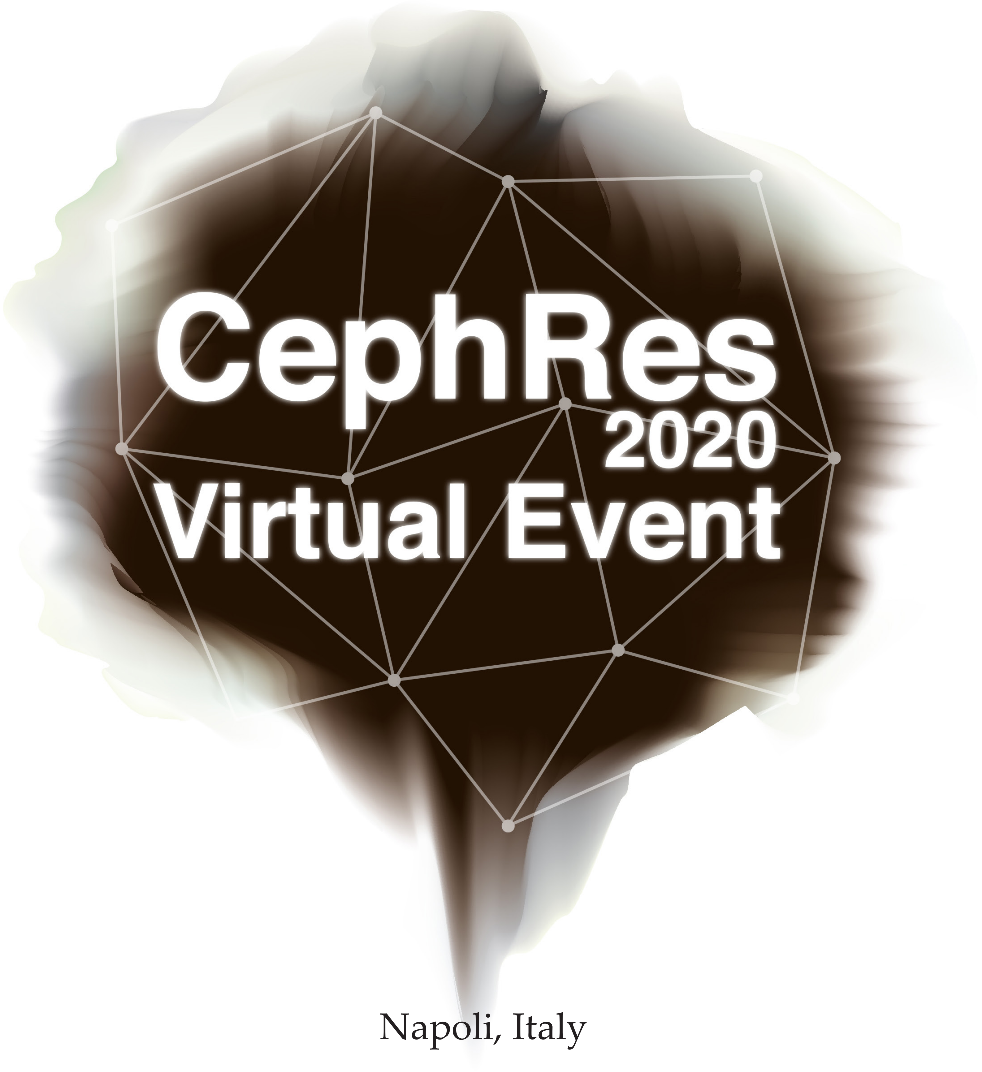
Biology and Life History of Cephalopods

an interim meeting of the international cephalopod community



CephRes 2020 Virtual Event

Napoli, Italy

16 - 21 September, 2020

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

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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.

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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Focus on Session: **RegenSea**

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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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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