Abstracts of Contributions Presented at EuroCeph 2011 "Cephalopod Biology Research in the 21st Century—A European Perspective"

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ABSTRACTS OF CONTRIBUTIONS

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"Cephalopod Biology Research in the 21st Century—A European Perspective"

Vico Equense, Napoli, Italy

April 7–10, 2011

Abstracts published in the following pages have been contributed to EuroCeph 2011 meeting. We thank the Editor in Chief, Sandra Shumway for her enthusiastic support and the Editorial Board and Publisher of *Journal of Shellfish Research*. We thank EuroCeph Program Committee and Local Organizers: Paul Andrews, Ludovic Dickel, Graziano Fiorito, Giovanna Ponte and Nadav Shashar.
This publication is funded by the non-profit association CephRes-ONLUS (www.cephres.org) aimed to support the advancement of biological research with particular reference to cephalopods and to facilitate this animal model in scientific multidisciplinary research.

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OVERVIEW, EUROCEPH 2011. Graziano Fiorito. Comitato Studi EuroCeph, Napoli, Italy; euroceph@gmail.org; CephRes-ONLUS, via dei Fiorentini 21, 80133 Napoli, Italy.

E-mail: info@cephres.org

EuroCeph 2011 meeting (Cephalopod Biology Research in the 21st century—"A European perspective") was held April 7–10, 2011 in Vico Equense, Napoli, Italy. There were 87 attendees from 18 different countries. The meeting consisted of thirty-two oral and thirty-six poster presentations. In addition, two special sessions were arranged: *i.* Saturday morning session to review on the "Revision of EU Directive 86/609: implications for cephalopod research in the EU", and *ii.* Sunday morning session to discuss "The Top 10 questions in cephalopod biology research".

In the last 100 years research in cephalopod biology, particularly octopus, squid and cuttlefish has made major contributions to several areas of science and neuroscience in particular. Cephalopod biology research is still very active in a number of countries.

The goal of EuroCeph 2011 meeting was to critically review current cephalopod research and specifically examine the potential of cephalopods as "model animals" to address a range of research questions from molecular neuroscience to ecology. The timing of the meeting was prompted by the revision to EU Directive 86/609 which will bring all cephalopods (adults and immature forms) within the legislative framework currently covering research using vertebrates. This legislation comes into force in January 2013.

With presentations ranging from basic neuroscience, genomics and systems biology to the biological basis of personality and consciousness, by way of robots and new materials, Euroceph in Vico Equense was a compelling and intriguing meeting. The great diversity of approaches managed to send a unified message and to show convincing evidence that cephalopods are not only fundamental model animals for physiology, but can in fact offer a common ground for researchers from different - even distant – fields.

At the same time, the meeting clearly highlighted some major challenges that the researchers will have to face in order to really exploit the great potential these organisms offer to science: in particular, the need to bring cephalopod research fully into the genomic era, and the need to share tools and protocols, and to create infrastructures that can support researchers in accessing these animals and working with them. The way these challenges will be addressed in the future will determine the development of this field of research.

A number of early career scientists and students were awarded for their contributions to the meeting; selection was made by the Nobel Laureate Torsten Wiesel and EuroCeph Program Committee.

The meeting was organized by Paul Andrews, Ludovic Dickel, Graziano Fiorito, Giovanna Ponte and Nadav Shashar. Financial assistance was also provided by Stazione Zoologica Anton Dohrn (Italy) and several sponsors and donations.

PRACTICAL IMPLICATIONS FOR CEPHALOPOD RESEARCHERS IN THE IMPLEMENTATION OF DIRECTIVE 2010/63/EU. P. L. R. Andrews. Division of Biomedical Sciences, St George's University of London, Cranmer Terrace, London, SW17 0RE, UK.

E-mail: pandrews@sgul.ac.uk

The inclusion of "all living cephalopods (adult and larval forms)" in the revised European Directive "on the protection of animals used for scientific purposes" which comes into force on 1st January 2013 has a number of practical implications for those undertaking research. These cover broad areas ranging from the welfare of the animals and the conduct/design of experiments to the likely expectation of Journals to require statements relating to compliance with the directive and more detailed descriptions of housing, husbandry and experimental protocols (e.g. ARRIVE type guidelines; Kilkenny. et al., 2010. PLoS Biology 8: 1-5).

This talk will focus on a number of specific issues and is based around issues discussed by a working party of the Boyd Group (http://boydgroup.wordpress.com/) in the UK with the aim of producing a response to a Home Office (the UK regulator, http://www.homeoffice.gov. uk/science-research/animal-research/) consultation on the implementation of the Directive. Implementation will require the development of guidelines covering a number of areas and this talk will focus on a few of these to illustrate some of the issues: Supply of animals; housing and handling; euthanasia; anaesthesia; Criteria for recognising pain, suffering and distress and application of humane end-points. It is clear that the next 18 months will require a considerable research effort to provide the evidence to support guideline development for the more commonly studied laboratory species (e.g. *Octopus vulgaris*, *Sepia officinalis*, *Loligo pealeii*, *Nautilus pompilius*).

A PRELIMINARY INVESTIGATION INTO HABITAT MODELLING OF COMMON CUTTLEFISH (*Sepia officinalis*) SPAWNING GROUNDS IN THE ENGLISH CHANNEL, USING PRESENCE ONLY DATA. I. Bloor, E. Jackson, C. Marshall. Marine Biological Association, The laboratory, Citadel Hill, Plymouth, Devon, PL1 2PB, UK and Marine Institute, University of Plymouth, Portland Square, Plymouth, Devon, Pl4 8AA, UK.

E-mail: isblor@mba.ac.uk

The common cuttlefish (*Sepia officinalis*) is an important commercial species in the English Channel. In spring, sexually mature adult cuttlefish concentrate in shallow coastal areas on both sides of the Channel to reproduce and spawn. These temporal aggregations are targeted by coastal metiers (e.g. pots and nets) within the commercial fishery. Whilst it is possible to identify broad scale spawning grounds from the location of these associated metiers, predictive habitat modelling will allow the locations of spawning grounds to be identified (predicted) independently and at a finer resolution. Information on the spatial and temporal

distribution of spawning grounds is essential for both stock inference and good fisheries management, especially for stocks such as *S. officinalis* whose reproductive capacity may be hampered through targeted exploitation of spawning adults and the additional loss of eggs from fishing traps. The use of habitat modelling is becoming widespread in the field of marine ecology and may provide a useful method to map potential spawning habitats, namely areas where environmental conditions are suitable for spawning to occur, for cephalopods. The aim of this study is to develop a species distribution model for *S. officinalis* allowing the predictive mapping of areas of suitable (potential) habitat for egg deposition along the UK coastline of the English Channel during the spawning season. In this present study a maximum entropy (Maxent) modelling approach is used to develop a model that relates key environmental variables (e.g. bathymetry, water temperature, salinity, wave exposure and sediment type) to occurrence data of spawning adults. The habitat model can be applied over several years to investigate whether inter-annual variability of the potential spawning habitat is detectable. Habitat-suitability maps of potential spawning habitats are generated and the model's performance evaluated using both internal (e.g. area under the curve AUC analysis) and external methods of validation (e.g. literature comparisons and direct observations). Knowledge of the spatial and temporal distribution of stocks is essential in designing strategies for fisheries management. The spatial analysis of suitable habitat from presence-only data using Maxent facilitates the identification of areas of spawning grounds in the English Channel. It is anticipated that accurate models and predictions of the distribution of spawning cuttlefish and or eggs will provide essential information for future management recommendations and stock monitoring.

***Sepia officinalis*: A NEW MODEL FOR ECO-EVO-DEVO STUDIES.** **A. Buresi[1], D. Franko[1,2], A. Andouche[1], Y. Bassaglia[1,3], S. Baratte[1,4], L. Bonnaud[1,2].** [1]Muséum National d'Histoire Naturelle, DMPA, Lab. BOREA. UMR MNHN CNRS 7208-IRD 207-UPMC, Paris, France; [2]Univ Paris Diderot, F-75013 Sorbonne Paris Cité, Paris, France; [3]Univ. Paris Est Créteil - Paris 12, Creteil, France; [4]Univ. Paris Sorbonne - Paris 04, F-75004 Paris, France.

E-mail: bonnaud@mnhn.fr

Lophotrochozoan models are crucial to elaborate hypotheses on body plan evolution at morphological and molecular levels. *Sepia officinalis* has been chosen for its particularities that are worth being better explored in an adaptive perspective: they share with other cephalopods but unlike other molluscs a direct development, a nervous system allowing cognitive processes, a brachial crown and funnel (derived from the foot). In addition, they show a complex body colored patterning and an internal calcareous shell. These characteristics confer an adaptive advantage and have been selected during evolution for its necto-benthic mode of life. They adopt immediately after hatching the same mode of life than adult. We study by a developmental approach the structural mechanistic and environmental relationships. Our aim is to characterize in an evolutionary perspective the molecular/biochemical pathways involved in the structures selected to escape predators and allowing and/or confering adaptive advantages. To reach that purpose we study 1) the spatio-temporal organisation of the body that takes place by a direct development 2) the central and peripheral nervous systems (NS), derived structures analogous to vertebrates and the associated muscular system, 3) the body colored patterns and the visual structures, linked to the neural network responsible of the complex behaviour. Several molecular methods are applied on the cuttlefish embryo, from the beginning of organogenesis to hatching. Thanks to ESTs database (CEA/Genoscope), expression coding morphogens, transcription factors, regulatory and structural molecules have been explored during the development (*Pax* family, *NK, shh, engrailed…*). Although the general features of these genes studied are conserved within metazoans, their role during the development of *S. officinalis* is different from that known in other species. Most studied genes show extended or restricted expression in the developing morphological novelties, arms, funnel, suckers, eyes, optic lobes, in the setting up and the orientation of the NS. Some other genes show unexpected expression in muscular structures. We show that the nervous system is differentiated and the ability to perceive the environment and regulate is in place very early before the brain is constituted. These results allow to gain insights into the molecular control of *Sepia* development and to enlighten the molecular pathway particularities of the cephalopod lineage. They underline the necessity to have a better evolutionary understanding of genetic structure/function relationships. Genome sequencing on cephalopod that possesses the highest genome among molluscs happens crucial for comparative genomics in lophotrochozoan lineages. *S. officinalis* being an ideally suitable eco-evo-devo model and because the ESTs data are increasing we propose to sequence its genome, the first one in cephalopod group.

EVALUATING AGE IN THE CEPHALOPOD MOLLUSC *Octopus vulgaris*: ANALYSIS OF GROWTH INCREMENTS IN THE UPPER BEAKS. **E. Canali[1], G. Ponte[1], P. Belcari[2], F. Rocha[3], G. Fiorito[1].** [1]Octopus Behavioral Biology, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy; [2]Dipartimento Scienze Uomo & Ambiente, University of Pisa, 56126 Pisa, Italy and Department of Physics and Astronomy and London Centre for Nanotechnology, University College London Gower Street, London WC1E 6BT, UK; [3]Department of Ecology and Animal Biology, University of Vigo, Campus of Lagoas-Marcosende, 36310 Vigo, Spain.

E-mail: giovanna.ponte@szn.it

To estimate age in *Octopus vulgaris*, we analyzed growth increments (rings) in the upper beaks of wild-caught specimens.

First, we report results of thermal marking experiments aimed to validate daily periodicity of deposition of rings in adult specimens; this approach was applied to *O. vulgaris* for the first time. Marking event induced a band on the ring corresponding to the day of thermal shock. We positively correlated the number of growth increments counted from the posterior edge to the darker ring with the days elapsed between the thermal shock event and the day of the sacrifice of the animals. Our results confirmed the hypothesis that one ring is produced in one day.

In addition, rings were counted on more than 700 specimens of *O. vulgaris* fished between 2002 and 2009 in the Bay of Napoli (Italy, Mediterranean Sea). Age of our specimens resulted to range between two to twelve months of life. The dataset provided a low correspondence between body-size and number of rings; in fact animals belonging to a broad range of body size resulted to have similar number of rings in the upper beak. This confirms the view that body size is a not a good index of age. The relationship between body size and number of growth increments resulted to be affected by season, thus confirming previous studies that suggest the coexistence of different octopus' cohorts in the Bay of Napoli and that temperature influence growth. Furthermore, in our dataset, distance between rings was clearly correlated to temperature oscillations due to seasonal effects.

CAN CUTTLEFISH USE POLARIZATION SENSITIVITY FOR ORIENTATION? L. Cartron[1,2], A.-S. Darmaillacq[1,2], C. Jozet-Alves[1,2], N. Shashar[3], L. Dickel[1,2]. [1]Univ Caen Basse Normandie, Grp Memoire & Plast Comportementale, EA4259, F-14032 Caen, France; [2]Centre de Recherches en Environnement Côtier, 54 rue du Dr. Charcot, 14530 Luc-sur-Mer, France; [3]Marine Sensory Ecology Research Center, Eilat Campus, Ben Gurion University, Israel.

E-mail: lelia.cartron@unicaen.fr

It is now well established that insects and birds can use sky polarization pattern in parallel of other reference cues to navigate but little is known on the use of underwater polarization pattern in aquatic animals. Cephalopods have been shown to have polarization sensitivity and to use it for predation and possibly for intraspecific communication. Here, we examined whether cuttlefish were able to find a shelter using the e-vector of a linearly polarized light in parallel of visual landmarks and then whether one of these cues was preferentially used when they gave contradictory directions. Experiments were conducted in 3 steps with eleven 7-month-old cuttlefish *Sepia officinalis* reared in captivity from hatching. In step 1, cuttlefish were trained to find a rewarded dark compartment at the end of the arm of a Y-maze which was illuminated with a linear polarized light (e-vector oriented 45 or 135°/starting position). In addition, two contrasted landmarks were placed at the entering of each maze arm which gave redundant information. They were given 5 trials per day in which the side of the rewarded compartment was randomly assigned. All the cuttlefish reached the learning criterion (8 out of 10 correct choices on 2 consecutive days) in 8±2 days. Percent of correct choices was significantly higher during the last session for each cuttlefish than during its first session. In step 2, cuttlefish were given two control tests, in 10 trials each, with only one type of cue available to solve the maze. They succeeded to orientate with the e-vector or with landmarks alone. In step 3, cuttlefish were tested with both cues indicating opposite directions (compared to step 1). Seven out of 10 cuttlefish followed the e-vector cue over landmarks to orientate, two cuttlefishes followed the landmarks and one did not show any preference. This study shows that cuttlefish can learn simultaneously, in parallel, to orientate with two visual cues, the e-vector of polarized light and landmarks, to find a shelter. Moreover, they are able to use each cue independently when one of the cues disappears or gives a direction different from the other one. Such cognitive capacities may be especially adaptive in a changing environment because the simultaneous acquisition of redundant kinds of spatial information allows secondary cues to be used as backup when primary cues are unavailable.

IMMUNE RESPONSE OF *Octopus vulgaris* AGAINST THE INFECTION BY THE GASTROINTESTINAL PARASITE *Aggregata octopiana*. S. Castellanos-Martínez and C. Gestal Instituto de Investigaciones Marinas (CSIC), Eduardo Cabello 6, 36208 Vigo, Spain.

E-mail: sheilac@iim.csic.es

Despite the ecological and economic importance of cephalopod the immune mechanisms are still scarcely known, even when viruses, bacteria and parasites have been recorded causing diseases in octopus. One of the main pathogen agents identified in the common octopus, *Octopus vulgaris*, from on-growing cages as well as in wild populations, is the intestinal protozoan parasite *Aggregata octopiana*. These coccidian causes a chronic infection associated with a malabsorption syndrome which may seriously diminish the productivity of the exploited individuals. Although molluscs lack an adaptive response, they possess an effective innate system composed by cellular and humoral elements. The haemocytes present in the hemolymph are the main effectors of the molluscs' response. Therefore, the best way to face against diseases in culture cephalopods is the study of the immune response and the searching of resistant animals. The purpose of the present work is to analyze the immune response capacity of *Octopus vulgaris* against *A. octopiana* at both functional and genomic level. Molecular characterization of the parasite was performed by amplification of 18S gene by PCR and an in situ hybridization of DNA was performed in sections of octopus tissue. Octopuses were collected in the Ria de Vigo (NW Spain) and kept in culture tanks of open seawater system at 15°C for 24h before experimentation. Octopuses were anaesthetized with MgCl2 and hemolymph was extracted from the

dorsal aorta and cell counting was carried out. The number of *A. octopiana* sporocyst per individual was counted and estimated per gram of digestive tract weight. Hemocyte population was determined by light microscopy, flow cytometry and scanning electron microscopy (SEM). Flow cytometry was also used to measure functional immune parameters such as phagocytic activity and reactive oxygen species (ROS) elicited by zimosán, while nitric oxide (NO) was measured by Griess reaction. After sequencing the *A. octopiana* 18S gene, parasites were positively recognized in tissue sections by in situ DNA hybridization. The infection was found in 97% of octopuses, mean infection found was 4.06E+06 esporoquistes/g (0-2.08E+07, D.S = 4.06e+06). A predominant hemocyte cell population with a nuclei U shaped and small cytoplasm was observed by flow cytometry, light microscopy and SEM.Maximum phagocytosis capacity of octopus hemocytes was 55%, being 10% the mean activity. The oxidative activity was highly variable from 111.6- 129.5%. Zymosan induced NO production was recorded in all octopus analyzed up to 30μM. NO production decreases with the increase of parasite infection. In contrast, phagocytosis increases with the number of sporocyst/g. These results show a correlation between *O. vulgaris* immune parameters and the infection intensity by *A. octopiana*. Moreover, a genomic approach is being carried out by cDNA's libraries creation via Differential Massive Sequencing in order to identify and characterize genes related to the immune response against the infection and to select candidate genes as biomarkers of resistance. A complementary proteomic approach in hemocytes and serum is also being performed to elucidate the presence of functional proteins differentially expressed in infected and non-infected octopuses.

SOME ASPECTS OF *Octopus vulgaris* EXPLOITATION IN THE EAST OF TUNISIAN COUNTRY. J. Chédia, K. Widien, B. Amina. Institut Supérieur de Biotechnologie de Monastir, Monastir, Tunisie.

E-mail: sirsina@yahoo.com

Octopus vulgaris is one of the main commercial and heavily exploited species of the demersal fishery of the coastal countries in Tunisia, particularly in the south of the country (Gulf of Gabès), It's exploited as well in the Eastern coast mainly in Sousse, Monastir and Mahdia.

Although, the value of common octopus, few studies have been done in Tunisia, essentially in the Eastern coasts.

Some biological and fishery and climatic aspect of *Octopus vulgaris* caught by trawlers and trammel net in the east of Tunisian country are studied. The analysis of size-frequency distribution evaluated the growth parameters from October to May. Time-series analysis of annual catches and catch per unit effort (CPUE) from 1995 to 2006 showed under-fishing state.

This work explored also the effect of environment on octopus CPUE during a 12-year period, through correlation analyses and the incorporation into surplus production models of sea surface temperature (SST) and rainfall data collected during cold (January–May) and hot (August–October) seasons.

VISUAL PERCEPTION AND CAMOUFLAGE BODY PATTERNING IN CUTTLEFISH. C.-C. Chiao[1] and R. T Hanlon[2]. [1]Department of Life Science, National Tsing Hua University, Hsinchu, Taiwan; [2]Marine Biological Laboratory, Woods Hole, USA.

E-mail: ccchiao@life.nthu.edu.tw

Cephalopods have the versatile capability to use body patterns for background matching and disruptive coloration. We qualitatively describe and quantitatively define the chief characteristics of the three major body pattern types used for camouflage by cuttlefish: uniform and mottle patterns for background matching, and disruptive patterns that primarily enhance disruptiveness but aid background matching as well. Although there is great variation within each of the three body pattern types, by correlating background statistics with those of the body pattern, we lay the groundwork to examine the visual perceptional rules of controlling camouflage patterns. Based upon laboratory testing as well as thousands of images of camouflaged cephalopods in the field, we note that size, contrast, and edges of background objects are key visual cues that guide cephalopod dynamic camouflage patterning.

AGE VALIDATION ON GROWTH LAMELLAE OF CUTTLEBONE FROM CULTURED *Sepia pharaonis*. M.-T. Chung[1,2], C.-H. Wang[2,3], H.-L. Chen[2]. [1]School of Ocean and Earth Science, University of Southampton, United Kingdom; [2]Earth Dynamic System Research Center, National Cheng Kung University, Taiwan; [3]Department of Environmental Biology and Fisheries Science, National Taiwan Ocean University, Taiwan.

E-mail: qentsam@gmail.com

Cuttlefish eggs (*Sepia pharaonis*) were collected off southwestern Taiwan. Hatchlings were immersed in alizarine complexone solution on the day of hatch and reared in 25°C and 30°C to investigate the temperature effect on their cuttlebone lamellae growth pattern. A higher deposition rate was found in lower temperature (3.29 ± 0.66 days per lamella in 25°C group) compared to 1.98 ± 0.35 in 30°C group. However, the cuttlebone from three-month-old juvenile *Sepia pharaonis* had a constant deposition rate at 1.75 ± 0.08 days per lamella, that 79% of the individuals formed 20-21 lamellae within 35 days culturing. Compared to juveniles, cuttlefish hatchlings reveal an unstable growth rate on the cuttlebone formation, that more lamellae corresponds to a larger mantle size and heavier body weight in the 25°C group. Although the cuttlefish in higher temperature environment generated more layers in cuttlebone, the septa width is smaller in 30°C group than

in 25°C. This result suggests that the cuttlebone deposition and growth rate in early life stage is strongly related to temperature. However, the reasons why the lamellae formation/deposition rate is slower at early life stage but reaches a stable state after juvenile stage are still unclear. Those issues should be addressed before applying lamellae counting methods on age estimation of cuttlefish.

UNUSUAL SQUID EYES SERVE GOOD VISUAL CAPABILITIES. W.-S. Chung[1], M. Keller[2], K. Fritsches[1], J. Marshall[1]. [1]Queensland Brain Institute, The University of Queensland, Queensland, Australia; [2]Centre for Advanced Imaging, The University of Queensland, Queensland, Australia.

E-mail: wensung.chung@gmail.com

Cephalopods are successful predators with sophisticated eyes and the most complex central nervous system in the whole invertebrate taxa. Their 'simple' eyes possess many superficial similarities with vertebrates, providing one of the best known examples of convergence in the animal kingdom. There are fundamental eye-design differences also, such as microvillar rather than ciliary based photoreceptors, the direction of the photoreceptors face and lens shape. Here we describe another fundamental difference, a large retinal distortion or bump in the tempero-dorsal region, that apparently renders a de-focussed image of around ¼ of the frontal visual field. Acute vision is vital for survival in cephalopods, mediating tasks such as finding prey, avoiding predators, and choosing a mate, so this is a surprising observation. Animals were examined in vivo, with conventional histological sections, and also with magnetic resonance imaging and high resolution ultrasound of anesthetized specimens to confirm the presence of the retinal bump. All methods confirm that this uncommon eye shape is found in four shallow water species and is associated with intrusion of the optic lobe pressing on the back of the eye. We also confirmed, using laser ray tracing that the spherical lens formed a focused image at a single distance in all directions. This unusual eye shape and resulting optical issues are difficult to reconcile with the required high acuity of cephalopod vision, especially in the frontal visual field. We suggest that this defocused area is partially compensated for by the unusual w-shaped pupil of squid such that the pupil slit provides different depths of focus in different eye regions. The largest depth of focus is in precisely the retinal bump region, corresponding to the narrowest pupil in resting condition. In other words, the w shaped pupil provides a progressive correction to the effects of the asymmetrical retina. Additionally, a specific behaviour may be associated with this defocused region. Squid bob up and down in the water prior to striking at prey. Unlike the bird head bobbing, to keep a stable visual scene, squid head pitching causes the visual scene to scan over the retina, in and out of the retinal bump region. Combined with other dynamic eye positioning during hunting, including increased binocular overlap, we suggest that the retinal bump may function in range-finding. Finally, initial examination of the retinal bump area photoreceptors suggests that several anatomical differences existed in this region, including longer photoreceptors, higher density, slower screening pigment migration speed, and non-othogonal photoreceptor arrangement compared to the rest of the retina. All these differences indicate that the retinal bump may act as a specialized detector in squid visual tasks.

CHOLINERGIC SYSTEM OF *Octopus vulgaris*: NEW CUES FROM CCHAT AND PCHAT IMMUNOHISTOCHEMISTRY. L. D'Este[1,2], A. Casini[1,2], R. Vaccaro[1], J.-P. Bellier[3], H. Kimura[3]. [1]Department of Anatomic, Histologic, Forensic and Locomotor Apparatus Sciences, Sapienza University, Rome, Italy; [2]Res. Ctr. ''Daniel Bovet'', Sapienza University, Rome, Italy; [3]Molecular Neuroscience Research Center, Shiga University of Medical Science, Otsu, Japan.

E-mail: arianna.casini@uniroma1.it

Cephalopods are considered to be the most highly evolved marine invertebrates with elaborate brain and complex behavior. Moreover, the arms of *Octopus vulgaris* show remarkable complexity of movements and thus represent a particularly interesting example of the dynamic musculoskeletal system provided by muscular hydrostatic support. The arms perform both motor and sensory functions related to the sucker, chromatophores and arm muscles. Acetylcholine is one of the most anciently known neurotransmitters, widely distributed in the animal kingdom and the cholinergic system besides playing an important role in motor system, is implicated in sensitive and in the complex processes regarding learning and memory. The presence of a large amount of acetylcholine in the nervous system of cephalopods is well known from several biochemical and experimental studies, but little is known about the precise distribution of cholinergic structures in octopus due to lack of a suitable histochemical technique for detecting acetylcholine. The most reliable method to visualize the cholinergic neurons is the immunohistochemical localization of the enzyme choline acetyltransferase (ChAT), the synthetic enzyme of acetylcholine, that has been widely used as a specific marker for cholinergic neurons. Two molecular forms of this enzyme, the common (cChAT) and the peripheral type (pChAT) have been recently distinguished and two polyclonal antisera specific for each of them have been produced by Kimura and coworkers. The cChAT is present in the central nervous system and the pChAT is mostly distributed in the mammalian peripheral nervous system. The presence of pChAT in vertebrates lower than mammals has been so far hardly demonstrated; the current idea is that the cChAT is the older phylogenetically form and the pChAT some recent splice variant of it. Our immunohistochemical study, using both antisera, reopens the debate because it shows that both enzymes could be easily demonstrated in the nervous

system of various invertebrates, including the cephalopod octopus. Our findings clearly indicated that the different parts of the octopus' nervous system show large differences with regard to their ChAT immunoreactivity distribution pattern. Therefore, *Octopus vulgaris* represents a useful invertebrate model to study peripheral and central nervous system, wherein the cholinergic system, as it occurs in higher vertebrates including mammals, plays an important role.

SEEING IS BELIEVING: EVIDENCE OF MODAL COMPLETION IN THE CUTTLEFISH *Sepia officinalis*. A.-S. Darmaillacq[1], S. Zylinski[2], N. Shashar[3]. [1]Groupe Mémoire et Plasticité comportementale, Université de Caen Basse-Normandie, Caen cedex, France; [2]Johnsen Lab, Biology Department, Box 90338, Duke University, Durham, NC, USA; [3]Department of Life Sciences, Eilat campus, Ben Gurion University, Beer Sheva, Israel.

E-mail: annesophie.darmaillacq@unicaen.fr

The detection of the boundary contours of objects is a fundamental problem of early vision. In the process of object recognition, it is common for some boundary segments of the object to remain undetected because they are partially occluded by an opaque surface. Such object borders are perceptually filled-in and so that one can recognize a whole object. There is evidence that mammals, birds and fish can make such completion. Cuttlefish, like other coleoid cephalopods, can instantaneously change their appearance to conceal themselves on various backgrounds. This behaviour has great flexibility, allowing the animals to produce a very large number of patterns, and is primarily visually driven. This provides us with unique access to cuttlefish visual perception. Recently, it has been shown that edge information alone is sufficient to elicit the body pattern known as Disruptive, which is the same camouflage response given when a whole object is present. Here, we present preliminary results investigating perception of subjective contours by measuring the expression of the body patterns in response to different visual conditions. We tested fifteen juvenile cuttlefish on six test stimuli (printed "objects" presented on a grey background). A uniform grey background, to which the animals usually respond with a Uniform body pattern, served as a negative control. White full discs and white circles were used as two positive controls of high-contrast objects of an area approximately 90% of the mean area of the test animal's white square component, which is known to elicit Disruptive patterns. Then, the cuttlefish were tested on white broken circles (made of four "pieces"), these same pieces of the circle rotated on their axis (turned at random), and with the pieces scattered. The expression of 32 body pattern components was scored in 540 images. We found there was no significant difference between the mean scores of cuttlefish on the positive controls and the broken circles. The mean scores on the negative control, the turned at random and scattered backgrounds were not significantly different. However, differences were found between the broken circle responses and the rotated and scattered pieces. These results suggest that cuttlefish can perceive subjective contours and fill in the missing parts. This may be particularly adaptive in case of low illumination conditions where objects are more likely to appear incomplete, and in a habitat where occlusions are common. Interestingly, this suggests such processes of perceptual completion have evolved independently in a variety of taxa as phyletically distant as cephalopods and mammals with basically similar mechanisms.

STRUCTURE AND ADAPTIVE FUNCTION OF SQUID IRIDOPHORES. D. DeMartini[1,2], M. Izumi[2], A. Tao[2], D. Morse[1,2]. [1]Biomolecular Sciences and Engineering, University of California, Santa Barbara, USA; [2]Institute for Collaborative Biotechnologies, University of California, Santa Barbara, USA.

E-mail: demartini@lifesci.ucsb.edu

Most cephalopods utilize iridescence to contribute to their overall body coloration and patterning. Such iridescence arises from optical structures contained inside specialized cells called iridophores. These reflective structures exist in several cephalopod tissues, for example, surrounding the eyes and ink sac, inside light organs and in the dermis. In many cases these reflective structures are static; however, some are adaptive, being able to modulate the wavelength of reflection. We are investigating the adaptive iridescence in the dermis of *Loligo opalescens* and *Loligo pealeii*. Dermal iridescence comes from large stacks of alternating high refractive index lamellae (membrane-bounded reflectin proteins) and low refractive index lamellae (extracellular space) that function as multilayer Bragg reflectors. Specific control over lamellae thickness provides the dynamic optical tunability that extends across the visible spectrum. Our work focuses on understanding the key features of this system that contribute to its adaptability, namely, cell morphology, protein composition, protein structure and post-translational modifications. We have examined the unique morphology of iridophore cells using scanning electron microscopy and confocal microscopy. We postulate that the complicated plasma membrane functions to provide uniform access and exchange of molecules between the reflectin-containing lamellae and the extracellular space. While static tissues contain typical reflectins, adaptive tissues are supplemented with unique reflectin types that contribute to adaptive functionality. We have characterized several of these adaptive reflectins by mass spectrometry and cDNA sequencing. These reflectin proteins show self-assembly characteristics that allow them to condense into a highly concentrated state enabling attainment of the high refractive index. This high refractive index provides the optical contrast necessary for reflection from the multilayer structures. Finally, we have characterized the post-translational modifications of reflectins by mass spectrometry and are seeking to understand how these

modifications drive the condensation of the reflectin platelets. The ultimate goals of our research are to elucidate the biochemical mechanisms controlling the adaptive optical function of iridophores and translate this knowledge to enable the development of new bio-inspired optically tunable materials.

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FOOD IMPRINTING AND VISUAL CATEGORIZATION IN CUTTLEFISH EMBRYOS. L. Dickel, M. Guibé, A.-S. Darmaillacq. Groupe Mémoire et Plasticité comportementale, University of Caen Basse-Normandie, France and Centre de Recherches en Environnement Côtier, Luc sur Mer, France.

E-mail: ludovic.dickel@unicaen.fr

Previous studies have shown the effects of early post-natal visual experience on predatory behaviour in young cuttlefish. We showed that early juveniles preferred crabs over shrimp when they have been visually familiarized to crabs during the first hours of their post-hatching life (this learning was demonstrated to be food imprinting). These results can be surprising, considering the very poor retention abilities of early juveniles of conditioning task. This suggests the occurrence of two separate visual memory-systems in very young cuttlefish.

One can wonder whether this learning may occur *in ovo*, in cuttlefish embryo. To clarify this question, we exposed cuttlefish embryos to crabs for at least a week before hatching. This crab exposure induced a subsequent visual preference for crabs in 7-day-old juveniles. These results show for the first time embryonic visual learning in animals. In a second time, cuttlefish embryos were visually familiarized to white crabs for at least one week. This exposure to white crabs induced a subsequent visual preference for white crabs over black crabs in 7-day-old juveniles. We demonstrated that the crab-type preference depends on the crab-type to which the cuttlefish were previously exposed. Additionally, young cuttlefish that were exposed *in ovo* to white crabs prefer black crabs over shrimp. This indicates that cuttlefish embryos are able of prey generalization and categorization.

Among others, these results show that complex cognitive skills can occur as early as the embryonic stages in cuttlefish. They address questions about the adaptive value of such early learning and memory abilities and their neural correlates. It also raises a crucial proceeding in cephalopod research: from which developmental stage cephalopod welfare has to be considered in experimentation?

HOW OCTOPUSES SEE THE WORLD AND OTHER ROADS LESS TRAVELED: NECESSITY VERSUS SUFFICIENCY AND EVOLUTIONARY CONVERGENCE IN THE STUDY OF ANIMAL CONSCIOUSNESS. D. B. Edelman. Experimental Neurobiology, The Neurosciences Institute, San Diego, CA 92121, USA.

E-mail: david_edelman@nsi.edu

Human consciousness is now considered a legitimate object of scientific study. But, the possibility of conscious states in non-human animals, though entertained by some, has not been investigated with any serious rigor. At least two factors might account for this. First, the term, "consciousness," is fraught with different associations across a variety of domains, from the spiritual to the philosophical. In fact, there seems to be no commonly accepted scientific definition of consciousness. Second, in contrast to the human case, researchers cannot rely on accurate verbal reports of experiences by non-human animals without the faculty of natural language. Nevertheless, certain properties of, and criteria for, conscious states can be extrapolated from the human case to non-human animal studies. In fact, a variety of neuroanatomical, neurophysiological, and behavioral evidence suggests that some non-human animals - in particular, non-human primates and certain birds - do indeed experience conscious states. But how can we approach the question of consciousness in animals that are far removed from the primate, mammalian, or even vertebrate, lineages? A helpful starting point would be to posit that there are major functional properties common to all nervous systems in which conscious states are instantiated. From here, we may dispense with arguments that are based on rigid structural homology in favor of those based on broad functional analogy. For example, in the case of an invertebrate species, it would be fruitful to identify neural structures that exhibit functions roughly analogous to those of cortex and thalamus (two structures seemingly critical for mammalian consciousness), as well as neurophysiological signatures (e.g., patterns of electrical activity) and behaviors resembling those observed in conscious vertebrates.

Endowed with the most complex nervous systems of any invertebrate, a sophisticated array of sensory and motor adaptations, and rich behavioral repertoires, cephalopod molluscs such as the octopus may represent rare instances in which consciousness was engendered in a group of animals quite distant from the vertebrates. Here, I will argue that, on neuroanatomical, neurophysiological, and behavioral grounds, the octopus in particular represents a good test case for the possibility of conscious states in an invertebrate. In making this argument, I will: 1) lay out a working definition for consciousness that may be extended beyond the vertebrate case; 2) describe structural and functional properties which may be the sine qua non of consciousness; 3) suggest evolutionary trends (e.g., the emergence of complex vision) that set the stage for the advent of conscious states in a variety of species; and 4) discuss the ethical implications of consciousness in non-human animals, including, quite possibly, the octopus.

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"MONCO": A NATURAL MODEL FOR STUDYING ARM USAGE AND REGENERATION IN *Octopus vulgaris*. M. Florini[1], G. Fiorito[1], T. Hague[2], P. L. R.Andrews[2]. [1]Octopus Behavioral Biology, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy; [2]Division of Biomedical Sciences, St George's University of London, Cranmer Terrace, London, SW17 0RE, UK.

E-mail: pandrews@sgul.ac.uk

Introduction and Aims: Arm and tentacle regeneration in several cephalopod species was described early in the last century (Lange 1920) and in the last decade (Rohrbach & Schmidtberg 2006) but the processes involved have not been studied extensively. Studies of arm regeneration have surgically amputated the limb under laboratory conditions but here we report a "natural experiment" documenting the incidence of arm damage in wild caught *Octopus vulgaris*, characterizing and measuring the regrowth of the arm together with behavioural studies only some of which are reported here.

Methods: *Octopus vulgaris* were caught by non-commercial methods in the Bay of Naples and carefully transported to the laboratory. Arm damage was assessed in all animals and the latency to attack a small crab was measured after an adaptation period to the holding tanks. The loss of an arm or part of an arm with no sign of regrowth at the time of capture was classed as "monco" the Italian word for "amputated" and these animals together with animals in which an arm was shortened but showing signs of regeneration were used to assess the total incidence of damage. A selected group of "monco" animals) was studied in detail with arm regrowth monitored over a period of 4 months (to date). Data are presented as mean ± sem).

Results: The incidence of damage (monco and regenerating) in 83 unselected animals was 51% with no difference in incidence between the sexes, equally represented in our population. In the animals showing any arm damage the rank order was L1 (57%), R1 (36%), L2 (34%), R2 (29%), L4 = R3 (20%), L3 (18%), R4 (16%).The incidence of damage to the dorsal pair of arms (L1,L2, R1, R2) was significantly greater ($p<0.0001$, Chi2) than that of the ventral pair (L3, L4, R3, R4). Measurements of the latency to attack a crab failed to reveal any significant difference between animals with and without damage and even in the "monco" animals showing at least 50% reduction in length of one arm no difference was detected although all 8 arms participate in prey capture in an intact animal. A key aspect of regeneration is the formation of a "bud" from which the new part of the arm grows. Buds are motile, have suckers and chromatophores although the bud is usually paler than the rest of the arm. The regenerating tip also shows some indication of increased sensitivity. In the sub group of "monco" animals (n = 6) the most severely damaged arm regrew from the starting length of 41 ± 7% to 69 ± 8% of the average length of the intact arms over a period of 16 weeks. The body weight doubled over this period (164 ± 10 g vs 334 ± 14 g, n = 6).

Discussion: Arm damage is common in this population and the higher incidence of damage in the dorsal vs the ventral pair of arms suggests that these may be used more in potentially damaging behaviours. The regenerative process appears slow for an animal with a relatively short life span but further studies are required to investigate the impact of age, season and sex on the process. The "monco" model in *Octopus vulgaris* may provide a novel tissue engineering model as regeneration involves coordinated genesis of muscle, skin, blood vessels and nerves.

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CLONING AND EXPRESSION OF SOX FAMILY GENES IN THE EUROPEAN CUTTLEFISH *Sepia officinalis*. L. Focareta and A. Cole. Biogem–IRGS, Ariano Irpino, Italy, 83031.

E-mail: agcole05@gmail.com

The Sox-family of transcription factors is an important class of DNA-binding proteins that are known to be involved in many aspects of cell differentiation. Using a degenerate primer strategy we have isolated partial coding sequence for three members of the Sox family of transcription factors from the European cuttlefish *Sepia officinalis*: SoSoxE, SoSoxB1, and SoSoxB2. The highly conserved high mobility group (HMG) DNA binding domain of SoSoxB1 and SoSoxB2 are identical at the amino-acid level, whereas the underlying nucleic acid sequences show 47% divergence in codon sequence. Analysis of expression patterns of all three genes during organogenesis reveals distinct, non-overlapping expression domains. SoSoxB2 expression appears to be restricted to the developing central nervous system. SoSoxB1 shows ectodermal expression, expressed throughout the developing epithelium. SoSoxB1 is excluded from the developing statocysts and eyes, as well as from all presumptive mesodermal territories. As development proceeds, SoSoxB1expression is gradually reduced in the medial epithelium of the developing head, and is no longer expressed within the mantle tissues. By stage 28, when most tissues of the embryo are in a phase of growth and maturation, SoSoxB1 expression is limited to two circular patches of cells located bilaterally on the surface of the head epithelium, and within the differentiating suckers of the arms. In contrast, SoSoxE is expressed in presumptive mesodermal territories, including the central-most tissues of the developing arms, the developing gills, cells within the developing eyes, cells that form a ring around the periphery of the developing head, a ring of cells surrounding the developing shell sac, cells located between the developing funnel folds, symmetrical patches of cells posterior to the stomodeum where the buccal mass will form, and a ring of cells located within the stomodeum. As development proceeds, SoSoxE shows a high level of expression within the developing circulatory system within the developing mantle and the head. A high level of expression is retained in the developing gills within the mantle cavity. Juvenile

cuttlefish also show a high level of SoSoxE expression within the nervous system. Our data suggests that SoSoxB1 is involved in epithelial development in the cuttlefish, in particular epithelial sensory and/or glandular structures. SoSoxB2, in contrast, is restricted to the developing nervous system. Members of the SoxE family in vertebrates are involved in the formation of mesodermal tissues, as well as playing an important role in neural crest specification. Our data suggest a similar role in mesodermal development in the cuttlefish. SoSoxE expression within the mature nervous system may represent a novel function for this member of the Sox family, although elements of the vertebrate PNS derived from the neural crest also involve SoxE transcription factors.

OCTOPUS ARM REGENERATION AND ITS POTENTIAL IMPLICATION IN REPARATIVE PATHWAYS. **S. Fossati[1], F. Carella[2], F. Benfenati[1], L. Zullo[1].** [1]IIT Italian Institute of Technology, Department of Neuroscience and Brain Technology, Genoa, Italy; [2]Federico II University of Naples, Department of Biological Science, Napoli, Italy.

E-mail: letizia.zullo@iit.it

The ability of regenerate whole-body structures have been long studied and still have a peculiar interest in stem cell biology for its therapeutic potential. A regenerative process implies a well-coordinated restoration of cells, tissues, and organs that have been physically or functionally lost. This reparative process must accomplish the recognition and recapitulation of missing structures, while simultaneously achieving functional integration between recently formed and pre-existing tissues, in order to direct physiological and structural alterations. Regeneration of arms in cephalopods and in particular in octopods has been the subject of several studies (Lange, 1920; May, 1933; Callan, 1940). In the invertebrate kingdom, cephalopods are the most evolved organisms. The anatomy of their circulatory and nervous systems makes them suitable models to study the mechanisms of regeneration. Octopus often loses arms or part of them throughout life and is endowed with a high regenerative power. His extraordinary regeneration capability can provide deeper insights into our understanding of latent basic pathways, which once unlocked can promote regeneration. The first study on the regeneration of the octopus arm demonstrated that all regenerated tissues, with the exception of the dermal connective tissue, are produced by the pre-existing tissues of the same kind (Lange, 1920). However, the details of the regenerative process in the octopus arm remain to be elucidated. Regeneration process in lower invertebrates and amphibians has been found to be regulated (promoting/inhibiting effect) by various neurotransmitters. Using *Octopus vulgaris* as model of regeneration, we are investigating cellular proliferation rate and the role of different cell types and neurotransmitters in the regenerative process. In order to follow cell replacement, we firstly assessed the expression of specific markers involved in cellular proliferation (PCNA, AgNOR). Then, since octopus has typical cholinergic innervations at the level of the arm neuromuscular system (Talesa et al., 1995), we focused on the possible role of the neurotransmitter acetylcholine in arm regeneration. We are testing the hypothesis that acetylcholinesterase (AChE) activity plays a major role in the regenerative process. Our preliminary data show that in arms at different stages of regeneration AChE activity is very low during the early phase of regeneration, then rose above the basal levels of the non-regenerating arm and eventually return to near normal levels. The evaluation of differences in the expression of AChE enzyme as well as of specific proteins during regeneration will allow us understand latent basic pathways which could be unlocked to promote regeneration.

LIPID COMPOSITION OF COMMON OCTOPUS (*Octopus vulgaris*) PARALARVAE FED WITH ALTERNATIVE PREYS AND A MICRODIET. **I. García-Herrero[1], D. Reis[2], C. Rodríguez[1], R. Riera[3], A. V. Sykes[2], B. C. Felipe[4], V. Martín[4], J. E. Villamandos[4], E. Almansa.** [1]Laboratorio de Fisiología Animal, Facultad de Biología, Universidad de La Laguna,Tenerife, Spain; [2]CCMAR-CIMAR L.A., Campus de Gambelas, Faculdade de Ciéncias e Tecnologia, Universidade do Algarve, Faro, Portugal; [3]CIMA SL, Arzobispo Elías Yanes, 44, La Laguna, Santa Cruz de Tenerife, Canary Islands, Spain; [4]IEO, Centro Oceanográfico de Canarias. C/ Gral. Gutiérrez N°4, C.P. 38003. Santa Cruz de Tenerife, Spain.

E-mail: asykes@ualg.pt

The aim of this study was to evaluate the effect of several live preys (*Artemia* sp. juveniles cultured with *Tetraselmis chuii*, *Palaemon elegans* zoeae and *Grapsus grapsus* zoeae) and one inert diet (with alginate) on the lipid composition of *O. vulgaris* paralarvae after nine days of rearing. The rearing of paralarvae was performed in 100L cylinder-conical fiber glass tanks, at a density of 1.5 paralarvae/L, and under a 12L:12D photoperiod. The total lipid content (TL), the lipid class (LC) and fatty acid (FA) profiles were determined on initial and 9 day-old paralarvae. No differences ($p > 0.05$) were found within the TL content of octopus after 9 days. Nonetheless, differences were detected within LC and FA composition of different diets. Newly hatched octopus presented higher polar lipid (PL) content (64.64 ± 1.64%), specifically phosphatidylethanolamine (PE – 32.73 ± 1.08%), and a high content in cholesterol (CHO – 32.27 ± 1.68%). After 9 days of culture, all paralarvae shifted its LC profile, being characterized by an increase in neutral lipid content ($p < 0.05$), especially in triacylglycerol (TG) and sterol ester (SE) contents and a reduction in PE contents. Principal component analysis (PCA), used to examine the multivariate structure of the data set, confirmed these trends of different LC profiles. The two components of PCA accounted for the 68% of data variation. PC1 component clearly separated lipid classes

which predominated in octopus hatchlings (PE), from those characteristic of 9 day-old paralarvae (TG and SE) demonstrating a significant separation between initial and 9 day-old parlarvae composition. Regarding FA profile, hatchlings presented a high content in 16:0, 20:5n-3 (EPA) and 22:6n-3 (DHA); and a DHA/EPA ratio of 1.55 ± 0.02. Nine day paralarvae presented a reduction in the DHA content ($p < 0.05$), which lead to a reduction in total highly unsaturated fatty acids (HUFA) and of the DHA/EPA ratio. Also, differences within 9 day-old paralarvae fed differently were observed. Octopus fed G. grapsus presented lower level of total n-3 and specifically EPA in contrast to the higher levels of total n-6 and specifically 20:4n-6. PCA analysis of FA showed that the two principal components accounted for 61.3% of variation. PC1 separated fatty acids representative of hatchlings (DHA, 16:0) from those characteristic of 9 day-old paralarvae grouping in three clusters: hatchlings, *G. grapsus* fed paralarvae and *Artemia*, microdiet and *P. elegans* fed paralarvae ($p < 0.05$). In summary, present results show a notable shifting of octopus paralarvae lipid profile after only nine days of feeding with live preys and inert diet. Regardless of the diet provided, there was a significant reduction in PE, DHA and HUFA content and regarding the DHA/EPA ratio.

FAST MOLLUSC WALKING: HIGH-SPEED SPECIATION IN COLD-WATER OCTOPUSES. I. G. Gleadall. International Fisheries Unit, Tohoku University Faculty of Agriculture, Sendai 981-8555, Japan.

E-mail: asykes@ualg.pt

Molecular sequencing of octopuses in the genus *Muusoctopus* (until recently, confused with the genera *Bathypolypus* and *Benthoctopus*) reveals that most species are very closely related, despite being widely distributed throughout much of the World Ocean. All species in this genus produce benthic young which develop directly, with no planktonic phase, so presumably they have achieved their wide distribution by walking along the seabed. Morphological and molecular sequence data are presented which demonstrate that the ancestors of one of these species probably walked through the gap between North and South America into the tropical Atlantic before closure of the Atrato Seaway. This species now has an amphi-Atlantic distribution. Another species has recently diverged into two subspecies, and it is argued that these were probably separated into two allopatric populations by physical and physiological barriers resulting from the Llanquihue Glaciation and the subsequent period of deglaciation. Estimates of the timing of these two vicariance events (separation of the Atlantic and Pacific populations, and separation of the two recently discovered subspecies) were used to calibrate a phylogenetic tree of species in the *Muusoctopus* group. The results strongly suggest that the mean rate of octopus speciation (in this group at least) has been progressing far more rapidly than previous estimations have led us to believe.

EXPLORING CEPHALOPOD OBJECT MANIPULATION: MODELING STUDIES OF NEURAL CONTROL OF OCTOPUS SUCKERS AND SQUID TENTACLES. F. W. Grasso. BioMimetic & Cognitive Robotics Laboratory, Dept. of Psychology, Brooklyn College CUNY, USA.

E-mail: fwgrasso@gmail.com

Cephalopods are able to perform complex grasping and manipulation of objects in structurally complex, 3-D environments (e.g., opening jars, piling stones, grooming egg fingers, etc.). These behaviors are comparable in functional complexity to those of higher vertebrates yet realized with fundamentally different brain and body architectures. The biomechanics of these behaviors in cephalopods are realized largely through the action of muscular hydrostat (MH) systems in which portions of muscular appendages are dynamically allocated to provide either contractile force or stiffness where needed to suite the manipulation. This implies substantial computational complexity in the neural systems controlling these behaviors and makes the principles of neural control found in cephalopod MH systems topics of great theoretical and practical interest. Serious technical challenges, however, limit our direct study of the integrated control of these systems in behaving cephalopods. As a parallel tool of inquiry, we developed the Artificial and Biological Soft Actuator Manipulator Simulator (ABSAMS), a physically and physiologically constrained computer simulation environment employed to study 3d models of MH systems (suckers, tongues, tentacles etc.) and their neural control. Muscle fibers are interlaced within the MH at many orientations but single motor neurons produce contraction only in small number of fibers. Active groups of motor units (MU) (muscle fiber groups innervated by a single motor neuron) control shape changes of the MH through cooperative and antagonistic action with other MUs. ABSAMS simulations allow us to explore the consequences of activation of single motor units acting alone or in coordinated groups on the shape of a cephalopod appendage. Evaluation of the similarity of the simulated shape changes with those of their biological counterparts provides a quantitative basis for evaluation of a model's performance. I report on the results from simulations of the squid tentacle strike and octopus sucker attachment as modeled in ABSAMS. The tentacle model quantitatively reproduced the 3d kinematics of tentacle strike observed in squid. The sucker model reproduced the attachment cycle of in a single octopus sucker and its relocation of a simulated object. Control of each model was implemented as a fixed action pattern: the sucker's triggered by contact with the object's surface, the tentacle by a simulated central command. In these studies a binary (as opposed to graded) activation of individual MU's was used. More sophisticated control schemes are possible and likely for the sucker.

The success in reproducing the ballistic tentacle strike is unsurprising and consistent with our understanding of tentacle biomechanics. Success of binary MU control in the sucker model is intuitively surprising because tactile and proprioceptive sensors are known to exist in the sucker. These results support the idea that sucker object manipulation can result from the execution of a single precisely timed, multi-component motor program lacking sensory feedback. MH systems are common amongst mollusks but in cephalopods their range and functional versatility are greatly advanced compared to other mollusks such as gastropods. These results raise the possibility that a computationally simple method of achieving flexible object manipulation evolved in cephalopods as its brain and soft body co-evolved.

THE *Octopus vulgaris* BRAIN IN NUMBERS: YOUNG (1963) REVISITED. A. M. Grimaldi[1,2], G. Fiorito[1], S. Herculano-Houzel[3]. [1]Octopus Behavioral Biology, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy; [2]Polo delle Scienze e delle Tecnologie, C.I.R.A.M, Università degli Studi di Napoli Federico II, Napoli, Italy; [3]Departmento de Anatomia, Istituto de Ciencias Biomédica, Universidade Federal de Rio de Janeiro, Brazil.

E-mail: annamaria.grimaldi@szn.it

The computation power of the brain is considered to be related to its numerical and cellular composition and network architecture; variables that contribute to determine brain size within and across species. In vertebrates, the number of neurons in the cerebral cortex is considered a better correlate of cognitive abilities across species than absolute or relative size of the brain. Can we assert the same for invertebrates?

Here we applied for the first time in the cephalopod mollusc *Octopus vulgaris*, an invertebrate, the isotropic fractionator, a fast and inexpensive method already applied to vertebrates for quantifying total numbers of neuronal and non-neuronal cells in brain or any dissectable neural region.

Brains were removed, dissected in parts (supra-, sub-esophageal masses and two optic lobes) and fixed in 4% paraformaldehyde in seawater. They were mechanically dissociated and pelleted nuclei were then suspended in PBS containing DAPI.

We obtained an estimation of total numbers of neuronal cell independently from brain volume and anisotropy. We found that the total number of cells in the adult octopus brain (N = 6) ranged between 192 and 353 million cells. We will present data referring to body size and will compare with figures provided by the classic study of Young (1963).

Our data, although preliminary, suggest that larger octopuses tend to have larger brains, which is consistent with the continued growth during adult life that octopuses are known to exhibit. Further experiments should be carried out to discriminate between neural components and supporting cells.

VISUAL PERCEPTION AND COGNITION IN CUTTLEFISH EMBRYOS. M. Guibé, S. Romagny, C. Bellanger, L. Dickel. Groupe Mémoire et Plasticité comportementale, Université de Caen Basse-Normandie, France and Centre de Recherches en Environnement Côtier, 54 rue du Dr. Charcot, Luc-sur-Mer, France.

E-mail: ludovic.dickel@unicaen.fr

Animal welfare is an important issue in animal experimentation but there is still a debate: from which developmental stage does it have to be considered? Embryonic development of cuttlefish has been precisely described; it consists in 30 well defined morphological stages. However, nothing is known about the functional development of sensory or cognitive systems in embryos. The present study focuses on the functional development of visual system in cuttlefish late embryos (*in ovo* response to visual stimuli, visual habituation processes and abilities to visual discrimination). Embryos have been exposed to light stimulus at stage 23, 25 and 30 for 150s. Responses were measured as changing of mantle contractions rhythms. Response to visual stimuli increases significantly from a basal rate at stage 25 and 30 but not at stage 23. Habituation to repeated light stimulations has been observed only at stage 30. To investigate abilities of visual discrimination learning in embryos, we used food imprinting paradigm; late embryos (stage 28-30) has been visually exposed to white crabs during one week. Seven days after hatching, food choices were offered to juvenile between white and black crabs. While naive cuttlefish prefer to attack black crabs, cuttlefish previously exposed to white crabs significantly prefer to attack white crabs. This result demonstrates abilities of embryos to discriminate categorize and memorize different crab-types on the basis of their contrast. Adaptive advantages of such early maturation of visual system in cuttlefish embryos, despite the presence of black envelops around the egg will be discussed. This study brings new evidence about the emergence of cognition in cuttlefish embryo: the future convention of animal welfare regarding to cephalopods should raise the problem of the first developmental stage from which it has to be considered for its application.

INLAND CLOSED SYSTEM LABORATORIES CHANCES AND CHALLENGES. T. Gutnick, J. Richter, B. Hochner, M. J. Kuba. Department of Neurobiology, Life Sciences Institute and the Interdisciplinary Center for Neural Computation,Hebrew University, Jerusalem 91904, Israel.

E-mail: michikuba@lobster.ls.huji.ac.il

While the majority of research on cephalopods has been conducted in laboratories close to the sea, recent advances in handling and keeping cephalopods has enabled inland facilities to be competitive with large, on shore, laboratories. The Jerusalem octopus laboratory is an example of how such a lab can be built and maintained. Our presentation will focus on what challenges a laboratory without permanent access to the sea faces. Distance

from the coast limits the availability of animals and adds the complication of transporting them safely to the off shore facilities. Not being able to rely on a permanent flow of fresh seawater filtering the water becomes a serious challenge, especially when dealing with large predators like octopus or cuttlefish. Specifically, in the case of the Jerusalem lab, additional problems arise due to the seasonal availability of octopuses; during the summer months, and at times up to mid-winter, animals are hard to obtain. Thus, stockpiling animals is necessary in order to ensure productive experiments year round. Maintaining large systems, in full capacity, requires the laboratory to place the outmost attention to the keeping and caring of the animals. When aiming to perform reliable behavioural studies, enrichment and low stress levels for the animals become crucial.

MOTOR CONTROL IN *Octopus vulgaris*. T. Gutnick, J. Richter, B. Hochner, M. J. Kuba. Department of Neurobiology, Life Sciences Institute and the Interdisciplinary Center for Neural Computation, Hebrew University, Jerusalem 91904, Israel.

E-mail: michikuba@lobster.ls.huji.ac.il

Octopuses use their eight highly flexible arms to perform a variety of tasks from exploring the environment to hunting, mating and cleaning. The motor control of the highly flexible eight arms of the common octopus (*Octopus vulgaris*) has been the focus of several recent studies. In our present studies that are part of the EU FP7 project OCTOPUS we investigate the ability of the octopuses motor output programs to adapt to new tasks. To do so we forced our animals to perform tasks challenging their standard repertoire of arm movements. We conducted a set of experiments that effected either, bend propagation and fetching movements, or required on-line central control of searching movements and an establishment of learning processes. In our first experiment we introduced a physical constrain to the base of the octopus arm. Animals were placed inside a transparent Perspex box ($40 \times 40 \times 40$cm) with a hole at the center of every surface that allowed the insertion of a single arm only (1.5 cm). During the experiment the subjects had to reach out through a hole to retrieve a food reward offered outside the box. The accuracy of the reaching towards a target movement did not improve in consecutive experimental sessions. However, the accuracy and speed of fetching movements improved both within and across sessions. A second set of experiments investigated the ability of octopuses to learn to turn their arm in a specific direction in an opaque Y shaped maze. The animals received neither chemical nor tactile information on the direction of the turn. Therefore the correct decision to turn left or right inside the maze could only be made based on proprioceptive information on the position of the arm. Five out of 6 subjects were able to successfully complete this task in less than 90 trials. These experiments show that octopuses are able to modify the execution of motor primitives as well as more searching and crawling related movements.

EVIDENCE FOR INTER-SUCKER COORDINATION DURING DIFFERENT ARM MOVEMENTS IN THE GIANT PACIFIC OCTOPUS. S. P. Hadjisolomou and F. W. Grasso. BioMimetic and Cognitive Robotics Laboratory, Department of Psychology, Brooklyn College, CUNY, Brooklyn NY, USA; Cognition, Brain and Behavior Program, The Graduate Center, CUNY, New York NY, USA.

E-mail: shadjisolomou@gc.cuny.edu

Coleoid cephalopods have suckers on their arms and tentacles. Squids and cuttlefish use their suckers primarily for adhesion by suction. Octopus suckers have different morphology, specifically the extrinsic muscles (Kier and Smith, 1990), which allow for more elaborate functionality. The flexibility of octopus sucker behavioral repertoires is well known (Packard, 1988), but the mechanisms for control are just beginning to be understood. Octopuses are able to use their appendages to manipulate objects with a virtually infinite number of degrees of freedom. The mechanisms by which they achieve fine and forceful control of grasping are realized through the coordinated action of the suckers and the arms. Recent evidence (Grasso, 2008) supports the hypothesis that the suckers of the octopus arm, acting as part of this hyper-redundant system, are not passive agents, but actively contribute to grasping and manipulation. In this study, we hypothesized that motions of neighboring suckers would be more coordinated during goal-directed arm movements compared with passive movements. To examine this hypothesis in closer detail, we tracked the movements of suckers from digitized video footage of single arms of Giant Pacific Octopuses, *Enteroctopus dofleini* (Hochberg, 1998) while they employed those arms to achieve different goals. In quantitative analysis, we calculated movements of sections of the arms carrying the suckers. We subsequently deducted the arm from sucker movements to estimate the individual sucker motions independent of the arm. We computed the pair-wise cross-correlation between the movements of 20–30 suckers along the arm. Significant correlations and anti-correlations ($p < 0.01$) were found that demonstrated neighborhoods of coordinated activity both locally and at distant intervals along the arm. The delays between these correlations were brief (<10.0 ms) and therefore unlikely to have included closed-loop communication between the sucker and the supra-esophageal ganglia. These patterns of arm-independent sucker activity varied with the task to which the octopus applied the arm. These results agree with earlier evidence indicating that sucker movements are not passively generated; they are coordinated with arm movements in order to assist specific goal-directed behaviors. These results provide functional evidence for the neuroanatomically demonstrated connectivity within the arm and an explicit demonstration of that information passing between suckers as that connectivity implies. Further investigations of the mechanisms underlying sucker functionality will help to better understand octopus behavior as well as the phylogenetic differences among octopuses, squids and cuttlefish.

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PRELIMINARY EVIDENCE FOR A NOCICEPTIVE REFLEX TO MECHANICAL, OSMOTIC AND ACIDIC STIMULI IN THE ISOLATED ARM OF *Octopus vulgaris*. T. Hague[1,2], M. Florini[1], E. Brown[1], G. Fiorito[1], P. L. R. Andrews[1,2]. [1]Octopus Behavioral Biology, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy; [2]Division of Biomedical Sciences, St George's University of London, Cranmer Terrace, London, SW17 0RE, UK.

E-mail: t_a_hague@hotmail.com

Introduction. There is evidence from invertebrates for the presence of nociceptors responding to mechanical, thermal and acidic stimuli (St. John Smith & Lewin, 2009). Studies in octopus have provided indirect evidence for the existence of nociceptors based on the induction of learned aversions (e.g. Boycott, 1953) and ultrastructure (Graziadei,1964). The inclusion of all cephalopods in EU 86/609/10 makes understanding the potential for these animals to experience pain suffering and distress essential in order that they can be minimized and humane end points identified.

Methods. Under terminal MgCl2 anaesthesia entire arms were removed from adult *Octopus vulgaris*. Isolated arms were maintained in chilled, gassed sea water and studied under three conditions: in chilled, gassed sea water; placed horizontally on a Perspex plate in a cool room with intermittent bathing with sea water; suspended vertically from the proximal stump in a cool room with intermittent bathing with sea water. In two suspended preparations the electromyogram (EMG) was recorded at two points (proximal and distal) from the arm. Stimuli used were: mechanical (via forceps applied to a point in the distal arm or tip); acetic acid (0.01- 5.0%) and tap water (hypotonic) were applied to ~1cm portion of the tip. Sea water was used as a control solution. Responses were recorded by video for subsequent analysis.

Results: No contractile responses were observed on application of sea water and no spontaneous movements were observed. Compression of the distal arm with forceps induced contraction of the proximal arm resulting in shortening of the arm in horizontal and vertically mounted preparations. Observation and EMG recording showed that the proximal arm muscles contracted before the distal arm muscles. Application of distilled water to the arm tip resulted in a rapid (<1s) withdrawal of the arm. Acetic acid induced a powerful withdrawal response (<1s) with the threshold concentration being 0.1%. Withdrawal responses often consisted not only longitudinal shortening of the arm but also formation of a "joint" at approximately 1/3 of the length of the arm and more complex "corkscrew" movements reminiscent of those seen during sucker cleaning. In suspended preparations the initial contractions subsided after 20–30s and the arm extended and withdrew again on contact with acetic acid or tap water but not sea water. Section of the nerve cord in the upper half of the arm prevented the withdrawal response to stimuli applied to the tip.

Discussion. Coordinated motor responses leading to retraction of the arm can be evoked by mechanical, hypotonic and acidic stimuli in the isolated arm. The nerve cord is responsible for transmission of the signal from the tip to the upper arm and it is presumed that the muscle activity is coordinated via the arm ganglia. The preparation requires refinement to investigate the neurophysiological correlates in detail but the results provide preliminary evidence for the existence of nociceptors (the first step in the pathway that can lead to the sensation of pain in other species) in *Octopus vulgaris*. The brain projections of the information from the putative nociceptors requires investigation before more definitive statements can be made about the ability of *Octopus vulgaris* to "feel" pain. However, the ability of an isolated arm to demonstrate a coordinated withdrawal from a noxious stimulus raises some issues for identification of pain in intact animals and criteria for surgical anaesthesia.

MODULATION OF LATERALIZATION BY PRE-HATCHING EXPOSURE TO PREDATOR IN CUTTLEFISH. M. Hébert[1,2] and C. Jozet-Alves[1,2]. [1]Groupe Mémoire et Plasticité comportementale, Université de Caen Basse-Normandie, France; [2]Centre de Recherches en Environnement Côtier, 54 rue du Dr. Charcot, 14530 Luc-sur-Mer, France.

E-mail: christelle.alves@unicaen.fr

Lateralization is a widespread and evolutionary ancient adaptation. It has been recently demonstrated in cephalopods, both in octopus and cuttlefish. However, the mechanisms underlying inter-individual variation remain unresolved. In fish, the degree of lateralization varies between populations of the same species that have been exposed to different ecological pressures. In our experiment, we investigated the existence of a modulation of lateralization by chemical signals of predation in cuttlefish. Three groups of cuttlefish embryos differed according to the type of olfactory stimulation that they were exposed to prior to hatching: predator odour (seabass), non-predator odour (sea urchins), no odour (blank water). Three days after hatching, lateralization was assessed by testing untrained side-turning preferences in a T-shaped apparatus. In each group, cuttlefish were either tested with predator odour, with non-predator odour or with blank water in the apparatus. Our study demonstrates a variation in the strength of side-turning bias in newly-hatched cuttlefish when tested with or without predator odour (i.e. seabass odour) inside the apparatus. Cuttlefish of the three groups display a significant

preference to turn to the left when tested with predator odour. That would suggest that cuttlefish are able to recognize predator odour as a chemical evidence of the potential presence of a predator nearby. The preference to turn to the left observed in cuttlefish tested with predator odour could well be the result of a greater motivation to hide. Indeed, lateralization has been described in the literature as being dependant of motivation. Whereas hatchlings that have not been exposed to predator odour during embryonic development (control and non-predator groups) showed no side-turning bias when tested with non-predator odour or with blank water, hatchlings that have been exposed to predator odour (predator group) preferentially turn to the left whatever the testing condition. The exposure to predator odour all the incubation long could possibly appear as an acute predicator of a high predation surrounding environment. Cuttlefish could thus behave as if a threat was potentially around even without chemical evidence of a predator nearby. Differences in strength of lateralization are likely to provide fitness benefits to cuttlefish that hatch in high-predation areas by enabling them to hunt and watch predators simultaneously. We here report the first example among invertebrates of modulation of lateralization. Research in cuttlefish would provide a valuable model system for investigating the mechanisms underlying inter-individual variation in invertebrates.

A COMPARATIVE STUDY ON THE HEARING ENDOGRANS AND AUDITORY PHYSIOLOGY OF A SQUID AND AN OCTOPUS. M. Y. Hu[1], W.-S. Chung[2], H. Y. Yan[2]. [1]Institute of Marine Science, IFM-GEOMAR, Kiel, 24105 Germany; [2]Sensory Physiology Laboratory, Marine Research Station, Institute of Cellular & Organismic Biology, Academia Sinica, Jiaoshi, I-Lan, 26242 Taiwan.

E-mail: hyyan@gate.sinica.edu.tw

This study aimed to examine whether cephalopods can hear underwater sound by using an oval squid (*Sepiotheutis lessoniana*) and an octopus (*Octopus vulgaris*) as experimental animals. Morphological structures of the hearing endogan, the statocyst, were examined with scanning electron microscopy (SEM) and the hearing thresholds in terms of intensity and frequency range were investigated with the use of auditory brainstem response (ABR) method. The squid statocyst consists of two chambers inside the cranial cartilage, with three sensory epithelia, localized in the anterior part of each chamber. Only the macula statica princeps (MSP) carries a well defined CaCO3 statolith which is movable in anterior and posterior direction on a spur in the center of the macula. The statocyst of O. vulgaris consists of a sac anchored with strands in each of the statocyst chamber. Inside this sac there is only one macula to which a cone shaped statolith is attached. The hair cell density in the macula of *O. vulgaris* is higher (104.78 ± 26.28) compared to that of *S. lessoniana* (58.22 ± 7.19) which is used to tune for a higher sensitivity towards gravitation and acceleration. On the other hand, shape, mass and the presence of different sized, smaller statoconia in the statocyst of *S. lessoniana* modulates for a wider detectable frequency range. The electrophysiological recording of acoustically evoked potentials showed that *S. lessoniana* had a hearing frequency range from 400 to 1500 Hz with sound pressure of 125–140 dB (re: 1 μPa) while *O. vulgaris* had a range of 400–1000 Hz and 400–1000 Hz, respectively. The best hearing frequency for both species is at 600 Hz. The thresholds of *S. lessoniana* were generally lower than those of *O. vulgaris*. Injection of neomycin (concentration: 0.8 nM; a known ototoxic agent which blocks signal transduction pathways of hearing sensory hair cells), into statocysts of both cephalopods ablated evoked potentials within 25–28 min after injection which demonstrated that sensory hair cells inside the statocysts are the functional units of sound perception. The differences in morphological structures of statocysts corroborate significant differences of hearing abilities between two species.

CAN CUTTLEFISH LEARN BY OBSERVING OTHERS? K.-L. Huang and C.-C. Chiao. Institute of Systems Neuroscience & Department of Life Science, National Tsing Hua University, Hsinchu, Taiwan.

E-mail: ccchiao@life.nthu.edu.tw

Observational learning is the ability to learn through observing others' behavior. The benefit of observational learning is apparent in that individuals can save time and energy without try-and-error, thus enhance the chance of survival and reproduction. Cephalopods (squids, cuttlefish, and octopus) have the most sophisticated central nervous system among invertebrates, and it is conceivable that cephalopods can develop some forms of cognition. Although it has been suggested that octopuses equip the capacity of observational learning, a previous study indicates that cuttlefish do not improve their predation tactics by observing conspecifics. Given that the danger avoidance is important for animal's survival, we sought to reevaluate whether cuttlefish show some form of observational learning under threatening conditions. Cuttlefish (*Sepia pharaonis*) were divided into two groups, the Self-Experience Group and the Observe-Other Group. In the training phase, a toy submarine was remotely controlled to expel the cuttlefish at its innately preferred place to establish the threat-place association in the Self-Experience Group. In the Observe-Other Group, the threat-place association was established by expelling the conspecific demonstrator at the observer's innately preferred place while the observer watched the whole process behind a transparent divider. In the testing phase, the choice of safe place in the absence of threat was used to probe the learning of cuttlefish in both groups. In the Self-Experience Group, we found that most animals chose the safe side more often than their innately preferred side, an indication of the association learning. However, in the Observe-Other Group, only a subset of animals showed this threat-place

association by observation. These results indicate that not all cuttlefish can learn by observing others, but individual difference exists. Nevertheless, this suggests that observational learning is possible in cuttlefish.

INVESTIGATION ON TROPHIC INTERACTIONS OF *Octopus vulgaris* (CUVIER, 1795) IN THE MOROCCAN ATLANTIC COAST. F. H. Idrissi[1], A. Berraho[2], N. Charouki[1], O. Ettahiri[2]. [1]Institut National de Recherche Halieutique (INRH), Département Halieutique, 2, rue Tiznit, Casablanca 20000, Morocco; [2]Institut National de Ressource Halieutique (INRH), Département Océanographie, Casablanca 20000, Morocco.

E-mail: idrissi_farah@yahoo.fr

Studies of the biological interactions of marine species are a fundamental aspect which permits to determine their importance in the trophic network. This study is dealing with feeding of *Octopus vulgaris* as a key species in the Moroccan Atlantic ecosystem. The feeding strategies are defined for octopus in the two zones, north and south of Boujdor (26°N), which are usually considered as different stocks and harvested by two different exploitation modes. By analyzing stomacal contents collected during the period 2001 to 2003, we estimated for the two populations some trophic indices, in occurrence the relative importance index (IRI), the trophic level (TL) and the omnivory index (IO). We conducted then statistical analysis to compare the trophic strategies of the two populations according to different biological parameters.

EMBRYONIC DEVELOPMENT OF THE CENTRAL NERVOUS SYSTEM IN *Euprymna scolopes* (CEPHALOPODA: SEPIOLIDAE). A. Kerbl. Department of Theoretical Biology—Morphology Section, University of Vienna, Althanstraße 14, 1090 Vienna, Austria.

E-mail: alexandra.kerbl@gmx.at

The nervous system in cephalopods has been a topic of interest for a long time in very different fields of biology, for example in neurobiology or in behavioural biology. In adults, the closely congregated ganglia form different lobes, as the original ganglia fuse to one mass. The pairs of cerebral, pedal and palliovisceral ganglia as well as the optic lobes form most of the central nervous system. The two stellar ganglia and the gastral ganglion are connected with the palliovisceral ganglion. The two components of the buccal ganglion are innerved by the cerebral ganglion. For *Loligo*, *Sepia*, *Vampyroteuthis* and *Nautilus*, some studies have drawn exact images of the "brains", detecting the different lobes and paying attention to their connections. The small squid *Euprymna scolopes* Berry 1913, would be an ideal candidate for an model organism due to its use in symbiosis research, but so far no studies have been carried out about the internal organization. Since genetic experiments have been conducted in *E. scolopes* investigating different parts of the nervous system, a histological background of this organ system is crucially needed. Using x-ray microtomography, the brain of eight developmental stages is analysed and described. The achieved data were used for volumetric calculations as well as computer-assisted 3D-reconstructions, which led to a combination of surface renderings and manual segmentation-based surface renderings. Ganglia are formed quite early in development and are preliminary to the formation of sensory organs like eyes and statocysts. In *E. scolopes*, the central ganglia (cerebral, pedal and palliovisceral ganglia) are present in an early stage (23) and already accumulated around the oesophagus. The optic lobes, lateral excrescences of the cerebral ganglion connected with the eyes, quickly become the most prominent parts of the ganglia system. The overall formation pattern appears to be largely similar among cephalopod species, although the temporal succession of individual processes such as ganglionic accumulation and lobe differentiation may differ. The ganglionic layouts in decabrachiate cephalopods are quite similar, whereas octobrachiate species differ somewhat, such as in the size of the optic lobes. In an early stage, the decabrachiate brain more closely resembles the nautiloid adult brain than the coleoid one. Therefore, investigating brain development in coleoid cephalopods such as *Euprymna* contributes to our knowledge about the ancestral conditions in this group and will answer questions about the development of complex brains.

HAEMOCYTE CHARACTERIZATION IN *Sepia officinalis* L. (MOLLUSCA CEPHALOPODA). C. Le Pabic, G. Safi, A. Serpentini, J.-M. Lebel, N. Koueta. Marine Molluscs Physiology and Ecophysiology Laboratory, University of Caen Basse-Normandie, 14032 Caen, France.

E-mail: charloox@yahoo.fr

Europe is a heavily urbanized area. The anthropogenic activities impact continental and coastal waters. In order to preserve the natural environment and manage its resources, it is important to evaluate the effect(s) of anthropic pollutants on live organisms. The European Common Cuttlefish *Sepia officinalis* is a growing resource in the seafood industry and its seasonal migrations in the English Channel are well documented. In the spring, adult cuttlefish migrate onshore to reproduce. After eggs hatching, juveniles stay in the coastal area for a few months where they benefit from warmer temperatures and large amounts of food. Consequently, it is during these first months that cuttlefish individuals are subjected to anthropic pollutants. As part of the European program CHRONEXPO, our studies focus on the impact of heavy-metal chronic exposure on the immune system of *S. officinalis*.

Consequently, the initial stage of this project consisted of characterizing the haemocytes of *S. officinalis* as this cell population plays a key role in the invertebrate immune response.

Haemocyte characterization was first undertaken using cytological staining, electron microscopy and flow cytometry. Primary culture trials were conducted to determine optimal maintenance conditions so that the impact of heavy-metals on haemocyte biology can later by assayed in vitro. Our first observations revealed a single haemocyte type characterized by a lobate nucleus and a high density of organelles and eosinophilic granules in the cytoplasm. Flow cytometer analyses did not isolate distinct sub-populations. Our in vitro approach was conducted using haemolymph from cuttlefish branchial hearts and a maintenance time of 10 (± 2) days in primary cell culture was observed. Notably, two distinctive adherent cell aspects were observed in culture: spread cells with long pseudopodia (fibroblast-like) and round non-spread cells (epithelial-like). The good survival rate of cuttlefish haemocytes over several days should allow to successfully studying the effects of anthropic pollutant chronic exposure on this type of cells.

EARLY VISUAL EXPERIENCE OF BACKGROUND CONTRAST AFFECTS THE EXPRESSION OF NMDA-LIKE GLUTAMATE RECEPTORS IN THE OPTIC LOBE OF CUTTLEFISH, *Sepia pharaonis*. **Y.-H. Lee[1], Y.-C. Chang[2], C.-F. Chuang[1], H. Y. Yan[3], C.-C. Chiao[1,4].** [1]Institute of Molecular Medicine, National Tsing Hua University, Taiwan; [2]Department of Optometry, Yuanpei University, Taiwan; [3]Institute of Cellular and Organismic Biology, Academia Sinica, Taiwan; [4]Institute of Systems Neuroscience, National Tsing Hua University, Taiwan.

E-mail: leeyihsin@gmail.com

Visual experience is an important factor in development of visual systems. Previous studies have demonstrated that dark rearing could affect the maturation of retina and visual cortex in several animals. It has also been shown that the expression of N-methyl-D-aspartate (NMDA) receptors was altered in the dark-reared animals. However, most of these studies lack behavioural correlates in response to the altered visual system development. Our previous studies showed that rearing background contrast influences the development of camouflage patterning and substrate preference in cuttlefish. In the present study, we aimed to examine if the expression of NMDA receptors in the optic lobe is affected by different rearing environments in *Sepia pharaonis*. The hatchlings were kept in two different rearing backgrounds (high and low visual contrast groups). In 4, 8 and 12 weeks, the spatial distribution and total expression of NMDA receptor NR2A subunit (an antibody that recognizes mammalian NMDAR2A subunit) in the optic lobe of both groups were examined. In the immunohistochemical experiments, the NR2A subunit immunostaining signal could be found in the granule layer and plexiform layers of the cortex as well as the soma and neuropile layers of the medulla area. In Western blots, the expression of the NR2A subunit in the low contrast group appeared to be stronger than that in the high contrast group in Week 4, but reversed in Weeks 8 and 12. This study indicates that the expression of NMDA-like receptors in the optic lobe is dependent of rearing background contrast, and the first two months are the most critical in development. This result provides a good correlate between the expression of NMDA-like receptors and its corresponding behavioural maturation in cuttlefish.

EFFECTS OF ENVIRONMENTAL FACTORS ON THE PRE-MATURE HATCHLING OF *Sepia pharaonis*. **C.-Y. Lin[1], Y.-H. Chen[2], I.-M. Chen[1,3].** [1]Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Taiwan; [2]National Museum of Marine Biology & Aquarium, Taiwan; [3]General education Center, Wenzao Ursuline College Languages, Taiwan.

E-mail: cephalopodnick@gmail.com

The cuttlefish *Sepia pharaonis*, a commercially important demersal trawl fishery species, distributes widely in the coastal waters of Taiwan. In term of morphology, the embryonic developments of *Sepia pharaonis* can be divided into 40 different stages. It is commonly found that some embryos hatched before completely depleting the associated yolks at the later developing stages. It is still not well understood that the survival rate of pre-mature hatchlings, and what and how environmental factors were involved in inducing such processes. The eggs were hatched naturally or artificially at different developing stages and the survival rate of hatchling at each test were compared consequently. The effects of different environmental factors on egg hatchings at different developing stages were examined as well. The studies shows that the hatchling artificially hatched at later developmental stages survived longer than those hatched at earlier stages. The feeding rates of those hatchlings artificially hatched after the 36th stage increased along with the developmental stages. The limits of temperatures on the embryonic development of *Sepia pharaonis* were not lower than 10° or higher than 35°, respectively. When the temperature were suddenly changed, the increases in temperature can more effectively induce the pre-mature hatching than did the decreases in temperature. The hatchings of pre-matured hatchlings were induced by either increasing or decreasing temperature for more than 10°C. Eggs were not hatched when the salinities were lower than 10 psu and some embryos cannot survive under the salinity lower than 20 psu. The pre-mature hatchings were induced when the salinities were suddenly declined from 33 to 10 psu. In order to get away from hostile environmental conditions, those embryos at 39th stage have a relatively higher percentage in pre-mature hatching than those at 36th stages which continued to grow until suitable conditions to hatch or die as unhatched eggs.

SPATIAL STRUCTURE OF THE METAPOPULATION OF CHOKKA SQUID *Loligo reynaudi* D'ORB., 1848 IN SOUTHERN AFRICAN WATERS. M. R. Lipinski, T. Stonier, W. H. H. Sauer, P. Shaw, D. Yemane. Fisheries Research, Department of Agriculture, Forestry and Fisheries, Cape Town, South Africa.

E-mail: mrlipinski@nashuaisp.co.za

The life cycle of *Loligo reynaudi* was in dispute. Published and accepted view held that this species spawns along the east coast of South Africa (between Plettenberg Bay and Port Alfred). The paralarvae are drifting then westward as they grow and juveniles & young adults feed on the Agulhas Bank. They then return to their original spawning grounds and the cycle repeats itself. Minor spawning grounds inshore westward from Plettenberg Bay and deepwater spawning were considered marginal. Eastward (spawning) migration was considered as a replenishment of the eastern fishing grounds. Management of the fishery however, was done on the assumption that eastern part of the resource is a ''black box'', with no input or output from/to outside.

Results of the DNA microsatellite work and morphometrics done along the distribution range of the species (presented in this study) helped to revise the old view. Significant differences were found between Angolan samples, samples collected on a greater Agulhas Bank, samples collected on the east coast (spawning grounds) and eastward tip of the range. Significance of the differences increased along the spatial range (distance-related). As a result of this, new (more fragmented and area-related) life cycle scheme for the species was proposed. Validity of morphological (phenotypic) analysis for the loliginid squid was confirmed as they naturally mimic the genetic differences.

REVISED EU LEGISLATION ON THE PROTECTION OF ANIMALS USED FOR SCIENTIFIC PURPOSES DIRECTIVE 2010/63/EU. S. Louhimies. The European Commission, Directorate-General for the Environment, Brussels, Belgium.

E-mail: susanna.louhimies@ec.europa.eu

Current scientific knowledge does not yet allow us to replace the use of animals for scientific purposes, which is our ultimate goal. Therefore, the EU has recently strengthened the legislation in this field. Directive 2010/63/EU on the protection of animals used for scientific purposes entered into force in November 2010. It revises and replaces the previous Directive which dated from 1986 (86/609/EEC). Member States have until November 2012 to align their respective national legislation towards achieving the objectives set in the Directive. It will take full effect on 1 January 2013.

The new Directive aims to ensure that the animals still used for scientific purposes receive appropriate care and humane treatment in line with Article 13 of the Treaty on the Functioning of the European Union. The scope of the new Directive is enlarged to cover animals used in basic research and in education and training. Cephalopods as well as foetal forms of mammalian species from the last third of their development are also covered by the new Directive. It tightens controls on animal use by introducing inter alia compulsory authorisation with an ethical evaluation for all projects using animals, more frequent risk based inspections and improved transparency. In addition, it sets compulsory standards for the housing and care of animals and introduces requirements for the education, training and competence of the personnel.

The principle of the Three Rs—the replacement, reduction and refinement of the use of animals in experiments—is firmly anchored in the new Directive. The Three Rs must be taken into account fully during all aspects of animal use and care. With the revision, the EU can claim the highest standards of experimental animal welfare in the world whilst promoting high quality, competitive science and research in Europe.

EFFECTS OF OCEAN ACIDIFICATION ON STATOLITH CALCIFICATION AND PREY CAPTURE IN EARLY LIFE CUTTLEFISH, *Sepia officinalis*. R. H. Maneja, U. Piatkowski, F. Melzner. IFM-GEOMAR, Leibniz-Institute of Marine Sciences, Duesternbrooker Weg 20, 24105 Kiel, Germany.

E-mail: upiatkowski@ifm-geomar.de

The influence of elevated seawater pCO2 on statolith calcification and prey capture was investigated in the early life stages of the common cuttlefish, *Sepia officinalis*. Cuttlefish were reared at 15°C and 35 psu in a flow-through seawater system under three pCO2 conditions, 700 µatm (control), 1400 µatm, and 4000 µatm during 63 days in June to August 2009. Both, embryonic and hatchling cuttlefish raised under 4000 µatm showed significantly reduced statolith calcification, whereas those grown under control and 1400 µatm did not. Reduced calcification was demonstrated by comparing 18 transects characterizing the anterior surface of the statoliths. The statolith morphometrics that showed the most remarkable changes between the different pCO2 conditions were total statolith length, rostrum transects, wing area and statolith weight. Statolith microstructure was significantly affected by irregularly arranged statoconia, which were typical in the statolith wing area, replacing the highly compact and well-arranged crystals in normal growing statoliths. This abnormal crystal structure can have profound effects on statolith density and consequently on its normal functioning as a tool for buoyancy, acceleration and movement. Changes in statolith morphology and microstructure may influence the prey capture efficiency of the early life cuttlefish. At 4000 µatm they showed a reduced ability to capture prey and were not able to successfully launch attacks against prey organisms. In order to verify these observations, a second experiment was conducted over 85 days in May to August 2010. Preliminary results showed that statolith morphology and microstructure differed again in the 4000 µatm group. On the other hand, prey capture ability of the hatchlings showed recovery during the experiment, indicating a possible acclimation.

A BIOENGINEERING APPROACH FOR IN VIVO MEASUREMENTS OF THE OCTOPUS ARMS. L. Margheri[1], B. Mazzolai[2], P. Dario[1,2], C. Laschi[1]. [1]The BioRobotics Institute, Scuola Superiore Sant'Anna (SSSA), Pontedera (Pisa), 56025, Italy; [2]Center for MicroBioRobotics@SSSA, Istituto Italiano di Tecnologia (IIT), Pontedera (Pisa), 56025; Italy.

E-mail: l.margheri@sssup.it

Octopuses are particularly interesting as ''animal models'' for innovative research in biorobotics science. Specifically, the octopus arms are totally soft structures with unique biomechanical characteristics and capabilities in term of compliance, stiffness control and dexterity. Bioengineering approach is here applied as method for a quantitative study of the mechanical performance of the octopus arms, as well as for translating their biomechanical principles into advantageous design concepts in soft robotics. To measure the octopus arm, we developed targeted setups to get in-vivo, non-invasive, and direct measurements of one arm at a time, based on a graduated transparent Plexiglas tube, joint to a supporting plate. The tool can be adapted to different purposes, by integrating sensors and mechanical components. A bait inside the tube is used to encourage the animal to insert one arm inside, and it is moved up for elongation measurements, attached to a load cell with a high resistance thread to measure isometric pulling force, or to a spring-sensor system to measure shortening and stiffening. To compare different arms and animals in vivo, we measured the length of the arms analyzing the backward movement by jet propulsion, where arms are trailed in line behind. We measured an average elongation of the arms of 70% (102 elongations, 24 *O. vulgaris*, 16 males, 8 females, Dorsal Mantle Length range: 72.5÷125.5mm), which corresponds to only a 23% of diameter reduction in a constant volume cone model, useful as advantageous mechanism. The isometric pulling force capability has been measured along the arm by changing the bait position during different trials. Results (928 measurements) showed that the whole arm has a ''fiber muscle-like'' trend, with a maximum of the isometric force in correspondence to an arm length close to the arm reference length, whereas decreases when the arm is too shortened or too elongated. We also recorded octopuses grasping the bait in the same position along the arm, making use of the distal quarter to grasp the bait and the proximal ¾ to exert the force. We measured a time of contraction to exert a peak of force of 1-2 sec, and fixed a mean force of 40 N for an arm length of 400mm as specifications for the longitudinal muscle-like actuators. By adding a spring to the load cell, we evaluated the relationship between the stress in the body (applied force measured with the load cell) and its change in length (through video analysis), obtaining the characterization of arm shortening and longitudinal stiffening. Data (559 measures) show that octopus shortens its arms of an amount of 20%, with a maximum strain of 50%, and with a mean strain rate of 17.1mm/s. The analysis of the results allowed deriving novel design concepts for soft robotics. Particularly, a totally soft arm prototype (silicone and cables driven), has been built using biomimetic approach. Experimental results bring evidence of the capability of the prototype to attain the same performances of the biological model. Besides, these bioengineering methods offer novel measuring instruments to the biological research, providing quantitative data and biomechanical information about the biological system, thus integrating knowledge from science and engineering.

NITRIC OXIDE DURING *Sepia officinalis* DEVELOPMENT. T. Mattiello[1], M. Costantini[1], A. Andouche[2], L. Bonnaud[2,3], A. Palumbo[1]. [1]Laboratory of Cellular and Developmental Biology, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121, Napoli, Italy; [2]Laboratory Biologie des Organismes Aquatiques et Ecosystèmes, UMR CNRS 7208 and Museum National d'Histoire Naturelle, DMPA, Paris, France; [3]University of Paris Diderot, Paris, France.

E-mail: matti@szn.it

Nitric oxide (NO) is an important physiological messenger involved in invertebrates in many processes, such as feeding, environmental stress, defence, blood sucking, bioluminescence, neural transmission, blood pressure regulation, immune response, swimming and regeneration processes. Increasing evidence reported in the literature indicate that its role is not limited to adults. Regarding molluscs, NO regulates feeding activity, locomotion and heartbeat during embryonic development of the pond snail *Lymnaea stagnalis*. In *Helisoma trivolvis* the gas stimulates ciliary beating of embryos. Moreover, NO is involved at different levels of the biological cycle, from fertilization to metamorphosis, in sea urchins, ascidians and some molluscs. In particular, NO inhibits metamorphosis in some snails. No informations are available on cephalopod molluscs that are characterized by a direct development without any metamorphosis phase. Among these, the cuttlefish *Sepia officinalis* is emerging as a new model for developmental studies for its biological features, wide natural distribution, commercial value and increasing availability of molecular biology tools. We report herein the presence of NO in embryos by detecting NO and nitric oxide synthase (NOS), the enzyme responsible of its formation, and by monitoring enzyme transcript levels during the various developmental stages. The spatial patterns of NO as well as of NOS is very dynamic and concerns superficial tissues as well as internal tissues and organs. In particular, its presence in some neural and epithelial tissues, as well in sensory cells at certain stages suggests the involvement of the gas in the first step of neural differentiation and in sensory cells maintaining. At later stages of development also gills and kidney are positive indicating a possible role of NO in the functioning of these organs. Temporal expression of NOS during development, followed by real-time PCR, shows that expression significantly changes during development and reaches maximum values at stage 26. These results provide the basis for understanding the role of NO in fundamental processes during *Sepia* development.

OCTOPUS AS SOURCE OF INSPIRATION IN ROBOTICS. **B. Mazzolai[1] and C. Laschi[1,2].** [1]Center for

E-mail: MicroBioRobotics@SSSA, Istituto Italiano di Tecnologia (IIT), Pontedera (Pisa), 56025; Italy; [2]The BioRobotics Institute, Scuola Superiore Sant'Anna (SSSA), Pontedera (Pisa), 56025, Italy.

E-mail: barbara.mazzolai@iit.it

Robotics is traditionally based on the concept of rigid links connected by joints, and robot control theories exploit geometrical parameters of the robot structure that are not supposed to change in time. To negotiate natural environments, robots need more flexibility and adaptability. Biological systems become a rich source of inspiration for robotics, especially for the compliance present in the physical structure of most animals and for the distribution of control between neural computation and morphology, called Embodied Intelligence [1]. In this context, soft robotics is an emergent field in robotics science and engineering, where biology is a source of inspiration for robotics but, on the other hand, robotics provides new tools for investigation in robotics [2][3].

The octopus represents an ideal source of inspiration for robotics, for the virtually infinite number of degrees of freedom and for its capability of controlling the stiffness, i.e. stiffening its arms for grasping or other tasks requiring the application of forces [4][5]. Furthermore, the octopus can be seen as an example of embodied intelligence: a biological demonstration of how effective behavior is tightly related to the morphology of the body. Our objective is investigating and understanding the principles that give rise to the octopus sensory-motor capabilities and at incorporating them in new design approaches and robotics technologies to build a robotic system, based broadly on the anatomy of the 8-arm body of an octopus, and with similar performance in water, in terms of dexterity, speed, control, flexibility, and applicability.

A bioengineering approach is applied for a quantitative biological study, i.e. for the direct measurement and characterization of the mechanical properties and anatomy of the octopus arms [6], and for the translation of the biological principles into robotics design concepts [7]. Results have been obtained on the arm elongation and force applied by one arm.

New technologies have been developed from the study of the octopus features and capabilities, which concern actuation (soft actuators) [8], sensing (distributed flexible tactile sensors), control and robot architectures, mechanisms (soft-bodied hydrostat structures), kinematics models. A prototype of octopus-like robot arm has been developed [9], composed of siliconic materials and SMA actuators.

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STOCK ASSESSMENT OF THE COMMON CUTTLEFISH, *Sepia officinalis* IN THE SOUTHEASTERN MEDITERRANEAN, EGYPT. S. F. Mehanna[1], H. M. Haggag[2]. [1]National Institute of Oceanography and Fisheries, POB 182, Suez, Egypt; [2]General Authority for Fish Resources Development, Port Said Branch, Egypt.

E-mail: sahar_mehanna@yahoo.com

The common cuttlefish (*Sepia officinalis*) is an important commercial resource and one of the most appreciated cephalopod in the Egyptian Mediterranean waters. It is a target species for the trawl fishery in Port Said fishing ground contributing about 10% of the total annual landings. The stock of *S. officinalis* in the southeastern Mediterranean (Port Said waters) has been assessed using a three years length frequency data (2008–2010) collected monthly from the trawl fishery at the fishing harbor of Port Said city. By applying the Y/R analysis on the pseudo-cohort 2008–2010 using the VIT program, it is noticed that this stock is over exploited, being the fishery operating below the optimal yield level since the current Y/R is lower than the maximum. Results suggest a decreasing trend in the average fishing mortality along the studied period by about 30–40%.

LOOKING BACK OVER CEPHALOPOD RESEARCH: WHAT HAVE WE LEARNED SO FAR? J. B. Messenger. Univ Cambridge, Dept Zool, Cambridge CB2 3EJ, UK.

E-mail: jbm33@cam.ac.uk

As we look back at research into cephalopods in the twentieth century we can recognise one line of inquiry that was patently 'successful' in that it led to discoveries fundamental to biology (and incidentally to a Nobel Prize). However another promising line of research could be deemed a failure, judged in the competitive culture of market-place science: years of research on the octopus did *not* reveal the physical basis of learning and memory in animals. Yet on the way to 'failure' we uncovered the remarkable visual and touch learning capabilities of octopuses, the fact that they can exhibit spatial and social learning and that they show some of the higher cognitive abilities usually associated with vertebrates.

But why are we studying cephalopods? Are we using them merely as models for research into problems of general biological interest? Or are we interested in them for their own sakes? Might not studying these fascinating and beautiful animals for themselves not only be worthwhile, but incidentally yield unexpectedly rewarding results?

TAXONOMIC STATUS OF *Rossia palpebrosa* OWEN, 1834 AND *R. glaucopis* LOVEN, 1846 (CEPHALOPODA: SEPIOLIDA) ON MOLECULAR-GENETIC DATA. A. R. Morov[1], A. V. Golikov[1], R. M. Sabirov[1], P. A. Lubin[2], A. A. Rizvanov[1], M. Sugimoto[3]. [1]Kazan Federal University, Kazan, Russia; [2]Polar Research Institute, Murmansk, Russia; [3]Okayama University, Kurashiki, Japan.

E-mail: morov_ars88@mail.ru

Rossia palpebrosa is one of the numerous species of cephalopods living in the Barents Sea. The species is widespread from the Canadian Arctic archipelago to the Kara Sea. According to K. N. Nesis (2003) *R. palpebrosa* is a circumpolar species. It can be found everywhere in the Barents Sea but for the south-eastern part with reduced salinity. In literature it's mentioned a similar species—*R. glaucopis*, that distribution is sympatric to *R. palpebrosa*. But *R. glaucopis* mainly occurs in the southern parts of area. The key morphological differences: suckers in proximal parts of *R. palpebrosa* arms are rearranged into 4 rows, papillae on the body are appreciable; the 4th row location of *R.glaucopis* suckers isn't clear, papillae are inconspicuous. But these features are variable. Data on taxonomy of *R. palpebrosa* and *R. glaucopis* are inconsistent. Some authors recognize a validity of both species (Muus, 1959; Frandsen, Zumholtz, 2004); others specify monotypic species *R. palpebrosa*, whereas *R. glaucopis* as its synonym (Filippova et al., 1997; Kantor, Sisoev, 2005; Gardiner, Dick, 2010). According to another point of view *R. glaucopis* is a bipolar species and occurs at the Patagonia coast (Jereb, Roper, 2005; Ibanez et al., 2009). Grimpe G. (1925) rejects it and considers this sepiolid could be confused with species of genus *Semirossia*. All species of sepiolids from Arctic regions and northeast Atlantic can be attributed to morphs of polytypic species *R. macrosoma* Delle Chiaie, 1830 (Costello et. al., 2004). In this study we conducted molecular-genetic analysis of sepiolids determined morphologically as *R. palpebrosa* and *R. glaucopis* for updating their taxonomy. Sepiolids were collected in 2008. 29 specimens as *R. palpebrosa* and 31 specimens as *R. glaucopis* were determined. Total DNA was isolated from arm tissues. 600 bp fragment of the 18S rRNA gene and 700 bp fragment of the mitochondrial cytochrome c oxidase (COI) gene were sequenced for both species. The comparative study revealed 93% homology with COI genes and 99% homology with 18S rRNA genes of arctic sepiolid *R. palpebrosa* in NCBI (National Centre of Biotechnology Information) database (accession numbers—AF000061.1 and AY557473.1 accordingly). The analysis of 18S rRNA and COI genes sequences showed their 100% identity. Phylogenetic tree constructions demonstrated the identical position of two sepiolids. Therefore, the molecular-genetic data show an accessory of both sepiolids to one species, which is *R. palpebrosa* according to a priority principle. *R. glaucopis* should be a subordinary synonym. On the basis of morphological and molecular-genetic data *R. palpebrosa* is a polytypic species, and it's presented by two ecological morphs: arctic «*palpebrosa*» and subarctic «*glaucopis*».

GENOMICS AND NEUROGENOMICS OF CEPHALOPODS: FROM GENES TO BEHAVIOR. L. L. Moroz[1,2], M.Citarella[1], F. Yu[1], C. Di Cristo[3], J. P. H. Burbach[4,5], A. Di Cosmo[3], K. Kokot[6], K. Halanych[4], A. B. Kohn[1]. [1]Neuroscience and [2]Genomic Institute, Univ of Florida, FL 32080, USA; [3]Univ of Naples, 80126 Napoli, Italy; [4]Univ Medical Center 3584 CG Utrecht, the Netherlands; [5]MBL, 5Woods Hole, MA, USA; [6]Univ of Alabama, AL 36849, USA.

E-mail: moroz@whitney.ufl.edu

In spite of the enormous diversity of molluscs and their importance for fundamental biomedical research, the genomic information from any representative of this phylum is very limited. Recently we, together with our collaborators (7 laboratories in the US and Europe), have initiated several large-scale molluscan transcriptome and three genome projects to support our physiological and evolutionary research. The 18 species selected represent all major branches of the molluscan evolutionary tree. This includes (i) sequencing the *Aplysia* genome, (ii) sequencing five cephalopod (*Nautilus*, 3 species of *Loligo*, *Octopus*) neuronal transcriptomes, and (iii) sequencing two cephalopod genomes using next generation technologies. As a result, we generated >40 Gb of sequencing information and established web-supported resources with novel and annotated genes and transcripts. In this talk I will describe the progress we have made focusing on cephalopod genomics. First, using phylogenomic approaches we were able to reconstruct deep molluscan phylogeny and reveal relationships among major molluscan classes. The obtained phylogenetic tree does not match with classical zoological classification

within the phylum of molluscs, suggesting polyphyly of selected lineages (including opisthobranchs) and an unexpected position for cephalopods as one of the basal molluscan branches (Kocot et al., 2011). An important corollary of the study is confirmation of our previous hypothesis (Moroz, 2009) that neuronal centralization leading to complex brains independently evolved at least 3-4 times among molluscan lineages. Surprisingly, we also demonstrated an enormous degree of parallel evolution in formation of shells and calcification in mollusks (Kocot et al., 2011). Second, we identified key developmental and neurogenic genes as well as more than 2,000 components of intracellular signaling in cephalopods. Specific attention in the presentation will be given to neuropeptides and protein hormones, known as ancient signaling molecules involved in virtually every activity of neural circuits and plasticity. 98 identified *Aplysia* neuropeptide precursors were used as an initial reference platform in this analysis. Here, using deep transcriptome profiling (454-Roche/Illumina sequencing) from CNSs and peripheral tissues of selected gastropod and cephalopod species we were able to identify more than 70 *Aplysia* prohormone homologs including a nociceptive class of peptides. These are evolutionarily conserved precursors for multiple classes of secretory peptides in molluscs, including many in the cephalopod lineage. This subset of molecular markers can be used to identify homologous neuronal populations across the major molluscan classes. Yet, our comparative analysis indicated that neuropeptides are amongst the fastest evolving group of intercellular signals, and cephalopods developed a number of lineage specific innovations in the repertoire of secretory molecules that might support their unique features and specification. Finally, I would like to discuss emerging opportunities to establish a cephalopod sequencing consortium that will target the completion of 4-6 novel genomes from representatives of the key phyletic branches within this class of Mollusca (including *Nautilus, Octopus, Sepia, Loligo*). In summary, combined with comparative and proteomic data from molluscs and other phyla, this genomic survey provides unique opportunities to reconstruct ancestral neuronal lineages, identify cell homologies across species and reveal trends in evolution within neural circuits.

MOLECULAR INSIGHTS IN THE RISE OF CEPHALOPOD ORGANIZATION: NEURONAL TRANSCRIPTOME IN *Nautilus*. **L.L. Moroz[1,2], A.B. Kohn[2], M. Citarella[2].** [1]Dept of Neuroscience, University of Florida, Gainesville, FL, USA; [2]The Whitney Laboratory for Marine Bioscience, St. Augustine, FL, USA.

E-mail: moroz@whitney.ufl.edu

Nautilus is a representative of one of the most ancestral lineages in Cephalopods. Here, using 454 pyrosequencencing we performed deep RNA-seq analysis of neuronal transcripts from 10 different regions of the *Nautilus* CNS, retina and chemosensory organs. It is estimated that we recovered more than 60–70% of all neuronal transcripts representing expression of at least 10,000 genes from this species. In this presentation, I will outline the identification of major classes of coding and non-coding RNAs in *Nautilus* and characterize their homologs in related cephalopods and other molluscs. As a result, the obtained genomic resources from this "living fossil" allow us to reconstruct a subset of evolutionarily conserved genes and transcripts in the phylum Mollusca. Moreover, we also found a number of novel genes and miRNAs that might present lineage specific innovations for this group of animals. Combined with comparative genomic data from other molluscs, this genome-wide survey provides an unbiased platform to reconstruct ancestral molecular toolkits underlying the very origin of the cephalopod organization in general, and evolution of the unique neuronal organization within this lineage in particular. I will present illustrated examples of genes and gene regulatory components that might be critically important for the rise and fall of various clades over half a billion years of cephalopod evolution.

CUTTLEFISH VISION IN CAMOUFLAGE. **D. Osorio[1] and S. Zylinski[1,2].** [1]School of Life Sciences, University of Sussex, UK; [2]Department of Biology, Duke University, UK.

E-mail: drcosorio@gmail.com

The huge flexibility of cuttlefish coloration offers a unique opportunity to study vision in an animal without language. This visual behaviour would be no use without commensurately sophisticated visual abilities, for example to characterise visual backgrounds to select the appropriate camouflage pattern. In contrast to most behavioural tests, which involve binary decisions, cuttlefish are able to independently control the expression of multiple behavioural components (or to mix body patterns). One can record responses on multiple behavioural dimensions to investigate how the animals integrate multiple cues to select coloration for particular background or behavioural context. We examine how the animals identify visual backgrounds as being made of discrete objects such as pebbles as opposed to a continuous patterned surface. This simple distinction seems to be an important factor in the choice of camouflage, and reveals insights into the classic visual problems of edge detection and figure-ground segregation. We find that cuttlefish like humans use multiple cues for these tasks including visual contrast, depth and texture differences.

THE GENTLE ART: PHYSIOLOGICAL FINDINGS THROUGH COOPERATION WITH THE LIVING ANIMAL. **A. Packard.** Stazione Zoologica Anton Dohrn, Villa Comunale, 80121, Naples, Italy *(retired)*.

E-mail: andrew@packards.de

In this talk, I give examples of fundamental physiological findings obtained using participant-observer procedures that have not, to my knowledge, been followed up in other laboratories or by other groups working with living cephalopods.

1) Lateral inhibition during morphogenesis of the chromatophore system

2) Patterns of neuro-muscular generation and regeneration in *Octopus vulgaris*
3) Nerve sprouting and midline questions (*Loligo vulgaris*)
4) Patterns of intercellular communication
5) Effects of anaesthetics on myogenic activity (*O. vulgaris*, *L. vulgaris*, *Sepia officinalis*)

All procedures are simple. Examples 2), 3) and 4) involve long-term unilateral denervation of the mantle. Surgery, imaging and pre- and post-operative handling methods will be described. Basics required of the experimenter: a) to know the ontogenetic history of the individual, b) to cooperate closely with individual animals over extended time intervals, c) to use the individual as its own control without sacrificing the animal.

I have referred to the method as ontophysiology (see http://gilly.stanford.edu/packard.html).

For discussion:

- what are the basic rules of ontophysiology?
- practical issues
- can the holistic approach be taught and how?
- should one be thinking in terms of intersubjectivity [between experimenter and experimental subject]?

A COMPARATIVE ANALYSIS OF DIFFERENT ANAESTHETICS IN OCTOPUS: TOWARDS TRUE ANESTHESIA? **E. Pagano[1+], G. Ponte[1+], P. L. R. Andrews[2], G. Fiorito[1].** [1]Octopus Behavioral Biology, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy; [2]Division of Biomedical Sciences, St George's University of London, Cranmer Terrace, London, SW17 0RE, UK. + *these Authors mutually contributed to this work*.

E-mail: giovanna.ponte@szn.it

In the common practice, anesthesia is achieved in cephalopods exclusively by immersion of specimens in a liquid: i.e. anesthesia by immersion. Various substances have been utilized: urethane, ethyl alcohol, chloral hydrate, magnesium chloride, phenoxyethanol, clove oil. In addition, by lowering the temperature of the seawater induce sedation and/or anesthesia in octopus. However, no-one of the substances utilized so far have met the requirements of an "ideal" anesthetic agent to be utilized during surgery and/or physiological investigations. To achieve this goal, they should, for example, be: non irritant; induce rapid onset stable general anaesthesia; not stop breathing or lower blood pressure; allow rapid recovery of normal behaviour (especially feeding) but with good analgesia. In addition a pharmacological study on the effects of various chemicals, above mentioned, is lacking in cephalopods.

Here we will review a series of experiments carried out at the Stazione Zoologica by using different anesthetic agents and explore their effects on the animal behavioral responses during induction and recovery. In addition we present a standardized protocol that could be used to compare the effects of anaesthetics by using a series of systematic tests that will monitor responses of octopuses to various stimuli and to assess any form of response to pain and distress. This also with the aim to explore possible sites of action of a given anaesthetic agent.

Finally, results of some preliminary trials using different substances widely utilized in other species will be presented.

REGULATORY ROLES OF NITRIC OXIDE IN *Sepia officinalis*: AN OVERVIEW. **A. Palumbo.** Laboratory of Cellular and Developmental Biology, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121, Naples, Italy.

E-mail: palumbo@szn.it

Over the past two decades, nitric oxide (NO) has emerged as an important physiological messenger distributed throughout the phylogenetic scale from invertebrates to mammals. In the course of our continuing studies on cephalopod biology we discovered that NO is involved at different levels in the defence system of *Sepia officinalis*. In particular, NO regulates the metabolic activity of the ink gland which is deputed to the continuous production of the black insoluble melanin that the animal accumulates in the ink sac. Biochemical experiments revealed that endogenously produced NO by NO synthase (NOS) activates tyrosinase leading to increased melanin production, and enhances secretion of ink constituents from the mature cells into the lumen. Recently, we found that NO is also produced in the skin at the level of chromatophores, the complex structures responsible of the variety of body coloration patterns. NO and NOS are detectable in pigment and muscle fibers in embryo, juvenile and adult chromatophore organs. Pharmacological bioassays and NO production monitoring on adult and isolated juvenile chromatophores revealed that NO induces chromatophore expansion at a slower rate than glutamate through the cGMP/cADP-ribose/ryanodine receptor signaling pathway, acting as an important messenger in the long term maintenance of the body coloration patterns. Finally, ongoing studies are disclosing NO production during *Sepia* development in superficial tissues as well as internal tissues and organs. Overall, these results provide an improved picture of the broad spectrum of roles played by NO in adult and developing cuttlefish.

PRELIMINARY RESULTS ON *Octopus vulgaris* JUVENILE AND ADULT COLD-WATER ANESTHESIA AND EUTHANASIA. **C. Perales-Raya[1], B. Felipe[1], A. V. Sykes[2], A. Bartolome[1], E. Almansa[1].** [1]Instituto Español de Oceanografía, Centro Oceanográfico de Canarias. C/Gral. Gutiérrez N°4, C.P. 38003. Santa Cruz de Tenerife, Spain; [2]CCMAR-CIMAR L.A., Centro de Ciências do Mar do Algarve, Universidade do Algarve, Campus de Gambelas, 8005-139, Faro, Portugal.

E-mail: asykes@ualg.pt

The objective of this work was to determine the effects of using cold-water as an anesthesia and euthanasia agent for the common

octopus, *Octopus vulgaris*. In addition these results were compared with anesthesia performed with clove oil in ethanol and at a concentration of 2 mL/L. A total of 60 wild caught octopuses were used. Animals were divided in juveniles (<0.75 Kg) and adults (>0.75 Kg) according to weight (W) and irrespective of sex. Thirty six octopuses (18 juveniles - JCW; and 18 adults—ACW) were used for cold-water anesthesia (performed at 3.3 ± 0.3°C), sixteen (7 juveniles—JE; and 9 adults—AE) for cold-water euthanasia (performed at -0.9 ± 0.3°C) and eight adults (ACO) for clove oil anesthesia (1:10 in ethanol). Induction and recovery times (in seconds; IT and RT, respectively) were verified by loss or recovery of suction activity and normal mobility, respectively. These times were registered individually and for every anesthesia agent. Induction plus loss of activity/reaction during 10 minutes were used to determine euthanasia. Weight and time data was transformed by logarithm. Linear correlations between weight vs induction time (WxIT), weight vs recovery time (WxRT) and induction time vs recovery time (ITxRT) were tested for anesthesia, while only the first was verified for euthanasia. Regarding correlations, these were only found between WxIT for JCW, and between WxRT for JCW and ACW. No linear correlations between ITxRT were found within similar groups and the correlations found had lower values. Cold water induced anesthesia in 210-230s (~3-4 min) for both groups. Recover was obtained in 196-264s (~3-5min) and IT and RT were similar between juvenile and adult octopus ($p > 0.05$). Regarding octopuses of similar weight (1-3Kg), IT were similar between cold-water and clove oil. However, octopus subjected to clove oil took more time to recover (mean RT of 193s vs 341s; $p < 0.05$). All octopuses survived both induction procedures. As for euthanasia, it took more time of induction to kill bigger octopi (mean IT of 441s; $p < 0.05$), with a maximum of 9 min being recorded. This means that, despite the increasing weight of octopus subject to anesthesia, no direct correlations with both times of induction and recovery may be linearly described. The reason for such a fact may be related to animal nutritional condition and physiology of different sexes. On the other hand, despite lower correlations were found regarding weight, the euthanasia procedure seems to be effective regarding time and stress (no ink ejection, erratic and/or violent behaviour was recorded). This is particularly important regarding the new Directive 2010/63/EU regarding the protection of animals used for scientific purposes.

CUTTLEFISH BODY PATTERN RESPONSE TO 3D OBJECTS. K. Perkins and D. Osorio. School of Life Sciences, University of Sussex, England.

E-mail: kerry.perkins@merlinentertainments.biz

Cuttlefish (*Sepia*) live in environments that consist of 3D objects (seaweed, reefs, rocks) in their visual vertical field as well as the horizontal (sand, stones). Due to the complexity of their environment cuttlefish often camouflage or mimic their surroundings using various chromatic components. This study investigated body pattern responses to 3D objects. Size and contrast of individual stones were varied on a low contrast sand background totalling ten different treatments. Cuttlefish reacted to the size and colour (High or low contrast) of the stone eliciting various chromatic responses. Proximity to the 3D object determined certain components, with the maximum distance away from the object producing substrate matching in most cuttlefish. Overall the proximity, size and contrast of a 3D object in the visual vertical field of a cuttlefish factor into the overall chromatic response.

CEPHALOPOD INTERNATIONAL ADVISORY COUNCIL (CIAC): 30 YEARS IN THE SERVICE OF CEPHALOPOD RESEARCH (1981-2011). Uwe Piatkowski. *on behalf of G. Pierce and CIAC.*

E-mail: upiatkowski@ifm-geomar.de

CIAC is the brainchild of Dr Malcolm Clarke, FRS, and traces its origins to two scientific workshops: one in March and the other in June 1981. During these meetings the idea of small consultative research body concerning cephalopods was tested and spread. CIAC was subsequently formalized at the meeting in Banyuls-sur-Mer in 1983. At that time, number of people working actively and continuously upon cephalopods was small. At the same time, interest in this group of invertebrates was growing exponentially. These facts have determined the format: self-electing body of senior researchers, with emphasis on geographic spread (not cephalopods, but scientists!) rather than the coverage of disciplines and specializations. Giant axon physiologists and paleontologists were not included as representing fields already covered by various scientific organizations. CIAC role as a consultative forum (reflected in the name) was never prominent; however, its input in teuthology was considerable. There was nine large symposia organized between 1985 and 2009 and with the one exception, large volumes of crucial scientific contributions were published after each gathering (every 3 years). Also, CIAC served as the information hub for the cephalopod-bound community, issuing a newsletter (1985–1993) and electronic means of communication and information, either directly or affiliated (FastMoll, Cephbase). There are at present ideas of transforming CIAC into the open society; however, these ideas are approached with caution because the working formula must be protected against possible fail of an experiment.

RESEARCH EFFORTS ON CEPHALOPODS: AN OVERVIEW OF THE LAST 5 YEARS. G. Ponte and G. Fiorito. Octopus Behavioral Biology, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy.

E-mail: giovanna.ponte@szn.it

In this talk I will present a sort of *"backstage"* of EuroCeph 2011 meeting. This in order to offer to all participants an overview on: *i.* the research efforts on cephalopods over the last five years

(2005–2010); *ii*. a glance of the activities and interaction with our website EuroCeph.org; *iii*. an introduction to the discussion on the Top Ten Questions in cephalopod biology research that is the topic of Sunday morning session of the meeting.

To start the organization of EuroCeph 2011 we decided to collect addresses and affiliations of people actively involved in cephalopod biology; this was done by collecting published works from the most common scientific databases (i.e. PubMed, Web of Knowledge, and Zoological Record). Our aim was to produce a database that will include data on scientific production, organisms, institutes and people involved. In this way, we estimated how many papers have been published on cephalopods, how many of these appeared every year and the countries that resulted to be more active on this taxon. This study provided also the source of data for contacting people for launching EuroCeph meeting and to map a geographical overview of the research carried out on cephalopods over the last five years (http://www.cephres.org/cephalopod-world-research).

After this overview on cephalopod effort, I will give an outlook on the interaction that people had with our website. This was achieved through Google Analytics and allowed us to discuss on some aspects of the worldwide interest for cephalopods and represents a sort of distribution of the currently active cephalopod community in the world.

Last, but not least, I will present the results of our survey on the "Top Ten Questions in Cephalopod Biology Research" to better focus on future directions. These questions have been analyzed from those suggested from the participants to the meeting. All these issues will help everyone of us to understand how the new European Directive 2010/63/EU will affect the research in this field, how many people are involved, and how many countries will be directly influenced by this new challenging avenue within the next couple of years.

ANTI-HRP EPITOPE IN *Octopus vulgaris* NEURAL TISSUE: THE FIRST AMONG LOPHTROCHOZOANS. G. Ponte[1], D. B. Edelman[2], G. Fiorito[1]. [1]Octopus Behavioral Biology, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy; [2]The Neurosciences Institute, San Diego, California, USA.

E-mail: giovanna.ponte@szn.it

Several invertebrates show a selective expression of membrane proteins on the surface of neural tissue that are recognized by antisera against the plant glycoprotein horseradish peroxidase (HRP). The epitopes recognised by anti-HRP in invertebrates are of carbohydrate nature. Until now, this carbohydrate epitope has been considered as a tissue-specific marker of Ecdysozoa (Paschinger *et al*., 2009) which, together with Lophtrochozoans, are two major clades of the Protostomians. This reinforced the split of these clades suggesting the lack in the evolutionary relationship among Protostomian animal phyla (Haase *et al*., 2001). HRP immunoreactivity is reported to be completely absent in the nervous tissue of Lophotrochozoans. The only exception are molluscs, where such epitopes has been found expressed in tissues of non-neural origin. Here we show, for the first time, a positive HRP reactivity in the neural tissue of *Octopus vulgaris*, a Cephalopod mollusc. Brain and arm of *Octopus vulgaris* were incubated with antisera against the plant glycoprotein horseradish peroxidase (HRP; 1:5000); immunoreactivity was visualised by secondary Ab conjugated to Alexa fluorophores A488, DAPI was used as a counterstain. As a control HRP antisera was omitted; this did not result in a positive signal. We found, that HRP immunoreactivity is detected in all brain lobes (soma and cellular processes) as a typical neural marker. In addition, HRP labeling was detected at the level of the octopus arm but again only in nervous tissues. These data suggest that HRP can be considered a novel important neural marker that helps to investigate the cellular architecture in *Octopus*. It is noteworthy to report that our results show variation in the expression of anti-HRP immunoreactivity in brain lobes, thus suggesting differences in their neural composition (neurons and support cells). The function of these molecules in neural and other tissues has to be further investigated also considering their role in the immune response.

OCTOPUS SYSTEMS NEUROSCIENCE: CIRCUITS & GENE TO BEHAVIOR. C. Ragsdale and S. Shigeno. Department of Neurobiology, Univ of Chicago, IL, USA.

E-mail: cragsdale@uchicago.edu

The frontal-vertical system of the octopus brain has classically been analogized to the cerebral cortex, specifically the hippocampus, of mammals. This analogy is based on the behavioral consequences of brain lesions, which include fairly specific learning and memory deficits. Whether the neural systems architecture of the frontal-vertical system exhibits any of the familiar features of cortical anatomy, such as the topographic maps of sensory and motor space seen in neocortical areas or the molecular differentiation of input and output territories demonstrated for cortical layers, remains uncertain. We have begun to address some of these questions with modern tract-tracing and histochemical methods, with a specific focus on the issues of somatotopy and molecular specialization within the frontal-vertical lobes. We have also investigated the gene regulatory networks involved in the early development of the frontal-vertical system. Our motivation is the recent claim from work on a non-molluscan lophotrochozoan, the marine annelid Platynereis dumerilii, that higher brain centers, including the cerebral cortex and the mushroom bodies, are generated by patterning mechanisms conserved from a common protostome-deuterstome ancestor.

CULTURE OF CUTTLEFISH (*Sepia officinalis*) AT THE MARINE BIOLOGICAL ASSOCIATION PLYMOUTH: THE SYSTEM AND METHOD. J. Rundle. Marine Biological Association of the United Kingdom, The Laboratory, Citadel Hill, Plymouth, PL1 2PB, UK.

E-mail: jru@mba.ac.uk

Since 1997 there has been a dedicated system for the culture of *Sepia officinalis*. At the MBA Laboratory, Plymouth, UK. Thousands of eggs have been produced from bloodstock, within and out season, and hundreds of hatchlings, juveniles and adults have been used for neurophysiology and animal behaviour research. This paper is on the aquarium systems and husbandry methods used at the MBA.

Historically the European cuttlefish *Sepia officinalis* has been a part of MBA research, but it was not until 1997 that a dedicated culture system was set up to supply eggs and animals for neurobiology and animal behavior studies. The paper details the systems, water quality, feeding regimes and breeding out of the natural seasonal cycle used to culture animals to adult generations.

The common European cuttlefish, *Sepia officinalis*, Linnaeus, 1758 is one of the most easily maintained of all cephalopod molluscs and therefore has been maintained, reared or cultured in a number of laboratories and public aquaria of Europe, and North America (Boletzky, 1983; Boletzky & Hanlon, 1983; Hanlon, 1987; Boyle, 1991; Forsythe, et al., 1991; Forsythe et al., 1994; Sykes et al., 2006). The purpose of this paper is to describe the systems and procedures used to culture *Sepia officinalis* at the Marine Biological Association. The culture project started in February 1997 and since this date has continued to deliver populations of cuttlefish from egg to full sexual maturity. Adult pairs of wild cuttlefish collected from the English Channel in the vicinity of Whitsand Bay, near Plymouth and cuttlefish that are bred in the laboratory are used for breeding stock.

Cephalopod History at the Marine Biological Association:

J. Z. Young in 1939 first described the giant fibre system in Loligo squid.

Hodgkin and Huxley's Nobel Prize work on the giant axons 1964.

Denton and Gilpin- Browns work on cuttlefish buoyancy.

COMPARISON OF THE PHYSIOLOGICAL PERFORMANCES OF CUTTLEFISH JUVENILES *Sepia officinalis* L. PRE-RECRUITS FROM DIFFERENT COASTAL AREAS OF THE ENGLISH CHANNEL. G. Safi[1], I. Bloor[2], M. Gras[1], E. Jackson[2], J.-P. Robin[1], N. Koueta[1]. [1]UMR M100 IFREMER-UCBN *Physiologie et Ecophysiologie des Mollusques Marins*, Esplanade de la Paix, 14032 CAEN Cedex, France; [2]Marine Biological Association, The Laboratory, Citadel Hill, Plymouth, Devon, PL12PB, UK and Marine institute, University of Plymouth, Drake Circus, Plymouth, Devon, U.K. PL48AA, UK.

E-mail: safigeorges@hotmail.fr

In spring, the European cuttlefish *Sepia officinalis* migrate in the English Channel, from the open sea to the coast, to lay their eggs. In autumn, after hatching, juvenile cuttlefish go towards the open sea. The aim of this work was to study the impact of different spawning sites from the French and English coasts on the physiology and the ecology of pre-recruit stages. Experiments with young recruits and with juveniles from each site reared in the same conditions were used to investigate the site impact on cuttlefish physiology. In 2009 and in 2010, eggs from two coastal French sites (West and East Cotentin) and one English coastal site (Tor Bay) were brought to the marine research station. Preliminary observations indicated that hatching and survival rate were identical whatever the site. Nevertheless, hatchling size was bigger and growth rate faster in specimen from Seine Bay (East Cotentin) and Tor Bay than from the West Cotentin site. Biochemical analyses showed higher intracellular enzymatic activities and faster alkaline digestion in juveniles from both Bays. Moreover, the juvenile biochemical composition changed according to their original site and the West Cotentin juvenile immune activities were higher than those of East Cotentin and Tor Bay. This 2-year survey showed an impact of the different spawning sites on the juvenile physiological performances. Since there is only one cuttlefish population in the English Channel, these observations suggest that this site impact should be related to the site environment parameters that eggs undergo during the incubation period.

NEEDS FOR GENOMIC APPROACHES IN CEPHALOPODS RESEARCH. R. Sanges, P. De Luca, G. Fiorito. Stazione Zoologica Anton Dohrn, Villa Comunale, 80121, Napoli, Italy.

E-mail: remo.sanges@gmail.com

Cephalopod mollusks present the most complex nervous systems outside the vertebrate lineage, therefore it has been often suggested that their genome sequences will provide useful insights into the evolution of complex brains. Recently, the number of noncoding genomic elements has been related to the complexity of the nervous system. Indeed, although the number of coding elements remains relatively stable across all metazoan species, the number of noncoding sequences significantly increases in relation to the complexity. Such elements have been shown to be enriched in proximity to brain expressed genes in mammals and it would be extremely interesting to isolate and analyze them in cephalopods. But we lack genome sequences.

Furthermore, classical strategies to understand the function of a gene investigate the territories and the timing of its expression as well as which biological processes are affected by mutating it. And almost every approach designed to study gene function requires sequence information. Finally, the discovery of new sets of genes also helps in establishing or strengthening phylogenetic relationships and allows to investigate functional differences between closely related species making possible population as well as ecological studies. So far cephalopod molecular tools have been developed mainly in *Aplysia californica* whose nervous system results relatively simple.

In order to tackle these issues we need to shed light on the molecular and structural organization of cephalopod genomes and genes, including the noncoding part of them. Genome sequencing has indeed always provided revolutionary insights into the knowledge of sequenced species and we believe this would be the case also for cephalopods. Their genome sequences will widely contribute to understand what lies at the basis of their incredible adaptive potentials and cognitive flexibility. Therefore here we want to propose a community effort in order to sequence the genomes of cephalopods species such as *Octopus vulgaris*, *Loligo vulgaris* and *Sepia officinalis*. The availability of such molecular tools to the community will, in coming years:

1. allow comparative genomics analyses among cephalopods and with other sequenced species, revealing functional properties and adaption;
2. open up the way to genetics and functional genomic studies, facilitating the design of strategies for the study of gene functions;
3. complement and integrate transcriptomic studies that are ongoing for these species;
4. contribute to place such organisms among well-established and widely adopted model species.

Analysis of the origin and evolution of neurons is crucial to reveal principles of organization of neural circuits and we predict that results from these studies will have important and unexpected implications for neurobiology, physiology, genomic sciences, biomedical applications and regenerative medicine.

ANALYSIS OF PAIRED-CLASS HOMEODOMAIN PROTEINS FROM THE EUROPEAN CUTTLEFISH *Sepia officinalis*. S. Sesso and A. G. Cole. Biogem-IRGS, Ariano Irpino, 83031, Italy.

E-mail: agcole05@gmail.com

Cephalopod mollusks possess a number of anatomical traits that often parallel vertebrates in complexity. Very little is known about the genetic mechanisms underlying patterning of the cephalopod embryo to arrive at these complex anatomical structures. Transcription factors that contain a DNA binding homeobox domain are critical genes involved in animal body-plan patterning. We have used degenerate primers designed against the conserved Paired-class homeodomain (PRD) to isolate PRD-class homeobox genes active during late-organogenesis from the European cuttlefish *Sepia officinalis*. The amplified 120 bp segment, which corresponds to the half of the conserved 60 aa homeodomain, was cloned into a commercial vector, and 50 bacterial colonies were sequenced. With this approach we have isolated three highly expressed PRD-domain genes: prophet of Pit1 homeobox (*Propx*: 20% of colonies sequenced), dorsal root ganglion homeobox (*Drgx*: 30%), and an aristeless-like homeobox protein *ARX* (37%). Gene identity was verified by multiple alignment of representative PRD-class homeodomains (http://homeodb.zoo.ox.ac.uk/) and neighbour-joining phylogenetic analysis. An additional 50 colonies were sequenced after screening for, and removal of, *SoDrgx* and *SoARX* colonies, leading to the identification of an additional PRD-class gene: visual system homeobox (*Vsx*), and two additional homeobox genes outside of the PRD-class: gastrulation-brain homeobox (*Gbx*) and *Lim3/4*. Preliminary phylogenetic analysis reveals a high level of conservation of the four identified PRD-class genes at the level of amino acids with homologous genes from arthropod lineages. This is in contrast with a similar analysis of ANT-class homeodomains, including *SoGbx*, which show higher similarity with deuterostome sequences. Sequences derived from other members of the Lophatrochazoan lineages (Mollusks, Annelids, etc) for these PRD-class genes are not publically available, and thus our results provide a first glimpse into the diversity of PRD-class homeobox genes in one of the largest, yet least studied, metazoan clades.

ELECTRONIC TAGGING OF CUTTLEFISH *Sepia officinalis* AND OCTOPUS *Eledone cirrhosa* TO STUDY COMMONALITIES IN BEHAVIOUR PATTERNS AND COMPARISONS WITH MARINE FISH. D. W. Sims, N. E. Humphries, M. McHugh, V. J. Wearmouth. Marine Biological Association of the United Kingdom, The Laboratory, Citadel Hill, Plymouth PL1 2PB, UK.

E-mail: dws@mba.ac.uk

Waiting is an integral part of animals' lives, including our own. How long animals such as cephalopods and fish wait between different activities or events is fundamental to the success of diverse behaviours such as foraging, avoiding predators or sharing information with group members. In the case of foraging, waiting for prey to move within striking distance offers a clear example of how waiting can present a distinct advantage to predators for attaining high rates of energy gain at minimal cost. However, although a great many studies have investigated how mobile animals move whilst searching for resources, resulting in a rich theoretical and empirical literature, surprisingly few studies have investigated the patterns and ecological significance of waiting times. It is possible that the patterns of burstiness in waiting (and, consequently, activity) represent strategies that are common across diverse taxa, and which may have evolved naturally to enhance encounter rate with resources such as prey. In this paper we describe studies to determine the behavioural structure and ecological significance of the intervals between activity (termed waiting times) in cephalopods (*Sepia*, *Eledone*) and fish (shark, ray, teleost fish). High-temporal resolution archival tagging of cephalopods and fish was used to record long-term time series of activity and waiting across scales from from seconds to years. Methods and success of tagging cuttlefish and octopus will be discussed. In addition, we present empirical results indicating similarities in the

statistical structure of burstiness across species, and simulation outputs suggesting their general ecological significance.

EFFICIENCY OF DIFFERENT ANAESTHETICS AS WELFARE PROMOTERS DURING HUMAN MANIPULATION OF EUROPEAN CUTTLEFISH (*Sepia officinalis*) JUVENILES. **A. V. Sykes[1], S. Taipina[1,2], R. Gonçalves[1], R. J. Bernardino[2], C. Aragão[1].** [1]CCMAR-CIMAR L.A., Centro de Ciências do Mar do Algarve, Universidade do Algarve, Campus de Gambelas, 8005-139, Faro, Portugal; [2]ESTM-IPL, Campus 4, Santuário Nossa Senhora dos Remédios, 2520 - 641 Peniche – Portugal.

E-mail: asykes@ualg.pt

Anaesthetics are frequently used to prevent injuries and stress and promote welfare while performing animal handling under experimental conditions. The efficiency of six anaesthetics was tested to determine the best agent and concentration in juvenile European cuttlefish (*Sepia officinalis*). Agents such as ethanol (5.0, 7.5 and 10.0 mL/L), 2-phenoxyethanol (0.10, 0.15, 0.20 and 0.30 mL/L), MS-222 (20, 30, 40 and 50 mg/L), clove oil (5.0 μL/L), hypothermia (sets of: 2–4, 4–10 and 10–15°C) and hyper-oxygenation (~400%) were tested. The choice of agents was preceded by a preliminary trial, where additional agents were used, such as AQUI-S (4 and 8 μL.L-1) and MgCl2 (30 and 60 g.L-1). Since AQUI-S shares a similar active compound to clove oil (Isoeugenol) and the higher concentrations needed to provide an effective use of MgCl2, these were discarded. In addition, a control without any agent was used. Anaesthetic induction was performed in 112 juvenile cuttlefish (16 groups of 6 animals plus 16 animals as control, with a mean weight of 31.1 ± 6.0g), in a plastic bucket containing 5L of hyperoxygenated seawater (177 ± 39%), plus the anaesthesia agent, and at a temperature of 26.4 ± 1.0°C. Afterwards, handling of animals was performed for 3 min, which consisted in handling and weighing procedures according to a developed CCMAR protocol. The animal was then placed in a recovery tank with flow-through hyperoxygenated water (149 ± 29%). All these procedures were filmed and documented a posteriori, and mortality registered after 48h. Results were analysed taking into account induction and recovery stages (table from Seol *et al*., 2007); quantity/concentration of agent needed; and stress, injuries and death promoted during the procedure. The best concentration for each anaesthetic, which promoted low mortality (<50%), was determined as being 10 mL/L, 0.2 mL/L, 40mL/L, and 10–15°C, for ethanol, 2-phenoxethanol, MS-222, and hypothermia, respectively. Hyperoxygenation did not induce anaesthesia and all animals exposed to clove oil died. From the best, hypothermia seemed to be the most appropriate anaesthetic agent for the species, promoting a rapid effect (phase A3 in less than 5 min), recovery (within 10 min) and no stress effects. In addition, this method is inexpensive and does not pollute water when compared with the remaining chemicals. Despite these results, the common procedure of not using any anaesthetic agent (control) did not seem to promote injuries neither stress effects, contrary to the use of some anaesthetics which promoted high ink jetting, erratic and violent behaviour and even death. So, is it worth using an anaesthetic agent, when trained personnel may perform normal handling in scientific procedures without causing stress? We believe not. In fact, we believe that anaesthetics should only be used for surgery and euthanasia in cuttlefish, since even the smallest error while using chilled water might promote animal death.

ON THE AQUACULTURE POTENTIAL OF CEPHALOPOD SPECIES: THE EUROPEAN CUTTLEFISH AS A SHOWCASE. **A. V. Sykes[1], P. Domingues[2], R. Gonçalves[1], M. Correia[1], J. P. Andrade[1].** [1]CCMAR-CIMAR L.A., Centro de Ciências do Mar do Algarve, Universidade do Algarve, Campus de Gambelas, 8005-139, Faro, Portugal; [2]Instituto Español de Oceanografía, Centro Oceanográfico de Vigo; Cabo Estai, Canido. 36390 Vigo, España.

E-mail: asykes@ualg.pt

There are many constraints in developing cephalopod culture technology due to the different species biology and ecology. The aim of this work was to provide a review on the aquaculture potential of cephalopod species by showing the advances on European cuttlefish (*Sepia officinalis*) aquaculture development over the last 10 years by CCMAR. Cuttlefish is cultured as an animal model for biomedical research (e.g. neuroscience), for aquaculture production and for aquariums/public exhibition. Recently, some of the species characteristics also point to an eventual use in cancer research. Until now, we have been following a multidisciplinary approach to develop culture methods based on knowledge regarding the species biology and ecology, which has proven to be successful. At this point, we are able to culture up to 6 consecutive cuttlefish generations based on a sole prey, the grass shrimp (*Palaemonetes varians*), to provide a basic culture methodology for all life stages and, recently, to early wean cuttlefish hatchlings on frozen grass shrimp from the first day after hatching. Although some of the already developed fish culture technology may be applied, cephalopod aquaculture practices usually need to be adapted to the species. So, in recent years, we added new fields such as nutrition and physiology as a support for understanding the obtained results. We are currently using the grass shrimp as a model prey and characterizing its nutritional composition and energetic content. In addition, we are also characterizing the cuttlefish enzyme content and performing the first radiolabelled studies to understand the species metabolism, at different life stages. In the years to come, we are planning to extend even more the complementary fields of research, which will include population genetics under captive conditions, behavior and animal welfare. All of them will contribute to develop a coherent culture methodology which must be animal friendly. Approaches, results and supportive lines of research will be discussed in detail regarding their contribution for the advance of cuttlefish aquaculture and the

existing bottlenecks. In addition, the application of the already established technologies to *Octopus vulgaris* and other cephalopods culture will be revised.

EFFECTS OF INCREASED TANK BOTTOM AREAS ON CUTTLEFISH (*Sepia officinalis*, L.) REPRODUCTION PERFORMANCE. **A. V. Sykes[1,2], D. Pereira[1], C. Rodríguez[2], A. Lorenzo[2], J. P. Andrade[1].** [1]CCMAR-CIMAR L.A., Centro de Ciências do Mar do Algarve, Universidade do Algarve, Campus de Gambelas, Faro, Portugal; [2]Departamento de Biologia Animal, Facultad de Biología, Universidad de La Laguna, La Laguna, Tenerife, Canary Islands, Spain.

E-mail: asykes@ualg.pt

The objective of this work was to determine the effects of using tanks with different bottom areas on the growth and reproduction of the European cuttlefish, *S. officinalis*. A total of 176 cuttlefish with a mean wet weight (MWW) of 46.1 ± 20.9g were used to test 2 types of tanks: 750 L (bottom area of 1.54 m2) and 9000L (bottom area of 6.67 m2). Tanks with a capacity of 250L (bottom area of 0.79 m2) were used as control. All cuttlefish were weighed every 15 days, until the start of reproduction, for growth data. Reproduction data was registered during this stage. In addition, egg samples were collected from each tank type for determination of egg proximate composition (total protein, lipid, carbohydrates, moisture and ash) differences and correlations with reproduction data. Regarding growth, after 105 days of rearing, there were no statistical differences between tank types. Nonetheless, absolute mortality was lower in 9000L tank replicates. Regarding reproduction, differences were found ($p < 0.05$) between tanks for mean egg (MEW), mean female (MFW) mean male and mean hatchling (MHW) weights. As for egg proximate composition, some differences ($p < 0.05$) between eggs of different tanks were also noted. Significant ($p < 0.05$) correlations were established between the duration of reproduction stage and number of egg batches ($r2 = 0.719$), MEW and MFW ($r2 = 0.833$), egg viability and MHW ($r2 = 0.774$), and MHW and egg ash content ($r2 = 0.750$). One of the 9000L tanks displayed the highest overall and individual fecundity (16593 and 1383 eggs, respectively) and egg viability (~72%) ever obtained in our facilities. Growth and MWW displayed similar values between different volumes/bottom areas and at the end of experiment, which lead us to suggest that cuttlefish, in space stressful conditions, might have the ability to adjust the amount of biomass present in each tank by competition. Circular tanks are perfect to promote mating between ripe male and female but might not be the better choice to promote growth of the species in captivity. The results obtained with 9000L tanks, despite being variable between replicates, point to a general increase in egg quantity and quality when these tanks were used. On the other hand, differences obtained within these tanks might be related to sex ratios of each tank and differences in weight between females and males. Differences in egg composition might have derived from spawners condition which depend on sex ratios and rearing conditions. To what extent each factor contributed to this remains to be determined. This means that cuttlefish reproduction in captivity has to be studied using a multidisciplinary approach, which will have to take several additional aspects into account such as population genetics, physiology and behavior. According to results of the present study, considering growth and reproduction, we advise the use of 9000L tanks as part of the spawners rearing protocol.

NEURAL REGULATION OF CHROMATOPHORE FUNCTION IN CEPHALOPODS. **N. Tublitz, E. Goodwin, Z. Zhang, J. Yacob, A.C. Lewis.** Department of Biology, University of Oregon Eugene OR 97403, USA.

E-mail: tublitz@uoregon.edu

One of the most amazing behaviors in the animal kingdom is Body Patterning Behavior in cephalopod mollusks. Cephalopods, which include octopus, squid, and cuttlefish, have the unique ability to generate numerous, highly complex body patterns. They use these patterns for camouflage as well as for intra- and interspecific communication. These body patterns are characterized by the richness of their detail, the speed by which they are formed (<1 sec), and the number of patterns produced. We have been studying Body Patterning Behavior in the European cuttlefish *Sepia officinalis*, a species which arguably generates the most detailed and varied body patterns in the animal kingdom. The skin elements responsible for these patterns, the chromatophore organs, consist of a pigment-containing chromatophore cell attached to a set of radially-arrayed muscles which, when contracted, cause chromatophore expansion. It is the coordinated activity of tens of thousands of chromatophores that give rise to the detailed body patterns exhibited by these organisms. Our goal is to understand the neural control of chromatophore function.

We have taken several approaches to explore this issue. On a molecular level, we have cloned, sequenced and analyzed a several novel genes in *Sepia officinalis* that codes for a family of neuropeptides involved in the control of chromatophore activity. At the cellular level, we have identified many of the major transmitters involved in controlling chromatophore muscle activity including glutamate and the family of FMRFamide-related peptides (FaRPs). We have recently developed a digital analysis system that enables us to measure the activity of multiple chromatophores simultaneously.

Another thrust in the lab has been to identify the principles underlying the process of chromatophore addition during maturation. New chromatophores are inserted into the cuttlefish skin continuously during growth. We have determined that new chromatophores are inserted at a higher spatial frequency than control chromatophores of the same color type. We also found that all chromatophores first appear as pale orange cells and slowly darken into their respective color types without passing through

intermediate color stages In addition, analysis of the appearance of individual chromatophore indicate that all chromatophores first appear as pale orange cells and slowly darken into their respective color types without passing through intermediate color stages These and other experiments will be discussed in this talk.

TEMPERATURE EFFECT ON STABLE ISOTOPE AND TRACE ELEMENT PARTITION IN STATOLITH AND CUTTLEBONE OF THE CUTTLEFISH *Sepia pharaonis*. C.-H. Wang[1], M.-T. Chung[2], H.-L. Chen[2], C.-F. You[2], C.-C. Chiao[3,4,5]. [1]Department of Environmental Biology and Fisheries Science, National Taiwan Ocean University, Taiwan; [2]Earth Dynamic System Research Center, National Cheng Kung University, Taiwan [3]Institute of Molecular Medicine, National Tsing Hua University, Taiwan; [4]Institute of Systems Neuroscience, National Tsing Hua University, Taiwan; [5]Department of Life Science, National Tsing Hua University, Taiwan.

E-mail: chwang99@mail.ntou.edu.tw

Seasonal temperature variation has the potential to dramatically affect the growth patterns and population structures of growing cephalopods. In particular, temperature significantly impacts the size that cephalopods can attain during the exponential growth phase. Stable isotopic ratios and trace element concentrations in marine calcium carbonate are commonly used as proxies for reconstructing past climate changes. However, validation experiments on temperature effect on microchemistry of cephalopods hard structure, such as statolith and cuttlebone are still lacking. Present study design a controlled experiment by rearing hatchling cuttlefish of *Sepia pharaonis* at three different temperatures (20, 25, 30 °C) for one month, and analyzing stable isotopic ratio and trace element concentration in rearing water, statolith, and cuttlebones, in order to find a suitable temperature proxy. Results showed that Mg/Ca and B/Ca on statoliths were positively linear related to temperature, while Sr/Ca and δ13C was negatively related. For cuttlebone, Li/Ca, Mg/Ca and δ13C was negatively related were negatively linear related to temperature. The combination of spatially resolved microchemical analysis and growth increment interpretation could be used to reconstruct life histories from wild cephalopod population in future.

CAMOUFLAGE WITHOUT COMPROMISE: CEPHALOPODS SWITCH BETWEEN TRANSPARENCY AND PIGMENTATION TO OPTIMIZE CRYPSIS IN THE DEEP. S. Zylinski and S. Johnsen. Biology Department, Duke University, Durham, North Carolina, USA.

E-mail: s.zylinski@duke.edu

The camouflage abilities of deep-sea cephalopods are generally considered unsophisticated compared to those of their shallow-water, benthic relatives. This view stems in part from an over-simplification of the visual problems faced in the low-light, structureless deep-sea. Additionally, there is a distinct lack of research focus in this area, due to the inherent difficulties associated with accessing, collecting, and maintaining animals that live hundreds of meters below the ocean's surface; to make formal behavioural observations is far from trivial. Here we present recent findings from two species of pelagic cephalopods, the octopus *Japetella diaphana* and the squid *Onychoteuthis banksii*, showing that they may use camouflage strategies which, although very different, might rival thise of benthic species in terms of speed and sophistication. We briefly introduce the four major camouflage strategies converged on by pelagic taxa—mirroring, transparency, counter-illumination, and red/black pigmentation—and discuss their major advantages and limitations, as well as their relevance to cephalopods. We then show, through data collected during ship-board behavioural experiments, that *Japetella* and *Onychoteuthis* can rapidly switch between the strategies of transparency and pigmentation, enabling them to retain the optimal camouflage under ambient down-welling light and biological searchlights (directed bioluminescence). We believe this is the first demonstration of rapid switching between these strategies, and that cephalopods can be crowned the kings of camouflage even in the deep sea!