two carcharhinids, the dusky and spinner shark (*Carcharhinus brevipinna*), further revealed an optimal reproductive size. This provides evidence for a trade-off between total maternal reproductive output and the condition of individual newborn animals with increasing size of the mother. Considering fisheries often target larger individuals, this has important implications for modelling the reproductive potential of a population and for structuring future management strategies.

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A1.5

10:30 Wednesday 1st July 2009

Waste not want not — Intestinal handling of solutes and water in elasmobranchs

Gary Anderson (University of Manitoba), Josi R. Taylor (University of Miami), Martin Grosell (University of Miami), Dirk Weihrauch (University of Manitoba)

In the iso/hyper-osmoregulating marine elasmobranch fishes intestinal handling of solutes and water is not well understood. Recent investigations have indicated a role for the intestine in relation to acid–base, nitrogen, ionic and osmotic balance in the spiny dogfish, *Squalus acanthias*. Previously we reported flux rates of solutes and water across the isolated intestine of the bamboo shark, *Chiloscylium plagiosum*. Here we present the solute concentration of plasma, stomach, intestine and colon in 3 species of elasmobranch fish, *C. plagiosum*, the clear nose skate, *Raja eglanteria* and the little skate, *Raja erinacea*. In all three species there was a high concentration of urea within the intestinal fluid which all but disappeared in the colon. Flux rates for solutes and water were measured in the intestine and colon in *R. erinacea*. In the isolated intestine flux rates were similar to values reported for the bamboo shark, and in the isolated colon, flux rates of solutes were much lower and all in an outward direction. In subsequent experiments using real time PCR we examined expression levels of both the urea transporter and a Rhesus-like protein (specific ammonia transport protein) in the intestine of *R. erinacea*. Expression profiles of these transport proteins tended to increase from the anterior to the posterior end of the intestine and this increase was significant in the case of the rhesus like protein. Results will be discussed in the context of renal and extra-renal expression of these transport proteins.

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A1.6

11:10 Wednesday 1st July 2009

Urea metabolism and transport: Regulation with changing environmental salinity

Patricia A. Wright (University of Guelph)

Marine elasmobranchs maintain high levels of urea and other osmolytes in their tissues in order to remain iso-osmotic with their environment. Urea synthesis occurs via the urea cycle, is present during early embryonic development and is found in both liver and muscle tissues of adults. Urea transport in elasmobranchs is dependent on both active and passive urea transport (UT) proteins. Recently, we discovered that UTs exist in the mitochondrial membrane of elasmobranch hepatocytes. As well, UTs are found in the brush border membranes of kidney cells and basolateral membrane of gill epithelial cells. These UTs play an important role in regulating gill and kidney urea permeability. Elasmobranch gills have a remarkably low urea permeability and most of the urea filtered in the kidney is reabsorbed before excretion of the final urine. Acclimation to lower salinity environments in some elasmobranchs results in modulation of the urea cycle, UT gene expression and net urea excretion, ultimately lowering tissue urea levels. In the past two decades, significant knowledge has been gained about the mechanisms of urea metabolism and transport in elasmobranchs, but there is still much to learn about the mechanisms of urea retention at different stages of development and in variable environments.

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A1.7

11:40 Wednesday 1st July 2009

Immunohistochemical localization and expression of aquaporin water channel membrane transport protein homologues in the osmoregulatory tissues of the dogfish (*Squalus acanthias*)

Christopher P. Cutler (University of Southern University), Gordon Cramb (University of St Andrews)

Very little data is available concerning the presence of homologues of members of the aquaporin water and small solute channel protein family in elasmobranches. Recently Cutler et al. (2005) and Meischke et al. (2007), identified an aquaporin homologue from the

euryhaline Bullshark (*Carcharhinus leucas*). This sequence data has since been utilized to identify four further aquaporin homologues from the dogfish (Cutler, 2007; *Squalus acanthias*). Affinity purified polyclonal antibodies were then generated against the C-terminal end of each dogfish aquaporin protein. Immunohistochemical localization studies were initiated to determine the cellular localization of these aquaporin isoforms in osmoregulatory tissues of the dogfish, including rectal gland, kidney, gill, oesophagus and intestine/rectum. Results from these studies will be presented as time allows.

Cutler, C.P. (2007) Cloning and identification of four aquaporin genes in the dogfish shark (*Squalus acanthias*). Bull. Mt. Des. Isl. Biol. Lab. 46: 19–20.

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Meischke, L., Cramb, G. and Cutler, C.P. (2007) Cloning and expression of aquaporin water channels in the euryhaline bull shark, *Carcharhinus leucas*. Comp Biochem. Physiol. 146: S93.

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A1.8

12:00 Wednesday 1st July 2009

Seasonal residency and migration of mature lemon sharks (*Negaprion brevirostris*) off the southeast Florida Coast

Steven T. Kessel (Cardiff University, Bimini Biological Field Station), Samuel H. Gruber (Bimini Biological Field Station), Todd Gedamke (NOAA), Rupert G. Perkins (Cardiff University)

In 2001 aggregations, of ~75, mature lemon sharks (*Negaprion brevirostris*) were discovered off the Jupiter coast, FL. The presence of concentrated groups presented the first opportunity to study wild lemon sharks of mature life stage. Feasibility studies were conducted from 2003 to 2005 revealing that these sharks could be caught for further study. During the subsequent winter seasons of January to March 2006–2008, sharks were caught on hooks using polyball droplines. Captured individuals were secured to the boat, measured, sampled for DNA and tagged (NOAA M-type dart tag and PIT tag). All mature lemon sharks received a Vemco V16H transmitter implanted in their coelom. These, in concert with an array of 18 VR2 monitors along the putative aggregation migration route were used to describe local movements, forming part of the Florida Atlantic Coast Telemetry (FACT) VR2 array consisting of approximately 123 monitoring stations, with 26 to date receiving hits from our study population. A male previously caught off Long Key, FL, and another previously caught in Winyah Bay, NC, were originally tagged under the NMFS co-operative shark tagging program. This demonstrated that mature lemons will undertake long migrations to join this aggregation. Results from the monitors showed that males left the array area around March/April 2007 then returned between December 2007 and January 2008. In contrast, the females produced hits on the array year round. The further deployment of two wildlife computer MK10 PAT tags (3 and 6 months release) should further reveal the longer-term movements.

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A1.9

13:20 Wednesday 1st July 2009

The unusual energy metabolism of elasmobranchs

Ben Speers-Roesch (University of British Columbia)

Elasmobranchs possess an unusual energy metabolism characterized by an increased reliance on ketone bodies rather than lipids as oxidative substrates. It was thought previously that elasmobranchs lacked extrahepatic fatty acid oxidation (FAO). Recently, however, we showed that the capacity for FAO, as measured by carnitine palmitoyltransferase (CPT) activity, is substantial in some extrahepatic tissues of elasmobranchs. We proposed a revised model of tissue-specific FAO in elasmobranchs with high capacity in the liver, kidney and rectal gland, and low or absent capacity in heart and skeletal muscle. Ketone body oxidation appears to be important in all tissues. We showed that holocephalans possess the same metabolic organization and thus so too did the common ancestor of chondrichthyan fishes, which lived ~400 Mya. A major challenge is to identify the proximate and ultimate causes of this metabolic organization. One hypothesis is that the high level of urea in chondrichthyans adversely affects the ability of lipid carriers such as albumin to transport fatty acids. Elasmobranchs lack albumin and have low levels of circulating free fatty acids. We tested this hypothesis by comparing the capacity for FAO in freshwater and marine elasmobranchs that vary in the amount of urea accumulated. There were no major differences between the species suggesting no role of urea in explaining the metabolic organization of elasmobranchs. Currently, we are investigating the determinants of the tissue specific expression of CPT in elasmobranchs. Surprisingly, we found that mRNA of CPT1 is expressed in dogfish heart despite the apparent absence of CPT activity or FAO.

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A1.10

13:50 Wednesday 1st July 2009

The influence of feeding and fasting on plasma metabolites in the dogfish shark (*Squalus acanthias*)

Chris M. Wood (McMaster University), Patrick J. Walsh (University of Ottawa, University of Miami), Makiko Kajimura (McMaster University, Wakayama University), Grant McClelland (McMaster University), Shit Fun Chew (Nanyang Technological University)

Dogfish sharks are opportunistic predators, eating large meals at irregular intervals. We will synthesize data, from both published and unpublished studies, on responses in plasma metabolites after natural feeding (at ration levels of 2.6% and 5.5%) and during prolonged fasting (56 days). Most responses were more pronounced at the higher ration level. These included increases in urea and TMAO concentrations at 20 h, followed by stability through to 56 days of fasting. Ammonia levels were low, exhibited little short-term response to feeding, but declined to very low values during the extended fast. Glucose and β-hydroxybutyrate both fell after feeding, the latter to a greater extent (up to 60 h), whereas acetoacetate did not change. During prolonged fasting, glucose concentrations were well regulated, but β-hydroxybutyrate increased to 2–3-fold control levels. Total plasma amino acid concentrations increased in a biphasic fashion, with peaks at 6–20 h, and 48–60 h after the meal, followed by homeostasis during the extended fast. Essential and non-essential amino acids generally followed this same pattern, though some exhibited different trends which will be high-lighted. Plasma non-

esterified fatty acid concentrations declined markedly after the meal. These data are interpreted in light of companion studies showing elevations in aerobic metabolic rate, urea production, rectal gland function, metabolic base excretion, and activation of ornithine–urea cycle and aerobic enzymes after the meal, and muscle N-depletion but maintenance of osmolality and urea production during long-term fasting (supported by NSERC Discovery Grants to CMW, PJW, & GM, and a JSPS Fellowship to MK).

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A1.11

14:10 Wednesday 1st July 2009

Divergent locomotor muscle design among thresher sharks

Diego Bernal (University of Massachusetts Dartmouth), Douglas Syme (University of Calgary), Jeanine Donley (Miracosta College), Chugey Sepulveda (Pfleger Institute of Environmental Research)

This study investigates several aspects of locomotor muscle function and design in the thresher sharks (Alopiidae). Threshers are a group of large, pelagic sharks easily recognized by their elongate upper caudal lobe. Alopiids represent the only genus to contain species with both lateral and medial positions of the red locomotor muscle (RM). Thus, the alopiids provide the ideal system in which to test the hypothesis that the medial RM position in the common thresher shark provides the basis for a propulsion mechanism similar to that found in the lamnid sharks and different from sharks with lateral RM. Sonomicrometry was used to quantify the *in-vivo* muscle dynamics during sustained swimming. Field studies on the RM and white muscle (WM) of 11 common threshers were performed. At a tailbeat frequency of 0.5 Hz, RM strain (at first dorsal fin) was consistently greater than that of the WM and decreased significantly during simulated swimming movements (when the RM was not stimulated; i.e., passive swimming). By contrast WM strain did not differ between active and passive swimming. A comparison of RM and WM phase during swimming showed instances in which RM shortening both led and trailed that of the surrounding WM, with no phase difference observed during the passive swimming experiments. This finding suggests that, similar to lamnid sharks, the common thresher RM sheers relative to the WM. Therefore, these results suggest that the common thresher may exhibit a similar uncoupling of RM shortening and local body bending as seen in the thunniform lamnids.

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A1.12

14:30 Wednesday 1st July 2009

Sexual segregation as a behavioural mediator of elasmobranch population dynamics

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Within-species sexual segregation is a widespread phenomenon among vertebrates; however, its causes remain a topic of much debate. Sexes may separate as a consequence of differential habitat use (termed habitat segregation) or when adults form single-sex groups due to social affinities or avoidance behaviour (social segregation). Female avoidance of male coercive mating attempts has the potential to influence the social structure of animal populations but has been largely overlooked as a driver of sexual separation. Here we show that sexual habitat segregation in the catshark, *Scyliorhinus canicula*, arises directly from female avoidance of male sexual harassment. In the wild, sperm-storing female catsharks form refuging aggregations in narrow-entranced, shallow-water caves for up to 70% of the time to avoid unwanted male mating attempts. Females compensate for higher metabolic rates experienced in shallow, warm-water refuges by remaining inactive. However, this thermal environment decreases egg production rate for ~20% of the time during summer months. Therefore, evasion of sexual harassment influences both the social structure and productivity of this population as individual females 'pay' to avoid aggressive males by trading-off potential injury and unsolicited matings with long term fitness (fecundity). This identifies sexual harassment as a persistent cost to females that mediates vertebrate population dynamics and indicates climate warming may further impact female fecundity.

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A1.13

14:50 Wednesday 1st July 2009

Modelling the movements and behaviour of sharks with Lévy statistics

Nicolas E. Humphries (Marine Biological Association), David W. Sims (Marine Biological Association)

Many aspects of the movements and behaviour of free-ranging marine predators such as sharks are poorly understood since direct observation is difficult. Recent advances in electronic tagging technology have provided new insights [Sims et al., 2008; Nature 451:1098–1102], but, by comparison, analysis aimed at exploring behavioural processes of sharks is less well developed. Identifying the behavioural 'rules' underlying movements may provide fundamental information on the diversity and evolution of behaviour in addition to providing data to parameterise more realistic, individual-based spatial population models for use in fisheries management and conservation. This paper describes an approach to the analysis of shark diving movements based on modelling with Lévy statistics. Optimal foraging theory predicts that free-ranging predators should adopt search strategies known as Lévy flights when prey is sparse and distributed unpredictably, so open-ocean sharks may have evolved to exploit Lévy flights. However, burgeoning empirical support has foundered recently because less accurate statistical methods have been used to identify Lévy flight behaviour in a range of organisms, from amoeba to human hunter–gatherers. Consequently, whether natural foragers exhibit Lévy flight search behaviour remains unclear. We present results using robust statistical methods (maximum likelihood estimation) to test for Lévy flight behaviour from the largest dataset of animal movements assembled for this purpose; nearly 12 million move steps across 14 species of open-ocean predators; principally pelagic and planktivorous sharks but also tunas, billfish, and sunfish.

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A1.14

15:30 Wednesday 1st July 2009

Elasmobranch models that have furthered our understanding of neural control of physiological function in vertebrates

Edwin W. Taylor (University of Birmingham)

Two studies of elasmobranch fishes have been pursued by zoologists from the University of Birmingham, both centred on the laboratory of the Marine Biological Association in Plymouth. They exploited the ease of exposing the brain via the chondrocranium in these cartilaginous fishes. Otto Lowenstein, working with Alec Sand from the Plymouth Laboratory, studied the labyrinth of the dogfish and skate. They recorded from the 8th cranial nerve while spinning or tumbling the isolated otic capsule of a skate, showing how the sensory cells of the semi-circular canals responded directionally to rotation. This was a ground breaking study of sensory transduction. Otto later studied gravity reception by otolith organs, working with Mike Osborne and Ray Thornhill to map the directionality of sensory hair bundles. Forty years later, working with Debbie Barrett, Pat Butler, Jenny Levings, Steve Short and Mike Young, we studied the role of the X^{th} cranial nerve, the vagus, in the parasympathetic control of the dogfish heart, exploiting its lack of a sympathetic supply and the relative ease of exposing respiratory and cardiac branches of the X^{th} cranial nerve via the posterior cardinal sinus. This work revealed the nervous mechanisms enabling the heart to be recruited by the respiratory rhythm. Both studies combined micro-anatomical with electrophysiological techniques to increase our understanding of fundamental processes in vertebrate and, therefore of course, human physiology.3

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A1.15

16:00 Wednesday 1st July 2009

Ecophysiology of neuronal metabolism in transiently oxygen depleted environments

Gillian M. Renshaw[a] (Griffith University), Graham Wise[a] (Griffith University Qld. Australia), Peter R. Dodd[b] (The University of Queensland Qld. Australia)

The interactions between coral reef topography, tide cycles and photoperiod provided a selection pressure that resulted in adaptive physiological changes enabling sheltered hypoxic niches to be exploited by suitably specialised tropical reef fish, including at least one species of shark. The epaulette shark (*Hemiscyllium ocellatum*) can withstand temporary cyclic hypoxia in its natural environment, several hours of experimental hypoxia at 5% of saturation (Wise et al., 1998) and even exposure to anoxia (Renshaw et al., 2002). The epaulette responds to cyclic exposure to 5% of saturation by entering a state of hypoxia-induced neuronal hypometabolism (Renshaw and Mulvey, 2000). Our data from GABA immunochemistry, HPLC analysis and receptor binding studies revealed that, in the cerebellum, there was an accumulation of GABA within neurons with no change in the concentration of GABA and this was accompanied by a significant increase in receptor density without any decrease in receptor binding affinity. While all hypoxia and anoxia tolerant teleosts examined so far, respond with significant elevations in GABA, the phylogenetically older epaulette shark did not, indicating that the novel mechanism may be used to elicit energy conservation. The increased receptor density is likely to protect the cerebellum from re-oxygenation damage.

Keywords: Elasmobranch; Hypometabolism; Hypoxia-tolerance; Ecophysiology

Renshaw, G.M.C., Mulvey, J.M. (2000) Neurosci. Lett. 290(1):1–4.
Renshaw, G.M.C. et al., (2002) Comp. Biochem. Physiol. B 131(2), 133–41.
Wise, G. et al., (1998) Comp. Biochem. Physiol. B 131(2), 133–41.

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A1.16

16:20 Wednesday 1st July 2009

Fluid flow in and around the olfactory organ of a hammerhead shark

Jonathan Cox (University of Bath), Richard Abel (National History Museum), Viet Bui Xuan (Simpleware Ltd), Ross Cotton (Simpleware Ltd), Philippe Young (Simpleware Ltd), Matthew Baker (University of Exeter), Gavin Tabor (University of Exeter), Timothy Nickels (University of Cambridge)

Despite their obvious and exotic appeal, there have been no detailed hydrodynamic studies of the olfactory organs of sharks. In a move to redress this situation, using high resolution computed X-ray tomography we have acquired a three-dimensional data set of the head of the golden hammerhead shark, *Sphyrna tudes*, and converted this data set into real and virtual models. With these models we have explored the fluid dynamics of flow in and around the olfactory organs, which are positioned on the anterior edge of the hammerhead's head. As flow between the extensive olfactory lamellae of the hammerhead is almost certainly generated by the pumping action of ciliated cells (difficult, but not impossible to model), we have focused on the anatomical features thought to be responsible for harnessing the oncoming flow experienced by the swimming fish in order to generate a steady continuous flow of water through the olfactory organ, ensuring that it is in continual contact with the odorants in its aquatic environment. These features include a narrow, pre-narial groove (absent in some hammerhead species), a shallow and previously unremarked-upon indentation in the roof of the incurrent nostril, the triangular excurrent nostril and the exquisite hydrodynamic integration of the olfactory organ into the unusual geometry of the hammerhead's head.

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A1.17

16:40 Wednesday 1st July 2009

Micromorphology and mechanics of the tessellated skeleton of cartilaginous fishes

Mason N. Dean (University of California Irvine), Kerin M. Claeson (University of Texas at Austin), Adam P. Summers (University of Washington)

The endoskeletal elements of sharks and rays are comprised of an uncalcified, hyaline cartilage-like core overlain by a thin layer of mineralized hexagonal tiles (tesserae), adjoined by intertesseral fibers. The basic spatial relationships of the tissue phases (unmineralized cartilage, mineralized cartilage, fibrous tissue) are well-known–endoskeletal tessellation is a long-recognized synapomorphy of elasmobranch fishes–but a high-resolution and three-dimensional

(3D) understanding of their interactions has been hampered by difficulties in sample preparation and lack of technologies for visualizing microstructure and microassociations. We have used cryo-electron microscopy, microfocus computed tomography and synchrotron radiation tomography to investigate the tessellated skeleton down to submicron thicknesses but without damage to the delicate relationships between phases or among tesserae. Our digital tomographic methods are non-invasive and thus provide non-destructive investigations of never before appreciated hard tissue anatomy on any virtual slice plane and in both extant and fossil species. Our data reveal structural features (e.g. canalicular passages linking cells within tesserae, mineralized cross-bridges connecting adjacent tesserae) that are likely important for the growth and mechanics of elasmobranch skeletons. We discuss these and other microanatomical features in the context of models we have generated of tessellated cartilage function and address where these "typical" morphologies are modified, such as high-stress or curved regions of the skeleton. We also highlight our 3D methodologies, which present several compliments and advantages to traditional dissection or chemical preparation of heterogeneous tissues such as tessellated cartilage.

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A1.18

Poster Session — Tuesday 30th June 2009

Does the stress associated with catch-and-release angling of bonefish (*Albula vulpes*) affect predation by juvenile lemon sharks (*Negaprion brevirostris*)?

Lorna J. Dallas (University of Plymouth), Aaron D. Shultz (Cape Eleuthera Institute), A. John Moody (University of Plymouth), Andy J. Danylchuk (Cape Eleuthera Institute), Katherine A. Sloman (University of Plymouth)

Predation by juvenile lemon sharks (*Negaprion brevirostris*) is the main cause of short-term mortality for bonefish (*Albula vulpes*) following catch-and-release angling. Previous studies suggested that these predators appeared to home in on stressed bonefish using olfaction. This study used a combination of field and laboratory work to investigate the possibility that lemon sharks detect stress chemicals associated with the angling event. To examine the response of predators to these chemicals, the behaviour of juvenile lemon sharks ($n=12$) held in captivity was quantified when exposed to 30 s pulses of ammonia (500 mM), cortisol (20 μg L^{-1}), lactate (6 mM), and urea (3 mM), as well as water from a cooler that had contained bonefish and control tank water. To quantify the release of stress chemicals, wild bonefish ($n=7$) were angled, fought to exhaustion, and exposed to air for 1 min. Bonefish were then placed in an aerated cooler and water samples were taken at 0, 1, 5, 10 and 30 min to be later analysed for ammonia, cortisol, lactate and urea. It was found that bonefish excrete both ammonia and urea during the first 30 min after angling; but cortisol and lactate were below detectable levels. Lemon sharks were significantly more active when exposed to ammonia and urea than when exposed to control water. Combined, these results show that products excreted by bonefish, particularly ammonia and urea, may provide an olfactory cue for the post-release predation of bonefish by lemon sharks during catch-and-release angling events.

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A1.19

Poster Session — Tuesday 30th June 2009

Development of embryonic gill vasculature in the yellow stingray, *Urobatis jamaicensis*

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As part of a series of studies examining gill vascularization in batoid elasmobranchs, we used corrosion casting to examine the development of gill vasculature in embryonic yellow stingrays, *Urobatis jamaicensis* (formerly *Urolophus jamaicensis*). The most marked changes in vascular configuration of the gills occur in the earliest castable stages of gestation. These changes included development of afferent external gill filament vessels and progression from paired dorsal aortae to a single fused dorsal aorta. Internal gill vasculature was found to nearly match that of an adult by the time the external gill filaments had fully regressed and yolk sac had been exhausted (>47 mm disk width). Examination of embryo casts also revealed characteristics of the branchial vasculature not previously reported in adult specimens. These include the presence of prelamellar sph_2 sphincters, intertrematic branches, afferent distributing arteries which supply blood to many afferent filament arteries, resulting in greater interconnection of the filaments, and observation that the afferent branchial artery in the first hemibranch supplies blood directly to afferent filament arteries on the dorsal half of this arch. The physiological significance of these early structures is not clear.

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Poster Session — Tuesday 30th June 2009

The role of the caudal fin in the common thresher shark, it's not just for swimming

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The thresher sharks comprise a monophyletic group of pelagic sharks most commonly recognized by their elongate upper lobe of the caudal fin. It has been hypothesized that thresher sharks utilize the elongate fin to stun small-schooling prey while feeding. The bi-functional role of the caudal fin for both thrust production and predation represents a unique adaptation that has not been described for any elasmobranch species. Despite the commercial importance of the thresher sharks, there are no published accounts on how the caudal fin is used during feeding. Field and laboratory studies were conducted to acquire video recordings of feeding common threshers, examine the caudal fin morphology, and investigate the ocular morphology which potentially enables vision in the posterior field. Of the 140 specimens captured 3% were hooked in the mouth while the remainder were hooked in the caudal fin. Video of feeding threshers yielded footage from 25 individual caudal fin-feeding events. Morphological examination of the common thresher caudal fin revealed that the upper lobe is predominantly comprised of tendinous and cartilaginous support tissues. The dissections also revealed the presence of locomotor muscle fibers throughout the entire length of the caudal fin. In general, the caudal fin structural architecture appears to be similar to that of lamnid sharks, but with

much larger dorso-ventral cartilaginous support elements. Preliminary findings also show that the eye has the capacity to rotate along the longitudinal axis in an anterior–posterior direction within the orbit which may enhance vision in the posterior field.

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Avoid the solvent abuse — An alternative method for the measurement of trimethylamine oxide

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In most vertebrate groups high levels of urea in the plasma are not tolerated. However, elasmobranchs have adapted a variety of mechanisms to counteract the potential harmful effects of the high concentration of urea (>300 mM) in their plasma. One such method is the retention of methylamines that counteract the toxicity of urea, the most notable of which is trimethylamine oxide (TMAO). A customary method for measurement of TMAO involves the reduction of TMAO to TMA followed by the extraction and analysis of the amine in its picrate form (Wekell and Barnett, 1991). In the alternative method TMAO was measured by ion exchange chromatography (Metrhom Peak). Briefly, plasma samples from the little skate, *Raja erinacea*, (10 ml) were diluted with 10 ml of MilliQ water and injected onto a cation exchange column (Metrosep 2-150) using a mobile phase of 4 mM tartaric acid and 0.75 mM dipicolinic acid at a flow rate of 1 ml min^{-1}. Cation detection was achieved using a Metrohm Peak IC 819 detector. TMAO was measured in the same samples using the method first described by Wekell and Barnett (1991) and results were found to be comparable. Additional methylamines and b-amino acids did not interfere with the measurement of TMAO and under these conditions TMAO did not interfere with the measurement of other ions from the same sample. This method was advantageous because"; much less sample was required for measurement; there was no requirement for strong solvents; and multiple cations were measured from the same sample.

Wekell and Barnett (1991), J. Food Sci. 56, 132–135.

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Metal toxicity in the spotted dogfish *Scyliorhinus canicula*

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Metal toxicity has been studied extensively in teleost fish, but much less is known about the effect of metals on elasmobranchs. In a previous study, examining the effects of metals on an elasmobranch, silver appeared to be 10 times more toxic to Pacific spiny dogfish than to similarly sized marine teleosts and in fact, sensitivity approached that of freshwater teleosts. This sensitivity coincides with high Ag accumulation rates in gill and other tissues. As in teleosts, toxicity appeared to be related to osmoregulatory disturbance; however, in this elasmobranch, failure of the urea retention mechanism played an important role in the osmoregulatory disturbance. Cu, which usually exert similar effects as Ag, did not induce this high toxicity or the high accumulation rates.

Despite the fact that normal background levels for metals in the marine environment are low, the differences in response between marine teleosts and elasmobranchs are intriguing. Therefore, the goals of the present study were to determine which metals showed high accumulation rates in another elasmobranch, *Scyliorhinus canicula*. For this purpose, we exposed the dogfish to 0.1 and 1 µM of Ag or to 10 µM Cd, Cu, Ni or Pb for 1 week and measured metal accumulation, metallothionein induction, and parameters related to osmoregulation.

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