CTENOPALOOZA

Organized by

Joseph Ryan, Whitney Laboratory for Marine Bioscience Christine Schnitzler, National Human Genome Research Institute Sidney Tamm, Marine Biological Laboratories

A Meeting of Ctenophorologists

March 14–15, 2016 Whitney Laboratory for Marine Bioscience



This meeting was funded in part by the National Science Foundation under Grant No. (1619712)

Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

Additional funding was provided by the University of Florida Office of Research and the Whitney Laboratory for Marine Bioscience

Other Contributions
Harvard University Press
EvoDevo

Cover:

"Ctenophorae" from Ernst Haeckel's Kunstformen der Natur, 1904. Cartoon on back cover courtesy of Sebastian Beroe Cartoons in this book are by Sidney Tamm

CTENOPALOOZA

Monday, March 14 – Tuesday, March 15, 2016

Monday	8:00 am	Registration & Breakfast
Monday	9:00 am	1 Fossils, Diversity, Phylogeny
Monday	10:30 am	Break
Monday	10:50 am	2 Nerve and Muscle Cells
Monday	12:00 pm	Lunch (provided)
Monday	2:00 pm	3 Ecology, Taxonomy, Behavior
Monday	3:40 pm	Break
Monday	4:00 pm	4 Poster Session I
Monday	5:30 pm	5 Poster Session II
Tuondov	0,00 am	Breakfast
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Tuesday	9:00 am	6 Metagenomics, Slime
Tuesday	10:00 am	Break
Tuesday	10:40 am	7 Genes, Cells, Development
Tuesday	12:00 pm	Lunch
Tuesday	2:00 pm	8 Evolutionary Genomics
Tuesday	3:00 pm	Group Photo / Break
Tuesday	4:00 pm	9 Benthic Ctenophores
Tuesday	5:00 pm	Keynote
Tuesday	6:00 pm	Drinks & Banquet



PROGRAM

MONDAY, March 14-9:00 AM

SESSION 1 FOSSILS / DIVERSITY / PHYLOGENY

Chairperson: Sidney Tamm,

Marine Biological Laboratories

<u>Joseph F. Ryan</u>, Whitney Laboratory for Marine Bioscience, Welcome and introduction to Ctenopalooza.

George D. Stanley Jr, The University of Montana Paleontology Center: A review of the fossil record of Ctenophora: New discoveries and problems.

<u>Larry Madin</u>, Woods Hole Oceanographic Institution: Diversity and distribution of ctenophores in the world ocean.

Steven H.D. Haddock, ¹ W.R. Francis, ¹ L.M. Christianson, ¹ A.D. Serrano, ² K. McConville, ¹ C.E. Mills, ³ ¹Monterey Bay Aquarium Research Institute (MBARI); ²Brown University; ³Friday Harbor Labs, University of Washington: A synthesized view of ctenophore phylogenetics and systematics.

<u>Claudia Mills</u>, ¹ Steven H. D. Haddock, ² ¹ Friday Harbor Labs, University of Washington ² Monterey Bay Aquarium Research Institute (MBARI): A previously unknown deep sea ctenophore from the NE Pacific, with an amusing feeding behavior/strategy.

MONDAY, March 14-10:50 AM

SESSION 2 NERVE AND MUSCLE CELLS

Chairperson: Andy Baxevanis,

National Human Genome Research Institute

KEYNOTE: <u>Mari-Luz Hernandez-Nicaise</u>, Université de Nice Sophia Antipolis, Nice, France: Revisiting ctenophore nerve and muscle cells.

<u>Timothy Jegla</u>, Penn State University: Ctenophores, cnidarians and the evolution of neuronal polarity.

<u>David Simmons</u>, Whitney Laboratory for Marine Bioscience, University of Florida: The general structure and development of the ctenophore nervous system.

MONDAY, March 14-2:00 PM

SESSION 3 ECOLOGY / BEHAVIOR / TAXONOMY

Chairperson: Claudia Mills,

Friday Harbor Labs

Christian Sardet, CNRS, Biodev Lab, Observatoire Océanologique, Villefranche sur Mer: From wandering nuclei in *Beroe* to Tara Oceans expedition.

<u>Jamileh Javidpour</u>, Juan Carlos Molinero, Thomas Larsen, GEOMAR- Helmoltz Centre for Ocean Research Kiel, Germany, Christian-Albrecht University Kiel, Germany: Fundamental trade-offs generating dispersal strategies in invasive ctenophores.

<u>Daniel Sasson</u>, ^{1,2} Joseph F. Ryan, ^{1,2} ¹Whitney Laboratory for Marine Bioscience, ²Department of Biology, University of Florida: Ctene-age wasteland: the influence of light, body size, and self-fertilization on the reproductive output of the sea walnut, *Mnemiopsis leidyi*.

Katharina Bading, ¹ Sarah Kaehlert, ¹ Xupeng Chi, ¹ Cornelia Jaspers, ¹ Mark Q. Martindale, ² and Jamileh Javidpour, ¹ ¹GEOMAR-Helmholtz Centre for Ocean Reasearch Kiel; ²Whitney Laboratory for Marine Bioscience: The effect of food supply on the regeneration ability in the ctenophore *Mnemiopsis leidyi*.

Otto M. P. Oliveira, Universidade Federal do ABC, Brazil: Ctenophora taxonomy: past, present and perspectives for the future.

MONDAY, March 14—4:00 PM

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Otar Akanyeti, 1 Joseph F. Ryan, 1 1 Whitney Laboratory for Marine Bioscience / University of Florida: How does the architecture of the Ctenophore statocyst relate to its function?	5
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Aino Hosia, ¹ Priscilla Licandro, ² Webjørn Melle, ³ ¹ Department of Natural History, University Museum of Bergen, Norway; ² SAHFOS, UK; ³ Institute of Marine Research, Norway: Cydippids rule - Jellies in VPR images from the Trans-Atlantic Euro-Basin cruise.	9

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Sofia Barreira, 1,3 Brian McStay,2 Cathal Seoighe,3 Andreas D. Baxevanis,1 National Human Genome Research Institute, National Institutes of Health, 2 Centre for Chromosome Biology, NUI Galway; 3 School of Mathematics, Statistics and Applied Mathematics, NUI Galway: Genomics of *Hydractinia*: Characterizing and Determining the Biological Relevance of Highly Repetitive Regions.

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<u>Stephen R. Bond</u>, Andreas D. Baxevanis, Computational and Statistical Genomics Branch, National Human Genome Research Institute, National Institutes of Health: Classification of pannexin orthogroups in ctenophores.

)

<u>Leonid L Moroz</u>, The Whitney Lab for Marine Biosci, Dept of Neurosci and McKnight Brain Institute, Univ of Florida: Neurobiology and System Biology of Dynamic Ctenophore Genomes: Molecular insights into origins of metazoan innovations, plasticity and diversification of body plans.

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The Core Adhesome.

TUESDAY, March 15-9:00 AM

SESSION 6 METAGENOMICS / SLIME

Chairperson: Tony Moss,

Auburn University

Richard M. Mariita, ¹ Mohammad J. Hossain, ^{1,2} Mark R. Liles, ¹ Anthony G. Moss, ¹ ¹Biological Sciences Department, Auburn University, ²The Department of Biological Chemistry, The Johns Hopkins University School of Medicine: Bacterial microbiota associated with *Mnemiopsis leidyi* and on the discovery of novel *Staphylococcus* species from their stomodeum.

Mya Breitbart,¹ Bayleigh E. Benner,¹ Parker E. Jernigan,¹ Karyna Rosario,¹ Laura M. Birsa,² Rachel C. Harbeitner,¹ Sidney Fulford,¹ Carina Graham,¹ Anna Walters,¹ Dawn B. Goldsmith,¹ Stella A. Berger,²,³ Jens C. Nejstgaard,²,³¹University of South Florida, ²Skidaway Institute of Oceanography,³Leibniz-Institute of Freshwater Ecology and Inland Fisheries: Discovery, prevalence, and persistence of novel circular single-stranded DNA viruses in the ctenophores *Mnemiopsis leidyi* and *Beroe ovata*.

<u>James Townsend</u>, ¹ Alison Sweeney, ¹ ¹University of Pennsylvania: The Slimes That Bind: Physiology and Biochemistry of *Mnemiopsis* Mesoglea.

TUESDAY, March 15—10:40 AM

SESSION 7 GENES / CELLS / DEVELOPMENT

Chairperson: Mark Martindale,

Whitney Laboratory for Marine Bioscience

<u>Jason S. Presnell</u>, William E. Browne, ¹ University of Miami: Expression and function of Kruppel like factors in *Mnemiopsis leidyi*.

Richard O. McCann, Mercer University School of Medicine: Origins of the Metazoa: Functional Constraints and Sequence Conservation in Coevolution of Proteins of The Core Adhesome.

<u>William Browne</u>, University of Miami: Patterns in ctenophore endoderm.

<u>Lauren E. Vandepas</u>, ¹ Kaitlin J. Warren, ² Chris T. Amemiya, ^{1,3} William E. Browne, ^{2,1}University of Washington, ²University of Miami, ³Benaroya Research Institute: Ctenophore cell culture preparation - methods and potential applications.

TUESDAY, March 15-2:00 PM

SESSION 8 EVOLUTIONARY GENOMICS

Chairperson: Mya Breitbart,

University of South Florida

Nathan Whelan, Auburn University: Body plan and lifestyle evolution in Ctenophora.

Christine E. Schnitzler,¹ Warren R. Francis,² Steven R. Bond,¹ Anh-Dao Nguyen,¹ David K. Simmons,³ Steven H.D. Haddock,⁴ Mark Q. Martindale,³ Joseph F. Ryan,³ Andreas D. Baxevanis,¹¹National Human Genome Research Institute, National Institutes of Health, ²Ludwig-Maximilians University, ³Whitney Laboratory for Marine Bioscience, University of Florida, ⁴Monterey Bay Aquarium Research Institute: Evidence of absence in ctenophore genomes and its impact on evolutionary thinking.

Andrea B. Kohn, ¹ Leonid L. Moroz, ^{1,2,3} ¹Whitney Laboratory for Marine Bioscience, University of Florida, ²Department of Neuroscience, University of Florida, ³McKnight Brain Institute, University of Florida: Epigenetic landscape in Ctenophora: from development to evolution.

TUESDAY, March 15-4:00 PM

SESSION 9 BENTHIC CTENOPHORES

Chairperson: Jamileh Javidpour,

GEOMAR: Helmoltz Centre for Ocean Research

Peter W. Glynn,¹ Shannon G. Moorhead,¹ Michael P. C. Fuller,¹ Tayla N. Fortson,¹ Patricia Blackwelder,¹ D. Abigail Renegar,² Brian Coffman,³¹Rosenstiel School of Marine and Atmospheric Science, University of Miami, ²Oceanographic Center, Nova Southeastern University, ³Fisher Scientific, Houston TX: Ubiquitous but Unseen -- South Florida benthic ctenophores: Some ecological research initiatives.

<u>George Matsumoto</u>, Monterey Bay Aquarium Research Institute: Benthic ctenophores: enigmatic, creeping, successful, and an increasing level of diversity.

<u>Gustav Paulay</u>, University of Florida: Benthic ctenophore diversity in the Indo-west Pacific.

TUESDAY, March 15-5:00 PM

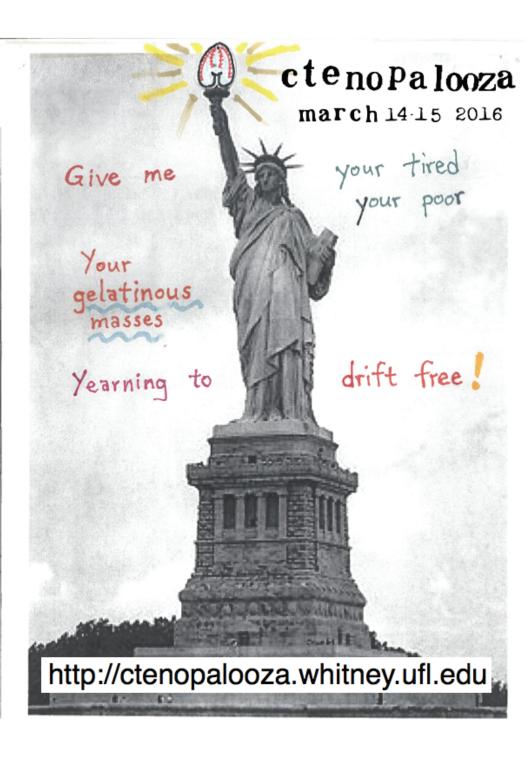
KEYNOTE 2

Casey W. Dunn, Brown University: Ten-four ctenophore.

TUESDAY, March 15-6:00 PM

Banquet / Awards Ceremony

DJs Ancient Deep spin rare funk, disco and soul at 45RPM



Beroese-English Dictionary

selections from the 7th Marine Edition

Compiled Underwater by: Dottore Sebastian Beroe, PhD, QED, FRS, ETC & Prof Sid Tamm, only PhD

Slurp (sssler-ppp): step 1 of quadrille of eating; when *Beroe* collides with another jelly to trigger opening of its big mouth.

Glurp (guh-ler-ppp): step 2; rapid expansion of stomodaeum and activation of macrociliary beating to ingest prey.

Gnish/Gnosh/Gnash (guh-ni-shh/guh-nahh-shh/guh-nass-shh): step 3; ripping and chewing of prey by stabbing, biting macrociliary teeth.

Burp (brr-eurr-ppp): step 4; end-product of digestion marked by release of bilious brown bubbles of odiferous gas; accompanied by seismic sounds.

Splashle (ss-plah-shh-luh): water turbulence caused by metachronal beating of rows of comb plates of a cruising *Beroe*.

Dashle (daa-shh-luh): obscenity uttered by *Beroe* upon missing prey. **Mashle** (mmm-ass-shh-luh): collision of *Beroe* with dock or ctenodipper.

Gashle (gas-shh-luh): laceration or decapitation of *Beroe* by boat propeller, kayak, or razorbill auk.

Brilligous (brill-le-gus): bright sunshine and calm weather inducing an up mood for finding prey.

Neemie Sushi (knee-mee sue-shee): *Mnemiopsis*, favorite food of *Beroe*, eaten raw with wasabi and ginger, or garnished with pink *Edwardsiella*.

Gooozzberrease (goo-zzzz-bear-eees): *Pleurobrachia*, another food, but less filling and with tough stringy tentacles.

Kantankible (can-tehk-a-bull): thunderstorms and big waves producing a down mood and high salt diet.

HiRentalKahndoze (high-wren-tahl-condos): glass bowl apartments in sea table with good views of lowly-paid indentured servants.

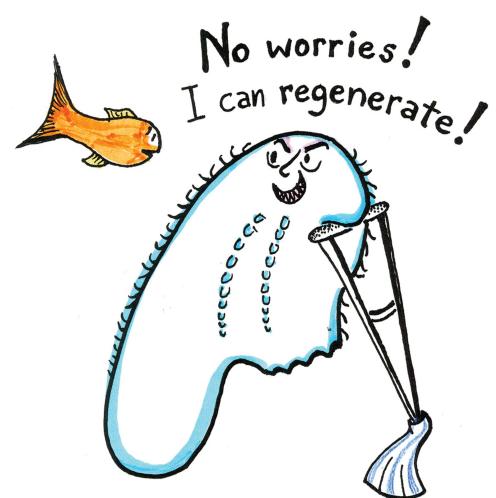
Heimatmeer! (hi-mott-me-yer! hi-mott-me-yer!): exclamation of a grateful *Beroe* escaping into the drain from a sea table.

Glurkle-Snottle-SkrewU! (gg-ler-kul ssknot-ul screw you!): cry of tortured *Beroe* waterboarded on a cteno-tilter while being videotaped by captor.

Izzitfancy? (iss-it-phan-see?): is this motel recommended by IPA (International Plankton Association)?

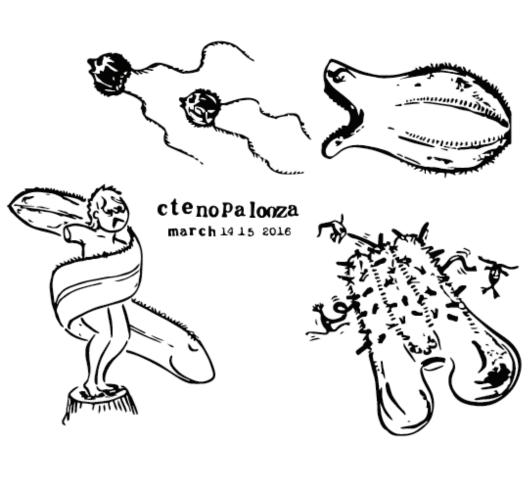
Delish? (dee-lischh?): does this restaurant serve gourmet cuisine with purple *Mnemiopsis* Jello?

BuckleSquirtleSloshh! (bb-kul-skwirr-tel-shh-laahh-shh!): please carry my bucket more carefully, as I have just eaten!



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ABSTRACTS

(sorted alphabetically by presenters last name)

Otar Akanyeti1; Joseph F. Ryan1, 1-Whitney Laboratory for Marine Bioscience / University of Florida: How does the architecture of the Ctenophore statocyst relate to its function?

The ability of a ctenophore to sense the gravitational field is critical for a range of behaviors including prey capture and predator avoidance. Ctenophores sense gravity using a dome-shaped statocyst, which contains a statolith supported by four mechanoresponsive balancer cilia. These balancers are differentially stimulated depending on the orientation of the animal. Recent work has shown that the statolith is superelipsoidal in shape and grows continuously throughout the development of the ctenophore. To better understand how statocyst architecture relates to function, we developed a simple mathematical model based on a two-legged table system that estimates the load distribution among the balancers. For the sake of simplicity, we assume that the legs of the table are massless, and the force associated with the weight of the table acts on a single point at the centre of mass (point load). We quantify the geotactic sensitivity as the difference in the load distribution between the two legs, with larger difference indicating higher sensitivity. We found that sensitivity can be described in terms of $2LWsin\theta/D$, where W and θ are the weight and orientation of the table, respectively. L is the length of each table leg and D is the distance between the two legs. Our analyses predict that the geotactic sensitivity decreases as the distance between the two legs increases. In Mnemiopsis, the four balancers are distributed in a rectangular arrangement, with a length-to-width ratio of 3:1, and the longer sides oriented parallel to the tentacular plane. Consistent with our predictions, it has been shown that the statocyst of *Mnemiopsis* is less responsive to perturbations along the tentacular plane than those along the saggital plane. Our mathematical model provides a link between form and function, and therefore it can tell us how geotactic sensitivity changes throughout ontogeny, and can be used to infer how morphological changes in statocysts have affected or been affected by the biology of ctenophores over evolutionary time.

<u>Katharina Bading</u>1; Sarah Kaehlert1; Xupeng Chi1; Cornelia Jaspers1; Mark Q. Martindale2, and Jamileh Javidpour1, 1-GEOMAR-Helmholtz Centre for Ocean Reasearch Kiel; 2-Whitney Laboratory for Marine Bioscience: The effect of food supply on the regeneration ability in the ctenophore *Mnemiopsis leidyi*

Ctenophores are known to readily heal and regenerate missing body parts upon injury; however, little is known to what extend environmental factors may influence the regeneration mode in real nature. In this study, we investigated the effect of food supply on the regeneration process in bisected larvae of *Mnemiopsis leidyi* under low (10 µg C.L-1) and high (100 µg C.L-1) prey supply over a recovery period of 10 days. The results show that the proportion of regenerating halves under high prey supply (~60%) was about twice as high than under low prey supply (~30%). In turn, this means an almost twofold increase of 'stable' half animals (not-regenerated but viable) under low prey supply. Regenerating back to a whole animal (original state) was only observed under high prey supply and took 8 days. Survival patterns were similar for stable and regenerating halves within prey supply levels with even higher survivability for stable than for regenerating halves under high prey supply. Overall, the findings indicate that the regeneration ability of larval M. leidyi is altered depending on external food supply and that the formation of 'stable' half animals might represent an energy-saving survival mode favored under adverse food conditions. Further studies are needed to elucidate the underlying facultative switch from mere healing to regeneration based on internally available energy supply, and to better understand the role of 'incomplete' animals on population level.

<u>Sofia Barreira</u>1,3; Brian McStay2; Cathal Seoighe3; Andreas D. Baxevanis1, 1-National Human Genome Research Institute, National Institutes of Health; 2-Centre for Chromosome Biology, NUI Galway; 3-School of Mathematics, Statistics and Applied Mathematics, NUI Galway: Genomics of *Hydractinia*: Characterizing and Determining the Biological Relevance of Highly Repetitive Regions

Repetitive elements comprise around two-thirds of the human genome. Large tandem repeats such as ribosomal genes (44kb), segmental duplications (<130 kb), and telomeric repeats comprise the short arms of acrocentric chromosomes. Importantly, these sequences are missing from the human reference genome. Establishing their organization and distribution is crucial to fully understand cellular function. To extend these regions, careful approaches are necessary to ensure accurateness of sequence information, orientation, and placement. Using these kinds of approaches, we have successfully extended the sequence content after the last human ribosomal gene (rDNA) repeat on the telomere side by 550 kb. Currently, our group is sequencing two *Hydractinia* species, organisms that have already shown great promise for the study of regeneration, early developmental processes, and bioluminescence. Since the overall repeat and AT-content of *Hydractinia* is quite high (47% and 65%, respectively), we intend to apply similar strategies as those used with the human genome sequence to identify important repetitive regions such as centromeres, telomeres, and rDNA in these de novo assemblies. This will not only enable us to offer a more complete assembly than those of any other available model organism but also provide a foundation for better understanding their origin and biological relevance.

<u>Stephen R. Bond</u>1; Andreas D. Baxevanis1, Computational and Statistical Genomics Branch, National Human Genome Research Institute, National Institutes of Health: Classification of pannexin orthogroups in ctenophores

Gap junctions (GJ) are a nearly ubiquitous feature of metazoan life, coupling the cytoplasm of adjacent cells into a partially selective syncytium. Connexins (Cxs) and pannexins (Panx) are two distinct GJ-forming protein families that are structurally analogous; pannexins are present throughout the Metazoa, while connexins are restricted to the phylum Chordata. The range of physiological and pathophysiological processes GJs are involved in is extensive, although most knowledge regarding GJ physiology is derived from studying the approximately two-dozen vertebrate Cxs orthologs. Panxs, however, are a more diverse family with hundreds of distinct orthologs. Presumably, Panxs are as integral to cellular function as Cxs, but a comprehensive and systematic survey of these molecules has not previously been performed. Interestingly, the phylogenetic relationship among the Panxs suggests a single founder sequence in the last common ancestor of the Metazoa, with expansion of the family occurring after the separation of groups leading to modern day phyla; e.g., there are no one-to-one orthologs among the Nematoda, Arthoropoda, Annelida, Chordata, Cnidaria, or Ctenophora. Unfortunately, the current state of Panx nomenclature fails to address this exquisite diversity. The historical convention has been to number genes by order of discovery in each new model organism, leading to a confusing scenario where many non-orthologous genes have essentially the same name. Drawing from the ever-increasing wealth of genomic and transcriptomic data, we are systematically reclassifying the Panxs into logical and extendable orthogroups using a novel method called Recursive-Dynamic Markov Clustering. Here we present the ctenophore pannexins as a case study.

Mya Breitbart 1, Bayleigh E. Benner 1, Parker E. Jernigan 1, Karyna Rosario 1, Laura M. Birsa 2, Rachel C. Harbeitner 1, Sidney Fulford 1, Carina Graham 1, Anna Walters 1, Dawn B. Goldsmith 1, Stella A. Berger 2, 3, Jens C. Nejstgaard 2, 3, 1 - University of South Florida, 2 - Skidaway Institute of Oceanography, 3 - Leibniz-Institute of Freshwater Ecology and Inland Fisheries: Discovery, Prevalence, and Persistence of Novel Circular Single-Stranded DNA Viruses in the Ctenophores Mnemiopsis leidyi and Beroe ovata

Gelatinous zooplankton, such as ctenophores and jellyfish, are important components of marine and brackish ecosystems and play critical roles in aquatic biogeochemistry. As voracious predators of plankton, ctenophores have key positions in aquatic food webs and are often successful invaders when introduced to new areas. Gelatinous zooplankton have strong impacts on ecosystem services, particularly in coastal environments. However, little is known about the factors responsible for regulating population dynamics of gelatinous organisms, including biological interactions that may contribute to bloom demise. Ctenophores are known to contain specific bacterial communities and a variety of invertebrate parasites and symbionts; however, no previous studies have examined the presence of viruses in these organisms. Building upon recent studies demonstrating a diversity of single-stranded DNA viruses that encode a replication initiator protein (Rep) in aquatic invertebrates, this study explored the presence of circular, Rep-encoding singlestranded DNA (CRESS-DNA) viruses in the ctenophores *Mnemiopsis* leidyi and Beroe ovata collected from the Skidaway River Estuary and Savannah River in Georgia, USA. Using rolling circle amplification followed by restriction enzyme digestion, this study provides the first evidence of viruses in ctenophores. Investigation of four CRESS-DNA viruses over an eight-month period using PCR demonstrated temporal trends in viral prevalence and indicated that some of the viruses may persist in ctenophore populations throughout the year. Although future work needs to examine the ecological roles of these ctenophoreassociated viruses, this study indicates that viral infection may play a role e n population dynamics of gelatinous zooplankton

William Browne, University of Miami: Patterns in ctenophore endoderm

The current paradigm of gut evolution assumes that early-diverging metazoan lineages either lack a gut (poriferans and placozoans) or have a sac-like gut (ctenophores and cnidarians), and that a through-gut originated either at the base of the bilaterian stem lineage or evolved multiple times independently within the Bilateria. An important group for understanding early metazoan evolution is the Ctenophora, widely thought to have diverged very early from the animal stem lineage. We demonstrate that ctenophores possess a fully-functioning through-gut, in direct contrast to the currently accepted archetype of a ctenophore specific 'flow-through system' containing one major opening for both ingestion and egestion, and conclusively settles a 170+ year old debate regarding the anatomical function of the aboral anal pores in ctenophores. Moreover, the terminal region of the tripartite ctenophore through-gut exhibits functionally sophisticated regulation of both muscle and cilia. The presence of a unidirectional functionally tripartite alimentary canal in ctenophores coordinated for the efficient intake and distribution of nutrients and elimination of waste present an intriguing evolutionary quandary. Either the ctenophore lineage has converged on an arrangement of organs forming a unidirectional through-gut remarkably similar to that in the bilaterian lineage or the unidirectional through-gut has its origins very early in the metazoan stem lineage. The assignment of a functional through-gut organ system to the Ctenophora thus provides new insights into character trait evolution associated with an organ system of fundamental importance to considerations of evolutionary trajectories within the Metazoa.

<u>Margaret L. Byron</u>, Matthew J. McHenry, University of California Irvine: Biomechanics of cyclippid ctenophores in turbulent flow

Ctenophores are fascinating animals, holding the interest of researchers in ecology, genetics, phylogeny, and biological oceanography. However, this interest need not be confined to biology—ctenophores can and should be studied from the perspective of engineers and physicists. As the world's largest ciliates, ctenophores occupy a unique niche in the world of underwater propulsion. The study of ctene rows may hold key insights for the stabilization and control of small underwater vehicles, and could advance our understanding of swimming at transitional Reynolds numbers. Our proposed experiments will quantify the agility and maneuverability of cyclippid ctenophores (*Pleurobrachia*) in complex flows, both turbulence and simple shear. We will subject animals to varying levels of shear (via a traditional kreisel tank) and turbulence (via a custom-built grid-stirred tank). The flows within these two tanks will be quantified using Particle Image Velocimetry (PIV). We will measure the effects of the flow condition on 1) agility, as measured via acceleration, turning radius, stopping distance, and other metrics, 2) preferred location within the tank(s), 3) animals' orientation with respect to gravity, and 4) prey capture attempts and success. We predict that animals will become less maneuverable in higher levels of turbulence and shear, and display avoidance of high turbulence and shear wherever possible. Additionally, we predict that animals will be unable to maintain a preferred orientation and will not be able to feed as effectively in high turbulence/shear. These experiments will shed light on the efficacy of macro-cilia (ctenes) as a control mechanism in complex flows.

<u>Brian Coffman</u>, University of Miami: Seasonal Modulation in a Benthic Ctenophore Population as Evidence for Reproduction

A newly described, shallow-water species of benthic ctenophore, *Coelo-plana waltoni*, has been monitored biweekly within a sampling area spanning over 40 miles from West Palm Beach, FL to Miami, FL. Several key findings have emerged that deal with annual variation in color, the settling of juveniles, and increases in overall population size. Late-fall through spring sees a relative low in population size and a distinct characteristic of only larger individuals. A vibrant pink to blood-red hue becomes nearly fixed among the adult population in the summer and fall months. Large adults exhibit a unique behavior wherein individuals pair off of and one individual extends its tentacles into the coelom of another via the tentacle sheath. Additionally, the presence of small juveniles (< 0.6 mm body length) becomes prolific and random among the octocoral community in the summer and fall months.

Amy Courtney1; Madeleine Lowery2; Mark Pickering1, 1- UCD School of Medicine, University College Dublin, Ireland; 2- UCD School of Electrical & Electronic Engineering, University College Dublin, Ireland: A low cost, flexible, modular microscopy system for investigating the nervous system in *Pleurobrachia pileus*

The anatomical structure of the ctenophore is challenging to image. However, it also offers many advantages, as their relatively small size and transparency permits the visualisation of intact 3D anatomical structures in live or fixed tissue. This can also be coupled with functional measurements such as electrophysiological recording or calcium imaging. In order to take advantage of these qualities we have designed a flexible microscopy system which can be adapted to the diverse imaging applications required when studying ctenophores. To achieve this we designed a modular, low-cost system constructed from simple optomechanical and custom 3D printed components. Fluorescent detection of nuclear markers, phalloidin and an antibody against tyrosylated α-tubulin in fixed *Pleurobrachia pileus* was possible with excitation provided by three independent high intensity LEDs. Oblique infrared illumination, which this system is also capable of, allows the visualisation of the 3D anatomical structure in a manner similar to differential interference contrast microscopy. Using a horizontal light path coupled to a vertically oriented objective by a pentaprism allows the system to be used in an upright or an inverted configuration. As all components are directly mounted to an optical table, including the XYZ translating specimen holder, other apparatus can be aligned and utilised with this system, such as electrode manipulators for electrophysiological applications, or a custom device we developed allowing the precise manipulation of Pleurobrachia pileus larvae and eggs. This microscopy system is a simple, cost-effective, versatile and powerful solution to the complex challenges faced when imaging ctenophores.

<u>Gen Dong</u> 1; Anthony G.Moss 1, 1- College of Science and Mathematics, Biological Sciences Department, Auburn University, AL 36849, USA: Spontaneous rhythmic spiking the tentacle and tentacular bulb of *Mnemiopsis leidyi*

Ctenophores have attracted considerable attention regarding their role in the evolution of nervous systems yet we know little about their nervous systems because of limited electrophysiological data. Previous studies of Pleurobrachia pileus by Moss and Tamm (1993) demonstrated an integrative center in the tentacular bulb. The present study used extracellular recording to examine the electrical activity of the tentacles and tentacular bulb of *M. leidvi*. We found spontaneous slow trains of robust biphasic action potentials (0.1-0.3mV, 0.25-1 spike s-1) in the tentacles and the tentacular bulb of adult animals. Similar rhythmic firing (0.02-0.04mV, 0.5-1 spike s-1) could be detected in tentacles of 4mm nonmetamorphic cyclippid larvae, i.e. bearing only two tentacles. Lesion analysis, in which the tentacular bulb was split in half, revealed the origin of the rhythmic spikes to be in the aboral half of the bulb. Furthermore, unlike in *P. pileus*, no rhythmic waves were ever detected on the surface of the tentacular bulb or pouch. In conclusion, we report here for the first time that spontaneous rhythmic action potentials likely original in the tentacular bulb of *Mnemopsis leidyi* and propagate into the embedded tentacle. The results are in contrast to those of Moss and Tamm with P. pileus, which does not exhibit spontaneous firing. Our data further suggest that there appears to be a central oscillator that drives spontaneous rhythmic activity in the bulb. Future efforts will focus on the cellular source(s) of the signal, the signal conduction pathway and its sensory and/or motor functions.

Casey W. Dunn, Brown University: Ten-four ctenophore

Ctenophores are frequently mischaracterized and misunderstood. These failures arise because their distinctive biology is often shoe-horned in with the biology of better known animals. This leads to a fixation on superficial similarities to other organisms and neglect of their unique traits. Things are starting to change as we get a better grasp on just how different ctenophores are from other animals. Instead of projecting our preconceived biases on them, it is time to do a better job listening to what ctenophores have to say.

<u>Michael P.C Fuller</u>1, 1-University of Miami, RSMAS: Seasonal Variation in the Population Structure of *Vallicula*

One of the more cryptic benthic fauna of South Florida is *Vallicula* multiformis, a tiny platyctenid species. Found living on the macroalga Acanthophora spp., among many other species, Vallicula is one of many metazoans that make up these benthic communities. In an effort to understand its typical population structure and reaction to the surrounding environment, weekly collections of Acanthophora spicifera and accessory aglae were made. Density was standardized using displacement volumes of algae as the volume in which the organisms reside, producing a measurement of animals per liter. These were then analyzed across seasons defined by temperature changes in south Florida waters. Results indicate that the densities seem to change through the year, though not necessarily on a perfectly seasonal basis. Between summer and autumn, the density was not statistically significantly different (p=0.0924), yet between summer/autumn and winter, it is (p<0.001). This is likely due to availability of substrate, as the amount of Acanthophora declined with the transition from autumn to winter. Another possibility is seasonality of food resources, though as of yet we do not have a definitive answer as to what exactly this specific population of Vallicula typically eats. Further data gathering and inclusion of prey availability will provide clearer results and allow for a qualitative assessment of the suggested abundance trend.

Peter W. Glynn 1; Shannon G. Moorhead 1; Michael P. C. Fuller 1; Tayla N. Fortson 1; Patricia Blackwelder 1; D. Abigail Renegar 2; Brian Coffman 3, 1-Rosenstiel School of Marine and Atmospheric Science, University of Miami; 2-Oceanographic Center, Nova Southeastern University; 3-Fisher Scientific, Houston TX: Ubiquitous but Unseen -- South Florida Benthic Ctenophores: Some Ecological Research Initiatives

Recent research initiatives into the biology and ecology of two south Florida platyctenid (Coeloplanidae) ctenophores -- Coeloplana waltoni Glynn, Bayer & Renegar 2014, and Vallicula multiformis Rankin, 1956 --Cw and Vm respectively, are summarized. These studies focus on (a) habitat niches; (b) population abundances and densities, and seasonal occurrences; (c) feeding behavior; (d) predators of benthic ctenophores, (e) reproductive biology, and (f) ecological roles in exposed, open-water octocoral (Cw) and protected, backwater pioneering/fouling communities (Vm). Cw is an obligate ectosymbiont of eunicid and muricid octocorals, and Vm is a facultative associate of macroalgae and diverse benthic invertebrates. Both species exhibit very high localized population densities of 102-103 individuals, particularly in the warm season. Minute zooplankters and particulate organic matter are captured by colloblastbearing tentacles; Cw possibly pilfers ingested food particles from octocorals by inserting their tentacles into the guts of their octocoral hosts. No gonadal development, brooding or spawning have been observed in either species monitored in weekly collections during the summer, fall, and winter seasons. Fragmentation of a large Vm individual occurred on one occasion. Of the numerous potential predators, experimental laboratory observations have revealed high consumption rates of Cw by a visual pygmy angelfish and a browsing gastropod mollusk; Vm was consumed by a decorator crab and sea anemone. Considering the extraordinarily high abundances of Cw and Vm, they likely play an important role in predator/prey interactions at microcommunity scales. Poster presentations offering more detailed informamation on these topics are identified by authors.

<u>Mari-Luz Hernandez Nicaise</u>, Université de Nice Sophia Antipolis, Nice, France: REVISITING CTENOPHORE NERVE AND MUSCLE CELLS

Since the first description of Ctenophore nervous system by Hertwig (1880), our understanding of "primitive nervous systems", together with our vision of the animal kingdom, have been dramatically changed by technological advances. Our aim is to reexamine through different images, how we progressed, from the first supposed "through conducting net" toward the current concept of a complex neural architecture: different subsets of neurons that communicate through synapses and may use various neurotransmitters. A variety of sensory receptors feeds these neuronal assemblies, which in turn command and coordinate the ciliated locomotory system, and various muscle fields. Our ultrastructural and cytochemical data indicate that neurotransmission in Ctenophores relies on exocytosis of synaptic vesicles and suggest possible cholinergic and aminergic mechanisms. Because muscle cells are linked to the emergence and evolution of the nervous system from the start, we investigated the structure and physiology of Beroe giant mesogleal muscle cells. By comparison with the organization described in smooth and striated fibres in Bilateria phyla, we tried to answer the following questions: Are myofilaments organized into myofibrils? Are the myofibrils composed of "hidden" contractile units? Where do the myofibrils attach, given the lack of dense bodies and attachment plaques?, and What are the molecular linkers of the actin filaments? We present evidence that questions 1 and 2 are partly answered. Questions 3 and 4 remain yet to be solved. Strategies of Ca 2+ management developed by these giant cells devoid of a T system are briefly reported.

<u>Steven H.D. Haddock</u>1, W.R. Francis1, L.M. Christianson1, A.D. Serrano2, K. McConville1, C.E. Mills3, 1-MBARI; 2-Brown University; 3-Friday Harbor Labs.: A synthesized view of ctenophore phylogenetics and systematics

Biologists have long known that the taxonomic classification of ctenophores is problematic. A reorganization proposed by Ospovat has been adopted by GenBank, but it still does not provide a satisfactory topology. In 1985, Richard Harbison posited an evolutionary scenario based purely on morphological traits. When our first molecular phylogeny, based on 18S rRNA, was published in 2001, it matched amazingly well Harbison's framework. Subsequent molecular studies have added taxa and additional genes, but the underlying picture has not substantially changed. We have developed a synthesized view of ctenophore diversity by generating transcriptomes from 35 species of ctenophores, and by sequencing 18S, 28S, COI from over 200 specimens. In addition to the gene-based trees, we have also produced a character-based morphological matrix and key for 78 species and morphospecies, including 34 undescribed deep sea soecies, many representing new families. These data sets provide new insights into the evolution of ctenophore traits, including the evolution of simple tentacles, and radiation into the deep sea.

Alexandra Hernandez 1,2; Joseph Ryan 1,2, 1-Whitney Laboratory for Marine Bioscience; 2-Department of Biology, University of Florida: Motorway to Roswell: Detecting alien genes in *Mnemiopsis leidyi*

The transfer of non-animal genes into animal genomes (horizontal gene transfer) is thought to have played an important role in the evolution of some lineages. However, identifying instances of horizontal gene transfer (HGT) has been difficult due to the scarcity in methodology. Towards this end, we have developed a program alien index to document HGT events (based on an algorithm used by Gladyshev and coworkers in the rotifer Adineta vaga). Besides rotifers, HGT has been documented in sponges, cnidarians, nematodes, molluscs, arthropods, but not yet in ctenophores. Using alien index, we have identified more than 30 horizontal gene transfers in the *Mnemiopsis leidyi* genome. Most of these genes have introns and are located on scaffolds with other intron-containing genes. These genes been transferred from bacteria, viruses, and non-metazoan eukaryotes. Many of them appear in transcriptome data from other ctenophore species suggesting ancient events. As more data become available from ctenophores and other animals, our alien index tool can be applied to provide more precise timing of HGT events. These events can be used as evolutionary characters and also be combined with functional experiments to identify key evolutionary innovations.

<u>Aino Hosia</u>1; Priscilla Licandro2; Webjørn Melle3, 1-Department of Natural History, University Museum of Bergen, Norway; 2-SAHFOS, UK; 3-Institute of Marine Research, Norway: Cydippids rule - Jellies in VPR images from the Trans-Atlantic Euro-Basin cruise

A towed MESSOR platform fitted with CTD-F, digitally-autonomous video plankton recorder (VPR), OPC, and multifrequency acoustics was employed between 0-400 m depth on 9 instances during the 2013 Trans-Atlantic Euro-Basin cruise between Bergen, Norway, and Nuuk, Greenland, in order to study the fine scale distribution of hydrography. fluorescence, plankton and fish. Resulting VPR images of gelatinous zooplankton were manually sorted based on morphology. Several taxa of gelatinous zooplankton were identified, although picture quality often precluded identification to species level. The most commonly observed gelatinous organisms were small cyclippid ctenophores belonging to several species. Numerous young Mertensia ovum were observed in the upper 100 m in cold (~1 °C) waters of Arctic influence. Also commonly observed were mesopelagic cydippids (or cydippid stage larvae) occurring in warmer water (~2.5-5 °C) at ~100-300 m depth. The species identity of the latter group has not been confirmed, but they do not appear to belong to any of the species commonly reported from the region. Small cyclippids did not contribute in comparable proportion to net samples collected during the same cruise, nor were the collected specimens in good enough condition to be identified even at this level, suggesting that this group is particularly poorly sampled using traditional methods.

<u>Jamileh Javidpour</u>1, Juan Carlos Molinero1, Thomas Larsen2, 1-GEOMAR- Helmoltz Centre for Ocean Research Kiel, Germany; 2-Christian-Albrecht University Kiel, Germany: Fundamental trade-offs generating dispersal strategies in invasive ctenophores

Understanding functional traits of invasive species in new environments is a central goal of invasion ecology. The comb jelly *Mnemiopsis leidyi* A. Agassiz, 1865 is acknowledged as one of the hundred disruptive marine invasive species with high dispersion potential. The proliferation of the *M. leidyi* in new habitats has been attributed to traits such as being eurythermic/ euryhalin, opportunistic feeding, stealth predation, and high fecundity. However, these traits alone cannot explain their success as an invasive species. I will provide evidence of cannibalism by *M. leidyi* and trade-offs in energy allocation in response to scarce food during post-bloom phase. Furthermore, I will debate whether the species may pose "Jack-and-master" traits conducive to the establishment of new populations.

<u>Timothy Jegla</u>, Penn State University: Ctenophores, cnidarians and the evolution of neuronal polarity

Functionally polar neurons with differentiated axons and dendrites underlie directional signaling in bilaterian nervous systems. Polar neurons feature a rich diversity of ion channels and have highly specialized cytoskeletal features that differentiate axons and dendrites. One of the major questions in evolutionary neurobiology is whether neuronal polarity co-evolved with nervous system centralization in the bilaterians or is a universal feature of nervous systems, present even in the comparatively simple nerve nets of cnidarians and ctenophores. Genomic and transcriptomic analysis of a broad diversity of early metazoans has yielded important clues to the evolutionary history of polarity. Ctenophores appear to lack key cytoskeletal features and many of the ion channels characteristic of typical polar neurons. Our current hypothesis is that ctenophores likely diverged from the eumetazoan lineage prior to the evolution of neuronal polarity. Ctenophores nevertheless have diverse behaviors and independently evolved ion channel diversity that suggest considerable neuronal complexity. Functional experiments will ultimately be necessary to determine whether and how cellular polarity plays a role in the ctenophore nervous system.

Andrea B. Kohn-1 and Leonid L. Moroz-1,2,3, 1-The Whitney Laboratory for Marine Bioscience, University of Florida, 9505 Ocean Shore Blvd, St Augustine, FL 32080, USA; 2-Department of Neuroscience, University of Florida, Gainesville, FL 32611, USA; 3-McKnight Brain Institute, University of Florida, Gainesville, FL 32611, USA: Epigenetic Landscape in Ctenophora: From Development to Evolution

Epigenomics regulation is emerging as a critical mechanism in virtually any biological functions from early development to cancer. However, little is known about evolution, distribution and functional role of these processes in basal metazoans. Here, we analyzed both DNA and RNA epigenetic machinery as well as developmental transcriptomes in ctenophores focusing on *Pleurobrachia* and *Mnemiopsis* as experimental models. We experimentally showed that a significant fraction of both ctenophore genomes is methylated. DNA demethylation is prominent during development, with differentiated both 5-hydroxymethyl cytosine (5-hmC) and its synthetic enzyme TET. Importantly, we identified the presence of RNA editing in ctenophores (Kohn et al, 2015). RNA editing is a process of targeted alterations of nucleotides in all types of RNA molecules (e.g., rRNA, tRNA, mRNA, and miRNA). As a result, the transcriptional output differs from its genomic DNA template. RNA editing can be defined both by biochemical mechanisms and by enzymes that perform these reactions. There are high levels of RNA editing detected in the mammalian nervous system, suggesting that nervous systems use this mechanism to increase protein diversity, because the posttranscription modifications lead to new gene products with novel funcctions. By re-annotating the ctenophore genomes, we found that the number of predicted RNA-editing enzymes is comparable to the numbers in mammals, but much greater than in other non-bilaterian basal metazoans. However, the overall molecular diversity of RNA-editing enzymes in ctenophores is lower, suggesting a possible "compensation" by an expansion of the ADAT1-like subfamily in this lineage. In two genera of ctenophores, Pleurobrachia and Mnemiopsis, there are high levels of expression for RNA-editing enzymes in their aboral organs, the integrative center involved in control of locomotion and geotaxis. This finding supports the hypothesis that RNA editing is correlated with the complexity of tissues and behaviors. Smaller numbers of RNA-editing enzymes in Porifera and Placozoa also correlates with the primary absence of neural and muscular systems in these lineages. In ctenophores, the expansion of the RNA-editing machinery can also provide mechanisms that support the remarkable capacity for regeneration in these animals. In summary, despite their compact genomes, a wide variety of epigenomic mechanisms employed by ctenophores and other non-bilaterian basal metazoans can provide novel insights into the evolutionary origins of biological novelties. Supported by NFS, NASA and NIH grants to LLM

<u>Lulu Leucothea</u> and Bettina Beroë, King Neptune U., Dept of Ctenophorology, AO: Learning and Yearning in Ctenophores

Learning and desirative motivational behaviour are widely believed to be qualities associated with higher animals. However, no studies have been performed on the first purported animals. A previous investigation on associative learning and behaviour of SpongeBob SquarePants (Porifera) using sitting as unconditioned stimulus (US) and chaffing as unconditioned response (UR) with tsunami as neutral stimulus (NS). yielded nada (ZERO, P = 0.000001). Subsequent studies on more than 186 species of Ctenophora from the planet's oceans, using remote submersibles, random BWR samplers, SCUBA diving, drag nets, dredging, FantaSea imaging, ctenodippers, and sunny marine stations (SMS) demon-strate a novel capability for associative learning and focused intentional behaviour in this phylum (J. Plank. Psych., in the press). Employing a newly developed underwater A-maze G-race (Proc. UnR. Sock. Lond. B, in the press) with copepods and/or young fishlings, krill, salt water taffy, or smoked pfeffer-makrele as the US and greedy/gobbling/slurping as the UR, with Hail Brittania played by the sub-Marine Band as the NS, it required only two pairings to achieve rapid and avid learning of conditioned stimulus and response (n = 83,591,752; classical conditioning and associative learning with exceptional motivational intensity (compared to boring sponges). The authors declare no conflicts of interest or pharmaceutical or commercial support. We just want to get back in the water and eat.

<u>Larry Madin</u>, Woods Hole Oceanographic Institution: Diversity and Distribution of Ctenophores in the World Ocean

Pelagic ctenophores are among the most beautiful, delicate and intriguing animals in the ocean. Apparently of very early origin, ctenophores have been a constituent of the pelagic environment for hundreds of millions of years. While now a clearly defined phylum with a distinctive position in metazoan evolution, ctenophores have always been a group of relatively few species, with a seemingly limited distribution in coastal surface waters. Early investigators like Carl Chun in Naples and Alfred Mayer in the Dry Tortugas expanded knowledge of ctenophore diversity with their rowboat-and-dipnet collections, but had no means to explore the open ocean or the deep sea. Relatively common and robust coastal species like *Pleurobrachia*, *Mnemiopsis* and *Beroe* became the textbook ctenophores, while other species remained largely unknown – too remote or fragile to be sampled and only rarely seen. Open ocean SCUBA diving began to change this view in the mid-1970s as divers found ctenophores to be widespread and abundant – for example, Cestum veneris, previously considered exotic – was the most widely distributed species in the North Atlantic. Soon after, explorations of the mesopelagic in submersibles, and later remote vehicles, revealed a whole new world of ctenophore species in the deep ocean, none of which had been collected with nets in the whole prior history of oceanography. My presentation reviews what we now know about the diversity and abundance of ctenophores throughout the world ocean and the opportunities to learn more about their adaptations and functions in the pelagic environment.

<u>Sanna Majaneva</u>1; Aino Hosia2; Steven Haddock3; Jørgen Berge1,4, 1-UIT Arctic University of Norway, Norway; 2-University of Bergen, Norway; 3-Monterey Bay Aquarium Research Centre, USA; 4-University Centre in Svalbard, Norway: Ctenophores – native aliens in Norwegian waters (GooseAlien)

The disproportionally fast warming of the Arctic together with massive reduction of sea ice thickness and extent mean that an ice-free Arctic summer is likely to occur within the next few decades. This, as well as increasing shipping traffic, will pose significant challenges for the Arctic organisms. There is strong evidence that southern species are expanding their distribution ranges northwards and increasing in population size. whereas some of the northerly species are declining. Shifts in biodiversity can directly and indirectly change species interactions and ecosystem processes, resulting in large cascading changes with implications for the entire Arctic ecosystem as well as for ecosystem services. The role of ctenophores in the Arctic and temperate systems is often ignored, although the few existing studies indicate more important ecological role than expected. At the same time, our taxonomical knowledge of the comb jellies in this region remains rudimentary at best. Here, we present preliminary results of an incipient project with the primary goal of describing and documenting the biodiversity of ctenophores in Norwegian waters, from the North Sea to the Arctic. Combining morphological and molecular methods, GooseAlien will increase taxonomic knowledge, which is a valuable first step towards establishing a baseline for future ecological studies, monitoring of climate impacts, and assessing the threat of introduced species in the High Arctic. By engaging the public audience in collecting samples, the project will increase the geographical area coverage and, in return, the laymen involved will come to appreciate completely new aspects of their own environment.

Richard M. Mariita 1, Mohammad J. Hossain 1, 2, Mark R. Liles 1, Anthony G. Moss 1, 1-College of Science and Mathematics, Biological Sciences Department, Auburn University, AL 36849, USA 2-The Department of Biological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, MD 21205-2185, USA: Bacterial microbiota associated with *Mnemiopsis leidyi* and on the discovery of novel *Staphylococcus* species from their stomodeum

Mnemiopsis leidyi is recognized as a driver of ecosystem change and key player in marine and estuarine ecosystems. As planktivorous predator and proven successful invader, M. leidyi plays an important role in marine ecosystem dynamics. Although extensively studied to address ecological and evolutionary questions, its associated microbiota remains poorly understood. The present study used shotgun metagenomic sequencing approach to study the structure, seasonal dynamics and functional capabilities of microbial assemblages associated with the stomodeum of *M. leidyi*. We demonstrate that the composition, structure and functional capabilities of M. leidyi microbiota changes shift seasonally, yet yearlong retains a distinct species (*Propionibacterium acnes*). The results contradict previous studies that suggest that *M. leidyi* does not have a constant microbiota, but supports them on the basis that they have seasonally variant microbial assemblages. Enrichments and isolations using a modified protocol adopted from Anacker and Ordal (1955) reveals novel stomodeum-inhabiting bacteria of Genus Staphylococcus. Characterization of this novel Staphylococcus was achieved through a polyphasic approach. Genus confirmation for the isolate was achieved though fatty acid, polar lipid and respiratory guinone analyses. Identification of species novelty was done using average nucleotide identity analysis, in silico DNA-DNA hybridization and multi-locus sequence analyses using multiple housekeeping genes. In conclusion, our data indicated that the associated microbiota of M. leidvi stomodeum harbors antibiotic resistance genes. Future efforts will involve a multidisciplinary approach investigation of the host-microbe interaction of M. leidyi. Funding: Alabama EPSCoR GRSP fellowship, AU-CMB fellowship, NSF EPS-1158862, USDA-Hatch 370225-310100 (AGM, ML)

Mark Q Martindale, Whitney Lab: Ctenophore regeneration: You CAN get there from here.

Ctenophores are a weird group of animals that show a diverse range of homeostatic properties. Benthic ctenophores divide asexually at will and some, but not all, adult ctenophores show impressive capacities to replace missing parts. Experimental tinkering (hacking them in to pieces) in the lobate *Mnemiopsis* (species whatever) reveals characteristics of their body plan organization, including the existence of "meta stable states" and the integration of apical and peripheral tissues in the regulation of regenerative events. All of this occurs in contrast to the highly determinative embryonic period that apparently all ctenophores display. Manipulations during embryogenesis reveal fundamental features of their homeostatic properties including subtle aspects of "positional information", the absence of a "stem cell" lineage, and the importance of endomesodermal cells to promote regenerative events. Finally, the expression of "germ line" genes suggests the common origin of somatic and germ line stem cellness.

<u>George I Matsumoto</u>, Monterey Bay Aquarium Research Institute: Benthic ctenophores: enigmatic, creeping, successful, and an increasing level of diversity.

Benthic ctenophores are an often overlooked and odd group within this remarkable and diverse Phylum. First reported in 1880 by Kowalevsky (*Coeloplana metschnikowii* - 1880), they have since been found in both shallow and deep waters around the world. The first observation in America (here in Florida) was in 1945 but was actually described from specimens in Jamaica (*Vallicula multiformis* - Rankin 1956). This presentation will provide an overview of the taxonomic groups and discussion of where further research is needed.

<u>Richard O. McCann</u>, Mercer University School of Medicine: Origins of the Metazoa: Functional Constraints and Sequence Conservation in Coevolution of Proteins of The Core Adhesome

The emergence of animal multicellularity was a major transition in evolution. The genome sequences of model unikont organisms representative of the base of the metazoan radiation have revealed that a group of conserved, interacting proteins comprises a core adhesome that was essential for the development of cell adhesion assemblies essential for animal multicellularity. These include integrin, talin, vinculin, α-actinin, and paxillin. As the complexity of these multicomponent adhesion assemblies increased during the metazoan radiation, functional constraints on the structure of the individual protein components of a specific adhesion complex should be reflected in changes in the apparent rate of amino acid substitutions in distinct adhesome components, compared to other proteins that are not part of complex assemblies. Thus, adhesome proteins from late-diverging animals are hypothesized to more similar to one another than those from extant descendants of early-diverging ancestors, which will have had much simpler consensus adhesomes. Comparisons of the molecular clocks of core adhesome components across 1.7 billion years of evolution are consistent with this hypothesis. Sequence similarity of core adhesome protein orthologs correlates directly with the level of adhesome complexity and inversely with divergence time and less adhesome complexity. These results provide a foundation for the study of adhesome protein structural and functional coevolution during the inferred prehistory of animal multicellularity and throughout the metazoan radiation, beginning with the ancestors of extant ctenophores. The approach described here will also be generally applicable to studies of protein coevolution in other multicomponent assemblies in other domains of life.

<u>Claudia E. Mills</u>1; Steven H. D. Haddock2, 1-Friday Harbor Labs, University of Washington; 2-Monterey Bay Aquarium Research Institute (MBARI): A previously unknown deep sea ctenophore from the NE Pacific, with an amusing feeding behavior/strategy

We report here a new genus and species of ctenophore, with a novel and amusing feeding behavior/strategy whereby it captures a new kind of prey. These one-cm ctenophores were not noticed during explorations in mesopelagic Pacific Ocean waters until 2004, but over the last 10-12 years, more than 350 of these colorless or golden ctenophores have been seen from MBARI ROVs off Monterey, California. A small number were also seen off central Oregon and the southern Gulf of California. We have observed only a couple of feeding events in the wild, but we are confident that we have discovered another novel morphology, feeding behavior and prey category in the Ctenophora, with our observations of this little deep-sea animal.

<u>Shannon G Moorhead</u>1; <u>Tayla N Fortson</u>1, 1- University of Miami, Rosenstiel School of Marine and Atmospheric Science: *Vallicula multiformis*: Habitat Niche and Predator-Prey Interactions

This study explored the habitat niche and predator-prey ecology of an urbanized south Florida population of the benthic ctenophore, Vallicula multiformis. Weekly collections of biotic substrates off floating dock slips in a marina were made to quantify and compare *V. multiformis* population densities on a variety of biotic substrates. V. multiformis were most numerous (per liter of substrate) in collections of Delicate Sheep's Wool bryozoan, Amathia distans, and the red macroalga Acanthophora spicifera. The high abundance of ctenophores on the bryozoan may have resulted from increased nutrient availability near the bryozoan's suspension-feeding gastrozooids, and perhaps even a symbiotic relationship between the ctenophores and gastrozooids. Potential predators of V. multiformis were identified from laboratory trials by exposing eighteen potential predators of ten species to two ctenophores simultaneously over a one to two day period. After the initial trials, six likely predators of four species were exposed to potential ctenophore prey in standardized time trials. Only four animals attempted predation upon *V. multiformis*: a decorator crab (Macrocoeloma trispinosum), both anemones (Aiptasia pallida) and the larger of two polychaete worms (Hesione vittigera). Only three animals actually consumed *V. multiformis*, both anemones and the decorator crab, suggesting that the ctenophore may be unpalatable for most species.

<u>Leonid L Moroz</u>, The Whitney Lab for Marine Biosci; St. Augustine, FL 32080; Dept of Neurosci and McKnight Brain Institute, Univ of Florida, Gainesville, FL 32610, USA: Neurobiology and System Biology of Dynamic Ctenophore Genomes: Molecular insights into origins of metazoan innovations, plasticity and diversification of body plans

Here, I will provide an overview of recent findings from our laboratory focusing on three research topics. (1) Reconstruction of early evolution of neuro-muscular systems and synapses – the current studies from more than 40 ctenophore transcriptomes and 4 ctenophore genomes strongly support the earlier hypothesis of independent origins of neural circuits (Moroz, 2009; Moroz et al 2014). (2) Neurogenesis and regeneration – this project led to identifications of genes and molecules controlling terminal differentiation, trans-differentiation and regeneration potential in ctenophores; (3) Genomic bases of diversification and evolution within the phylum Ctenophora - this program includes NASA-/NSF-sponsored voyages with direct genome-scale sequencing aboard of oceanic ships or Ship-Seq. The ongoing Ship-seq approach is critical to study many fragile ctenophore species at remote oceanic locations with advance tools of real-time genomics. Combined with information from other basal metazoan lineages, it is now possible to decipher molecular innovations underlying major body plan transitions which occurred during 550-600 million years of evolution. Collaborative research and data sharing are vital to cost-efficiently use growing genomic resources from many ctenophores for better understanding of numerous innovations in this enigmatic group of animals. Supported by NSF. NIH NASA

Anthony Moss, Department of Biological Sciences, Auburn University: Structure and novel biomineralization of *Mnemiopsis leidyi* and *Beroe ovata* lithocyte concretions (LCs) as revealed by polarization (LC-POL), scanning electron microscopy (SEM) and electron dispersion spectroscopy (EDS)

Ctenophore statoliths are uniquely formed and organized (Curr. Biol. 24:R951; Biol. Bull. 227:7). Here I test whether ctenophore lithocytes are calcareous, and so expected to be sensitive to ocean acidification. Lithocyte concretions were prepared by: 1) microsurgical collection, washed w/0.2 µm filtered sea water (FSW) followed by 5 diH2O rinses in a deep well dish: 2) fixed in 2.5% glutaraldehyde or 1% paraformaldehyde-2.5% glutaraldehyde in 10 mM pH 7.8 HEPESbuffered FSW, and either microsurgically released or 3) released with 50% Chlorox/FSW). LCs were analyzed by EDS at 3 and 10 kVe and viewed for SEM at 20 kVe on a Zeiss Supra 40 VP equipped with INCA (Oxford). LCs examined by LC-POL revealed no birefringence. LCs viewed by SEM were either a lumpy mass (M. leidyi) or smooth ellipsoid (B. ovata). SEM of LCs showed shattered and released overlapping, multi-oriented crystals. EDS of LC crystals from both species collected by any method lacked Ca, Mg and Mn. Predominant elements were Na, O and S in ratios suggesting that LCs contain mixed sodium sulfates. The unique composition of ctenophore statoliths may have been critical for their 600+ million years persistence (J. exp. Biol. 218:592) including survival through multiple global extinctions and related ocean acidifications. Thanks to L. Kerr, R. Oldenbourg, S. Mehta, A. Verma, M. Tran, A. Kuzirian and S. Tamm for stimulating discussion and technical advice. EDS reagent standards were curtesy of L. Amaral-Zettler, J. Huber and K. Gribble (Bay Paul Center/MBL), Funding: AU PIL Program, NSF-EPS-1158862.

Otto M. P. Oliveira, Universidade Federal do ABC, Brazil: Ctenophora taxonomy: past, present and perspectives for the future

Since the first species descriptions, the identity of ctenophore General are the solid base within the group taxonomy. However, supra-generic arrangements (at Family, Order and Class levels) do not reflect the phylogeny of the group and much must be done to refine identifications at the Species level. The first taxonomical ordination, proposed by Eschscholtz in 1827, divided the Phylum in two Classes (Tentaculata and Nuda), based on the presence/absence of tentacles. This arrangement have been used for some authors up to today. An alternative arrangement, creating the classes Cyclocoela and Typhlocoela, based on the presence/absence of the peristomal system of gastral canals, was proposed by Ospovat in 1985. Both classifications disagree with the phylogeny of the group, proposed by Harbison in 1985 and later by Podar and colleagues in 2001. That revels the urgency for the proposal of a new supra-generic arrangement for the Phylum Ctenophora. In the other hand, species diversity seems to be overestimated. The description of new species based on color variations and the presence/absence of pigment spots, instead of other morphological characteristics, were very common in the past, creating a great number of potential synonymys. Color variation can be related the presence/absence of alimentary pigments or even with the association with commensal microorganisms. The molecular identification of species, associated with accurate morphological studies, is the tool we must use to access the identity of the ctenophore species.

Mariana P. Almeida1; Mickey Charteris2; Otto M. P. Oliveira1, 1-Universidade Federal do ABC; 2-Caribbean Reef Life: Ctenophores from the Caribbean Sea of Honduras

Ctenophora is one of the lesser-known invertebrate phyla in the Caribbean Sea and there are no previous records from Honduras. Mayer's study on the taxon, made in the Tortugas Laboratory of the Carnegie Institution of Washington in the beginning of the last century is the closest reference for the faunistic of the area. In this study, we present a catalog of ctenophore species from Roatan Island, Honduras, based on specimens identified through photographic records made by professional divers since 2009. Seven species were photographed alive in the Roatan Marine Park's western edge, located between 16°15'N 86°36'W and 16°20'N 86°34'W. We found Beroe forskalii, Bolinopsis infundibulum, Eurhamphaea vexiligera, Leucothea multicornis, Mnemiopsis leidyi, Ocyropsis crystallina, Ocyropsis maculata. All of these species can also be found in coastal waters along the tropical and sub-tropical coast of the Americas. From the nineteen species registered by Mayer to Tortugas, five are present in our observations. Also Mayer's Leucothea ochracea is probably a synonym of *L. multicornis*. *B. forskalii* was never recorded for the Caribbean Sea before. Based on other records for the tropical Atlantic, we can expect a higher number of species still to be found in the area.

<u>Gustav Paulay</u>, University of Florida: Benthic ctenophore diversity in the Indo-west Pacific

Described marine diversity is around 240K species, yet <10K species are known from the pelagic biome, the largest habitable space on Earth. Ctenophores are generally thought of as pelagic clade, but benthic species, albeit rarely observed and nowhere as abundant as their pelagic kin, are almost as diverse. As for marine life in general, this great benthic diversity even in this primarily pelagic group, reflects on the much greater diversity of habitats, niches, and opportunities for speciation offered by the ocean floor. This presentation will provide an overview of some of the benthic ctenophore diversity I have encountered in the IWP. Benthic ctenophores are common and diverse on most IWP reefs. Species of Coeloplana are epibiotic on diverse sessile organisms, while Benthoplana meteoris and Lobatolampea tetragona live on expanses of soft bottom between reefs. A few species of Coeloplana have conspicuously colored, large bodies and are visible during the day, but most are cryptic and best seen at night when their tentacles are extended and give them away. Macroalgae, octocorals, sea urchins, and sea stars are common hosts. All harbor cryptic species, and at least octocorals and sea stars hold colorful species. Most Coeloplana appear to have a narrow host-range, and several species co-occur on western Pacific reefs. While platyctenes are diverse in reef habitats, Lobatolampea is monotypic and widespread across the IWP. It is a remarkable benthic lobate that retains an ability to swim and broods larvae.

<u>Jason S Presnell</u>1; William E Browne1, 1-University of Miami: Expression and function of Kruppel like factors in *Mnemiopsis leidyi*

Kruppel-like factor (KLF) genes encode transcription factors characterized by a highly-conserved C2H2 zinc finger DNA binding domain. KLFs are involved in various biological processes, namely metabolism, stem cell maintenance, and balancing cell proliferation and differentiation. However, most of what is known about this gene family is restricted to bilaterian model systems. Our previous work characterized KLF complements found in a variety of eukarvotic genomes including KLFs in four non-bilaterian metazoan phyla. We inferred a pattern of gene duplication to account for the expansion of KLFs from the reduced complement observed in non-bilaterians to as many as 23 KLFs in vertebrates. To gain insight into the potential role of non-bilaterian KLFs, we are characterizing the expression and function of KLFs in *Mnemiopsis*. We found three KLF genes in the *Mnemiopsis* genome. MleKLF5a and MleKLF5b group with metazoan KLF5 orthologues, while a third homolog, MleKLFX, is a highly divergent sequence with no clear orthology to other KLF sequences. Our expression results suggest embryonic expression is associated with areas of high cell proliferation, including in the developing pharynx, apical organ, and gastrovascular cavity. In late stage embryos, strong MleKLF5a expression is restricted to endodermal regions of the developing gut. It is known that KLF5 promotes endoderm differentiation through inhibition of mesoderm, and in mammalian systems KLF5 is associated with cell proliferation in the gut. Thus, an early role for KLFs during metazoan diversification could have been in balancing cell proliferation and differentiation during the specification and development of gut endoderm.

1-Susan Rashid, 1-Richard M. Mariita, 1-Anthony G. Moss, 1-Biological Sciences Department, College of Science and Mathematics, Auburn University, AL 36849, USA: Zinc-dependant peptidases could be critical in wound repair in *Mnemiopsis leidyi*: An investigation into the evolution of matrix metalloproteinases

Matrix metalloproteinases (MMPs) are either secreted or membraneanchored peptidases found in animals (J Biol Chem 290(8):4728-40). They have multiple functions, among them wound repair. By time lapse, DIC and oblique illumination microscopy, we demonstrated that in Mnemiopsis leidyi, repair of a standardized 3 mm incision in the outer epithelium occurs, nearly without scarring, in approximately 30 minutes. Repair is blocked by addition of millimolar EDTA in Ca-reduced artificial sea water, hinting at MMP involvement in the wound repair process. We targeted membrane-associated proteinases, gelatinases and proteases involved in broad ECM-related breakdown. Ten species representing evolutionarily divergent organisms (M. leidyi, Ambystoma mexicanum, Ciona intestinalis, Drosophila melanogaster, Homo sapiens, Hydra vulgaris, Nematostella vectensis, Rattus norvegicus, Schmidtea mediterranea and Strongylocentrotus purpuratus) were used for the analysis. Genome mining provided insight into the evolution and phylogeny of MMPs. Gene sequences for the different classes of MMPs were concatenated separately and used for Multilocus Sequence Analysis (MLSA). Based on JTT-corrected matrix-based models, our analysis revealed distinct evolutionary trajectories in the different classes of MMPs. Despite this, the characteristic Zn-dependant peptidase region was found consistently in each class, likely highlighting its critical regulatory activity in wound repair. Our results are in agreement with previous work (Wound repair regen. 15:2) that indicated that zinc is an important co-factor in wound repair. Further molecular analysis is underway to investigate the diversity of MMPs and healing responses in *M. leidyi* under treatments that vary divalent cations.

<u>Thomas Reese</u> and Carolyn Smith, NINDS, National Institutes of health: *Trichoplax* feed on algae, but lack a mouth, gut, or synapses

Trichoplax adhaerens is the sole named member of the Placozoa, an ancient metazoan phylum. This coin shaped animal glides on ventral cilia to find and digest algae on the substrate. It has six cell types, all but two incorporated into the epithelium that encloses it. The upper epithelium is thin, composed of a single cell type. The lower epithelium is thick and composed primarily of ciliated cells that power locomotion. Interspersed among these cells are two types of secretory cell: one containing large lipophilic granules that, when released, lyse algae under the animal; the other, less frequent, is replete with smaller secretory granules containing neuropeptides. Cells in the epithelium are joined by belts of adherens junctions that hold cells in place and limit the passage of large molecules across the epithelium. Feeding behavior is more complex that might be expected from the simple construction. When a Trichoplax encounters a cluster of algae cilia cease beating and the animal becomes immobile, then lipophil cells in the vicinity of the algae secrete their large ventral granules, rapidly followed by lysis of the algae. The fluorescent material released from the lysed algae disappears over the course of minutes, presumably due in part to uptake by endocytosis by epithelial cells, as very little diffused out from under the *Trichoplax*. Once most of the algal material has disappeared, the animal begins to move again. Neurosecretory cells, which contain peptides such as endomorphin, may have a role in coordinating the cells during feeding.

Fernando Ruiz-Escobar1; Diana Karen Valadez-Vargas1; Otto M.P. Oliveira2, 1-Universidad del Mar, campus Puerto Ángel, Oaxaca, México; 2-Universidade Federal do ABC, São Bernardo do Campo, SP, Brasil: Ctenophores from the Oaxaca coast, with an overview of studies in the Mexican Pacific

Ctenophora is one of the lesser-known invertebrate phyla in the Tropical Eastern Pacific. Studies in the region are scarce, with the most notorious work on Ctenophora systematics dating back more than a hundred vears. Records of ctenophores in the Mexican Pacific have been mainly provided from the Gulf of California, while in the Oaxaca coast, their occurrence has only been briefly mentioned at the genus level. With this work, we provide the first records of ctenophores from the Oaxaca coast. Near-surface specimens were collected at five localities of Oaxaca, using homemade nets, photographed inside an aquarium, fixed in 1% trichloroacetic acid and preserved in 1% formalin. Identifications were done using a key to the species of the Northeastern Pacific. Three species were identified: Beroe forskalii, Bolinopsis vitrea and Ocyropsis maculata. Preservation of *Bolinopsis vitrea* was unsuccessful, thus, we only provide photographic evidence of its record. Voucher specimens of the other two species were deposited in the scientific collection of the Laboratorio de Sistemática de Invertebrados Marinos of Universidad del Mar. campus Puerto Ángel, Oaxaca. With this work, the number of species known from the Mexican Pacific increases to eight, highlighting the poor state of knowledge of the phylum in the region.

<u>Joseph F. Ryan</u>1,2; Marta Chiodin1,2, 1-Whitney Laboratory for Marine Bioscience; 2-Department of Biology, University of Florida: There goes my neuron: How sponges and placozoans may have lost neural cell types

Recent phylogenomic evidence suggests that ctenophores may be the sister group to the rest of animals. This phylogenetic arrangement opens the possibility that sponges and placozoans could have lost neural cell types or that the ctenophore nervous system evolved independently. We critically review evidence to date that has been put forth in support of independent evolution of neural cell types in ctenophores. We observe a reluctance in the literature to consider a lost nervous system in sponges and placozoans and suggest that this may be due to historical bias and the commonly misconstrued concept of animal complexity. In support of the idea of loss (or modification beyond recognition), we provide hypothetical scenarios to show how sponges and placozoans may have benefitted from the loss and/or modification of their neural cell types.

Rachel Sanford, Andrea Kohn, Leonid L Moroz, The Whitney Lab for Marine Biosci; St. Augustine, FL 32080; Dept of Neurosci and McKnight Brain Institute, Univ of Florida, Gainesville, FL 32610, USA: Genomic Basis of Regeneration in the Ctenophore, *Mnemiopsis leidyi*.

The amazing ability of regeneration in ctenophores has captured the interest of biologists for centuries. The morphological features of ctenophore regeneration have been documented, but the molecular and cellular components behind this phenomenon have remained a mystery. With this study we have performed RNA-seq analyses of the regeneration dynamics using next generation sequencing technologies. The data show evidence for the involvement of several dozen of evolutionarily conserved and ctenophore-specific signal transduction pathways in the regeneration in *Mnemiopsis* including novel secretory peptide candidates, their receptors, components of downstream Ca2+-dependent and MAP-kinase cascades as well as energetic and cell adhesion components. In addition, we identified a unique subset of transcription factors involved in the regeneration including homeobox, forkhead, transcriptional enhancers and inhibitors. Our data on several related ctenophore species suggest that many of these transcription factors are upstream regulators of the pathways involved in regeneration. Combined experimental and comparative allowed us identity candidate molecules involved in activation and repression of the regeneration in ctenophores. In summary, the identification of hundreds of ctenophore-specific genes and molecules associated with regeneration illuminate novel evolutionary mechanisms and strategies underlying morphogenesis and origins of evolutionary innovations in Metazoa, Supported by NSF, NIH, NASA grants to LLM

<u>Christian Sardet</u>, CNRS, Biodev Lab, Observatoire Océanologique, Villefranche sur Mer: From Wandering Nuclei in *Beroe* to Tara Oceans expedition

25 years ago intrigued by the Chun's observations on Beroe embryos in Naples (1880) and the experiments of Gary Freeman on *Mnemiopsis*, my colleagues and I decided to reinvestigate fertilization and "mosaic development" in the large eggs (1.5 mmm) of Beroe ovata a species which was then abundant in the bay of Villefranche sur mer in the spring. We discovered the amazing "intracellular behavior" of female nuclei probing different male pronuclei before fusing with one. We further showed that the site of pronuclear fusion determined the oral aboral axis via a cell-cycle driven wave (see 1,2, 3). Although Beroe is a unique and exceptional model in which the establishment of a unique primary axis can be investigated we stopped working on it because it became increasingly difficult to collect this species concentrating instead on our developmental studies of ascidians (Phallusia, Ciona) and jellyfish (Clvtia). I kept a keen interest in Ctenophores though and when we organized a global survey of plankton in all oceans (Tara Oceans expedition 2009-2013) with my son Noe we made a film about these wonderful creatures part of our popular « Plankton Chronicles » site (4). Much remained to be done to study the abundance and biodiversity of Ctenophores and exploit the extraordinary genomic resources made available by the expedition (5).

- 1) Rouvière et al. (1994) Cell Mot. & Cytoskel. 29, 301-311
- 2) Houliston, E. et al.(1993) Development. 117, 75-87
- 3) Carré, D. et al. (1991) Dev. Biol. 147, 381-391
- 4) http://planktonchronicles.org/en/episode/iridescent-ctenophores/
- 5) http://www.embl.de/tara-oceans/start See Publications link

<u>Daniel Sasson</u> 1; Joseph Ryan 1, 1-University of Florida: Ctene-age wasteland: the influence of light, body size, and self-fertilization on the reproductive output of the sea walnut, *Mnemiopsis leidyi*

Interest in the ctenophore *Mnemiopsis leidyi* has rapidly increased due its use as a model system to study animal evolution and development and its impact on fisheries in introduced waters. With this growing interest comes the need to understand the reproductive ecology of M. leidyi. In this study, we assess the influence of light cues on spawning, the effect of body size on spawning, and the cost of self-fertilization on offspring viability in M. leidyi. Our attempts to spawn M. leidyi using existing protocols had little success. Instead, our results suggest that M. *leidyi* spawning is more strongly influenced by circadian rhythms than by specific light cues. Additionally, we found that body size positively correlates with both spawning likelihood and reproductive output. Finally, we tested whether self-fertilization is costly by comparing offspring viability in *M. leidyi* that spawned alone to those that spawned in pairs. We found significantly lower viability after 24 hours in embryos from selfing individuals, suggesting that self-fertilization may lead to inbreeding depression. Such a cost to self-fertilization may be especially impactful for *Mnemiopsis* populations expanding into new geographic areas that would likely be more reliant on self-fertilization. Surprisingly, we also found that *M. leidyi* in pairs did not spawn more eggs than *M. leidyi* that spawned in isolation, suggesting that *Mnemiopsis* reproductive behavior may be more complex than often assumed. These results provide insight into the reproductive ecology of *M. leidyi* and should be a useful resource to researchers working with these animals in the laboratory.

Christine E. Schnitzler1; Warren R. Francis2; Steven R. Bond1; Anh-Dao Nguyen1; David K. Simmons3; Steven H.D. Haddock4; Mark Q. Martindale3; Joseph F. Ryan3; Andreas D. Baxevanis1, 1-National Human Genome Research Institute, National Institutes of Health; 2-Ludwig-Maximilians University; 3-Whitney Laboratory for Marine Bioscience, University of Florida; 4-Monterey Bay Aquarium Research Institute: Evidence of absence in ctenophore genomes and its impact on evolutionary thinking

The sequencing and public release of several ctenophore genomes in recent years has provided a new framework for exploring the genetic and genomic underpinnings of this poorly studied and enigmatic group of non-bilaterian animals. Having access to whole-genome and transcriptome sequences enables orthology prediction, phylogenetic analyses, studies of gene gain and loss, and identification of lineage-specific genes - all of which are being used to address evolutionary questions that ctenophores are uniquely poised to answer. Unfortunately, the level of 'completeness' of the assemblies and accuracy of the predicted ctenophore gene models may negatively influence the outcome of comparative studies. With this in mind, we have now compared the two publicly available ctenophore genomes (from Mnemiopsis leidyi and Pleurobrachia bachei) and an additional unpublished genome (from Beroe ovata), together with RNAseg-based transcriptomes from each species. CEGMA and BUSCO were used to assess 'completeness' of the assemblies and the gene models predicted from these genomes. Clusters of orthologous genes were then predicted from 37 taxa, including 34 metazoans and three outgroup species using OrthoMCL; the resulting distribution of 'missing' orthologs in each ctenophore species was examined. Examples of manual improvements to ctenophore gene models will be presented. along with an updated assessment of ctenophore-specific genes and putative gene losses in the ctenophore lineage. The impact of the current state of ctenophore genomic data on our ability to draw conclusions about evolutionary questions – particularly discussions of gene absence or loss - should not be overlooked, and will be discussed in light of our current results.

<u>Simmons DK</u>, Martindale MQ, University of Florida: The general structure and development of the ctenophore nervous system

Ctenophores, regardless of their contentious phylogenetic position with respect to sponges, can confidently be regarded as the earliest extant animal with true neurons. Genomes of two ctenophore species, *Mnemi*opsis leidyi and Pleurobrachia bachei are now publicly available and have shed light on the genomic complement of homologues to bilaterian neural genes present in these two representatives of the phylum ctenophora. Ctenophores share a more similar bilaterian neural gene content to that of the sponges (which possess no neurons), than to placozoans and chidarians. As such, many questions regarding the ctenophore nervous system remain unanswered and poorly researched using modern molecular biology techniques. Using the single celled transcriptome technique CEL-Seq, we have produced ½ hour (during gastrulation) to hourly interval developmental transcriptomes of two species of ctenophores, Mnemiopsis leidyi and Beroe ovata fl. This data has allowed us to track the developmental expression and timing of potential neural patterning genes from the time of cleavage to early cydippid stages (0 to 20 hours postfertilization). Combined with evidence from cell lineage experiments showing the developmental origin of neural tissue types and Immunohistochemical staining of developmental stages clearly showing the presence of classically described neural cell types. This research provides much needed insight into the timing and development of the ctenophore nervous system.

George D Stanley Jr, The University of Montana Paleontology Center: A Review of the Fossil Record of Ctenophora: New Discoveries and Problems

Ctenophores are represented in the fossil record but their gelatinous nature and fragility make it difficult to understand their occurrence as fossils. Fossil ctenophores come from unusual deposits where soft tissues are preserved. Examples from the Devonian Hunsrück Slate of Germany (408-400 Ma) reveals comb rows and tissues. The Middle Cambrian Burgess Shale of B.C. Canada (505 Ma) reveal taxa of unconventional morphologies, difficult to reconcile with Ctenophora. Older material of Early Cambrian age comes from the Chengjiang biota (520 Ma) of Yunnan, China. Tiny phosphatized fossils from the Early Cambrian Meishucun assemblage (540 Ma). Shaanxi, China, vielded late-stage ctenophore embryos and Raman spectroscopy revealed eight comb rows and an absence of tentacles. The hypothesis could be presented that these Lower Cambrian Chengjiang fossils represent a stem group of Ctenophora. While some of the Chengjiang material may represent the remains of ctenophores. other material does not. Accepted ctenophoran fossils fall into several taxa exhibiting eight comb rows, tentaculated and non-tentaculated forms and possible tentacle sheaths. Soft tissue preserved in one specimen may represent former digestive organs. These ancient ctenophores do not represent a stem group of the phylum. Despite their Early Cambrian age, some are surprisingly modern and likely exploited pelagic life styles comparable to living taxa. The alleged Middle Cambrian Burgess Shale specimens were not considered in the comparison because features of their anatomy suggested unknown groups of metazoans unrelated to ctenophores. Some fossils reported as ctenophores probably are cases of mistaken identity, compounded by taphonomic change and the vagaries of preservation. Comb rows illustrated among Chengijang fossils require further study and more detailed comparisons with living taxa. Some resemble living taxa but others, examples like Gemmactena, Thaumactena and Batofaciculus have presumably rigid and bizarre angular plates, struts and spoke-like structures unlike any known ctenophore. Reports that Chengjiang ctenophores evolved skeletonized or "armored" comb rows need to be reexamined because almost identical structures show up in unrelated Cambrian cnidarians. Alleged "Comb rows" in other Chengliang taxa, like the unusual Stromato*veris*, do not hold up to scrutiny. The ctenophore stem group may extend further back in deep time, possibly to the Early Precambrian where their fossils have yet to be discovered. The Precambrian (Ediacaran) rooted Rangea and the spiral armed Eoandromeda, both dubiously attributed to the Ctenophora, belong instead to unrelated organisms. Before any sense can be made from these fossils about ctenophore phylogeny an appreciation is needed for the role of taphonomy during preservation of soft tissues. Careful scrutiny also is needed by ctenophore biologists.

S. L. Tamm, MBL: The statocyst and control of geotaxis in ctenophores

The ctenophore statocyst is bilaterally symmetric in the sagittal and tentacular planes about the oral-aboral axis. The four mechanoresponsive ciliary balancers are arranged in a rectangle parallel to the tentacular plane, and support a superellipsoidal statolith with its major axis in the same plane. Refractile lithocytes arise on opposite sides of the epithelial floor along the tentacular plane, migrate apically, and bud off next to balancers which transport them distally by ciliary surface motility to form the statolith. The non-radial symmetry of the statocyst has functional consequences during the righting response of ctenophores to gravity. Cteno-tilting of negatively geotactic animals at orthogonal orientations to gravity shows that the activated lowermost comb rows beat faster when an animal is tilted in the sagittal plane than in the tentacular plane. Micro-tilting of isolated statocysts shows that the amount of downward displacement of the statolith and the degree of deflection of the lowermost balancers by the statolith are greater in the sagittal plane, explaining the observed higher beat frequency of comb rows in this plane. A hardware model demonstrates that the matching geometric asymmetry of load and supporting elements accounts for the differences in mechanical and motile outputs observed between tilting ctenophores and isolated statocysts in sagittal vs. tentacular planes. Important questions remain: is the righting response of free animals in the sea stronger in the sagittal than in the tentacular plane? If so, is this behavior advantageous for ctenophores? How?

<u>James Townsend</u>1; Alison Sweeney1, 1-University of Pennsylvania: The Slimes That Bind: Physiology and Biochemistry of *Mnemiopsis* Mesoglea

Ctenophores are principally composed of a gelatinous tissue known as the mesoglea which is thought to be derived from the extracellular matrix of the enclosing epithelium. Despite being 10~1000 times more voluminous than the epithelium, the mesoglea is presently one of the least understood features of ctenophore physiology. Ctenophores are approximately 95% water and 4% salt by weight. The remaining 1% of biomass accounts for all of the animal's physical features, including the mesoglea. Furthermore, mesogleal tissue is largely acellular, with cells making up approximately 1% of the tissue's volume in larvae. Yet, despite the apparent sparsity of this tissue, mesoglea is about as stiff as mammalian brain tissue and plays an active role in feeding and metabolism. We posit that this apparently featureless hydrogel is anything but, and present a variety of physical, biochemical, and anatomic data on the mesoglea of Mnemiopsis leidyi in support of this notion. Our investigation so far combines novel physical measurements, such as calorimetry, rheometry, and MRI, of mesoglea in adult *Mnemiopsis* with a detailed study of the microscopic anatomy of their larvae. The resulting picture is one in which mesoglea, while still mysterious, appears to be markedly physiochemically distinct from other metazoan tissues.

<u>Lauren E. Vandepas</u>1; Kaitlin J. Warren2; Chris T. Amemiya1,3; William E. Browne2, 1-University of Washington; 2-University of Miami; 3-Benaroya Research Institute: Ctenophore cell culture preparation - methods and potential applications

In light of recent molecular studies suggesting that Ctenophora, one of the most ancient animal lineages, may be sister to the rest of Metazoa, ctenophores have garnered increasing attention as a critical taxon for understanding the early evolution of animals. While molecular techniques continue to be developed and optimized in ctenophores, cell culture has not yet been widely employed despite the pivotal importance of cell culture-based in vitro studies for addressing problems among the major developmental model systems. We have improved upon classic techniques for the isolation and maintenance of multiple cell types from primary cell cultures of the lobate ctenophore *Mnemiopsis leidyi*. These cells can then be utilized for a wide variety of studies that do not require whole animal assays. With our innovative methods, we have enhanced current studies and widened possibilities for future cellular-level research on ctenophores.

ABSTRACTS (cont.)

<u>Kaitlyn Warren</u>1; William Browne1, The University of Miami: Characterization of endodermal and muscle cells in Mnemiopsis leidyi primary cell cultures

We have developed a primary cell culture system that can be utilized to isolate adult somatic cell types from *Mnemiopsis leidyi*, a lobate ctenophore. Our primary cell cultures are derived from tissue explants and maintained in a complex undefined media. Approximately 24 hours after explant removal, cultures are screened for the presence of desired cell types. These primary cell cultures can be reliably maintained and visually monitored for a variety of parameters including proliferation, changes in cell morphology and/or differentiation. Exemplar cell types that are easily isolated from primary cultures include cells derived from endoderm containing large pigmented vacuoles and giant smooth muscle cells exhibiting inducible contractile properties. In parallel we have also derived 'tissue envelopes' which contain sections of endodermal canal that are used to monitor targeted cell types in an in vivo context. Experiments further characterizing these primary cell cultures will facilitate the analysis of ctenophore development from a cell biological perspective.

ABSTRACTS (cont.)

Nathan V. Whelan, Auburn University: Body plan and lifestyle evolution in Ctenophora

Relationships among non-bilaterian phyla have received much attention in recent years, but relationships among major ctenophore lineages are still poorly understood. A robust phylogenetic hypothesis of ctenophore relationships is needed to better understand morphological evolution within ctenophora. Past studies suggest that some traditional, morphology based groups (e.g. Cydippida, Lobata) are not monophyletic, but relationships among many lineages have yet to be resolved. Past molecular studies also conflicted with morphology-based hypotheses, especially in regards to the placement of species lacking tentacles. Robustly resolving relationships among ctenophore lineages will be essential for understanding the evolution of ctenophore body plans and general metazoan characteristics such as muscles, neurons, and benthic versus pelagic lifestyles. We used Illumina and Ion-Proton platforms to sequence 26 new ctenophore transcriptomes and combined these with eleven publicly available ctenophore transcriptomes to assemble a phylogenomic dataset of ctenophores representing most major lineages. Maximum likelihood analyses on a partitioned dataset using RAxML recovered a well-supported phylogeny. In agreement with recent phylogenetic hypotheses, two higher-level Ctenophora groupings, Lobata and Cydippida, were recovered non-monophyletic. Bayesian ancestral state reconstruction suggests that the ancestor to extant ctenophores was pelagic and likely had a Cydippida-like morphology. Ancestral state reconstruction also supports a single loss of tentacles, but a benthic, or semi-benthic, lifestyle appears to have evolved at least twice within Ctenophora. The transcriptomic-based approach used here has resulted in a robust picture of ctenophore relationships, and our findings suggest that many different morphologies have evolved from pelagic, Cydippidalike ancestors.

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Sebastian Beroë's RULES for a happy life ~

Drift dreamily together.

Avoid propellors.

Know up from down.

Coordinate your activities.

Eat while the sun shines.

Gulp your food.

Brush your macrocilia.

Sleep in the deep.

Yell in the swell.

Don't cry in the sea.

