Biology and Life History of Cephalopods

an interim meeting of the international cephalopod community

Napoli, Italy

16 - 21 September, 2020

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CephRes2020 Virtual Event

Biology and Life History of Cephalopods

behavior, cognition, evolution, ecology, fisheries, genomics, neuroscience, taxonomy

an interim meeting of the international cephalopod community

a CephRes initiative
endorsed by the CIAC (Cephalopod International Advisory Council)

Book of Abstracts

doi: 10.53124/cephres.202001

CephRes2020 Virtual Event

Concept, General Overview and Direction

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(*short Bio*)

Hannah Schmidbaur is a PhD student at the University of Vienna in Dr Oleg Simakov lab (Austria), interested in the evolution of animal diversity. She wants to understand how morphological diversity is linked to molecular mechanisms using her background in genomics and zoology.
Her current work focuses on regulation and synteny in cephalopods and how genome rearrangements can lead to the evolution of novelties.

Xitong Liang is a Postdoctoral Fellow from Gilles Laurent group in Max-Planck Institute for Brain Research, Frankfurt (Germany). He received PhD in Neuroscience from Washington University in St. Louis, USA and BS in Biology from Peking University, China.
His research interest is the neural basis of behavior diversity.

In the following pages Abstracts of the Invited Lectures are listed in chronological order within the Scientific Program

Nervous Cnidarians started it all…

Ferdinando Boero

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The Cnidaria are usually described as having neither a brain nor a central nervous system, but just a simple nerve net that reacts to stimuli and commands muscular cells. If nervous cells are monophyletic, then the Cnidarians are the simplest living animals that possess them, albeit with a "loose" architecture. The Bilateria sit on this evolutionary innovation that became very elaborate in the "higher phyla". This story can be told in a different way, though.
The cnidarian life cycle comprises a bilateral planula, with a front and a rear part, a radial benthic polyp that often forms modular colonies, and a radial planktonic medusa that is usually a single individual (with noticeable exceptions in the Siphonophora).

1: Planulas can have a brain and a central nervous system. The planula of the hydrozoan *Clava multicornis* has a frontal concentration of nervous cells and a nervous chord that departs from it and reaches the posterior end. This is the simplest, and the first, central nervous system. According to some theories on metazoan evolution, paedomorphic planulas that attained sexual reproduction resemble the simplest Bilateria: acoelous turbellarians (Acoelomorpha).

2: Polyps usually have a nervous net (and a head). Once the planula has found a settling spot, it metamorphoses into a primary polyp and the central nervous system becomes a network. Radial polyps receive stimuli from all directions and are not polarized as bilateral organisms, such as the planula and the rest of the bilateria. Polyps are mostly benthic and can have very simple sense organs that receive stimuli in form of vibrations, being also able to perceive chemicals dissolved in the water. It is suggestive that the solitary and freshwater *Hydra* is the paradigmatic cnidarian in textbooks: the exception becomes the rule! *Hydra*, however, has a "head" in the form of a concentration of nervous cells around the mouth (some call it a "brain").

3: Medusae have elaborate sense organs and a circumesophageal centre of control (nerve ring). Medusae are free living and swim with an open body cavity that is lined with striated muscles (a mesoderm) and do have a circle of tentacles on the bell margin, where, according to species, sense organs

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are present in form of eyes, statocysts, or rhopalia (compound sense organs comprising both eyes and statocysts). Some cnidarian eyes are rather simple, but others (e.g. those of the Cubozoa) are very complex. Pax Genes, the control genes that command the formation of eyes, are shared by the Cnidaria and the rest of the Metazoa. Medusae do have two nerve rings along the umbrellar margin, directly connected with the sense organs. They receive stimuli from the sense organs and send commands to the striated muscles of the subumbrellar cavity and to the tentacles. An interstitial Hydrozoan, Otohydra, has a closed subumbrellar cavity that becomes a brood pouch, the mouth is encircled by the tentacles and the ring of statocysts around it is connected with a nerve ring that is a circumesophageal brain. The two rings of medusae are a brain, and this architecture is optimal for a radial organism that, being a predator, performs elaborate behaviors.

4: The evolution of the nervous system started with the Cnidaria and deserves more attention in comparative neurology: to understand the apexes, the roots must be considered carefully, and fully understood.

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Making sense of alien genomes – synteny and regulation in cephalopods

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Cephalopods, with their sophisticated camouflage, extraordinary intelligence and highly modified body-plan can almost seem other-worldly. But not only their morphology and behaviour is fascinating, cephalopod genomes also show some unique features. Recent studies indicate large-scale genomic rearrangements before the split of Octo- and Decapodiformes. Local gene-order (synteny), which is widely conserved between many distantly related species, has largely been lost in cephalopods through this event. Interestingly, however, we can find hundreds of novel, cephalopod-specific gene clusters, which have been retained in different cephalopod species for the last 300m years. Chromosomal-scale analyses show that those novel syntenic clusters formed through a complex fission and fusion history of ancestral linkage groups. So far it is unclear if and how these cephalopod-specific micro-syntenies contributed to the formation of novelties in cephalopods. Through state-of-the-art methods including Hi-C and ATAC-seq we can now reconstruct the evolutionary history of the formation of recent cephalopod chromosomes, the emergence of novel, cephalopod specific-local gene clusters and their regulation during development. We find large topologically associated domains (TADs) of an average size of 2.5Mbp in the Hawaiian bobtail squid Euprymna scolopes. Both the organisation of cephalopod-specific micro-syntenies within these TADs and their co-expression profiles indicates that the clusters are functional, co-regulated units. Their function however seem to be more complex than a simple contribution to a particular cephalopod-specific tissues, indicating novel regulatory mechanisms associated with their emergence.

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Hannah Schmidbaur *Early Career*

Hannah is a PhD student at the University of Vienna in Dr Oleg Simakov lab, interested in the evolution of animal diversity. She wants to understand how morphological diversity is linked to molecular mechanisms using her background in genomics and zoology. Her current work focuses on regulation and synteny in cephalopods and how genome rearrangements can lead to the evolution of novelties.

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Neural control of 2-d skin patterning in two species of cephalopods

Xitong Liang, Gilles Laurent

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Coleoid cephalopods (octopus, squid, and cuttlefish) have the ability to change their skin patterns instantaneously for camouflage or communication. Their skin patterns are generated in great part by an extensive array of variable-sized pigment cells (chromatophores), controlled individually by radial muscles. Using this system, the cuttlefish *Sepia officinalis* generates skin 2d textures that match certain statistics of its surrounding visual environment. How thousands to millions of chromatophores are controlled in parallel and coordinated to generate different textures is an open and fascinating question. A different cephalopod, the bobtail squid *Euprymna berryi*, camouflages by covering itself with sand. Its chromatophores change size mostly synchronously, switching entire animal between transparency and dark pigmentation.

To study the neural basis underlying these divergent chromatophore dynamics, we compare the neuronal and network properties of chromatophore motor control between these two species. By tracing their axons in descending nerves, we identified chromatophore motoneurons in both species. Although those motoneurons show similar electrical properties, the ratio of motoneurons to chromatophores is ~9-fold higher in *Sepia* than in *Euprymna*. Electrical stimulation further suggested a somatotopographic organization of motoneurons in *Sepia* posterior chromatophore lobes, while such maps were absent in *Euprymna*.

We developed the first preparation to carry out whole-cell recordings and calcium imaging on chromatophore motoneurons, in conjunction with observing the dynamic activity of the chromatophores, elicited by stimulating the upstream visual system. This approach begins to uncover the principles of organization of neural circuits generating high-dimensional motor output, and may reveal how such neural circuits have diverged adaptively during evolution.

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Xitong Liang *Early Career*

Xitong Liang is a postdoc from Gilles Laurent group in Max-Planck Institute for Brain Research, Frankfurt, Germany. He received PhD in Neuroscience from Washington University in St. Louis, USA and BS in Biology from Peking University, China. His research interest is the neural basis of behavior diversity.

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Ceph Gastrophysics

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In contrast to many places in the world, e.g., South East Asia and Southern Europe, there are regions such as the Nordic countries where there is no tradition for using cephalopods as food, although there is an abundance in the waters and, e.g., squid is only brought in by the fishermen as a bycatch. In order to promote a cephalopod cuisine in Northern Europe we have initiated a research programme to develop a cephalopod cuisine, combining the science of gastrophysics with gastronomy, culinary innovation, and design. Our main focus has been exploring flavour (umami) and texture of Loligo forbesii, attempting to use most parts of the organism. We have also performed consumer testing.

Along the way we pay attention to the special role cephalopods may play in sustainable, flexitarian eating behaviour and for promoting a green transition, engage in a new research programme relating humane slaughtering methods to cephalopod eating quality, as well as reaching out to children and young people on our communication platform Taste for Life.

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From giant brains with tiny neurons to tiny brains with giant neurons; the brains of Nudibranchs

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Unlike cephalopods, nudibranchs have brains composed of fewer than 10,000 neurons. Many of these neurons are individually identifiable and have large somata, allowing neural circuitry to be worked out with pairwise microelectrode recordings. Furthermore, homologous neurons can be recognized, allowing neural circuitry to be compared across species. My lab has studied the neural circuitry underlying swimming behavior in several nudibranch species and has found that these circuits can be quite small, consisting of as few as four neurons. The same neurons can play different roles across species. Moreover, homologous behaviors can be produced through different neural mechanisms, suggesting the behavior and neural mechanisms are independent levels of biological organization with their own evolutionary histories.

Recently, we have begun a connectomics analysis of the brain of the nudibranch, *Berghia stephanieae*. Initial results suggest that although the brain contains a small number of neurons, a much larger number of axons enter the brain from the periphery.

This raises the possibility that nudibranchs, like cephalopods, have "embodied intelligence", with peripheral processing playing a larger role than previously appreciated.

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Orchestration of motor and other behaviour by the two biogenic amines, tyramine and octopamine

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Tyramine and Octopamine are two biogenic amines which occur in all invertebrates.
These play an important role as neuromodulators and/or neurohormones for motor and other behaviour.

Most studies were carried out in insets and, therefore, this talk will mainly focus on functions in the insect nervous system but will also consider other invertebrates.

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https://www.nwg-info.de/de/council/elections/2013/pflueger

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Other Minds: Similarities and Differences

Peter Godfrey-Smith

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I will discuss how recent work on octopus behavior bears on the question of whether, to a significant extent, animals with very different brain designs share cognitive similarities.

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In the following pages Abstracts are sorted by their attribution to Sessions in the scientific program

NeuroCeph - Cephalopod Neurophysiology a CephRes2020 Virtual Event Focus on Session

Main Organizer: Dr Letizia Zullo (Italy)
Co-Organizers: Prof. Binyamin Hochner (Israel); Dr Michael Kuba (Japan)

Cephalopod body and nervous system have been study 'models' in Neuroscience from before the early twentieth century. Many fields of investigations from Behavior to Neuroanatomy and Neurophysiology have been developed so far. In recent years, cephalopods turned to be exciting animals for understanding the independent evolution of neurophysiological processes involved in mediation of complex behavior and as an outstanding demonstration for highly efficient motor behavior in soft-bodied animals. We will attempt at presenting the major 'hot' topics, including some newest discovery and challenges in the field of brain and muscle Neurophysiology with a special attention on the current methodological advances and bottlenecks.
Spanning from single cell to collective recording of excitable cells activity, we aim to favour a collaborative bench-work aiming at providing new neurophysiology methodological strategies for data collection and interpretation to be beneficial to cephalopod research.

[*abridged from the original proposal*]

A novel NO-dependent 'molecular-switch' mediates memory acquisition in the vertical lobe of *Octopus vulgaris*

Binyamin Hochner[1], Naama Stern-Mentch [1 2], Flavie Bidel,
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The octopus vertical lobe (VL), a brain area that controls the sophisticated learning of this invertebrate, demonstrates a robust activity-dependent long-term potentiation (LTP) which was shown to be important for memory acquisition (Shomrat et al, 2008).
We show here that the presynaptic expression of LTP involves activation of nitric oxide synthase (NOS) and that nitric oxide (NO)-dependent reactivation of NOS functions as a 'molecular switch' mediating the very long, protein synthesis-independent, LTP maintenance (> 10h). While NADPH-diaphorase histochemistry supports the presence of NOS in the VL, we could not find any indication for the involvement of the canonical NO-dependent cGMP cascade in LTP. Additionally, NO-donors and NO-scavengers had no effect.
These negative results suggest the possible involvement of processes that function at high NO concentration (e.g., s-nitrosylation). We then measured NO concentration amperometrically and found that induction of LTP is accompanied by a long-term increase in the amperometric signal that corresponded to NO's oxidation potential (750 mV). This increase, on μM ranges, was much higher than the one found for the activation of cGMP cascade. We therefore hypothesize that a process such as s-nitrosylation could serve as an effective mediator of a local retrograde message for ensuring specificity in presynaptic LTP.

Support: ISF, BSF and the National Institure for Psychobiology (to TS and NN)

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Deciphering Octopus brain spiking activity

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Octopuses possess the most complex brain organization outside of vertebrates. They exhibit a rich behavioral repertoire that involve various forms of learning. The octopus' central brain integrates a large amount of sensory information from the optic lobes and the peripheral nervous system of the arms and issues commands to lower motor centers. It was shown that the octopus appears not to use a somatotopic representation of body parts in the higher motor centers (the basal lobes) in the central brain. This raises the question whether there is a somatotopic representation of the sensory information in these higher brain centers.
In the current work, different stimulation modes (tactile and visual) were given to freely behaving animals while extracellular multi unit recordings from different areas in the central brain were acquired. Here we developed a computational methodology to perform spike sorting and clustering analysis that enables characterizing the neuronal units that are activated by the different simulation modes. The results suggest that as was found with motor representation (Zullo et al., 2009) there is no somatotopic organization of sensory information in the higher motor centers. Moreover, the clustering analysis suggests that units representing different sensory modalities are integrated into different clusters depending on the stimulus delivered to the animal.

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Unrestrained EEG recording in Cephalopods

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The present study aims to establish the first-ever electro potential recordings from the brains of behaving unrestrained octopuses, and the first-ever multi-channel recordings from a cephalopod. Previous studies (Brown et al 2006, Zullo et al 2009) were limited to animals with implanted wired single-channel electrodes directly connected to an amplifier and recording device outside of the water. An octopus has eight sensitive and maneuverable arms, which can grab and extract an electrode even if just a fragment protrudes from the skin, yielding displacement of the probe, unstable recordings, electrode damage, and abortion of the experiment.
The neurologger is fully contained within the animal, and does not rely on protruding cables, so that the animal can't tamper with wires or corrupt the recording by altering the location of the electrodes.
We have been able to collect the first multi-channel EEG recordings from the brains of behaving unrestrained octopuses. Our current research has found differences in the EEG power spectrum during different activity states. Using the neuro-loggers together with standard electrodes embedded trough the cartilaginous capsule into the brain, we can EEG activity with electrodes either remaining above the brain or inside the brain. This and future recordings will help us to better understand the evolutionary origins of sleep behaviour and chronobiology. Additionally, knowing the activity cycle of these animals will be important for any attempts regarding sustainable marine culturing of this highly important species.

This work was supported by a KAKENHI grant "Brain activity of cephalopods during active and rest states." https://nrid.nii.ac.jp/nrid/1000010790280/ and by the Okinawa Institute of Science and Technology (OIST) Physics and Biology Unit.

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Insight into the connectome of the octopus learning and memory system

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Cephalopod molluscs exhibit sophisticated cognitive abilities mediated by a pivotal brain structure: the vertical lobe (VL). It is believed that the VL shows a connectivity organization typical of learning and memory network, however the anatomical considerations are limited to the work of Young and Gray in the 1940-70's.

The current study aims to produce detailed description of the synaptic connections of the various neurons comprising the VL, using recent progress of volume electron microscopy imaging. Accordingly, an *O. vulgaris* VL was fixed, serially sliced (30nm sections), imaged with a Scanning Electron Microscope at 4 nm pixel resolution and aligned into a traceable 3D stack (260x390x30µm). Extraordinary rich and highly dense synaptic profiles, with various type and combinations of synaptic vesicle types, are observed across the VL neuropil. All synaptic outputs of several superior frontal lobe (SFL) axons, the main input to the VL, were reconstructed revealing that in addition to the known connection to the amacrine interneurons (AM), they also innervate two yet undescribed cell types or subtypes.

Our results show that the AMs are structurally exceptionally simple receiving just a single synaptic input from only one SFL axon, thus suggesting an interneuron with no integration role. In contrast, the newly uncovered SFLs postsynaptic partners display complex dendritic arborizations receiving multiple synaptic inputs from the SFL axons. Overall our results reveal a larger diversity in VL cell types together with a connectivity scheme more complicated than previously described including unprecedented mono-presynaptic input in central synapse.

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Visual coding and functional organization in the octopus optic lobe

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Cephalopods are highly visual animals who use vision to detect predators and prey, as well as to drive unique behaviors like their rapid body pattern camouflage displays. Strikingly, the cephalopod visual system evolved independently from those of other highly visual species, so that both the eye and the underlying neural circuitry are evolutionarily distinct. However, there have been no direct recordings of visual responses in the cephalopod central nervous system, so it is unknown how this independently evolved visual system encodes the visual world. In this study, we used two-photon calcium imaging to record visually evoked responses in the primary visual processing center of the octopus central brain, the optic lobe, to identify the features extracted by the octopus visual system and determine how information is organized in the central brain. Similar to most visual systems, we found neurons with spatially localized receptive fields responsive to both ON and OFF stimuli locally clustered within the optic lobe that were organized in a retinotopic order. We also identified unique properties, such as size suppression for light stimuli but size summation for dark, that may have evolved to suit the specific demands of the visual world of the octopus. This study represents the first insight into the neural coding and organization of this highly capable but poorly understood visual system.

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Octopus arm biomechanics

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Cephalopods are highly evolved marine invertebrates that colonized almost all the oceans of the world at all depths. This imposed the occurrence of several modifications of their brain and body whose muscle component represents the major constituent. Moreover, modern cephalopods are soft-bodied animals that manifest, during movements, considerable variations in their body shape alongside with reversible modification of their stiffness/softness ratio. These properties are especially interesting for their translational aspects. Hence, studying their muscle physiology may give important hints not only in the context of animal biology but also in the emerging field of soft robotics. Here we present the major features of the octopus arm musculature. We will show their physical constraints within the arm embedding and how these muscles are adapted to work in relation to their use in motion. This information may provide a bench work for designing new soft materials with muscle-like properties employed in soft robotics.

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CephDev - Cephalopod developmental biology a CephRes2020 Virtual Event Focus on Session

Main Organizer: Prof. Eve Seuntjens (Belgium)
Co-Organizer: Dr Camino Gestal (Spain)

We aim to cover research within the broad field of cephalopod developmental biology, including the early larval stages. Given the technological advances in sequencing, genome editing and 3D imaging, a novel era of descriptive and functional developmental biology is expected to provide interesting novel insights into major developmental biology questions.
The session spans from genomic studies and novel tools such as CRISPR genome editing, organ development and embryonic to early larval physiology and behavior including different cephalopod species.

Within the physiological research, developmental biology is one of the most integrative fields, covering topic from genetics and epigenetics over stem cells and cell biology, and yielded several Nobel prizes. Novel technological break-throughs have the potential to open up cephalopod research to developmental biology, yet many hurdles still need to be taken. Bringing together researchers in this field is of utmost importance to boost this exciting cephalopod research domain.

[*abridged from the original proposal*]

Observing cephalopod embryonic development: from Naef to now

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In the beginning of the twentieth century, Adolf Naef described the embryonic development of cephalopods in great detail during two stays at the Stazione Zoologica Anton Dohrn in Napoli, Italy. Being a representative of the "idealistic morphologists" he carefully observed cephalopod development to develop his view on evolution, phylogeny and systematic morphology. His work became the work of reference for embryologists that followed his trace to study cephalopod development, and is -still today- a highly valued resource.
Using *Octopus vulgaris* as an example, this short presentation will discuss the main characteristics of cephalopod development, based on Naef's data as well as on our own observations recently obtained using contemporary microscopy.

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Transcriptomic study of early developmental stages of *Octopus vulgaris*

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The common octopus *Octopus vulgaris* has been proposed as a good candidate for aquaculture operations due to its fast growth and short life cycle. Settlement of animals has been the main bottleneck to reach the adult stage under captivity. However, recent achievements allowed the obtaining of samples to decipher the molecular basis that regulates the intricate early stages in this species.

In this study, *O. vulgaris* samples at different stages were sequenced by RNA-seq for a massive *de-novo* transcriptomic analysis following the TRINITY protocol. Four main comparisons were carried out considering a mix of different embryonic stages and paralarvae at 0, 10, 20 and 40 days post-hatching (dph). A total of 2300 up-regulated transcripts were found in newly hatched paralarvae (0 dph) compared to embryo whereas the number of overexpressed transcripts reached 2336, 3013 and 2580 in 10, 20 and 40 dph paralarvae, respectively, compared to newly hatched animals.

Gene ontology enrichment analysis revealed common terms at all ages such as chitin metabolism and peptidase related activities. Terms related to proteolysis and catabolism were significantly more represented in 0, 10 and 20 dph paralarvae whereas mobilization of proteins into the nucleus was more represented at 40 dph. Neural development and response to stimulus and activation of immune response were also differently represented attending to the age of the animals.

Altogether these results represent an overview of the different biological functions at paralarvae stage and will help to decipher at what age relevant functions are activated.

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Generation of Transgenic Lines in Cephalopods: Development of CRISPR-based tools in *Euprymna* spp. early embryos

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Cephalopod and vertebrate lineages split about 600 million years ago. Despite this evolutionary distance, both lineages independently evolved complex nervous systems and behaviours. This makes cephalopods ideal non-vertebrate animals to study and compare the design features of complex nervous systems across phyla. Cephalopod neuroscience and behaviour would greatly benefit from the establishment of powerful genetic tools. However, no transgenic animals have been recorded in this taxon.

Our goal is to generate CRISPR-based transgenic lines in *Euprymna berryi*. Because of its small size, ease of culture, short reproductive cycle and transparent embryo, this species has emerged as a potential genetic model organism among cephalopods. To integrate a transgene in the genome successfully, we plan to use the CRISPR/Cas9 system to induce knock-in through homology-directed repair. Target endogenous genes are cephalopod orthologs of SNAP25, VGLUT and VGAT, while possible transgenes are eGFP and GCaMP6. Genome editing involves several processes, such as phylogenetic analysis, design of sgRNAs, microinjection of dyes, mRNAs and CRISPR solutions into early embryos, as well as phenotyping and genotyping. We are at an early stage of testing these components.

In parallel, we aim to solve several challenges related to the propagation of genetic lines, by improving the husbandry of embryos, paralarvae and adults, sexing, identification tagging, evaluation of anaesthesia and euthanasia agents, and live video monitoring.

Ultimately, this study will generate powerful molecular tools to produce stable and tractable transgenic lines in cephalopods, that express genetically encoded calcium indicators and optogenetic actuators to image and control neural circuits.

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The octopus' solution to building a complex brain

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The development of the cephalopod nervous system from neural cords eventually forming mature lobes has been described on the morphological level. It is however still unclear where the progenitor cells are located and what the sequence of neurogenesis (from stem cell to postmitotic neuron) precisely is.
We used histological techniques and molecular markers to label dividing cells, neural stem cells, intermediate progenitors and neuroblasts on tissue sections of *Octopus vulgaris* embryos. Our results indicate that progenitor cells are located outside the optic, cerebral and palliovisceral cords in the lateral lips adjacent to the eyes, leading to the hypothesis that newly formed neurons should migrate into the cords.
(Neural) migration is one of the most complex cellular behaviors in the animal kingdom. In the developing vertebrate nervous system, extensive migration over long-range paths allows neurons born in distinct regions to integrate in a network. This process is essential to establish proper circuits and brain function. Little evidence exists for such neural migration in invertebrates and reported trajectories are limited to few cell lengths. To test our hypothesis, we performed lineage tracing experiments. Using light sheet imaging at different time points after dye injection in the lateral lips, we found that neurogenic progenitors are indeed located in these lips and that they divide multiple times before integrating into the cords.
The findings that octopus neural progenitors divide multiple times and migrate over such long distances are reminiscent of vertebrate neurogenesis and emphasize the complexity of the octopus brain even more.

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Awareness of danger inside the egg: early behavioral competencies in the cuttlefish

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The embryos of many species are extremely sensitive to prenatal stress. This stress in particular has deleterious effects on the cerebral and behavioral development of the young. O'Brien et al. (2018) have shown that there were no such effects in cuttlefish *Sepia officinalis*. Marthy et al. (1976) suggested the existence of a tranquilizer in the perivitellin liquid (PVF) of the eggs of the squid *Loligo vulgaris*, another cephalopod. Indeed, the locomotor activity of squid larvae decreases when they are placed in PVF taken at the end of embryonic development. The squid's PVF also has a tranquilizing effect in crustaceans. The existence of such a tranquilizer in cuttlefish eggs could reduce the effects of stressors in the embryo. Adaptively, the presence of a tranquilizer would be particularly relevant in the cephalopod species that develop in transparent eggs. In the absence of parental protection, it would act as a buffer against a stressful environment (e.g. presence of predators) and reduce the movements of embryos decreasing the probability of being detected by visual predators or premature birth.

He we will review the development of the sensory systems in embryos of two cuttlefish species. We showed that embryos can innately respond to predatory cues and can learn to recognize an odour as a threat by classical conditioning. Last, we showed that embryos are not sensitive to direct artificial or natural stress although a maternal stress impairs juveniles behaviours.

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Molecular pathway of light photosensitivity in cuttlefish embryo

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Background: Cephalopods are well known for their performing eyes and visually guided behaviour. Extraocular photosensitivity have also been evidenced in several species of adult cephalopods: in the skin, luminous organ or stellate ganglia. Photosensitivity is allowed by photosensitive molecules belonging to the same conserved gene families: opsins and cryptochromes. Behavioural studies have demonstrated that photosensitivity appears during development before hatching.
Aim: We looked for the molecular systems implicated in photosensitivity during development. We determined in a first step the diversity of the photosensitive molecules in the cuttlefish, then tested their expression (level and localization) in eye and two extraocular tissues, skin and central nervous system (CNS).
Results: We identified six (6) opsins, two (2) cryptochromes and one (1) visual arrestin in *Sepia officinalis*, more than previously shown. In embryos, some of these genes are expressed only in the eyes but not in the skin or CNS. Expression in these extra-ocular tissues appears in juvenile and adult *S. officinalis*.
Conclusion: These results suggest that *1)* the molecules studied (possibly in association) could have a role in both visual and non-visual ability to perceive light, *2)* extra-ocular photosensitivity sets up after hatching, reflecting a complex maturation in light perception of the cuttlefish.

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Emergence of cephalopod genomic novelty in developmental and organismal expression modules

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Studies on cephalopod genomes have revealed various levels at which genomic novelty evolved in these fascinating animals. However, how such novelties translate to regulatory and ultimately organismal innovation remains answered.
Using comparative genomic and transcriptomic approaches, we studied how extensive rearrangements and the emergence of novel genes in cephalopod genomes have contributed to the organismal evolution during developmental transitions, from early embryogenesis to adult. We conducted developmental time-course RNAseq in the Hawaiian bobtail squid *Euprymna scolopes* and found that novel cephalopod genes are preferentially expressed at later developmental stages and contribute to specific sets of adult tissues (e.g., orphan genes in the accessory nidamental gland, GPCR cephalopod-specific duplicates in the eyes). We also found that the ancestral bilaterian and molluscan genomic regions still retained in cephalopods showed a depletion of novel genes. Conversely, novel cephalopod syntenies and non-syntenic regions had a higher amount of novel gene formation. This suggests a relaxation of selective pressure potentially aiding novel gene emergence. Moreover, to study regulatory innovations we reconstructed 38 putative co-expression modules active during development using a weighted gene correlation network analysis (WGCNA), characterized the number of novel and syntenic genes in those modules, and profiled their expression in adult tissues.
Altogether, these results give us first insights into the potential role of novel genes during cephalopod development and their importance for particular organ functions.

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RegenSea- Regeneration in cephalopods: past approaches, present trends and future directions a CephRes2020 Virtual Event Focus on Session

Main Organizer: Dr Pamela Imperadore (Italy)
Co-Organizer: Dr Letizia Zullo (Italy)

Regeneration, a process restoring structure and function of damaged tissues, is widely distributed across the animal kingdom, spanning from early branching cnidarians to vertebrates. Cephalopods show impressive regenerative abilities, known since antiquity. Recent studies are supporting the advantage to further investigate this phenomenon in these organisms. Although molecular and cellular machinery involved remain largely uncharacterized in cephalopods, increased availability of genomic, transcriptomic and proteomic tools are helping shedding light on this complex process. Recent progress in the field is pushing this expansion, allowing for the identification of the main pathways involved in this phenomenon, so prominent in cephalopods. In addition, a comparison with other organisms is aimed to facilitate growing of the area, possible strategical networking, and to help in deciphering common and divergent mechanisms of regeneration, crucial tool in regenerative medicine.
We aim to draw a line between the past and the future directions of these studies with an insight in other invertebrate models.

[*abridged from the original proposal*]

Center and periphery. The early history of regeneration experiments on cephalopods (ca. 1900-1950)

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The occurrence of regeneration in cephalopods has been an established fact since antiquity, but could only be systematically studied in the second half of the XIX Century. Thanks to the special conditions provided by marine stations, the impressive breadth of regenerating capabilities of cephalopods could be explored, as well as their species-specific mechanisms and limits. From a physiological perspective, in the first half of the XX Century researchers engaged in exploring the potential of cephalopods not only as specific instances of regeneration, but also in a wider sense, as plausible research models for general mechanisms, common to both vertebrates and invertebrates.

This talk will provide an overview of the first ca 50 years of regeneration research in cephalopods (esp. *Octopus vulgaris*), with a special focus on the nervous system. Through analysis of the earliest works of Adolf Naef, Mathilde Lange, Enrico Sereni and JZ Young, I will illustrate the first theories of regeneration, their consequences in the interpretation of the anatomy, physiology and general biology of the animal, and the early attempts at establishing parallels between invertebrate and vertebrate regeneration.

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Convergence between octopus arm regeneration and development

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Cephalopod mollusks offer a particularly viable alternative to canonical limb regeneration models due to their similarities in early arm development to vertebrates, their complex arm structure and function, their fast and efficient regenerative capabilities and the relatively simple animal maintenance and handling. Due to its high regenerative power, *Octopus vulgaris* (the common octopus) has become of increasing interest for studies on regenerative processes.
Here we will present currently available information on the "mechanistic" events and molecular pathways underlying octopus limb formation during embryogenesis and regeneration.
We show that cephalopods share some aspects of the morphology of early stages of regeneration with other animals. This is particularly interesting in light of the recent advances in the field of cephalopod genomic and might help establishing a framework of where morphogenetic and more specifically regenerative processes occur in this animal species.

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Healing, regeneration and re-wiring in *Octopus vulgaris*: from imaging to biology

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Regeneration is a process restoring structure and function of damaged tissues. Cephalopods appear to be master in this phenomenon, in contrast to adult mammals, where recovery is often unsatisfactory. In *Octopus vulgaris* the pallial nerve and the arm appear to be interesting systems to investigate regeneration. The pallial nerves connect the brain to the periphery (mantle), allowing for the control of breathing and body-patterning. Injury of one nerve leads to loss of neural control on these functions. The eight arms are flexible muscular hydrostats lacking hard skeletal supports, which the animal largely use for exploring and interacting with the environment, thus exposing them to potential damages. Both the abovementioned structures are endowed with the capacity of fully healing and functionally regenerating after severe and repeated injuries.

Direct imaging of injured tissues has always represented an advantageous approach in regenerative studies. This technique in cephalopods has been limited by the reduced number of markers commercially available. New microscopy methods recently applied to vertebrate models help in overcoming these issues.

Here we present the results of the application of label-free approaches, i.e. multiphoton microscopy, to the regenerating nerves and arms of octopus. We identified structures usually not revealed through classical staining: hemocytes building up scars and phagocytizing debris (through CARS), degenerating fibers and muscles (TPEF) and the formation of a leading connective tissue bridge (SHG) involved in axons guidance.

Our data provide helpful morpho-chemical information; the techniques confirmed to be species-specific independent, thus appearing promising for regenerative studies in non-model species.

Support: This work has been supported by the Association for Cephalopod Research (PI)

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Tissue crosstalk is required to induce a stem cell based regenerative response in the Anthozoa Cnidaria *Nematostella vectensis*

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Regenerated body parts are essentially identical to the parts that were developed Little is known about the origin of the inductive signal that translates the amputation stress into a cooperative cellular response.
By studying the process underlying the reformation of lost body parts in the anthozoan cnidarian *Nematostella vectensis*, we identified a regeneration-inducing structure that, via a tissue crosstalk, is responsible for the initiation of the repair program. We further revealed for the first time in anthozoan cnidarians, that fast and slow-cycling/quiescent stem cells respond to the amputation stress and actively participate in the reformation of lost body parts. Importantly, a synergic interaction of both stem cell populations is required to complete the regeneration process.
Our findings suggest that the emergence/loss of structure complexity/compartmentalization influences the proprieties of tissue plasticity, changes the competence of a tissue to reprogram and, in the context of regeneration, the capacity of the tissue to emit or respond to a regeneration-inducing signal.

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Whole body regeneration requires a rewired embryonic gene regulatory network logic

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Regenerated body parts are essentially identical to the parts that were developed during embryogenesis. This observation is at the origin of a century old hypothesis proposing that regeneration utilizes developmental processes originally set aside for embryonic development. If this is true, the genetic interactions driving these two processes are predicted to be largely overlapping.
In order to address this hypothesis, we used the sea anemone *Nematostella vectensis* that is perfectly suited to compare the gene regulatory networks underlying embryogenesis and regeneration. After characterizing in detail the regenerative capacity and the principal events underlying oral regeneration of *Nematostella*, we performed a high-resolution temporal RNAseq time-course spanning this process and compared it to embryonic RNAseq data. Combined with molecular analysis obtained from signaling pathway perturbation experiments, we show that regeneration is a partial and rewired re-deployment of the embryonic GRN rather than a complete recapitulation of the embryonic program.

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Genome skimming clarifies the evolutionary relationship of bobtail squid

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Bobtail squid are small benthic, nektonic, or nektobenthic species; abundant in the Mediterranean Sea, the Indo-Pacific Ocean, and the Atlantic Ocean. However, their species relationship remains unclear. Here, we used low-coverage whole-genome sequencing to retrieve the complete mitochondrial genome and nuclear loci, from 24 different species that comprise the two genera within family Sepiidaridae and the three accepted subfamilies within the Sepiolidae, to resolve their phylogenetic relationship.

Our results based on mitochondrial genes support the sister relationship between the subfamilies Heteroteuthinae and Rossiinae, and indicate that some species need to be re-assigned, particularly within the Sepiolinae. The available reference genome of the Hawaiian bobtail squid *Euprymna scolopes* also proved useful to retrieve over 600 conserved loci. These conserved loci supported the relationship of subfamilies within the family Sepiolidae, similar to that based on mitochondrial genes alone, yet some relationships are in discordance.

Our study resolves the relationships of the main clades within the Sepiolidae and highlights the utility of low-coverage whole-genome sequencing to assess the relationship of cephalopods by using mitochondrial genes and nuclear loci, particularly when a reference genome of a closely related species is available.

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Deciphering cephalopods along south coasts of India using genetic markers

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Cephalopods are the third-largest group in Mollusca and the subclass Coleoidea, includes squids, octopuses and cuttlefish. There are about 60 species of cephalopods recorded from Indian waters. The annual fishery of cephalopods from Indian waters is estimated around 220,844 tonnes, with 6-7% of the total marine fish landing. There are a lot of taxonomic ambiguities within the Cephalopods and the accurate identification of species is essential for the sustainable fishery, ecosystem management and conservation measures. Though many studies are there elsewhere on molecular identification of cephalopods, from Indian region, molecular characterisation studies are very few. With a view to identify and genetically catalogue the Cephalopod species of Indian coasts, specimens were collected from landing centres of Southern Indian waters. Molecular characterization was done with partial sequence information of mitochondrial gene Cytochrome C oxidase-I (COI) gene. Genetic analysis showed, a total number of 14 species belonging to four families Loliginidae, Octopodidae, Sepidae and Sepiolidae. Phylogenetic analysis using maximum likelihood approach revealed that all 4 families are monophyletic. Interspecific distance between octopus, cuttlefish and squid was in the range 11-19%, 15-22% and 14-27% respectively. However, *Sepiella inermis* collected from East and West Coast showed 7% difference, hence warrants the need for finding any cryptic species available within the species.

Unlike other loliginids, *Sepioteuthis lessoniana* (~20%) showed more genetic similarity with Sepidae family than loliginidae family, and this also needs further exploration to understand the evolutionary relationship among the cephalopods.

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Molecular tools are contributing to the discovery of new cephalopod species in the northern Gulf of Mexico

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Two programs focusing on assessing biodiversity for the midwater fauna of the Gulf of Mexico, the Offshore Nekton Sampling and Analysis Program (ONSAP, 2011) and the Deep Pelagic Nekton Dynamics of the Gulf of Mexico (DEEPEND, 2015-2019), have provided the research team with over 12,000 cephalopods ranging in size from paralarvae to adults. These samples have been used for numerous studies that presently include: trophic dynamics, contaminants (PAH's), vertical distribution patterns, and genetic connectivity to other ocean basins. One exciting outcome to this work has been the discovery of at least four new cephalopod species.

The team has been using both morphological and molecular tools to analyze potential new species. One of the surprising discoveries made was finding three new Bathyteuthid species to the Gulf of Mexico and north Atlantic Ocean which has been recently published in the Bulletin of Marine Science (April 2020).

The discoveries from these programs will be discussed in this presentation including a new description of a *Helicocranchia* species.

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Adaptive evolution at mRNA editing sites in soft-bodied cephalopods

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Adaptation of low-polymorphic populations is limited by their genetic variability, and heritable epigenetic modifications could be an additional source of selected variants due to the involvement of otherwise neutral mutations in the evolutionary process. One example of such modification is a widespread type of mRNA editing where adenine nucleotide is substituted with inosine, which is subsequently recognised by the cellular machinery as guanine. mRNA editing, while potentially important, very rarely alters the protein sequences in all lineages studied so far. An exception are soft-bodied cephalopods (coleoids), where the numbers of re-coding editing sites exceed those in other lineages by orders of magnitude, which is interpreted in terms of editing sites being useful for sophisticated phenotypic adjustments in complex coleoid nervous systems.

We propose, that, apart from conservative editing sites, which comprise up to 47% of sites and could bear a regulatory function, there are non-conservative sites, comprising more than 53%, which could temporarily mask beneficial adenine-to-guanine substitutions until an actual substitution to guanine happens, and, as coleoids are rather low-polymorphic, such scenario could be a way for them to promote adaptation. We show, that non-conservative edited adenines are indeed frequently substituted to guanines and for heavily edited sites there is positive selection acting on this transition, and, as we further show, that editing depends on mutations of the local context, we propose, that mutations enhancing editing could be indeed a source of variants needed for selection.

Our study provides the first transcriptome-wide example of an epigenetic process that contributes to the increased expressed genetic variability and hence facilitates adaptation.

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Architeuthis dux genome: a search into cephalopod gigantism and key-adaptations to deep-sea environments

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The giant squid (*Architeuthis dux*; Steenstrup, 1857) is an enigmatic giant mollusc with a circumglobal distribution in the deep ocean, except in the high Arctic and Antarctic waters. The elusiveness of the species makes it difficult to study, the lack of fresh samples hinders genetic studies, and the complexity of the cephalopod tissue creates technical challenges in the laboratory. Still, having a genome assembled for this deep-sea dwelling species will hopeffully allow unlocking several pending evolutionary questions.

I will present our strategy and describe how we produced a draft genome assembly that includes 200 Gb of Illumina reads, 4 Gb of Moleculo synthetic long-reads and 108 Gb of Chicago libraries, with a final size matching the estimated genome size of 2.7 Gb, and a scaffold N50 of 4.8 Mb. In addition, we sequenced the proteome of the same individual and RNA from three different tissue types from three other species of squid species (*Onychoteuthis banksii*, *Dosidicus gigas*, and *Sthenoteuthis oualaniensis*) to assist genome annotation. We annotated 33,406 protein coding genes supported by evidence and the genome completeness estimated by BUSCO reached 92%. Repetitive regions cover 49.17% of the genome.

This annotated draft genome of *A. dux* provides a critical resource to investigate the unique traits of this species, including its gigantism and key adaptations to deep-sea environments.

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Establishment of a Model Association: Investigating the Squid-*Vibrio* Symbiosis Research from 1970s

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Model organism approaches gained considerable attention by historians and philosophers of science as they have been very prominent in life sciences. One of the recent trajectories in historical and philosophical scholarship is looking beyond individual species and focusing on model systems research involving multispecies parallel to recent trends in biological research.
Here I argue that multispecies model systems are beyond the reach of 'model organism' and propose 'model association'. This work has two aims: ***1.*** A case study is developed on the squid-*Vibrio* model system from 1970s offering a detailed historical account of the development of this model association which the literature is lacking despite the important status and wide use of squid-*Vibrio* system in symbiosis research. **2.** Notion of model association inherently assumes a relationship between more than one organism, so the methodological analysis answers the critique of scientists regarding the neglect of the multi-organismic nature of life and their emphasis on the microbiome.
Accordingly, I first offer a historical description of how the partnership of the squid *Euprymna scolopes* endemic to the shallow coasts of Hawaiian archipelago and the bacteria *Vibrio fischeri* became a prominent model with the work of McFall-Ngai and colleagues. This draws on earlier challenges of introduction and adjustment of the squid into the laboratory and later establishment of the model system and the formation of a research community around it. Secondly, based on the case study, I identify the practical, philosophical, and social conditions turning a model system into a model association.

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“It just stares back at you!”. The early history of psychophysiological experimentation on Octopus

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By focusing on selected examples of experimental psychophysiology of *Octopus vulgaris* in the XX Century, this work will follow three main routes.
Firstly, I will consider the weight of casual observations and long-established “octopus myths” on the choice of this animal for conditioning and learning experiments.
Secondly, I will reconstruct the development and refinement of experimental settings, in relation to both the paradigm and the technical setup.
Finally, I will pose the question of the “contribution” of the octopus to psycho-physiological theories at different degrees of generality, as an exception, an example or a model. In this connection, special attention will be devoted to the framing of the octopus in a comparative perspective with vertebrates, in terms of both specific experimental paradigms and general theoretical interpretations.
The construction of the octopus as an “exceeding” mollusk will be put in relation with more recent debates about its legal status of protected animal in many countries.

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The Octopus and Consciousness: *What Can We Learn*?

Sidney Carls-Diamante

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This talk presents philosophical insights that arise from empirical research on octopuses, focusing on those significant for the scientific study of consciousness. The results presented are the outcome of integrating empirical findings about the octopus nervous system with philosophical theories about the mind.

Due to the extent of decentralization of the octopus nervous system, and the ensuing anatomical distribution of cognitive substrates and routines, octopus consciousness may not exhibit a unified structure. This possibility is significant, as it challenges the received view that where consciousness exists, it is unified (i.e. what is experienced by the subject is a single, integrated field of consciousness). Consequently, the need arises for theorists to reevaluate presuppositions and other commonly held notions about the mind, such as the association of complex intelligence and behaviour with unified consciousness, or that the "default" or "normal" structure of consciousness is that it is unified.

The broader significance of philosophical investigations such as these is that they address the issue of how cognitive science can expand its explanatory toolkit to better accommodate forms of intelligence that are not as familiar as those of well-studied vertebrates.

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Visual attack on the moving prey by cuttlefish

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Visual attack for prey capture in cuttlefish involves three well characterized sequential stages: attention, positioning, and seizure. This visually guided behavior requires accurate sensorimotor integration of information on the target's direction and tentacular strike control. While the behavior of cuttlefish visual attack on a stationary prey has been described qualitatively, the kinematics of visual attack on a moving target has not been analyzed quantitatively.

A servomotor system controlling the movement of a shrimp prey and a high resolution imaging system recording the behavior of the cuttlefish predator, together with the DeepLabCut, a pose estimation method based on transfer learning with deep neural networks, were used to examine the tactics used by cuttlefish during a visual attack on moving prey.

The results showed that cuttlefish visually tracked a moving prey target using mainly body movement, and that they maintained a similar speed to that of the moving prey right before making their tentacular strike. When cuttlefish shot out their tentacles for prey capture, they were able to either predict the target location based on the prey's speed and compensate for the inherent sensorimotor delay or adjust the trajectory of their tentacular strike according to the prey's direction of movement in order to account for any changes in prey position.

These observations suggest that cuttlefish use the various visual tactics available to them flexibly in order to capture moving prey, and that they are able to extract direction and speed information from moving prey in order to allow an accurate visual attack.

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Mapping and understanding the function of RNA in the Octopus brain

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Coleoid cephalopods possess the most complex nervous system among invertebrates. Recent studies have identified high levels of RNA-editing in the cephalod nervous system. Thus, there may be a link between RNA biology of the coleoid cephalopods and the cognitive success of this group. However, previous studies have focused on polyadenylated transcripts and thus the knowledge about other RNA species and their relation to the nervous system is still missing.
Here, we report the assembly of a draft transcriptome (adenylated and non-adenylated) of the *Octopus vulgaris*. To accomplish this, we have performed full-length RNA transcript sequencing ("Flam-seq", Legnini et al, Nature Methods 2019) of 18 different tissues from adult animals. In addition, we have performed small RNA sequencing of the same tissues.

Computational analyses of these data suggest that we have created a useful catalogue of RNA species in this animal. We believe that this catalogue will aid to generate insights into unique RNA-mediated mechanisms of cephalopods.

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Mapping location, projections, spatial arrangement and response properties of peripheral nociceptors

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Identifying and characterizing different neuronal cell subtypes has always been challenging in cephalopods, due to the complexity of the nervous system and lack of validated methods for identifying functional divisions within discrete brain regions. We have previously used behavioral assays, pharmacology and electrophysiology to identify nociceptive sensory afferents in peripheral ganglia and CNS in squid and octopus, but anatomical arrangement and functional properties of individually-identified cells has proven elusive.

In this project we are using calcium imaging of peripheral ganglia in semi-intact preparations of the squid, *Euprymna scolopes*, to map response properties, spatial arrangement and local circuit involvement of primary afferent nociceptors. We show that primary nociceptors and 2nd-order neurons are located in the stellate ganglion, and that there is somatotopy of sensory neurons both in the medial-lateral and rostral-dorsal planes.

Ongoing work is aimed at revealing whether somatotopy of primary afferents is preserved into higher order brain structures, and if central nociceptive circuits articulate with the higher cognitive centers of the brain.

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The evolution of intelligence in cephalopods

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Theorists working on the evolution of brains and intelligence have argued that sociality has been key to the evolution of complex cognition. However, the tests of these theories have relied on data from more social animals—primates, birds, and recently cetaceans. These theories and empirical tests predict that big brains co-evolve with long lives, long juvenile periods and complex social organization.

Coleoid cephalopods, with their short lives, little to no nurturing of young, and at most simple social organization, pose a challenge to these social brain theories. A new theory, the cultural brain hypothesis, tries to generalize theories of brain evolution and has suggested another pathway of asocial learning with different predicted brain correlates, such as a contracted juvenile period and lack of relationship with group size. Coleoid cephalopods are an ideal test of these predictions. We are beginning a research project to compile a large database of publications on Coleoid starting with brains, bodies, behavior, life history, sociality, reproductive patterns, and ecology, assisted by the existing data of Borrelli (2007). Using this dataset, we hope to learn more about the evolutionary roots of cephalopod intelligence. Such a dataset will be useful in itself, but will also show gaps in our knowledge and point out patterns in the evolution of intelligence that we might not have expected. The assistance of the community of cephalopod researchers will be most helpful in targeting areas and emphases, and identifying caveats and challenges that we may miss in this project.

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Squid camouflage to substrate

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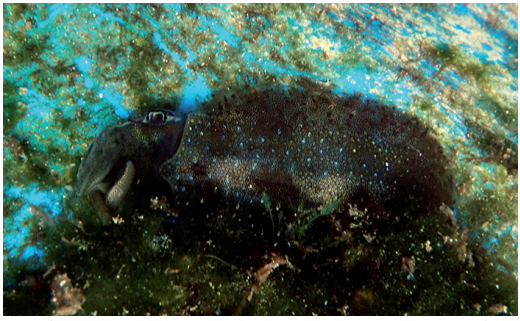
Coleoid cephalopods are known to rapidly camouflage to its environment to protect themselves from predators. They achieve this mainly by expanding and contracting neurally controlled pigment containing cells called chromatophores in their skin. Thus far, studies concerning cephalopod substrate camouflage were covering almost exclusively benthic species such as European cuttlefish, *Sepia officinalis* species complex and pharaoh cuttlefish, *Sepia pharaonis* species complex. These cuttlefish spend much of their early life sitting on a substrate without much movement. It makes them ideal subjects for quantifiable analysis of cephalopod camouflage.
In contrast to benthic cuttlefish, oval squids of *Sepioteuthis lessoniana* species complex are considered to represent semi-pelagic animals that spend most of their time in a water column and use countershading as primarily defense mechanism.
In this study, we demonstrate that *S. lessoniana* Sp.2 (Shiro ika, white squid) from Okinawa archipelago (Japan) in laboratory settings display two different types of substrate camouflaging behavior: 1) substrate camouflage in motion (dynamic motion camouflage), and 2) situational substrate camouflage.



In the motion camouflage, chromatophores expansion follows the substrate reflectivity. In the situational camouflage, the squids deploy body pattern including three chromatic expression (uniform, mottled and disruptive), five postural (hovering, resting, curled arms, spread arms, straight arms, upward curl) and corrective formation (alone, group, touching and piling).

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Get The F(ish) Out: octopuses punch during collaborative interspecific events

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Cooperation and collaboration are ubiquitous in nature, providing immediate direct benefits and/or future indirect benefits to participating partners. Octopuses and various species of fishes are known to form multi-partner hunting groups, where the formers pursues prey within rock and coral crevices, while the latter scour the sea floor and guard the water column. Groupers use referential gestures to signal hidden prey locations to octopus partners, thus these groups increase prey opportunities for all participants. However, in heterogeneous multi-specific groups, some species may benefit more than others, which can lead to conflicts over the distributions of investment and payoffs.

Here we report a series of observations where different *Octopus cyanea* individuals engage on active displacement of partner fish during collaborative hunting. To this end, the octopus performs a swift, explosive motion with one arm directed at a specific fish partner, which we refer to as "punching" (n=8 events). From an ecological perspective, actively punching a fish partner entails a small energetic cost, but simultaneously imposes a cost on the targeted fish partner, i.e. subtraction of an immediate opportunity to catch prey, relocation to a more external or less advantageous location in the group, or even eviction. Thus, punching serves as a control mechanism, the nature of which (e.g. sanctions, aggression, punishment) is dependent on the ecological context of the interaction, and on how the benefits are yielded from inflicting costs to fish partners.

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Experiments exploring the limits of cognition and observational learning in *Octopus vulgaris* using mechatronics and video enrichment

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Experiments with *Octopus vulgaris* have shown that this species is capable of using electromechanical sensors to control electronics and robotic devices even when not motivated by food, and that *O. vulgaris* also can correctly interpret videos of crabs as showing prey, or conspecifics as rivals. Animals are able to intensely watch these videos over long periods of time from a distance while understanding (after trying unsuccessfully) that the crabs or conspecifics in the video cannot be reached because of the glass wall of the aquarium. *O. vulgaris* also has been shown to be duped by toy crabs, and experiments with mammals (up to Gorillas and Orang Utans) as well as birds (penguins and parrots) have shown that even these highly intelligent animals are duped and/or intrigued by clearly artificial robotic lookalikes of conspecifics.

The combination of these observations allows to conduct repeatable, scientifically rigorous experiments that unambiguously prove the existence of social observational learning of *O. vulgaris*, opening up an efficient way to explore the upper limits of cognitive and learning abilities of the species by using training videos that are animations of life-like but artificial "teacher" octopuses, thereby allowing to teach real octopuses advanced cognitive skills that no real octopus has ever learned by other means before, because a real "teacher" octopus that learned these skill the slow, natural way, is not needed anymore.

It also allows to experiment with simplified animations of "teacher" octopuses that are less real life-like and more cartoonish.

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Cephalopod Cognitive Evolution

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Large-brained vertebrates (e.g. apes, cetaceans, corvids) share slow life histories and challenging social environments, suggesting that these two factors play a key role in the evolution of intelligence. However, coleoid cephalopods (octopus, cuttlefish and squid) question this view. These shell-less molluscs evolved large brains supporting strikingly rich behavioural repertoires (e.g. tool use, complex anti-predatory strategies and mating tactics) although they do not engage in complex social bonds and have fast life histories (e.g. lifespan < 2 years, terminal reproduction). It has been proposed that cephalopod cognitive evolution may have been shaped primarily by predatory and foraging pressures, but a challenging mating context may also have played a role. The disappearance of the shell in coleoids may have resulted in higher rate of unavoidable mortality (due to stronger predation), thereby preventing the evolution of slow life histories. However, future research will be essential to test the influence of life history and of different selective pressures in cephalopod cognitive evolution. In parallel, a systematic investigation of cephalopod cognition is also needed to quantify the cognitive complexity in these molluscs.
Ultimately, these lines of research have the potential to shed light on alternative routes for the emergence of cognitive sophistication in non-human animals.

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Embodied organization of motor control in a soft-bodied animal – *Octopus vulgaris*

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Octopuses provide an outstanding example of successful motor behaviors expressed in a flexible body. In skeletal animals, the interfacing of sensory and motor information for motor planning is based on representation in body-part coordinates. In contrast, in hyper-redundant soft-bodied animals like the octopus, this control approach is impractical as it would require infinitely large number of variables (DOFs) to represent the soft body. Octopuses overcame this difficulty through the coevolution of unique features at all levels of body and brain; from the neuromuscular system of the arms up to the organization of the central brain's higher motor centers. The overall embodiment of all these unique properties helps explain how the "alien"-looking body of the octopus simplifies locomotion control and how the special distribution of the central and peripheral nervous system simplifies control of goal-directed arm movements. It also helps explain why higher control centers in the brain are not organized somatotopically as in vertebrates and why arm coordination in locomotion involves probabilistic control rather than a deterministic CPG. Finally, the overall embodiment can explain why motor learning employs 'strategy-learning' rather than 'skill-learning'.
The octopus demonstrates that embodied organization, a concept developed in robotics, is an important evolutionary principle showing how adaptation of the body to the task helps simplify its control.

Support: the European Commission EP7 projects OCTOPUS and STIFF-FLOP and by the Israel Science Foundation (ISF)

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A journey around Cephalopod Research and possible future avenues

Graziano Fiorito
talk delivered on behalf of coworkers

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Cuttlefish, squid and octopus provided fascinating cases for studying biological, neural and behavioral plasticity.
I will surf around some of the most recent interesting findings including: genomes (namely: *Octopus bimaculoides*, *Callistoctopus minor*, *Octopus vulgaris*, *Euprymna scolopes*, *Octopus sinensis*, *Architeuthis dux*)[1], an huge effort about five years; the role of extraocular photoreceptors in light-activated chromatophore expansion; RNA-editing capabilities; *Dosidicus gigas* use of bioluminescence to illuminate "semantically complex" communication patterns and in foraging. Cephalopod genomes are also mentioned taking into account on-going research around orphan and cephalopod specific genes. Further examples are provided based on: ***i.*** the exploration of the organization of 'brains' in cephalopods and their complexity; ***ii.*** neural cell numbers and their variations among individuals; ***iii.*** the neural plasticity and underlying molecular fingerprint; ***iv.*** marked inter-individual variability in behavioral performances arising through the reciprocal influence of environmental and behavioural factors.
Following J.Z. Young (1985), exploration of the properties of aggregates of neurons should be explored, with octopus' "various neuropils" providing "the material ... needed, just as the giant fibers of the squid ...testing of new methods for the study of membranes". Exploring the biological plasticity in cephalopods may help to study modular organization of the brain and its evolution, orchestration of neuromodulators (single cells sequencing recent approaches may be one example), cross-modality, and central vs periphery (what this 'means' for cephalopod/mollusc nervous systems).
It is suggested to act as an interdisciplinary forum for scientific interaction that would further our knowledge and understanding by facilitating cross-talk and collaboration among groups of scientists from very different fields who otherwise might not have opportunities for exchanging ideas so directly. My ultimate goal is to stimulate discussion around future efforts and possibly facilitating further some unexplored research avenues.

Supported by: the Stazione Zoologica Anton Dohrn; the Association for Cephalopod Research 'CephRes'; Fondazione Banco di Napoli; RITMARE FlagShip Project

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1. listed in order of year of publication

A metabarcoding approach for assessing the diet of the glass squids (Oegopsida: Cranchiidae)

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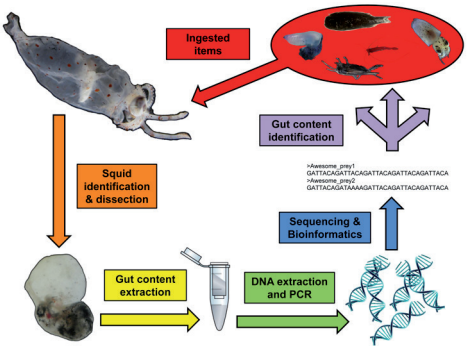
Members of the family Cranchiidae Prosch, 1847, commonly known as glass or bathyscaphoid squids, range from small translucent species to the massive-sized colossal squid. Those cephalopods are widely distributed in all oceans, also representing one of the more diverse families of squids and, despite their ecological importance, their diet is almost unknown. Cranchiids represent a significant prey for many organisms in the mesopelagic zone, from fishes to marine megafauna, as sharks, swordfishes and whales. Their trophic role is mostly unknown and only few studies are available.

Aim: characterize for the first time the diet of cranchiid squids through DNA metabarcoding.

Methods (see graphical abstract of the workflow): gut contents of 62 squids collected in the Atlantic, belonging to 10 genera were used to amplify a fragment of the mitochondrial gene cytochrome c oxidase subunit I, and sequenced in the high-throughput sequencing platform Illumina. Sequencing reads were identified using published sequences.



Results: A preliminary bioinformatic analysis of the reads from the gut contents shows a wide diversity of eukaryotic sequences. The more represented reads belong to Malacostraca, Actinopterygii, Cephalopoda, Hydrozoa and Insecta.

Conclusion: Molecular assessment of the diet of 10 of the 13 described genera of cranchiids revealed the taxonomic spectrum of their ingested items, helping to understand the role of these squids in the mesopelagic realm.

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Enigmatic parasites from an uncommon habitat: Diversity of ciliates and mesozoan parasites of renal appendages of cephalopods

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The renal sacs of cephalopods are an uncommon habitat for two phylogenetically distant wormlike parasites, dicyemid mesozoa and ciliate chromidinids. These are organisms, which can reach a few millimetres in length and are attached to the renal epithelia by their anterior part. Information was mainly based on morphological criteria and little is known about the biodiversity and taxonomy of these enigmatic parasites. In this study, we have undertaken to study these two groups with regard to their biodiversity in cephalods from the Mediterranean Sea and the Atlantic Ocean. Cephalopods from sampling campaigns revealed a weak prevalence of the infection by chromidinids ciliates probably biased by the sampling method since sampled hosts were predominantly benthic, while chromidinids mainly infect pelagic cephalopods. Hence, the diversity is underestimated, even if we were able to describe two new species and obtain the first time molecular data from the 18S rDNA marker.
Phylogenetic analyses show that chromidinids belong to the apostome ciliates and to the Oligohymenophorea. Prevalence of the infection by dicyemids was shown to be more important. A major problem in the dicyemid systematics is that a large number of named morpho-species are based on incomplete morphological descriptions, causing ambiguities in their identification. We therefore redescribed all stages of the development and assigned an 18S rDNA marker sequences to the species of sampled dicyemids species.

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A comprehensive review of early life stages in Cirrata (Cephalopoda: Octopodiformes)

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Living cephalopods (Mollusca: Cephalopoda) exhibit a wide range of reproductive strategies, that can be classified into simultaneous terminal, polycyclic, multiple, intermittent terminal, as well as continuous spawning. While information about reproductive, and ultimately life history strategies are readily available for most neritic taxa, significantly fewer data has been gathered on deep-sea cephalopods. This applies in particular to the Cirrata (Octopodiformes: Octopoda), an enigmatic group of semi-gelatinous benthic, benthopelagic, and pelagic octopods that occur down to over 7,000 m depth. Within this taxon, females have an ostensibly derived reproductive biology, whereby the eggs are coated with a protective layer that hardens when in contact with water and thus also serves in attaching the embryo permanently to a wide range of surfaces.

Here we provide the first comprehensive overview of biogeographic, morphometric, and morphological data gathered for representative taxa from all eight extant cirrate genera. The insights derived from this comparative analysis are based on results obtained through a broad literature search, the application of non-invasive as well as invasive imaging techniques, and online database queries. The data obtained on cirrate eggs, embryos, hatchlings, and juveniles are compared with documented characters from selected octopodiform outgroup taxa and provide further evidence for the uniqueness of the cirrate reproduction strategy among cephalopods.

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Evolution of the hectocotylus in Sepiolinae (Cephalopoda: Sepiolidae), a sound base for generic revision

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The subfamily Sepiolinae (Mollusca: Cephalopoda: Sepiolidae), currently containing the genera *Sepiola*, *Euprymna*, *Inioteuthis*, *Rondeletiola* and *Sepietta*, is characterized by the hectocotylization of the left dorsal arm, i.e. its transformation into a copulatory organ due to modifications of some sucker/pedicel elements. The hectocotylus morphology varies to a great extent across genera and species. In particular, one to several pedicels in its proximal third are suckerless and highly and diversely modified in shape to constitute the copulatory apparatus. An evolutionary gradient was observed in copulatory apparatus morphology, from the simple modification into a papilla of one pedicel from the third element of the ventral sucker row (some nominal *Euprymna* species) to a quite complex structure involving several variously modified pedicels in both the ventral and dorsal sucker rows (*Inioteuthis*). In some species, elements in the distal portion of the hectocotylus may also be highly modified, such as the *Euprymna* columnar suckers. Nine groups of species that do not match the current generic subdivision of Sepiolinae can be distinguished on the basis of hectocotylian diversity. Additional morphological characters (number of sucker rows on arms, female bursa copulatrix, occurrence and shape of visceral light organs, etc.) corroborate the subdivision of Sepiolinae into nine subtaxa, i.e. genera. Accordingly, a cladogram may be drawn to describe the possible phylogenetic relationships among these nine clades. It corroborates the need to redefine all extant genera and describe some new ones.

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Effect of water turbidity on visual abilities of cuttlefish (*Sepia officinalis*)

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In the English Channel the level of turbidy changes seasonally and daily in seawater. As a consequence, most visual abilities based on intensity of contrast are useless. On the contrary, in such environment, polarization sensitivity may help individuals detecting preys and predators. Almost all studies use cuttlefish coming from breeding systems in clear water, but according to species history, one can expect that cuttlefish sensory systems are better adapted to turbid environment, for example with development of polarized light sensitivity. To tackle this question, hatchlings from eggs laid by wild cuttlefish were reared one month under three conditions: clear water, slightly turbid water (0.1 g/l of clay) and highly turbid water (0.5 g/l of clay). The visual capacities of the cuttlefish were tested at 7 days and 1 month with an optomotor apparatus in clear or turbid water, using three patterns (contrasting stripes, polarized stripes and uniform gray).
Preliminary results suggest that the breeding conditions had no significant effect on the visual performance of cuttlefish of both age. Surprisingly turbidity seems to reduce ability of all cuttlefish to respond to polarized patterns but have only few or no effect on contrasting patterns. Whether this result is due to species history or to individual experience needs further investigations. Our results may differ from previous literature because we use clay to obtain turbid water instead of fine sand. The size and shape of the particles can change light polarization pattern in the environment.
In conclusion, cuttlefish should probably use both polarization and intensity contrast depending on the property of light in water.

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Cephalopods under climate changes: from Palaeozoic to recent years

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Cephalopod molluscs exhibited a range of responses to environmental drivers throughout their evolutionary history. These responses included: 1) shift in reproductive strategies (e.g. during Turonian global warming and after C/T mass extinction); 2) change of the lifestyle (e.g. Jumbo squid Dosidicus gigas in two recent decades); 3) mastering to live and reproduce in new habitats (e.g., *Illex coindetii* in recent years); 4) use of short – term climatic opportunities to explode in abundance (e.g., *Todarodes sagittatus* off NW Africa in 1974 and in NE Atlantic in early 1980-ies); 5) switch to another level of abundance and establishing a new role in the ecosystem (e.g., *Octopus vulgaris* off NW Africa in 1970-ies).

Climate–driven shifts of species ranges might be a common case of cephalopod life histories due to high flexibility of their reproductive strategies. When cephalopods were not able to cope with large-scale environmental changes, they were subjected to mass extinctions (e.g. at various Palaeozic and Mesozoic acidifications).

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Feeding rates of *Octopus* aff. *vulgaris* paralarvae fed with different prey types and densities

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Nutrition and the lack of an established feeding protocol for Octopus vulgaris paralarvae are bottlenecks for large-scale cultures. Herein, we aimed to obtain *Octopus* aff. *vulgaris* paralarvae daily feeding rates (FR) by testing different live prey types (*Acartia lilljeborgii* copepods, *Callinectes sapidus* zoeae, *Artemia nauplii*) and densities (20, 40, 60, 80, 120 and 160 prey L^{-1}). Experiments to estimate FR were performed at 22°C with 1 and 8 day-old paralarvae and lasted 24h, after which the number of prey ingested was recorded to estimate FR. For each density, five replicates were used, with one paralarva each. Mean FR obtained under different prey densities were analyzed through one-way ANOVA. The results showed that FR increased with increasing prey density, for both 1 and 8 day-old paralarvae. Individual variability was high in all experiments and FR ranged from 0 to 69 prey day^{-1}. The highest FR were observed with *Artemia nauplii* as prey for 8 days-old paralarvae (63-69 prey day^{-1}), at a density of 160 prey L^{-1}. For 1 day-old paralarvae, the highest FR was recorded for zoeae (33 prey day^{-1}), while the lowest was recorded for copepods (0-9 prey day^{-1}) for 8 days-old paralarvae. Our results provide important information on daily FR of paralarvae on natural preys, such as copepods and zoeae. Predation by paralarvae is favored by increasing prey density up to 160 prey L^{-1}.
Overall, these results may serve as an important reference for rearing paralarvae and have ecological value in estimating the impact of paralarvae predation in nature.

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Feeding ecology of the cephalopod *Octopus vulgaris* illustrated by a stable-isotope approach

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Cephalopods play a key role in the marine environment, but studies of their feeding ecology are still limited. Stomach content analysis have been the main method used for studying the diet of cephalopods, but despite being time-consuming, the prey is often difficult to determine. This technique allows the determination of species eaten, using hard parts that tend to resist digestion and remain in the stomach for longer period of time or hard parts which resist digestion, such as crustacean exoskeletons, cephalopod beaks, fish otoliths, and bones.

A stable isotope study was carried out seasonally to investigate the feeding ecology of the cephalopod *Octopus vulgaris*, and its potential prey species were identified according to the species that appeared in pots collected by fisherman. The food items identified were the fan mussel (*Atrina fragilis*), Algarve volute (*Cymbium olla*), Henslow's swimming crab (*Polybius henslowii*), and the red-band fish (*Cepola macrophthalma*).

The stable isotope mixing model SIAR indicated that *O. vulgaris* is predating mainly on the bivalve *A. fragilis* (mean value: 70%). *P. henslowii* could have a contribution of 18% to the cephalopod diet, whereas the gastropod *C. olla* and the fish *C. macrophthalma* were much less important to the consumer diet (7 and 5%, respectively).

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Ontogenetic changes in stable isotope composition (δ13C and δ15N) in two widespread species of the Arctic cephalopods

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Stable isotope analysis (SIA) has recently been established as a powerful tool in trophic ecology studies, with the most used isotopes in marine environment being δ13C and δ15N. Trophic level (TL) could be estimated based on δ15N using specific equations, and δ13C is largely a proxy of habitat utilization capabilities. Two species were studied using SIA: pelagic squid *Gonatus fabricii*, the most abundant Arctic cephalopod and the only Arctic squid, and nekto-benthic sepiolid *Rossia palpebrosa*, the most abundant nekto-benthic cephalopod in the Arctic. Lower beak SI values were used in our studies, and 4.8‰ correction was used while estimating TL, as suggested in the literature. Trophic levels were estimated with scaled approach to trophic enrichment factor.

Significant geographic differences in δ13C values were found in both species, with δ13C values increasing from the Barents Sea to West Greenland through East Greenland. Significant ontogenetic increase in δ13C values was found in *G. fabricii* only, and no ontogenetic pattern revealed in *R. palpebrosa*. Significant ontogenetic increase in δ15N values and TLs was found in both species. The increase in *G. fabricii* was more steep and pronounced, than in *R. palpebrosa*: 10.0‰ and 2.6 TLs vs 5.4‰ and 1.5 TLs.

These findings suggest: a) more migratory life style in *G. fabricii* vs supposed absence of migrations in *R. palpebrosa*; b) different patterns of ontogenetic changes in δ15N values and TLs suggest different scenarios of possible climate change-related adaptations (as Arctic cephalopods were repeatedly proven to be influenced by climate change).

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Influence of pH and diet on mercury bioaccumulation in cuttlefish

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Mercury (Hg) remains one of the most problematic metallic contaminants in the marine environment due to its high bioaccumulation capacity in organisms, its biomagnification within food webs, its high toxicity and elevated potential health risks linked to the consumption of seafood. As both predators and prey of many species, cephalopods have a pivotal role in trophic webs making them a vector of Hg towards top predators. Despite an evident Hg accumulation in their tissues, the bioaccumulation dynamic of Hg remains poorly documented in cephalopods. Bioaccumulation processes of Hg is influenced by biotic (e.g. size, habitat) and abiotic factors (e.g. temperature) but, to date, the effect of environmental pCO_2 are not yet fully explored. The present work aims at characterizing the bioaccumulation kinetic of Hg in the common cuttlefish *Sepia officinalis* in the context of future ocean acidification. The bioaccumulation kinetics and the organotropism of both waterborne inorganic Hg (iHg) and dietary methylmercury (MeHg) were studied using the 203Hg radiotracer. In addition, the influence of the pCO2 on their kinetics were investigated. Finally, the influence of the prey type, either fish or shrimp, on the trophic transfer of MeHg was also characterized. Our results demonstrate that the pCO_2 had no significant influence on Hg accumulation either for iHg waterborne or dietary MeHg. In contrast, the diet composition plays a major role.
Despite a MeHg assimilation efficiency close to 100% whatever the prey, the shrimp-derived biological half-life of MeHg is about 25 days while MeHg from fish remained tightly retained in cuttlefish tissues (half-life not significantly different from infinity). Thus, diet composition is expected to have a major role on Hg concentrations in cuttlefish tissues.

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Cuttlefish Buoyancy in Response to Food Availability and Ocean Acidification

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Carbon dioxide concentration in the atmosphere is expected to continue rising by 2100, leading to a decrease in ocean pH in a process known as ocean acidification (OA). OA can have a direct impact on calcifying organisms, including on the cuttlebone of the common cuttlefish Sepia offcinalis. Moreover, nutritional status has also been shown to affect the cuttlebone structure and potentially affect buoyancy. Here, we aimed to understand the combined effects of OA (980 uatm CO_2) and food availability (fed vs. non-fed) on the buoyancy of cuttlefish newborns and respective cuttlebone weight/area ratio (as a proxy for calcification).

Our results indicate that while OA elicited negative effects on hatching success, it did not negatively affect the cuttlebone weight/area ratio of the hatchlings—OA led to an increase in cuttlebone weight/area ratio of fed newborns (but not in unfed individuals). The proportion of "floating" (linked to buoyancy control loss) newborns was greatest under starvation, regardless of the CO_2 treatment, and was associated with a drop in cuttlebone weight/area ratio. Besides showing that cuttlefish buoyancy is unequivocally affected by starvation, here, we also highlight the importance of nutritional condition to assess calcifying organisms' responses to ocean acidification.

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Conditioned place preference reveals tonic pain in Octopus

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Despite the absence of conclusive evidence for pain or suffering, invasive procedures on cephalopods are now subject to ethical review in most research nations. However, practical efforts to improve welfare during invasive procedures are hampered by lack of knowledge about how similar cephalopods' pain experience might be to vertebrates', or how such pain might be identified, measured and alleviated. There is therefore an urgent and critical need to establish robust protocols for measuring not only the reflexive, peripheral nociceptive component of pain, but also the centrally-mediated negative affective state. Negative affect is the foundation of pain experience and suffering. To date, no experimental procedure has successfully captured negative affect in any invertebrate species. Conditioned Place Preference (CPP) is considered one of the most unambiguous measures of the affective component of pain in vertebrates.

Here we show preliminary evidence that a CPP assay originally designed for rodents reveals a negative affective state (i.e., ongoing pain) in octopuses. This is the first unequivocal evidence that any invertebrate is capable of experiencing the emotional component of pain. We present data showing evidence for pain, discuss opportunities to further refine the assay for testing other cephalopod species and other aspects of welfare, and provide a validated protocol for assessing centrally-acting and local analgesics as welfare promoters for cephalopods.

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Poster Session

Temperature effects on the embryonic development of common octopus (*Octopus vulgaris*)

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The effect of temperature in embryonic development of *Octopus vulgaris* has been revised based on bibliography data. Higher temperatures implies higher energy requirements and higher yolk consumption which was linked to a decrease in embryos survival rate, an increment of premature paralarvae and smaller hatchlings with lower paralarval survival rates under starvation. Hard structures such as the beak were also affected. In addition, studies on different biomarkers showed the sensitivity of RNA/DNA, heat shock protein (HSP70) and glutathione S-transferase GST to temperature raise with an increase on its content and activity during late embryonic and paralarvae stages. The raise on rearing temperature increase the consumption of total lipid content from embryos, nonetheless its fatty acid composition remained similar between different rearing temperatures. Regarding the length of embryonic development, warming shortened this period. When the rate of development (day-1) in ectotherms is plotted against temperature (°C), an increasing linear pattern arise for a range of permissive temperatures and regression procedures allow to calculate a threshold temperature (Th) and a thermal constant (K, days-ºC) for the embryonic development. Previous studies, and a new experimental data reported in the present work, allow the calculation of Th and K for different populations of *O. vulgaris*. Plots for *O. vulgaris* coming from different geographical origins lead to Th ranging 7.3-12.6°C, and K ranging 370-455 d-°C". Eggs from Canary Islands did not survive above 23°C, whereas eggs from Senegal can finish development above 25°C. The comparison of thermal parameters of different populations suggests geographical adaptations.

Poster Session

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Predator *Oncaea mediterranea* (Copepoda: Oncaeidae) attacking SD complex paralarvae in the Gulf of California

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Studies on cephalopod paralarval abundance in Mexican Pacific waters, indicates that the SD complex (paralarvae ≤3.0-4.0 mm of mantle length of *Sthenoteuthis oualaniensis* and *Dosidicus gigas*) is the most abundant taxa in the cephalopod community. Attacks of zooplanktonic predators upon these ommastrephid paralarvae have been registered in samples collected in the mouth of the Gulf of California. Predation results were obtained from two out of 10 oceanographic cruises. These were made in November 2004 and 2006 on board of the SSV Robert C. Seamans and along 2014-2017 in one coastal station at Cabo Pulmo National Park (CPNP).

Zooplankton samples were taken with a CalCOFI net towed obliquely (200 m depth, 19 samples) and with Neuston net (1 m wide x 0.5 m height, 335 μm, 15 samples) tows. The copepod *Oncaea mediterranea* was the only predator observed attacking paralarvae of the SD complex. During 2004 predation was recorded in two oceanic stations (10% of the total samples), in 2006 in 46% of the samples, and sporadically in the CPNP. In 2006, predation was observed in the same region that during 2004 but also in front of Bahía de La Paz where it was higher. Most attacks were associated with high abundance of paralarvae collected in surface, and occurred in organisms averaging 1.2 mm of mantle length. Relative importance of *Oncaea mediterranea* predatory activity by station (1 to 30%), support the hypothesis that this copepod species account for mortality of recently hatched paralarvae to a high degree.

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Poster Session

Observations on mating and spawning in *Sepiola affinis* and *Sepiola intermedia* (Cephalopoda: Sepiolidae: Sepiolinae)

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Several papers have described mating in different sepiolines. In this paper, three copulatory acts are described in captive *Sepiola affinis* (two acts) and *Sepiola intermedia* (one act; first description of mating in this species). No substantial differences were detected between the two species. In all cases, the male grabbed the female with its arms and positioned it in the "male-to-female neck" position, that is female ventral side lying on male dorsal side. Hence the male inserted its copulatory arm (hectocotylus) into the female mantle cavity to transfer spermatophores. In this position the hectocotylus, left dorsal arm, is lined up with the bursa copulatrix, in the left mantle cavity of the female. Copulations lasted between 30 and 60 minutes. There are few reports of spawning in Sepiolinae. Egg deposition by a female of *S. intermedia* is described here for the first time. This female deposited its eggs individually, one after the other. To spawn each egg, the female placed itself with the arms joined at their tip to form a cone whose apex pointed to the chosen place where to lay the egg. Following waves of mantle contractions, an egg came out from the arm cone and was attached to the ground. Each spawning act lasted ca. 30". In all some 100 eggs were laid.

It is known that sepiolines are multiple spawners, therefore the group of eggs deposited in the described act represents just one of the batches that this female would have spawned during its maturity life.

Poster Session

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Thysanoteuthis rhombus: egg mass first record and paralarvae distribution in the northeastern Tropical Pacific

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An egg mass portion and one paralarvae of *Thysanoteuthis rhombus* found during summer 2008 in the northeastern Tropical Pacific and autumn 2014 in the Gulf of California, Mexico, respectively, represent the first evidence of reproduction of this species in the region. The egg mass portion was constituted by a gelatinous matrix in disintegration and contained 31,552 hatchlings from which 96.2% were premature, and 1,192 eggs at different developmental stages.

Head chromatophore patterns, buccal crown pigmentation, arm and tentacles morphological indexes, and other morphological characteristics complement previous descriptions of *Thysanoteuthis rhombus* paralarvae. Zooplanktonic fauna associated to the egg mass included predatory copepods, fish larvae, and amphipods, among other.

The composition of the egg mass portion and the oceanographic conditions suggest early stages of *Thysanoteuthis rhombus* may show a fast embryogenesis with early hatching, being able to exploit a wide environmental range. These characteristics, support its worldwide tropical-subtropical distribution. Also, that predation by copepod and probably other cephalopods may contribute to a high mortality of paralarvae and juveniles.

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Poster Session

An Atlas of Embryonic Development of the Brain of *Octopus vulgaris*: Preliminary Data

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Octopus vulgaris is an exceptionally intelligent cephalopod species with a quite wide behavioural repertoire. Previous comparative studies on the embryonic development of the brain in coleoid cephalopods have suggested that brain development is highly conserved amongst them. While the adult anatomy of *O. vulgaris* brain has been substantially described, the morphological, cellular and molecular steps of *O. vulgaris* embryonic brain development are less well known. Previously, it was suggested that the embryonic development of its brain develops following multiple spherical ganglia, however, recent studies indicate that the central brain has a cordal origin. Towards the end of the embryonic development, the central brain consists of multiple paired lobes, of which the optical, cerebral, visceral and pedal lobes are the most prominent ones. The cerebral lobes lead to the formation of supraesophageal mass, and pedal and visceral lobes form the suboesophageal mass where both masses are connected to the laterally positioned optic lobes via the optic tract.
The main aim of this study is to establish an annotated histological atlas of embryonic brain development of the common octopus, *O. vulgaris*, up to hatchling, according to the most recently published staging atlas. Different conventional histological methods have been combined to provide an optimized imaging data set of brain sections obtained from *O. vulgaris* embryos from stages VI to XX.

Poster Session

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Genome and specific biodiversity of oceanic squids assessed through Next Generation Sequencing

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Oceanic squids of the order Oegopsida Orbigny, 1845 have great ecological and evolutionary importance, and some of them also are important economic resources. Despite that, they are among the most mysterious groups of pelagic organisms. There are still many knowledge gaps, such as the actual relationships between the 22 oceanic squid families, and the actual biodiversity hidden within many of the putatively "cosmopolitan" species of the group. Starting in October 2019, the 2-year postdoc project GOIPD/2019/460 "Genome and specific biodiversity of oceanic squids assessed through Next Generation Sequencing", funded by the Irish Research Council, aims to address these two scientific questions.

Aim 1: to understand the evolution of oceanic squids. To answer this question, we will use a phylogenomic framework based on a shallow whole genome sequencing method known as genome skimming using samples of 34 oegopsid species representing 86 % of the oegopsid biodiversity at the family level and 100 % of the described subfamilies. This will provide a better understanding of the evolution of several morphological and physiological traits that these animals developed to live in the water column.

Aim 2: the assessment of biodiversity patterns in pelagic oceanic organisms using squids as models. We will address this using samples from 13 oegopsid species collected from Brazil to Iceland, and the Mediterranean Sea. Based on newly developed and commonly-used molecular markers, we are going to test the effect of several oceanic barriers to the population connectivity of these squids.

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Poster Session

Nociceptive changes during senescence as welfare indicators for Giant Pacific Octopus, *Enteroctopus dofleini*

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When octopuses undergo their terminal reproductive phase and senescence, they experience extreme physical decline over a period of weeks or months before eventual death. With an increasing demand for welfare standards in zoos, aquariums and labs, the need for research examining physiology is increasingly necessary. However, studies examining physiological changes occurring during senescence and their implications for welfare are uncommon.

This study aims to understand if the giant pacific octopus (GPO), *Enteroctopus dofleini*, experiences significant changes in nociceptive physiology during senescence, and how this may affect their welfare. Nociceptive behavior and sensory thresholds were measured using a series of 5 von Frey filaments in ascending series to several body locations. In a multi-site, longitudinal study of GPOs held in public aquaria, changes in nociceptive behavior were measured from when animals were healthy and compared to behavior as they declined through senescence. Additionally, we measured apoptosis and necrosis in peripheral nervous, muscle, and skin tissues fixed at the point of euthanasia. Preliminary results suggest that E. dofleini experiences hypersensitivity as animals near death, which may indicate a negative welfare state, and then a complete absence of behavioral nociception response the day or two before death, suggesting severe, terminal degeneration of tissue that is nociceptive to *E. doflieni*. Novel perspective on how senescence may compromise welfare will be pivotal to setting guidelines and recommendations on ethical care of animals in terminal decline.

Poster Session

The role of ionotropic receptors in behavioural alterations at elevated CO_2 in a squid

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Projected future carbon dioxide (CO_2) levels in the ocean can alter the behaviour of marine animals. Disrupted functioning of the γ-aminobutyric acid type A (GABA-A) receptor is suggested to underlie CO_2-induced behavioural changes in fish, however, the mechanisms underlying behavioural changes of marine invertebrates at elevated CO_2 levels are not well understood. Here, we aimed to assess the role of GABA-A-like and other chloride (Cl^-) channel receptors in behavioural alterations at elevated CO_2 in a cephalopod.

We exposed two-toned pygmy squid *Idiosepius pygmaeus* to ambient (~450 μatm) or elevated (~1,000 μatm) CO_2 levels for seven days. Squid were treated with sham, gabazine (GABA-A receptor antagonist) or picrotoxin (Cl^- channel blocker) immediately before measurement of conspecific-directed behaviours and activity levels upon mirror exposure. If disrupted function of GABA-A-like and/or other Cl^- channel receptors underlies the behavioural changes, we predicted that gabazine and picrotoxin would attenuate the behavioural changes at elevated CO_2. Elevated CO_2 increased squid activity levels and altered some, but had no meaningful effect on other, conspecific-directed behaviours. Gabazine and picrotoxin attenuated some of the behavioural changes at elevated CO_2, indicating altered GABA-A-like and Cl^- channel receptor functioning may underlie these behavioural changes. However, gabazine and picrotoxin had the same effect at both CO^2 levels on other behavioural traits, suggesting altered function of GABA-A-like and Cl^- channel receptors was not responsible for other behavioural changes at elevated CO_2.

Our results suggest multiple mechanisms may be involved, which could explain variability in the effects of CO_2 and drug treatment across behaviours.

Poster Session

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Identification of demersal egg masses and spawning grounds in commercial squid in the Northwest European waters: *Loligo vulgaris* vs *L. forbesii*

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Two commercial squid species genus *Loligo* (*L. vulgaris* and *L. forbesii*) with nearly coinciding species ranges inhabit waters around Europe from the east Mediterranean to the North Sea. Both species reproduce all year round with distinctive peaks, mostly in the cold season, and mature females of both species are often captured together in the same hauls. Their spawning grounds overlap spatially: *L. vulgaris* lay eggs mostly at 20-50 m, down to ~100 m while *L. forbesii* is slightly further offshore - at 10-150 m, occasionally down to 300-700 m. Egg size increases several times during embryonic development making this feature unreliable for species identification without additional information.

Our study summarises existing literature data on egg capsule length, number of eggs per capsule and egg/embryo size at the particular developmental stage throughout the species range, together with information from genetically identified squid egg masses collected in 2017-2019 in the West English Channel and Celtic Sea. Resulting guidance will allow future identification of egg masses, including information gathered by recreational divers during The Cephalopod Citizen Science Project.

Measurement and counts of egg clusters used in this study (depth 18-155, mean 66 m) as well as historical data from the same area, January to August (Holme, 1974 J. mar. biol. Ass. U.K. 54, 481-503), revealed that all sampled egg clusters belonged to *L. vulgaris*. No *L. forbesii* spawning was yet observed in the Western English Channel and surrounding waters though this species potentially might reproduce there.

Poster Session

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Contribution to knowledge on biometric data and morphology of *Octopus cyanea* *i*n Zanzibar coastal waters

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A total of 137 *Octopus cyanea* samples (63 females and 74 males) were collected from artisanal fishers between June to November, 2019 in Zanzibar. Purposive sampling was adopted to include individuals of all sexes of different sizes. This study aimed to add knowledge on biometric parameters to describe morphological and general biology of species. Samples from artisanal fishers were taken to laboratory where biometric data such as length (arm length AL, total length TL and dorsal mantle length DML) and weight (total weight TW, eviscerated weight EW, Gonad weight GW, beak weight BW and weight of ink sac ISW) were measured to nearest 0.01cm and 0.001g respectively. All individuals were sexed and suckers were carefully counted. Data were analyzed by SPSS software and Microsoft excel. Total lenght of organisms ranged from 31cm (50g) to 128cm (2459g) for females and from 23cm (37g) to 126cm (2427g) for males. Number of sucker ranged from 1948 (mean: 231±6.251 suckers/arm, SE) to 3360 (mean: 420±8.206 suckers/arm) for males and from 1992 (mean: 249±7.163 suckers/arm, SE) to 3956 (mean: 496±14.093 suckers/arm, SE) for females. The male hectocotylized arm is short, muscular with enlarged suckers accounting 40-60% of other arms suckers. GW ranged from 0.03%-1.5% of TW and is influenced by reproductive stage and sex of animals. ISW and BW contribute 2-5% and 0.0.2-0.08% of TW respectively while average eviscerated weight is about 91%. The information will add information for species identification but also be linked to other biological data to advance best management undertakings.

Poster Session

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Future–planning abilities in the common cuttlefish

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Recently, the cognitive abilities of *Sepia officinalis* - in particular its memory - have been the subject of several studies. As memory is thought to be intrinsically directed toward the future, cuttlefish may possess the ability of foresight. However, some claims that animals cannot anticipate their future needs as they cannot escape their present state.
To challenge this hypothesis, we conducted an experiment on 13 captive-reared juvenile and 18 wild-caught adult cuttlefish. Each cuttlefish was tested in a Y-maze, where they were fed until satiety. Afterwards they were proposed a choice between two arms: one with a shelter, but without any prey, and the other without a shelter but with a prey. They were confined inside the chosen arm overnight. The following day, after being fed until satiety, the same choice test was undertaken. If cuttlefish were bound to their current needs, they should choose the shelter on the two tests. However, if they anticipated their future needs (hunger during the following night) irrespective of their current needs (hiding), they should prefer the arm with the food the second day. All cuttlefish but two went to the shelter the first day, a choice consistent with their current state of motivation. The second day, whereas juvenile cuttlefish and control adults still chose the shelter, half of the adult cuttlefish preferred the arm with the food.
Although the number of adults was too low to reach statistical significance, these results provide the first indication of an ability of adult cuttlefish to anticipate their future.

Poster Session

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Observational Learning in *Octopus laqueus*

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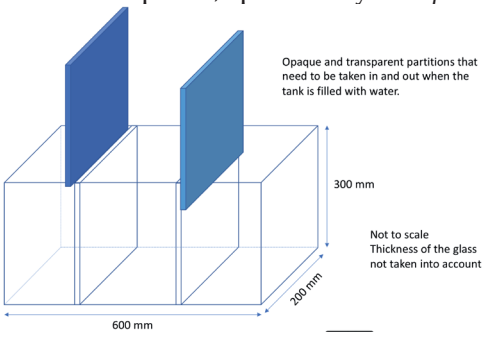
The evolution of coleoid cephalopods from sessile, shelled mollusks to soft bodied animals with potentially infinite degrees of freedom in movement and posture is thought to have led to changes in how they perceived and had agency in their environment. The nervous system of modern octopuses is decentralized, that is, two-third of their neurons are distributed in their eight arms (review in Hochner, 2012). Octopuses are known for their unexpectedly complex and variable behavioral repertoire. This is exceptional both because it appears to have evolved independently from similar faculties in other highly intelligent animals, and because octopus life histories do not have the classic hallmarks of cognitively complex animals such as sociality and long lifespans. Octopuses have been shown to be capable of associative learning and there has been some indication that *Octopus vulgaris* can learn to perform observational learning. However, this result has not been adequately reproduced, and has been mostly studied in only one species of octopus. The aim of this study is to investigate whether octopuses, specifically *Octopus laqueus*, can learn to perform a task by watching other conspecifics. We have designed a tank in which this experiment can be carried out (see experimental tank design in figure). This experiment are expected to be conducted at the Okinawa Institute of Science and Technology over the upcoming months and we will present our progress at the conference.



This work may provide insights into the cognitive capabilities of octopuses and help establish them as a model for the study of more complex forms of embodied learning, imitation and cooperation.

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Poster Session

Neurally underdeveloped cuttlefish newborns exhibit social learning

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Learning can occur through self-experience with the environment, or through the observation of others. The latter allows for adaptive behaviour without trial-and-error, thus maximizing individual fitness. Perhaps given their mostly solitary lifestyle, cuttlefish have seldomly been tested under observational learning scenarios.

Here we used a multi-treatment design to disentangle if and how neurally-immature cuttlefish *Sepia officinalis* hatchlings (up to 5 days) incorporate social information into their decision-making, when performing a task where inhibition of predatory behaviour is learned. In the classical social learning treatment using pre-trained demonstrators, observers did not register any predatory behaviour. In the inhibition by social learning treatment, using naïve (or sham) demonstrators, more observers than demonstrators learned the task, while also reaching learning criterion in fewer trials, and performing less number of attacks per trial. Moreover, the performance of demonstrator-observer pairs was highly correlated, indicating that the mere presence of conspecifics did not explain our results by itself. Additionally, observers always reported higher latency time to attack during trials, a trend that was reversed in the positive controls. Lastly, pre-exposure to the stimulus did not improve learning rates.

Our findings reveal the vicarious capacity of these invertebrate newborns to learn modulation (inhibition) of predatory behaviour, potentially through emulation (i.e. affordance learning). Despite ongoing changes on neural organization during early ontogeny, cognitively-demanding forms of learning are already present in cuttlefish newborns, facilitating behavioural adaptation at a critical life stage, and potentially improving individual fitness in the environment.

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Poster Session

Polarization vision allows cuttlefish to see sharper in partly turbid waters

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Polarization vision is well documented in cephalopods, where it is used for a range of tasks, from navigation via prey detection to communication. In a series of laboratory and field experiments we examined the effects of polarization vision on the very nature of an image, meaning the contrast distribution in it.
Results indicate that in clear to semi-turbid waters, polarization contrasts remain sharper at longer optical distances as compared to intensity contrasts. These results indicate that polarization vision allows cephalopods in general and cuttlefish in particular, to see sharper images, meaning seeing more details, as compared to intensity vision alone.

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Poster Session

Gentle, precise, fast, and simple method to measure the volume and mass of octopuses without the need to take them out of their tank

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The 'Slany' method allows routinely measuring the volume and mass of octopuses with high precision without taking them out of their tank. It does not require any special handling or immobilization of octopuses, besides common husbandry practices such as feeding. The method thus is particularly well suited to gently handle animals without harming them physically or emotionally, in fact it is rewarding and enjoyable for the octopuses. It can be carried out without the need for any special equipment. A sufficiently large bin is immersed into the tank. The border of the bin is higher than the water level in the tank. The bin can be transparent or opaque. Tankwater is inserted into the bin up to a level that leaves enough space in the bin so that the octopus, once inside the bin, does not make the bin spill over. The octopus is shown that a crab has been inserted into the bin. The octopus will, attracted by the prey, go over the border of the bin and enter it. The difference between the water level in the bin before the octopus enters it and after it entered corresponds exactly to the octopus's volume, and can be easily measured with a builtin scale or similar methods. Mass is then computed by multiplying with the known density of octopuses (depending on species, but mostly they have neutral buoyancy).
The border of the bin can be more or less high depending on the species and to allow getting rid of water.

Poster Session

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Mirror experiment of self-recognition and tool-use of *Octopus vulgaris*

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Training 1: A transparent bin contains otherwise identical blue and yellow balls. They are linked through a fishing line which is threaded through an eyelet so that the octopus can only take one ball up to the hole. The octopus receives a reward when she first takes the blue ball to the hole. Control 1: Bin's open side is attached to a large opaque plate. The octopus cannot look around the plate to visually direct its arm. A mirror is placed outside of the aquarium glass so that the octopus can see her arm in the mirror image, but the octopus has never encountered a mirror. The choice of the ball remains random over many repetitions.

Training 2: The mirror is placed as an enrichment in the middle of the tank. A training regimen is used to make the octopus understand what the mirror image shows, among many others by parading various objects around the mirror and letting the octopus see the backside of the mirror. The mirror is kept in the tank for several days and can be freely moved by the octopus.

Test 1: Like Control 1, but the mirror is placed in such a way in the tank that the octopus can see its arm in the bin in the mirror. Control 2: Control 1 without mirror. The sides of the tank are made non-reflective during this phase.
Test 2: Like Test 1, but the mirror is initially placed so that the octopus cannot see its arm.

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Molecular organization of the octopus visual system determined by single cell RNA-sequencing

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Octopuses, like vertebrates, have camera-like eye structures despite the two lineages having diverged evolutionarily over 500 million years ago from a common ancestor. Furthermore, anatomical evidence suggests that octopuses and vertebrates may have similar neuronal cell types within their visual system. However, little is known regarding the molecular determinants of visual system organization in octopuses. We performed single cell RNA-sequencing of the optic lobes of juvenile *Octopus bimaculoides* with the goal of developing a molecular atlas of the octopus visual system that we could connect to anatomical cell types. Cells were sequenced using the 10X platform, sequencing data was aligned to improved gene models generated through a HISAT2/Cufflinks bioinformatic pipeline, and clustered using the Seurat package. Initial analysis has identified ~40 different neuronal cell types based on their unique transcriptional profiles. Within these, distinct cell types correspond to different neurotransmitter and/or neuropeptide identity, providing a framework for the neural circuit organization of the optic lobe. Furthermore, a number of cell types also express markers that may mediate cell fate or connectivity, such as transcription factors and adhesion molecules. Based on sequencing results, we used fluorescent RNA in situ hybridization to confirm several of these transcriptional cell types, as well as demonstrate their corresponding anatomical organization within the optic lobe.

This study begins to illustrate the molecular determinants underlying neural circuits in the octopus visual system, which can, in turn, provide insight into development and function of the optic lobe as well as evolutionary influences on visual processing.

Poster Session

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Octopus protocadherins in neural development

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The nervous system of an octopus consists of 500 million neurons, which are all intricately and efficiently wired to enable higher cognitive function and behaviour. The question of how these neurons form millions of appropriate synapses remains unanswered. Recent genome sequencing of several cephalopods has revealed expansions of a specific transmembrane protein family, the protocadherins, which are essential for mammalian neural development. Although it has been shown that octopus protocadherins expanded independently in cephalopods and vertebrates, the molecular building blocks that make up a protocadherin appear to be conserved. Based on QuantSeq data, we show that some protocadherins are highly expressed during early embryogenesis and might be important during neurogenesis. Other protocadherins are more expressed later during development and can participate in processes such as axon terminal formation and dendritic self-avoidance. Via in situ hybridisations on different stages during *Octopus vulgaris* development, we show that cephalopod protocadherins are expressed in distinct neuronal populations and/or cell types and are mostly limited to post-mitotic neural areas, as has been observed for vertebrate protocadherins. Intriguingly, structurally distinct protocadherins appear to be expressed in different areas and probably exert different functions. By analysing the expression and the function of the octopus protocadherin gene family we hope to decipher the molecular blueprint for a complex brain and shed light on mechanisms such as neuronal recognition.

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Poster Session

Comparison of micro-CT scanning and traditional dissections for cirrate octopods

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Computed tomography (CT) scanning allows for accurate 3D imaging of specimens and their internal structures in a non-invasive, non-destructive manner, which is particularly important for precious specimens, i.e. type specimens or for rare or difficult sample specimens such as cirrate octopods. When successful, micro-CT scans of specimens allow for the identification of characters without the need for dissections.

We aim to compare the information gained from micro-CT scans of cirrate specimens to that of traditional dissections with regards to the time and effort required to process a sample and the information gained on the position and size of organs.

Twenty-two cirrate specimens were CT scanned at the Imagine Centre in the Natural History Museum of London. Visualisation of the CT scans and three-dimensional renderings of the internal organs were obtained using manual segmentation. Of the 22 specimens, we succeeded in obtaining usable micro-CT scans from only eight specimens, due to issues with specimen preservation, the resolution of the CT scans and movement of specimens during scans. Therefore, careful consideration must be taken when choosing micro-CT scans over traditional dissections, due to the limitations of this method.

Depending on the objectives, one method may be more informative than the other, for example as micro-CT scanning allows for visualisation of the organs in as natural state as possible it may help determine the function of an organ and the exact shape, volume and position. However, traditional dissections are more useful for visualisation of small characters, i.e. the optic nerve in cirrates.

Poster Session

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SWATH-based proteomic analysis of the impact of the incubation temperature on the embryonic development of *Octopus vulgaris*

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The common octopus (*Octopus vulgaris*) is an excellent candidate for aquaculture diversification. It is known that temperature has a great effect on early life stages of cephalopods, drivng the development of embryos, yolk absorption rate and growth rate of paralarvae, which increases with the temperature water. In this study, the impact of temperature incubation on octopus' embryos (EMB, stage XV) and paralarvae after hatching (PL0) were investigated using the proteomic SWATH-MS approach to identify proteins or protein profiles as biomarkers to evaluate the welfare in early stages of O. vulgaris. Two temperatures of incubation were compared: high (H, 21 °C, commonly used in paralarvae aquaculture) and low (L, 16 °C, as representative of natural conditions).

The proteomic analysis quantified a total of 2352 proteins. For EMB, 24 proteins showed significant differential expression between high (H) and low (L) temperature, whereas in PL0 a total of 127 proteins presented significant differences. PCA analysis of all quantified proteins showed a clear separation between development stages (EMB and PL0) and temperature. The up-regulated proteins by high temperature in EMB (11 up) were mainly related with signaling pathways (signal transduction) scavenging and binding activity (metal, ions, tubulin and chitin). Whereas in PL0, the up-regulated proteins (64 up) by high temperature were involved mainly in proteolysis (hydrolysis activity related with protein metabolism), carbohydrate metabolism, nervous system development and neurotransmitter transport, lipid metabolic process, detoxification, extracellular organization and binding (chitin, metals and ATP). These results allow defining characteristic proteomes for EMB and PL0 that are involved in different biological processes associated to temperature.

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Poster Session

Life cycle and reproductive biology of *Argonauta nodosus* from Brazilian waters

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In spite of its cosmopolitan distribution, little has been reported on the life cycle and reproductive biology of *Argonauta nodosus* Lightfoot, 1786. This study aims, therefore, to improve understanding of the life cycle and reproductive biology of this species from southern Brazilian waters. All stages of the life cycle, from hatchlings to adults were examined, including 27 shells with eggs, 19 adult females and 28 males, which were collected with bongo nets (60 cm mouth diameter), rectangular midwater trawl (opening area 8m²) and throw nets between 28°09' and 33°S. Females were measured and the mantle cavity was opened to check for the presence of hectocotyli. The majority of females (N = 17, 21 to 46 mm mantle length-ML) had stored several hectocotyli (up to 11 in a single female), however, the number found appeared uncorrelated with female ML. The presence of multiple hectocotyli in some females suggests that males could potentially be more abundant. Among the males examined, no one had deployed the hectocotylus, and the largest male retaining an intact hectocotylus was 6.8 mm ML. The hectocotylus diameter increased linearly in relation to the mantle in males <5 mm ML, but became considerably larger in larger males, indicating the hectocotylus develops quickly in males > 5 mm ML. The eggs found inside the shells had a mean diameter of 1.46 mm and were at different developmental stages, indicating intermittent spawning, which corroborated with the presence of hatchlings in plankton samples year around.
These findings reveal important aspects of the life history of *A. nodous*, which are presented and discussed in the poster.

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Poster Session

CephRes2020, a summary

CephRes2020 Virtual Event was an extravaganza of interdisciplinary science. It brought together researchers from neuroscience, genomics, behaviour, ecology, evolution, history of science and more. Its open collaborative feel was initiated by two free-to-attend pre-conference satellite workshops, that took place in the two days preceding the main event, focusing on the diverse topics of cephalopod early life stages and cephalopod genomics. Using a digital teaching platform specifically designed to facilitate breakout groups, these workshops were productive and interactive. The first, attended by 29 researchers, progressed aspects of a community effort towards a revised guide to juvenile and paralarval cephalopods (a timely update of the "Larval" and juvenile cephalopods: a manual for their identification). The second '*Cephalopods in genomics era*' had over 30 registered participants from over a dozen countries. During the satellite[1], the need to share DNA (RNA) extraction protocols, genomic and transcriptomic information, assembly approaches, gaps in the phylogenetic sampling were the topics discussed, among others.

The main virtual event was spread over five shortened 'days' comprising European afternoons and evenings, to facilitate attendance from as many different time zones as possible. Each day featured an invited speaker, each selected to broaden our horizons, and showcasing the cnidarian nervous system, the cephalopod genome, cephalopod neuronal control, neuronal modulation in invertebrates, cephalopod consciousness, and cephalopods as gastronomic delights.

We enjoyed a 'focus-on' session on three of the days, each a series of short talks looking at current hot topics more deeply. Featuring cephalopod neurophysiology, cephalopod development, and tissue regeneration, each session showcased the latest developments in the field, from neuro-loggers to transgenic bobtails, and stem cell response to amputation stress.

Other talks were loosely combined into themes (genomics and evolution; models of life and mind; neural networks to behaviour; others) and were broad and diverse in their subject areas. Evolution and ecology talks covered the globe, reporting on cephalopods from the Indian, the Pacific and Atlantic Oceans, from shallow waters to the deep sea, and from the Palaeozoic to the present day, with appropriate focus on how cephalopods are impacted by and responding to the pressures of the Anthropocene.

1. https://www.cephalopodresearch.org/2020-events/satellite-scientific-events/#event3

The focus of behaviour talks ranged from single behaviours to the evolution of cognition and intelligence.

Backing up the talks was a series of posters, available for view on the virtual platform but also highlighted by flash talks throughout the programme. Again, these ranged from across the world's oceans, but also featured cutting edge techniques such as single-cell RNA sequencing and micro-CT scanning, and were a testament to the diversity and creativity of the cephalopod community.

A feature of the five-day event was its focus on networking and collaboration. Three current networking initiatives were showcased, each broad in their ambitions. Cephs and Chefs aims to develop new markets for cephalopod food products while increasing sustainability of the fisheries. MOLLUSCience is a slack channel for molluscan researchers. EUVEN is a new COST Action aiming to encourage venom investigations across Europe.

Extensive discussion also centred around a potential new networking initiatives whose focus would be the invertebrate brain.

Together, we proved that we could be as inspiring, productive and effective virtually as we can in person, which bodes well for the future of cephalopod science in a changing world.

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CephRes2020 Virtual Event has been realized
through CephRes CISCO Webex Suite

a CephRes publication - Reference Doc

published in 2022
logo designed by Sara Sossi © 2020 for CephRes