

BOOK OF ABSTRACTS

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Recent Advances in Cephalopod Science



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Oral presentations

Session: Behavior

November 10, 0845-0915 [1], Opening talk

Cephalopod behavior: Recent advances and future challenges

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The acknowledged hallmarks of cephalopods include their large brains, sophisticated sensory and motor systems, and complex behaviors combined with fast growth and short life cycle. This is a very “behavioral” animal group yet in some ways their behavior is difficult to study because they are often hard to study underwater and many of them need specialized facilities for laboratory studies. Yet since 1996 there have been several hundred papers published on some aspect of cephalopod behavior. There have been major advances during the past two decades in understanding mechanisms of visual perception and defense, as well as many aspects of sexual selection and reproduction. Some of these studies have found applications in fisheries management and bio-inspired approaches to engineering and materials science. The genome of octopus has just been published and several more species are about to be completed, laying the groundwork for future advances in the physiology and neurobiology of cephalopod behavior. I will review these advances and speculate on the potential for a “new era” in which burgeoning technologies and experimental approaches can lead to major advances in evolutionary biology and in specific fields such as ethology, behavioral ecology and neurobiology.

November 10, 0915-0930 [2]

Octopus behavioral biology: the last 30 years of research at the Stazione Zoologica Anton Dohrn

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²This presentation is on behalf of more than 50 students, alumni and collaborators joining the experience of working on *Octopus vulgaris* at the Stazione

Octopus vulgaris is an extraordinary, challenging animal investigated at the Stazione Zoologica Anton Dohrn (SZN; Napoli, Italy) since the mid XX century for the extreme plasticity of predatory responses with the aim to serve as basis for the search of a ‘model of a brain’. I will review, from a behavioral perspective, the studies carried out by J.Z. Young and coworkers at the SZN that provided the ground for our subsequent work carried out over the last three decades.

I will present our current knowledge on the neural circuitry underlying behavioral and cellular plasticity in these animals. Focus will be provided on: **i.** inter-individual variability in behavior, including the so-called personality, and intervening factors possibly causing this variability; **ii.** shy-bold continuum; **iii.** learning and memory recall; **iv.** knowledge on organization of the neural-‘connectome’ and **v.** of neuro-modulators operating in the ‘brain’ to control behavioral and physiological responses; **vi.** nociception; **vii.** response to stress; and **ix.** regeneration phenomena. Recent data on cephalopod genomics will also briefly overviewed.

November 10, 0930-0945 [3]

Hijacking, hitchhiking and burglary behaviors of pelagic octopuses

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Pelagic octopuses are a highly specialized group of octopuses that have secondarily left the seafloor and evolved a holopelagic existence. One of the most striking adaptations amongst a suite of related pelagic octopus families (superfamily Argonautoidea) is their associations with gelatinous zooplankton (jellyfishes and salps). Here, we report footage of a never-before-seen interaction in nature (here coined as “hijacking” behavior), between a male octopus (*Haliphron atlanticus*) and a venomous jelly (*Pelagia noctiluca*) at the surface ocean. The peculiar orientation of this encounter and size of the intruder opposes the pelagic “hitchhiking” and the “burglary/weapon stealing” strategies observed to date in these pelagic octopuses. The “hijacking” behavior is a more complex interaction. While maneuvering the jelly (and possibly ingesting it), *H. atlanticus* appears to be using the jelly’s marginal nematocystic tentacles to hunt. This constitutes the first evidence that all four octopod families of the Argonautoidea display opportunistic associations with gelatinous zooplankton, and establishes a new category of biotic associations.

November 10, 0945-1000 [4]

Conservation and convergence in the neural organization of the cephalopod learning and memory system

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Octopuses achieve advanced, vertebrate-like behavior with a different and relatively simpler invertebrate brain. Do they utilize conservative molluscan learning and memory (L&M) mechanisms or have they evolved novel mechanisms, possibly through convergent evolution with vertebrates? Answering this question may reveal evolutionary and biological principles of L&M systems. The vertical lobe (VL) of *Octopus vulgaris*, an brain region exclusive for L&M, functions in parallel to the circuitry controlling the stereotypic attack behavior of the octopus. The VL has the connectivity typical of a simple feed-forward ‘fan-out fan-in’ association networks with non-NMDA activity-dependent LTP at the fan-out synapses. The *Octopus vulgaris* LTP involves a unique adaptation of the typical invertebrate nitric oxide (NO) system to mediate the maintenance phase of the LTP. We also have studied the way typical molluscan neuromodulators regulate the activity dependent LTP. 5-HT has conserved its facilitative effect on molluscan synaptic transmission in the VL but has ‘lost’ its long-term modulatory effect that was demonstrated in the *Aplysia* defense reflex. However, it indirectly enhances the activity-dependent LTP induction. Octopamine (OA) has a similar short-term facilitatory effect but, unlike 5-HT, it attenuates LTP induction and depotentiates consolidated LTP. Thus, 5HT and OA may convey opposing reinforcement signals. In summary, the octopus VL shares global cellular properties with complex L&M systems of vertebrates. Yet its synaptic plasticity is based on extensive adaptations of conserved molluscan molecular mechanisms. This suggests that convergence to complex L&M systems can be achieved in multiple independent ways.

November 10, 1000-1015 [5]

Facing the challenge, how the octopus can control its flexible arms and use them to extract sensory information

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Living in a sensory world very different from our own, octopuses provide a challenge and inspiration for generations of researchers. Not only are their bodies boneless and flexible but they come with a unique advanced visual system for an invertebrate, analogous to the vertebrate model. Despite of several years of research many open questions remain. To better understand and decipher the behaviors and abilities of the octopus we used a series of maze apparatuses that force the animals to rely on one certain aspect of its sensory repertoire to solve a learning task?

Using a modification of Y shaped mazes we investigated two different sensory modalities. First we investigated the ability of octopuses to learn to turn their arm in a specific direction in an opaque Y shaped maze excluding specific tactile chemical or visual stimuli. Therefore the correct decision to turn left or right inside the maze could only be made based on the information obtained on the position of the arm. 5 out of 6 subjects were able to successfully complete this task in less then 90 trials. A second set of experiments was carried out using a similar maze apparatus this time testing tactile discrimination learning – for the first time in intact animals. Eight out of nine animals learned this task in 60 to 90 trials. All animals showed a characteristic multi-peaked learning curve. Our present work sheds new light on tasks the octopus can conduct using a single arm. This raises questions to what extend octopuses might be aware of the position of their highly flexible bodies.

November 10, 1015-1030 [6]

Field observation of mating behavior in *Abdopus* sp. (Cephalopoda: Octopodidae), and its effect on arm loss

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I observed the mating behavior of shallow-water octopus *Abdopus* sp. in the wild. The mating occurred at night with low tide. Male *Abdopus* sp. tracked and copulated by stretching the hectocotylyzed arm to female's mantle cavity. Male often repeated the insertion and extraction of the hectocotylus during mating because the mated female actively moved to forage at the same time. The mating behavior lasted a maximum of more than 25 minutes. I also found a positive size-assortative mating in this species. In some cases, a few males stayed around a mating pair to gain mating opportunities. The transient or sneaker males were smaller than the paired and guarding ones. Moreover, the loss of arm and arm regeneration appeared more frequently in transient or sneaker males. On the other hand, mating success by an unusual male which had lost all arms except for the hectocotylyzed arm was observed in the wild. These results suggest that male physical condition such as arm loss and regeneration might have strong influence on the mating behavior and mating success in this species. This is important in considering life history strategy for arm-regenerating octopuses.

November 10, 1100-1115 [7]

Behavior and personality of giant Pacific octopus (*Enteroctopus dofleini*) in simulated social contexts

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Recently, social behaviors have been noted, but not formally analyzed, in several species of octopus. Social interactions have not been reported in giant Pacific octopuses (*Enteroctopus dofleini*) outside of mating and male-male competition. However, we have observed changes in an individual octopus' personality in the presence of conspecifics of different sizes, suggesting that conspecific size may be used as a behavioral cue in a social context. We exposed seventeen isolated *E. dofleini* to high-definition video stimuli to simulate a variety of functional contexts. Individuals readily responded to video stimuli with highly variable behaviors. We compared reactions of the focal octopus to stimuli before and after exposure to a conspecific, reactions to prey items and small conspecifics, and reactions to predators and large conspecifics. Octopuses demonstrated strong predatory responses toward the small conspecific, but did not uniformly display the same avoidant, anti-predator behaviors toward a large conspecific as with a predatory marine mammal. Thus, the threat of cannibalism may not be automatically assumed in an interaction of two mature *E. dofleini*. This study is the first to demonstrate the influence of a non-reproductive social context on octopus behavior using a standardized conspecific stimulus, indicating that octopuses may be more socially aware than previously established.

November 10, 1115-1130 [8]

Influences on prey choice of *Octopus cyanea* in Moorea, French Polynesia

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There are many influences on prey choice of a generalist predator such as the octopus. We wanted to test the influence of two factors, diversity of habitat and personality of individual animals, with *Octopus cyanea* on the island of Moorea, which has a large variety of potential prey species. Twelve octopuses from five different study sites were tested for their position on the shy-bold scale of personality. After testing, we surveyed the area for twenty meters around each octopus den, to evaluate the proportion of thirteen different habitat types. Hard shells remains of crustacean and molluscan prey were collected from the area near each den, initially and as long as the den was occupied. No relationship was found between personality and prey diversity but there was a relationship between habitat complexity and prey diversity. This suggests that the number of prey species available in many different habitats is a major influence on prey choice of octopuses.

November 10, 1130-1145 [9]

Behavioral ecology, reproductive biology, and public display of an undescribed cirrate octopus from the northeastern Pacific

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The Monterey Bay Aquarium opened the first exhibit devoted to exploring cephalopod diversity in April 2014. Located adjacent to the Monterey Submarine Canyon, which cuts through the continental shelf allowing for access to deep-water habitats just off the coast, the aquarium planned from the beginning to include the rarely seen but fascinating squids, octopuses, and *Vampyroteuthis* that inhabit great depths. The aquarium worked with its sister institute, the Monterey Bay Aquarium Research Institute, to collect and care for these cephalopods. One species of interest is a small cirrate octopus in the genus *Opisthotenthis*, which are commonly referred to as flapjack octopuses because of their flattened appearance when sitting upon the benthos. On multiple occasions we have successfully collected this species using Remotely Operated Vehicles, housed them under conditions that mimic their natural environment, and delighted aquarium visitors by displaying them. In the course of finding, collecting, and caring for these octopuses, our knowledge of their ecology and behavior has greatly improved, and both morphological assessments and molecular techniques are being used to compile a species description. In addition, a number of individuals laid fertilized eggs, indicating that these deep-dwelling octopuses can store sperm just as many shallow-water cephalopods do. Very little is known about the early life stages of cirrates, so we are anxiously waiting through the long development period for these hatchlings to arrive.

November 10, 1145-1200 [10]

Movement patterns of the California two spot octopus, *Octopus bimaculatus*, using acoustic telemetry

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Octopuses have significant ecological roles and predatory impacts on prey populations, yet their movement and activity patterns are very rarely included in studies of habitat selection and usage. During August 2014 on Catalina Island, CA, USA, nine *Octopus bimaculatus* were caught on SCUBA and tagged with continuous transmitters. Of the nine tags, six stayed on for the duration of the tags' battery lives; two tags fell off immediately, and one fell off after five days. Six of the octopuses were actively tracked for a 24 h period, and daily GPS locations were recorded for all individuals. *Octopus bimaculatus* is a very mobile octopus compared to other octopus species. On average, octopuses inhabited an activity space of 6000 m² over the course of the study. Octopus movement was highly variable between individuals. There was no difference in movement between day, night, or crepuscular time periods or between sexes. Larger octopuses moved more, and individuals changed den locations every other day on average. This study is the first of its kind in California, and one of the first successful octopus acoustic telemetry studies. Understanding octopus movement will provide insight to habitat choice and the intersection of octopuses and anthropogenic activity.

November 10, 1400-1415 [11]

Analysis of characteristics of live preys evoking hunting behavior in pygmy squid, *Idiosepius paradoxus*

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Squids are predators and hunt living preys. To recognize live preys, visual information is important for squids. However, it is still unclear which visual characteristics of live preys evoke hunting behaviors in squids. In order to understand the characteristics of live preys, we employed pygmy squids, *Idiosepius paradoxus*, in the present study. First of all, we established a system to evaluate the hunting behavior of the pygmy squids and examined if visual stimulus was enough for evoking the hunting behavior in pygmy squids. As the results, pygmy squids showed hunting behaviors against a movie of a live Mysid shrimp displayed in a monitor, indicating that the visual information in the monitor was enough for pygmy squids to evoke the hunting behavior. In addition, pygmy squids also showed the hunting behavior against an artificial moving object following swimming pattern of a Mysid shrimp. Interestingly, pygmy squids showed escape behaviors when the size of the artificial moving object became larger. These findings suggest that size of prey is also important for pygmy squids to recognize live preys. Taken together, swimming pattern, i.e., motion and size of an object are key characteristics for pygmy squids to recognized the object as a live prey.

November 10, 1415-1430 [12]

Use of ink for predation by Japanese pygmy squid (*Idiosepius paradoxus*)

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Inking is a well-known defence method used by cephalopods. Here we report that Japanese pygmy squid (*Idiosepius paradoxus*) also may use ink during predation. Squid predatory behaviours toward three prey species (the mysid *Neomysis intermedia*, the shrimp *Latreutes acicularis*, and the prawn *Palaemon serrifer*) were observed in the aquarium. Squid most often (68.3%) succeeded in preying on *N. intermedia* and least often (25.3%) succeeded in preying on *P. serrifer*. Inking behaviour was observed 17 times in 322 trials, and squid attacked prey after inking in 13 of these cases (8 with *L. acicularis* and 5 with *P. serrifer*). Ink use during attacks can be divided into two types: (1) squid release ink between themselves and the prey and then attack through the ink cloud; and (2) squid release ink away from the prey and then attack from another direction. Squid never used ink to attack *N. intermedia*. The success rate of attacks using ink was higher in *L. acicularis* (75.0%) than in *P. serrifer* (11.1%). These behavioural observations suggest that Japanese pygmy squid may use ink for predation, and that this process is clearly distinct from that involving the use of ink as an anti-predator defence, which occurs together with hide or escape behaviours

November 10, 1430-1445 [13]

Time varying friction drag on the swimming squid, *Doryteuthis pealeii*, from flow visualization data

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We used visualization of flow within millimeters of the surface of swimming squid combined with kinematic analysis of the body to determine the fluctuation of friction drag and the character of the boundary layer through the locomotory cycle. Swimming speeds ranged from 0.25 to 2 body lengths per second, or 10 to 60 cm/s for long-finned squid, *D. pealeii*, ranging in body length from 30 to 40 cm. We report the results in the context of previous works estimating the friction drag, energy budget, and efficiency in swimming squid by other experimental and theoretical methods.

November 10, 1445-1500 [14]

Cephalopod intelligence, sociality and communication I) Prologue: Social recognition in squid

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Unlike other mollusks, the coleoid cephalopods (squids, cuttlefish and octopuses) possess highly developed nervous systems with huge brains equivalent in size to some vertebrate brains. They exhibited highly intelligent and complicated behavior like impressive memory, learning, and body patterning. The reason why cephalopods have developed such huge brains and cognitive ability is still unknown. Understanding the recognizing abilities and social interactions of cephalopods would provide answers to this question. In the series of presentations, we will talk about our recent advancement for behavioral experiments on some cephalopods inhabiting at the tropical ocean of the Ryukyu Archipelago, Japan. We will discuss these experiments in light of the diversity of intelligence and sociality. In addition, we will challenge to enlarge our idea to evolution of communication in animals including human. At the beginning, I will focus on some aspects of social recognition in oval squid (*Sepioteuthis lessoniana*), which include specific reactions to the mirror reflection, school caste like behavior and some aspects of group dynamics. I will also mention a possibility for cephalopods being an interesting model to understand evolution of intelligence in animal kingdom; an idea is originated from the statement by J-Y Cousteau, “Cephalopods, primates in ocean”.

November 10, 1500-1515 [15]

Cephalopod intelligence, sociality and communication II) Social structure and dynamics of the oval squid (*Sepioteuthis lessoniana*) school

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Although it has been mentioned that teuthoid squids form school with social function, many of them are just originated from the episode from field observations. To exhibit empirical evidences for the sociality of squid, we have surveyed schooling behavior of oval squid (*Sepioteuthis lessoniana*) in captivity and from nature. In shore area around the Okinawa-jima Island of the Ryukyu Archipelago, Japan, oval squid showed various formations of school. In the school, a particular schoolmate behaved as a sentinel and/or a scout, which had been also reported for the related species (*S. sepioidea*). Through the series of long-term captive experiments with identified individuals, we were successful to analyze the social structure of oval squid school. First, we found rank as dominance hierarchy among the schoolmates. Second, based on the analysis of social network, we found the complicated social structure of schoolmates. In the school, some individuals have strong relation with many mates, which are defined as hub typed squid, while many remaining individuals connect with one mate, which are defined as peripheral typed squid. The hub position were temporally stable but were changeable by permutation of school membership. The hub typed squid affects the behavior of other remaining schoolmates. For example, in the case of offense, many schoolmates often followed the hub typed squid. In case the hub typed squid was removed from the school, remaining schoolmates became to being more defensive.

November 10, 1515-1530 [16]

Cephalopod Intelligence, Sociality and Art VIII) Epilogue: How close can it get? Observations of predatory strategies of the pharaoh cuttlefish, *Sepia pharaonis* in captivity.

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Coleoid cephalopods (cuttlefish, squids and octopuses) are formidable and flamboyant predators. They have an ability to instantaneously change their visual appearances which is achieved through neurally controlling extensive repertoires of chromatic, textural, postural and locomotor components. The varieties and flexibility of each component produces an incredible range in total body patterns expressed in a truly 3D environment. Using such ability, cephalopods demonstrate unique behavioral systems including adaptive coloration, mimicry and intra/interspecies communications. In this study, we focused on the predatory strategies of the pharaoh cuttlefish, *Sepia pharaonis* from the coastal waters of Okinawa Island, Ryukyu Archipelago, Japan. More specifically, we have created eight different environmental conditions (white sand, white sand with obstacle, black sand, black sand with obstacle, black and white sand, black and white sand with obstacle, no sand and no sand with obstacle) with equal numbers of sapphire devils, *Chrysiptera cyanea* as prey. The predator and prey interaction between two species were recorded for video analysis. During this study, we identified four predatory strategies (ambushing, stalking, masquerade and aggressive mimicry) which are described in detail using the species' ethogram. *S. pharaonis* also showed preference in strategy selection based on different environmental conditions. Our findings may help better understand the dynamics between environmental conditions and behavior of the animal.

November 10, 1600-1615 [17]

Understanding bending and stiffening properties of the muscular hydrostat using the ‘arm slap’ of octopus, *Abdopus sp.*

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Octopuses can produce a variety of flexible arm movement patterns using principles that govern muscular hydrostats. Bending and stiffening are two consequences of the key principle that muscular hydrostats maintain constant volume. Various degrees of static localized bending and stiffening of the arm are involved in the most stereotyped movement patterns - ‘reach’ and ‘fetch’. Gutfreund et al. (1996) described reaching as extension of the arm by stiffening and propagating a bend to the tip. Fetching uses quasi-articulated structures to combine localized stiffening with multiple bend points that behave like temporary joints (Sumbre et al., 2006). We are interested in understanding the relationship between static bending and stiffening in dynamic movement patterns. One pattern that has not been heavily studied but was observed in the field with octopus, *Abdopus sp.*, involved the octopus’s reaction toward a goby fish by using an ‘arm slap’, first described by Mather (1992). We found the arm is initially tightly coiled at the base that upon use, rapidly uncoils using a combination of bending and stiffness propagation to quickly hit the fish target. We observed variations in bending and stiffness propagation such that some slaps exhibited complete transmission to the tip while others stopped prior to the tip. In the latter case, the more distal part to the bend stiffens and is hypothesized to optimize force/mechanical amplification more than distance/kinematic amplification. We show that systems can use principles of levers to maximize either displacement or force for a bending muscular hydrostat.

November 10, 1615-1630 [18]

Motion camouflage in relation to varying background and prey in *Sepia officinalis*

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Being cryptic is often effective when stationary due to strategies such as background matching, however once the animal moves the concealment that may have existed is often lost. In *Sepia officinalis* the ability to change camouflage rapidly within their environment allowed us to investigate what techniques may be employed to reduce detection while in motion. Chromatic and behavioural components were analysed to determine how background and prey influence camouflage decisions. Behaviour differed dependent on distance, substrate and prey, with shorter distances having one or two preferred capture techniques compared to an array when distance was increased. Greater distances also yielded greater chromatic changes which differed dependent on background. Certain chromatic components were reduced when in motion, where as other components were expressed only during the hunting process.

Session: Biodiversity and conservation

November 10, 1630-1645 [19]

Twelve years of changes to the known cephalopod fauna of the western central Atlantic Ocean

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In 2003, I authored a guide, published by the United National Food and Agriculture Organization, to the cephalopods of the western central Atlantic Ocean. That publication listed approximately 121 species in 72 genera (32 families). During the dozen years since publication, numerous trawling and submersible cruises, as well as some monographic revisions, have added substantially to our knowledge of that fauna. In addition to numerous name changes, the known fauna has increased by two families, seven (possibly eight) genera, and 14 species.

November 10, 1645-1700 [20]

Recent advances on the abundance and diversity of oceanic cephalopods from the eastern tropical Atlantic Ocean

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Oceanic cephalopods are an important part of the ecosystem of the eastern tropical Atlantic forming the link between zooplankton and top predators. To better understand ocean cephalopod communities in the eastern tropical Atlantic, and to monitor their potential changes as a result of exposure to stressors, it is necessary to obtain baseline data on diversity, distribution and abundance patterns. We analyzed the cephalopod fauna captured during oceanic trawl surveys in the eastern tropical Atlantic on the FRV *Walther Herwig III* in 2014 and 2015. The surveys sampled the epi- and mesopelagic communities in three regions of the eastern tropical Atlantic; the equatorial region, the pronounced oxygen minimum zone area south east of Cape Verde and the moderate oxygen minimum zone north of Cape Verde. More than 2,000 cephalopod specimens from at least 18 families were collected with a large midwater trawl at 19 stations, mostly during the night between 50-600 m of depth. A multisampler in the cod end allowed for the discrete sampling of three depths, which were chosen based on the depth of the scattering layers. While the upper layers were characterized by small individuals of the families Cranchiidae, Enoploteuthidae and Pyroteuthidae, deeper tows also captured larger individuals including mature specimens of *Mastigoteuthis*, *Bathyteuthis abyssicola*, *Ancistrocheirus lessneri*, *Octopoteuthis sicula* and *Liocranchia reinhardti*. Diversity and abundance comparisons between the three regions are presented and discussed.

November 10, 1700-1715 [21]

Large-scale spatio-temporal patterns of Mediterranean cephalopod diversity

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Species diversity is widely recognized as an important trait of ecosystems' functioning and resilience. Understanding the causes of diversity patterns and their interaction with the environmental conditions is essential in order to effectively assess and preserve existing diversity. In this work we present spatio-temporal trends of cephalopod diversity across the entire Mediterranean Sea during the last 19 years, analysing data from the annual bottom trawl survey MEDITS conducted by 5 different Mediterranean countries using standardized gears and sampling protocols. The influence of local and regional environmental variability in different Mediterranean regions are analysed applying generalized additive models, using species richness and the Shannon Wiener index as diversity descriptors. While the western basin showed a high diversity, our analyses do not support a steady eastward decrease of diversity as proposed in some previous studies. Instead, high Shannon diversity was also found in the Adriatic and Aegean Seas, and high species richness in the eastern Ionian Sea. Overall diversity did not show any consistent trend over the last two decades. Except in the Adriatic Sea, diversity showed a hump-shaped trend with depth in all other regions, being highest on the 200-400 m depth. Our results indicate that high Chl_a concentrations and warmer temperatures seem to enhance species diversity, and the influence of these parameters is stronger for richness than for Shannon diversity.

November 10, 1715-1730 [22]

Conservation status of *Nautilus* and *Allonautilus* in the South Pacific Ocean

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Although represented by just two extant genera (*Nautilus* and *Allonautilus*), nautilus shells and jewelry are sold all over the world. Since the 1970's, nautilus fisheries collected the ornamental shell with little to no regulation or management, resulting in anecdotal reports of population decline. In 2010, with still no scientific data to rely on, the United States Fish and Wildlife Service funded the first expeditions to assess the current status of nautilus populations. Our team traveled to several sites in the South Pacific to compare fished populations (Philippines locales) to non-fished populations (Australia, Fiji, American Samoa, Vanuatu, and Papua New Guinea). We used a combination of catch per unit effort (CPUE) and baited remote underwater video systems (BRUVS) to assess populations. Overall, our data showed significant differences between the Philippines catch rate in the 1970's to present day. We also found significant differences in population abundance between fished and non-fished sites. The most parsimonious explanation of the difference is that unregulated fisheries negatively affect nautilus populations. An unexpected outcome was the relatively small populations of all nautilus populations, suggesting that all may be vulnerable to not only fisheries but potential anthropogenic changes. In particular, we found that *Allonautilus* may be even rarer than previously thought; a scary proposition as 'rarity' is so often coveted by shell collectors. For the first time, we have data illustrating the great need for protection and conservation of nautilus. Now is the time to act to save one of the oldest lineages on the planet.

Session: Reproduction

November 11, 0830-0900 [23], Keynote talk

Cephalopod reproductive ecology: from individuals to populations

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The short-life span of most cephalopod species means that spawning in each year must occur given the absence of 'storage' of multiple generations in the population to protect from reproductive and recruitment failure. At the population level, cephalopods have adopted a range of strategies to ensure reproductive success and knowledge of these strategies has allowed the adoption of fishery management strategies to reduce the risks of over-fishing and to allow spawning. However, often the devil is in the detail and in particular the details of behaviours and physiology and the influence of environmental factors. Knowledge of processes occurring at the individual and sub-individual level are going to allow better predictions of how environmental change, especially those related to climate change, will affect these processes.

November 11, 0900-0915 [24]

The phenomenon of monocyclic or semelparous ontogeny in coleoid cephalopods: terminology, definitions and variety of patterns

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There is the problem of denomination and definitions of terminology for general reproductive strategy of all coleoids. The pairs of opposite terms – monocyclic (=semelparous) and polycyclic (=iteroparous) are widely used in literature on reproductive biology without clear and operable definitions. The terms monocyclic (MC) and semelparous are synonyms, same is correct for terms polycyclic (PC) and iteroparous. The MC means that each animal during ontogenesis has only one reproductive cycle i.e. one cycle of reproductive system developing, maturation and functioning, and after single spawning period they died. In the PC ontogeny after first complete reproductive cycle take place the reiteration of several or many incomplete reproductive cycles (i.e. with the exception of developing stage), and the reproductive system after each spawning period revert to immature state. As rule PC has seasonal cyclicity. The origin of coleoids in the Carboniferous was associated with the transition from PC to MC, which probably allowed compensating for the increased mortality due to loss of external shell. All studied coleoids are MC, i.e. “big-bang” reproducers. However this metaphor for coleoids has multiple-valued sense: it really varied from one “big-bang” to a series of small fractional “bangs”. There is a high variability in individual spawning period duration (from 5 to 50% of ontogenesis) and type of spawning from one-short to extend intermittent with multi-batches. On the MC base coleoids formed uniquely diverse range of r-K reproductive strategies by which they successfully inhabited at nearly all of the adaptive zones of the World Ocean.

November 11, 0915-0930 [25]

Strategic sperm release from spermatophores in loliginid squids responding to sperm competition risk

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To maximize fertilization success, males should allocate their limited sperm resources strategically according to the conditions of each mating possibility. Sperm competition risk is one of the most important factors affecting such sperm allocation, and theory predicts that males allocate more sperm when sperm competition risk is high. In loliginid squid, males have alternative reproductive tactics (consort and sneaking tactics), and females have two sperm storage sites (near or inside the oviduct and near the mouth, respectively). We previously showed that males produce spermatophores of different sizes associated with the adopted mating tactic. However, spermatophore size is a limited indicator of direct sperm allocation, because sperm are continuously released from the spermatangium after the spermatophoric reaction and the instantaneous number of sperm at fertilization depends on the release rate. We measured number of sperm in the spermatophore, including sperm release rate, in two loliginid species (*Heterololigo bleekeri* and *Loligo reynaudii*) with different sperm competition risk (less promiscuous and more promiscuous, respectively). Both in consorts and sneakers, *L. reynaudii* spermatophores contained double the number of sperm of those in *H. bleekeri*. In *L. reynaudii*, consort spermatophores released 97% of the sperm within 30 minutes, whereas sneaker spermatophores released only 66% of their sperm. In stark contrast, in *H. bleekeri* only 1% and 27% of sperm (respectively) were released within 30 minutes. These results suggest that males strategically allocate their sperm according to their sperm competition risk.

November 11, 0930-0945 [26]

Complex adaptive ejaculate traits in relation to alternative reproductive tactics in *Heterololigo bleekeri*

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In *Heterololigo bleekeri* and other sister species, female promiscuity allows polymorphic male mating behaviors (head-to-head, male parallel and sneaking), resulting in insemination polymorphism in place and time. Hence, ejaculates inseminated at different locations and different times should take different routes to reach ova, which could give an opportunity for adaptive trait evolution in ejaculates. We found a tight relationship between sperm lengths and insemination sites; i.e., larger consort and smaller sneaker males produce spermatozoa with short and long flagellum, respectively. Spermatozoa of sneaker males are capable of cluster formation in response to self-emitted carbon dioxide. Comparative proteomic analysis identified biased (>10-fold) protein abundance in sneaker (61 proteins) and consort (55 proteins) sperm among total of 4302 proteins detected. Notably, a large number of metabolic enzymes are listed in these biased groups, thus we examined sperm energetics such as energy sources and metabolic pathways. We found that sneaker sperm store large amount of glycogen and consume them during the period between ejaculation and storing in the female seminal receptacle. We propose that adaptive forces to insemination environment drive sperm trait evolution in relation to design, action and survival.

November 11, 0945-1000 [27]

Fertilization mechanisms in the male-dimorphic squid *Doryteuthis plei*

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Recent evidence suggests that male intrasexual dimorphism in squids involves distinct fertilization strategies between male morphs. We investigated alternative fertilization mechanisms in the male-dimorphic squid *Doryteuthis plei* by conducting mating experiments, *in vitro* spermatophore experimentation and light and electron microscopy analyzes. Our data indicate completely distinct fertilization scenarios for each male morph. Consort males place spermatophores near the oviduct opening, which lacks a sperm storage organ, sperm release from consort spermatangia being diffuse and lasting up to 2h. Sneaker males transfer spermatophores to the female buccal membrane, which bears a seminal receptacle provided with extrinsic and intrinsic musculature, as well as with a diverse array of secretory cells differing in their histochemistry and ultrastructure, suggesting a complex organ for sperm uptake, storage and release. Sperm release from sneaker spermatangia is not only significantly longer than in consorts (up to 5h), but also involves a characteristic aggregative behavior, in which the released sperm aggregate in a motile spherical mass near the spermatangium's tip. Sneaker spermatangia might also enter and block the opening of the seminal receptacle, suggesting a strategy for preventing the use of stored sperm from rival sneaker males. In conclusion, although consort males might have increased fertilization success, there is a temporal constraint for copulation with each female (i.e., egg-batch laying period). Sneaker males might engage in more copulations, with a sperm release strategy possibly associated with sperm storage in the female, and might also employ a hitherto unknown “sperm plug” tactic presumably involved with sperm competition between sneakers.

November 11, 1000-1015 [28]

Male dimorphic allometry in the squid *Doryteuthis plei*

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Sexual selection exerts a major influence on reproductive success of individuals. Under the pressure of paternity dispute, males less successful on fighting other males and guarding females often adopt alternative reproductive tactics, such as sneaking copulation. In many species, males who employ alternative tactics present not only behavioral differences, but also divergences in morphological and physiological aspects and are often called dimorphic males. In squids, alternative mating tactics are exhibited by two male morphs, known as consorts and sneakers. In the loliginid squid *Doryteuthis plei*, one of the most abundant cephalopods on the continental shelf off southern and southeastern Brazil, there has been growing evidence that males may adopt alternative mating tactics, but such tactics have not yet been associated with dimorphic males. Here, we investigated the presence of male intrasexual dimorphism in *D. plei* analyzing male individuals collected off the northern littoral of São Paulo state, southeastern Brazil. Based on the bimodal size distribution of mature males, we identified a morphological switch point between morphs at mantle length 155 mm. Using standardized major axis regression, we estimated the allometric coefficients of regressions between body size and body weight against internal and external characteristics for both classes of male. There were significant differences on hectocotylus length, testes weight and spermatophoric complex weight allometries between classes, with smaller males showing a steeper slope than larger males. Our results indicate a body size-related dimorphism in males of *D. plei*, possibly associated with alternative tactics, and a potentially distinct reproductive investment between morphs.

November 11, 1015-1030 [29]

Observations of multiple pelagic egg masses from small-sized jumbo squid (*Dosidicus gigas*) in the Gulf of California

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Ommastrephid squids lay pelagic gelatinous egg masses that are rarely observed in the wild. To date, a single egg mass has been found and documented from jumbo squid, *Dosidicus gigas*, in 2006. Female squid reach two or three size classes at maturity depending on environmental conditions as paralarvae. While the only egg mass documented to date was from a large-sized *D. gigas*, we present observations of six egg masses from small-sized *D. gigas* captured while blue-water diving in the Gulf of California, Mexico in 2015. This study reveals new information about the allometry of egg masses from different-sized females as well as novel observations on ommastrephid reproductive biology. Egg masses were found at a shallow thermocline and pycnocline. They were smaller and less fecund than those produced by large-sized *D. gigas* indicating that egg mass size and egg batch fecundity scale with body size. Embryonic densities in the gelatinous masses were less than those produced by large-sized individuals due to the larger relative size of nidamental glands in small-sized individuals. Egg masses were found within a 3-day period but were in a variety of ontogenetic stages indicating that females likely spawn at a similar but not simultaneous timeframe. Additionally, amphipods and ciliates were found within the egg masses; thus casting doubt on the effectiveness of egg mass jelly to deter all predators and parasites.

November 11, 1100-1115 [30]

New insights in reproductive regulation of *Octopus maya* inferred by RNA-Seq analysis of the optic lobe

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Temperature affects critical fitness components in *Octopus maya*. This enhances the importance of octopus female capacity of sperm storage and fertilization delay until optimal environmental conditions arise. The mechanisms that regulate this process remain unclear; nevertheless evidence suggests that different lobes in the nervous system may be involved. The aim of this study is to describe genes transcribed in the optic lobe (OL), with emphasis in the reproductive process and the temperature effect. Transcriptome assembly and RNA-Seq analysis were used to assess the effect of temperature and female reproductive stage on the genetic expression of regulatory genes in *O. maya*. Essential processes involved in embryonic development and reproduction (gametogenesis and hormonal process) were represented by 1,136 contigs, of which 328 were associated with gene ontology terms related to sperm (capacitation, motility and integrity maintenance). Results suggest that female OL could be involved in late sperm maturation, sperm re-mobilization, as well as in fertilization with transcription of genes required for sperm-egg recognition and interaction. OL may also participate in estrogen production and its activity seems to be regulated by progesterone fluctuations during reproductive process. Temperature stress and reproductive stage provoke important changes in optic lobe gene expression. Differentially expressed genes are not directly participating in reproduction or embryo development; however they could represent important molecular markers for identification of thermal stress during female reproductive process, and could be applied in monitoring the effects of thermal anomalies in this species.

November 11, 1115-1130 [31]

Control of egg-laying in the cuttlefish *Sepia officinalis*: structural and functional characterization of regulatory peptides

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In the English Channel, egg masses of the cuttlefish *Sepia officinalis* are spawned in specific mating and spawning coastal areas where mates aggregate between April and June. Environmental cues are clearly involved in the aggregation process, but chemical communication also plays a determining role in these mechanisms. Our studies show that the regulation of the successive steps of egg-laying is monitored by three classes of regulatory peptides: (1) neuropeptides (integration of environmental cues), (2) ovarian regulatory peptides (ORPs) (modulation of the activity of the genital tract), and (3) sex pheromones expressed by the oviduct gland (aggregation of mates and stimulation of mating).

Biochemical and physiological approaches first led to the identification of neuropeptides and ORPs modulating oviduct contraction. The recent development of NGS allowed us to sequence large batches of tissue samples: 26 transcriptomes sequenced by *de novo* RNAseq (Illumina) yielded around 360,000 unique annotated sequences.

We structurally characterized 38 neuropeptide families. Expression patterns associated to neuropeptide tissue mapping by mass spectrometry allowed us to identify neuropeptides involved in the successive steps of egg-laying.

Transcriptomics also revealed that ORPs were cleaved from a single yolk protein expressed in the oocyte, and that the three recently identified sex pheromone precursors were processed to release small peptides (1-8 kDa), but also large 22-26 kDa polypeptides/proteins mixed to egg capsule proteins.

After this major effort on the structural characterization of regulators, a thorough functional study will have to ensue to clarify the role of each class of regulators in the control of spawning.

November 11, 1130-1145 [32]

Group-synchronous ovarian development in the Argentine shortfin squid *Illex argentinus* (Cephalopoda: Ommastrephidae)

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Ovarian development images substantial spawning pattern of animals, especially in the semelparous cephalopod species. Theoretically, cephalopod species carry continuous, intermittent, or multiple spawning strategies as undergoing different oocyte maturations, ovulation and spawning during the ovarian development. In this paper, the ovarian development and oocyte growth of Argentinean shortfin squid *Illex argentinus* were investigated using samples collected from Chinese squid jigging fleets in the high seas of southwest Atlantic during January to March 2013 and April to June 2014. The ovary of *I. argentinus* presented monocyclic development and six distinct histological phases could be found among the ovaries, based on the histological examination of 108 ovarian tissues. For each ovary histological phase, the frequency distribution for both numbers and occupied areas of each oocyte stage were unimodal, and gradually moved forward along with the ovarian development. Additionally, size-frequency distribution and fecundity of the oocyte were analyzed based on the reproductive system of 19 females from macro-maturity maturing to active spawning. Oocyte development was growing as a multiple-batch group-synchronously during the period of ontogeny; ripe eggs in the oviducts was about 8.57% of fecundity among mature animals, but up to 20.91% of fecundity in the active spawning ones. In summary, our results show that *I. argentinus* carry out the group-synchronous ovarian development, with a multiple-batch oocyte growth and intermittent ovulation.

November 11, 1145-1200 [33]

Hermaphroditism in molluscs with the first case of finding a hermaphrodite specimen in cephalopods

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Molluscs have the most diverse spectra of reproductive strategies from dioecious animals to sequential and simultaneous hermaphrodites; from expelling sexual cells in the water and external fertilisation to complex inner and outer genital organs with internal fertilisation and even self-fertilisation, from direct egg development with larval phase to embryonisation and hatching juveniles that look like adult animals. Cephalopods are considered to be completely dioecious (=gonochoristic) animals. However, rare cases of pseudohermaphroditism (aberrant development of another sex' genital organs) were reported in some squid and octopus. In May 2015, one unusual specimen of the squid *Illex argentinus* was caught on the shelf of the Falkland Islands (Southwest Atlantic). It was a healthy maturing specimen of 24.5 cm mantle length. On the left side it had a fully developed spermatophoric complex of organs, with the Needham sac containing about 80 normal spermatophores with spermatophoric sacs filled with sperm. On the right side, it had a normally developed spiral oviduct (without eggs) with the oviducal gland. It also had a pair of normally developed nidamental glands. The gonad had a different texture, being loosen and greyish in the posterior part and compacted and white in the anterior part. Morphological characters and results of on-going histological and biochemical studies of the gonad of this hermaphrodite specimen were discussed in the context of sex determination, as well as occurrence and ecological significance of hermaphroditism in molluscs.

November 11, 1200-1215 [34]

Characterisation of a new family of egg case proteins in the cuttlefish: *Sepia*ECPs

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Sepia officinalis egg protection is ensured by a complex capsule produced by the female genital glands and the ink bag. To understand how eggs are formed, we studied the nidamental glands and the egg case. Nidamental gland transcriptomes associated to proteomic analysis allowed us to identify some of the proteins involved in egg formation. We thus characterized a new egg-case protein family produced by the main nidamental glands: SepECPs (*Sepia officinalis* Egg Case Proteins). SepECPs exhibit high sequence homology, especially in the signal peptide and the three cysteine-rich domains. Illumina sequence assembly analysis demonstrated identical 5'-ends corresponding to the signal peptide, suggesting alternative splicing from a single gene. SepECPs contain a high number of cysteines with conserved motifs involved in their 3D-structure. SepECP dimerization was evidenced by SDS-PAGE and then confirmed by TEM observations that revealed a protein network. This network is similar to the egg capsule network. It associates these structural proteins with polysaccharides, melanin and bacteria to form a narrow mesh. It provides the embryo physical protection through its resistance and elasticity. In addition, SepECPs also have bacteriostatic antimicrobial activity on GRAM - bacteria. The observation of the SepECP / *Vibrio aestuarianus* complex in SEM demonstrated that these proteins could agglomerate bacteria and thus inhibit their growth. These original proteins identified from the outer egg case provide physical and chemical protection to the developing embryos. They are a first clue towards understanding how embryos can survive in the environment without any maternal protection.

Session: Genetics and Evolution

November 11, 1400-1415 [35]

Coleoid gladiuses - homologies or homoplasies?

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One of the central homology problems in coleoid cephalopods represents the gladius (English "pen"; French "plume"; German "Schulp"), the unmineralized derivate of a molluscan shell that is jointly responsible for the most powerful mode of jet-propulsion among cephalopods. A gladius occurs in Loliginida, Oegopsida, Bathyteuthoidea, Sepiolida, and Idiosepiidae as well as the deep sea vampire squid *Vampyroteuthis*. As modern phylogenies suggest, gladiuses might have emerged 4-5 times. How is it possible that such a key innovation developed multiple times in the evolutionary history of the Coleoidea? Are there different formation mechanisms or is it instead possible to reconstruct a single ancestral gladius? Is the gladius possibly an adaptive feature related to the life habitat? The present contribution explores these questions by looking at neontological and palaeontological evidence and therefore at the evolutionary shell transformations that might have led to the modern gladius types. We will re-consider the ideas of pioneers in coleoid research, differentiate between *a priori* and *a posteriori* approaches, have (where possible) a closer look at the morphogenetic roots of the different gladius types, and elaborate similarities and dissimilarities between the different gladius types.

November 11, 1415-1430 [36]

Deciphering the venom proteome of the Southern blue ring octopus (*Hapalochlaena maculosa*) and the southern sand octopus (*Octopus kaurna*)

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We present a combined transcriptomic/proteomic analysis identifying 2,307 proteins from the posterior salivary gland (PSG) of the southern blue ring octopus, *Hapalochlaena maculosa* and 1,196 proteins from the southern sand octopus, *Octopus kaurna*. These are the first venom proteomes to have been characterized from any member of the Octopodiformes. The PSG proteomes of these two species were broadly similar. Examination of gene ontology molecular function terms revealed enrichment for peptidases congruent with digestive and potentially toxic functions within both species. Toxin candidates were identified from among salivary gland proteins by filtering according to criteria (i.e. short length, >2% cysteine percentage, at least three cysteines, presence of a signal peptide and homology to a known toxin). This process revealed a total of 33 toxins from *H. maculosa* and 28 from *O. kaurna* spanning ten protein families with the following putative toxin representatives: acid phosphatase, CAP (cysteine rich secretory proteins, antigen 5, pathenogenesis related), chitinase, DNase, hyaluronidase, metalloprotease, phospholipase, serine protease, short cysteine rich proteins and tachykinin. Absolute expression measurements of putative toxins showed that in both species a bias toward serine proteases was present, with chitinases the second most expressed family in *H. maculosa*. All other putative toxins were present at an order of magnitude lower abundance. Putative toxins identified in this study are of interest for further inquiry as novel proteinaceous toxins derived from venoms may contribute to pharmaceutical design.

November 11, 1430-1445 [37]

Cephalopods and the evolution of metazoan genome architecture

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Cephalopods have large, complex genomes, and thus have so far eluded comparative whole genome studies. In our recent work, we have sequenced and analyzed the 2.7Gb genome of *Octopus bimaculoides* [1]. Through comparison to other lophotrochozoan genomes [2] and other metazoans in general, we find, apart from some very notable exceptions, that *Octopus* lineage has surprisingly retained most of the ancestral bilaterian features of gene content (family size and representation) and structure (domain architecture, intron, and indel). Despite such conservation on the gene level, octopus genome had undergone a significant syntenic reshuffling, for example losing well-known linkages, such as Hox, Forkhead, or Wnt. The loss of synteny correlates with distinct (in the time domain) expansions of transposable elements (TEs), that constitute around 50% of the genome length. We differentiate between different classes of TEs contributing to either genome reshuffling or evolution of novel regulation and tissue-specific expression. These results provide the first insights into the genomic architectural changes that occurred in the evolutionary path leading to the octopus. They suggest that comparative studies with other cephalopod genomes will lead to a new understanding of the relationships between genomic variation and morphological novelties in metazoan evolution.

[1] Albertin CB, Simakov O, Mitros T, et al. "The octopus genome and the evolution of cephalopod neural and morphological novelties." Nature 2015, in press

[2] Simakov, O., F. Marletaz, S. J. Cho, et al. "Insights into bilaterian evolution from three spiralian genomes." Nature 2013

November 11, 1445-1500 [38]

Transcriptome and proteome analyses of the *Nautilus*' shell matrix proteins: Insights into their evolution in Mollusks

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Conchiferan mollusks are known for their prominent external calcified shell. Research on the genomic aspects of biomineralization of this group might provide insights to understand the shell's evolutionary success. Interestingly, although cephalopods are systematically conchiferan mollusk, they have secondarily lost their external mineralized shells. However, phylogenetically basal cephalopods, the nautiloids, still retain their true shells. This makes studies on nautiloid shell biomineral-proteins important and interesting, since insights from the nautiloids will allow for the identification of core components of conchiferan shell matrix proteins, and help to elucidate the evolution of shell proteins through comparisons with other conchiferan biomineral-related protein data.

Here, we report our result of the genomic explorations to identify biomineralization-related proteins in the nautiloid *Nautilus pompilius*. To do so in our research, we first determined the total transcriptome sequences from the mantle tissue by Ion-PGM pyrosequencing, while simultaneously did a total proteome analysis of the shell's hydrophilic proteins by orbital-trap mass-spectrometry. We then conducted a transcriptome-proteome comparative analysis to identify the hydrophilic components of shell biomineral-related proteins in the *Nautilus*, and we identified 61 distinct shell specific EST/protein sequences. Besides that, from the transcriptome analysis, we also found members of early development-related signaling pathways thought to be involved in shell formation. In this presentation, we are going to discuss about how these findings might provide insights into the evolution of mollusk shell formation and biomineralization genes/proteins.

November 11, 1500-1515 [39]

Insights from the transcriptome into the embryonic development of *Octopus bimaculoides*

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Cephalopods have a highly derived body plan and a suite of innovations with no obvious correlates in other molluscs, making them a compelling model for the study of the evolution of novelty. One of the most striking novelties in cephalopods is their embryogenesis, which lacks any trace of the spiral cleavage pattern found in non-cephalopod molluscs. To identify gene networks important for the development of the octopus body plan, we analyzed transcriptomes of five developmental stages, including pre-epibolic and epibolic stages, and stages during and after the emergence of organ primordia, of the California two-spot octopus, *Octopus bimaculoides*. The *O. bimaculoides* genome contains only a single complement of the metazoan developmental “toolkit” genes, including homeodomain transcription factors and signaling ligands [1]. Notably, bioinformatics-based differential expression analysis reveals that these highly conserved “toolkit” genes appear to be expressed primarily during or after the emergence of the body plan, but not before. This analysis has, however, identified suites of genes that show dynamic changes and stage-specific expression profiles. Our data indicate the power of combining RNAseq with the newly available *O. bimaculoides* genome in addressing key questions about the evolution of cephalopod novelties.

[1] Albertin CB, Simakov O, Mitros T, Wang ZY, Pungor JR, Edsinger-Gonzales E, Brenner S, Ragsdale CW, Rokhsar DS. 2015. The octopus genome and the evolution of cephalopod neural and morphological novelties. *Nature* **524**:220-224.

November 11, 1515-1530 [40]

Genome-wide single nucleotide polymorphisms reveal patterns of population structure in two widely co-occurring cryptic lineages of reef squid

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Diversification in the marine environment has long been assumed to be driven by primarily allopatric (i.e. physical) barriers to gene flow. However, recent studies indicate that this explanation is too simplistic and that a multitude of both physical and ecological drivers exist and interact to generate and maintain diversity. However, previous studies only provide limited insight into these mechanisms as most focus on benthic organisms. Moreover, while data from 'last-generation' sequencing methods are useful, they often lack sufficient power to illuminate fine-scale patterns of population structure. In light of this, this study uses a comparative population genomic approach to examine barriers to gene flow in two co-occurring, cryptic species of the big-fin reef squid (*Sepioteuthis* cf. *lessoniana*), a neritic organism within the center of marine biodiversity (the Coral Triangle). We used a RADseq generated dataset (using the 2b-RAD method, Wang et al. 2012) consisting of ~2,000 single nucleotide polymorphisms (SNP) sampled from individuals collected from 5-7 geographic locations across the Coral Triangle for each lineage. Comparing patterns of genetic breaks and connectivity between these two species indicates differing levels of overall population differentiation. Lineage B is significantly more structured over the Coral Triangle than lineage C ($F_{ST}=0.3$ vs. $F_{ST}=0.05$, $p<0.001$ for both). Furthermore, we observe that not all common phylogeographic breaks defining this region are observed for both lineages. Strikingly, there is no differentiation over the Sunda Shelf, indicating significant levels of gene flow between the Indian and Pacific Oceans. However, in lineage C, divergence is indicated between the Ryukyus and the Coral Triangle, as well as over the Maluku Sea region. These breaks likely indicate that deep ocean basins and sea surface currents pose substantial barriers to gene flow for *S. cf. lessoniana*.

November 11, 1600-1615 [41]

***Octopus vulgaris* genome survey**

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⁴ Presentation on behalf of members of the CephRes project for the genome survey of the common octopus

Cephalopods genomics has been promoted after 2011 and efforts achieved during these years provided the first cephalopod genome ever sequenced (i.e. *Octopus bimaculoides*). Despite similarity, octopuses are a very diverse set of species, with different developmental, physiological, possibly morphological and behavioral adaptations, including challenging differences in the richness of behavioral repertoire. We present genome sequencing (i.e. 30X genome survey) of *Octopus vulgaris* achieved through a CephRes collaborative project (with BGI and other partners). The project achieved to update the current available estimation of genome size of the species, heterozygous rate and repeat content of this genome. The availability of *O. vulgaris* genome sequence data will complement the one available from the other species further facilitating studies on behavior, genetic diversity, physiology, and evolution.

November 11, 1615-1630 [42]

The planktonic stage of *Octopus vulgaris* paralarvae in the Canary current Eastern boundary upwelling ecosystem: distribution, dispersal and diet

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The planktonic stage of *Octopus vulgaris* is the least studied part of their life cycle and is the bottleneck in making their aquaculture viable. All octopus paralarvae captured to date over the continental shelf (<200 m depth) were early stages with only three suckers per arm. Captive studies have shown that octopuses settle with around 23 suckers per arm. So, where are octopus paralarvae growing? What do they eat? Two surveys were carried out in the upwelling systems of NW Iberian Peninsula (CAIBEX-I) and Morocco (CAIBEX-III), where 99 and 35 octopus paralarvae were found respectively. A positive relationship was found between octopus size and distance to shore. Up to 74 octopuses were found over the continental slopes off Iberian Peninsula (n=48, 3-6 suckers) and Morocco (n=26, 3-15 suckers) with bottom depths 780 - 3110 m. These facts reveal that planktonic *O. vulgaris* paralarvae have an oceanic strategy rather than the coastal-shelf strategy of other neritic species (loliginids and sepiolids). The ontogenic changes in the diet were studied using next generation sequencing by comparing 1) the paralarvae collected over the shelf (3 suckers) and open ocean (>3 suckers); 2) the paralarvae transported from the coast by upwelling filaments more than 200 km offshore. Contrasting differences were found between surveys and along the coastal-oceanic gradient mirroring the sharp change in zooplankton communities. This study features the intricacies of *O. vulgaris* planktonic stage opening new rearing possibilities for aquaculture and encouraging the need to reproduce these oceanic conditions in captivity.

November 11, 1630-1645 [43]

Evaluation of the 5' end of the 16S rRNA gene as a novel DNA barcode marker for the Cephalopoda

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Using short fragments of the genome, DNA barcoding represents a powerful tool for species identification important in many ecological, conservation and taxonomic studies. Cephalopods are not the exception and are represented by a wide range of species with economic and ecological importance worldwide. With the aim to propose a novel DNA barcode marker for the cephalopoda, the 5' end region of the 16S rRNA gene is tested among 28 different species. Based on the alignment of the full-length 16S rRNA gene from 28 species including the extant Nautiloidea and Coleoidea subclasses, we designed novel degenerate primers. Except for the PCR failure in *Octopus dofleini*, we were able to group the remaining 27 species into well supported clusters in accordance with contemporary morphological classification. Furthermore, the average interspecific divergence of our proposed region among coleoids is almost 2 and 4 times higher than the commonly used mtDNA COI and 3' end 16S rRNA markers, respectively, whereas slightly similar values were found at the intraspecific level. In addition, two well supported clusters in *Sthenoteuthis oualaniensis*, *Sepia pharaonis* and *Loliolus japonica* were found, indicating the possibility of cryptic species. This study provided evidence of the high polymorphism of this region, which can be incorporated in further barcoding studies.

November 11, 1645-1700 [44]

Population structure and geographic boundary of the Upper Spencer Gulf giant Australian cuttlefish

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Giant Australian cuttlefish, the largest cuttlefish species in the world, is widely distributed across southern Australian waters. Through most of its range it breeds on inshore rocky reefs, but it is renowned for forming the single largest known breeding aggregation of any cuttlefish species in the world in Upper Spencer Gulf (USG), South Australia. We aimed to determine fine scale population structure, resolve the systematic status of the USG population to determine the extent of its geographic boundaries and to assess population viability. Fine scale population structure was determined by analysing elemental chemistry along profiles of the statoliths and by assessing the extent and pattern of genetic differentiation of cuttlefish in South Australian waters using next generation DNA sequencing. Our results suggest that two major clusters of individuals occur in South Australian waters that are related to geography; one cluster comprised only specimens from USG, while the other comprised all specimens from elsewhere in Spencer Gulf and Gulf St Vincent waters, and some specimens from USG. The range of the two genetic clusters overlapped across 0.7° of latitude, and there was some evidence of recent hybridisation. Our results suggest that individuals found on the breeding aggregation in USG are genetically isolated from individuals found elsewhere suggesting that a greater conservation focus on the species is required. Results also highlight the importance of identifying the ecological/environmental factors that are driving the observed changes in population abundance and whether certain life-history stages are more vulnerable than others.

November 11, 1700-1715 [45]

The genome and transcriptome of *Idiosepius paradoxus*

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Cephalopods are among the most advanced invertebrates, having sophisticated brains and eyes, dexterous chemo-sensitive arms, and exquisitely controlled dynamic coloration. Cephalopods are great models for understanding not only the evolution of these novelty but also as a unique and independent comparison to complex features in vertebrates. Advanced sequencing technologies and bioinformatic methodologies now enable genomics in non-model organisms. With genomics, we can move quickly to elucidate the genes, networks, and systems that underlie novel and convergent features in Cephalopods. We have completed first draft genome of pygmy squid, *idiosepius paradoxus*, and obtained stage and tissue specific RNAs. Pygmy squids are known to be the smallest of all the Cephalopods, and diverged early in the squid. The genome and Transcriptome of the pygmy squid, together with other cephalopods genomic information, could reveal genetic differences associated with phenotypic and behavioral diversification among cephalopods.

Session: Physiology

November 12, 0830-0900 [46], Keynote talk

Sperm whales and large mesopelagic squids - a talk in memory of Malcolm R. Clarke

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I stayed at the Plymouth Marine Biological Laboratory for three months from October to December, 1978. I was in the first year of my doctoral course at Hokkaido University, and Dr. Okutani helped arrange for me to go to Plymouth as a foreign student under the direction of Dr. Malcolm Clarke. He taught me statolith morphology and analysis. In 1981, Dr. Clarke organized a beak workshop at MBL, and Dr. Okutani and I were invited to attend. He stressed the importance of squids in the diet of large predators. This workshop was a milestone for my research on feeding habits of various marine animals, such as seabirds, sharks and marine mammals afterward. In 1983, Dr. Clarke offered a comprehensive map showing the family composition of squid prey in the diet of sperm whales caught in various regions of the world. But his map did not include information from the western North Pacific.

From 2000 to 2013, the stomach contents of 49 sperm whales were examined from the western North Pacific, and 33 species of cephalopods from 12 families were identified. The whales were found to have fed on a variety of unusual mesopelagic large squids, some of which I will introduce in my talk. The dominant prey were *Belonella borealis*, *Histioteuthis dofleini*, *Galiteuthis phyllura* and *Taningia danae*. Detailed analysis will be shown in a poster presented during the poster session. This research offers information on the squid prey of sperm whales in the western North Pacific, an area missing from Dr. Clarke's map.

Dr. Louise Allcock informed us about the sad news that Dr. Clarke passed away on May 11, 2013 at Pico Island, Azores, Portugal. He was 82 years old. I would like to express my heartfelt condolences on his death, as well as my cordial thanks for his helping guide my cephalopod studies, encouraging my research in Plymouth and afterwards, and accepting me as a member of his family.

November 12, 0900-0915 [47]

How the brain represents the body: neural circuitry and transcription factor codes in octopus

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The systems circuitry of how sensory and motor information are integrated and processed in the brain remains uncertain. Our studies, carried out on California two-spot octopus *Octopus bimaculoides* adults and hatchlings, employed carbocyanine dyes to trace neural connections. We asked to what extent are the sensory and motor signals represented as maps in the brain. Our findings establish that in octopus brain, as in mammalian brain, sensory and motor maps are a characteristic feature of brain organization. Interestingly, though, sensory maps are not maintained in all brain processing centers and in particular are substantially reorganized or absent in supraesophageal centers implicated in higher sensory processing and cognitive tasks. We also used nucleic acid histology to study the expression patterns of region-specific transcription factors known to be important in brain development in other taxa. Our molecular studies showed that the octopus brain shares some of the molecular profiles seen in the brains of other animals. Strikingly, the supraesophageal centers lacking sensory and motor maps also showed clear departures from the developmental control gene profile reported for mammalian cerebral cortex and the mushroom bodies of insects. Our findings establish substantial molecular and cellular novelty in the chemical and circuit architecture of the octopus supraesophageal brain.

November 12, 0915-0930 [48]

Cuttlefish venomics: The multiple roles of posterior salivary glands in *Sepia officinalis*

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In unconventional models, recent advances in next-generation sequencing have made it possible to obtain large-scale molecular data validated by mass spectrometry identification of mature products. In the cuttlefish *Sepia officinalis*, transcriptomics associated to proteomics allowed for the identification of the salivary gland proteome.

These experimental approaches showed that saliva secreted by the venomous posterior salivary glands (PSGs) contains neurotoxic proteins such as CRiSP (Cystein Rich Secreted Protein) or cephalotoxin (a 110-kDa protein specifically expressed in decabrachia), and hydrolytic enzymes that paralyze and pre-digest preys.

In addition, *in silico* analysis of the PSG transcriptome revealed the occurrence of salivary proteins involved in immune defense (LBP/BPI, PGRP, lysozyme, macroglobulin), similar to those described in mammals. As saliva is also associated to spermatozoa storage and egg capsule elaboration, salivary proteins could be closely involved in the external layer that protects gametes or facilitates fertilization. Interestingly, some genes are differentially expressed in male and female PSGs, suggesting a true involvement of saliva in the reproduction process.

November 12, 0930-0945 [49]

Body pattern control in the optic lobe of oval squids *Sepioteuthis lessoniana*

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Cephalopods have the most sophisticated dynamic skin coloration for rapidly camouflage in nature. The pair of the optic lobes located bilaterally in their brain plays a key role in controlling the expansion of chromatophores for generating diverse body patterns. However, the functional organization of the optic lobes and their neural control of various body patterns have not been examined systematically. We applied electrical stimulation in the optic lobe to investigate the neural basis of body patterning in oval squids *Sepioteuthis lessoniana*. After anesthetizing, a stimulus electrode was inserted into their optic lobes, and the dynamic changes of body patterns evoked by electrical stimulation were recorded by a video camera from above. We have observed that the optic lobe mediated ipsilateral expansion of chromatophores was dominated on mantle skins but not on head or arms. The expanded area of skins during electrical stimulation were positively correlated with increasing voltages and depths. Furthermore, we analyzed 14 elicited body pattern components and their corresponding stimulation sites in the optic lobe, and found that many body pattern components have multiple motor command units in the optic lobe and subsets of body pattern components can be co-activated by stimulating the same area in the optic lobe. Based on our findings, the hierarchical organization in the medulla of the optic lobe is likely to be responsible for determining the expanded area and component expression on skins, thus the body pattern control.

November 12, 0945-1000 [50]

Short and long-term effects of anesthesia in *Octopus maya* (Cephalopoda: Octopodidae): Oxygen consumption, growth rate and hemolymph parameters

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The present study was designed to establish the type and level of anesthetics that must be used to manipulate juveniles and adults of *Octopus maya*. First, we investigate the oxygen consumption before, during and after exposure of *O. maya* juveniles to different levels of hypothermia, ethanol, magnesium chloride, ethanol in combination with magnesium chloride and clove oil. After the exposure, animals were weighted and manipulated for 180 seconds before the recovery in oxygenated seawater. Additionally, we registered induction and recovery times and if animal ingested food to determine an optimal recovery. After those experiments, the better anesthetic dose of each anesthetic was selected to test their effects on *O. maya* adults. As in the juvenile experiments, we evaluated the induction and recovery times. We also evaluated if such doses affected the hemolymph parameters before euthanasia. Results on oxygen consumption revealed that juveniles exposed to anesthetics showed, before induction, a metabolic rate higher than animals only manipulated suggesting that the substances tested provoked more metabolic demand than only manipulation. We also observed that juveniles were not affected by the manipulation and substances tested (except clove oil) suggesting that short-term manipulation is not enough to provoke long-term effect on growth rate. To manipulate adults (>500g), we recommend ethanol 3% to anesthetize *O. maya* for hemolymph sampling and to facilitate handling during weighting. Analysis of hemolymph parameters indicated that magnesium chloride modified levels of protein, lactate, cholesterol and acylglycerides suggesting that this anesthetic should be used with caution when those parameters are measured.

November 12, 1000-1115 [51]

Establishing a link between personality and resting metabolic rates in the dumpling squid, *Euprymna tasmanica*

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Inter-individual variation in behaviours allows a description of personality syndromes in cephalopods, but processes maintaining or facilitating these are unknown. Correlations between physiology and behaviour examine the capacity of individuals and populations to respond to changes in the physical and biological environment. More active individuals that take greater risks to obtain food may do so because they need energy to support faster metabolic rates. The aim of this project was to identify if a relationship between metabolic rates and personality occurs in the dumpling squid *Euprymna tasmanica*, and to determine if personality and metabolic rates were altered when individuals experienced stress associated with a reduction in ration. Personality was tested under threat and feeding scenarios; mass-specific resting metabolic rate was estimated from an individual's based on oxygen consumption rate while at rest. Once personalities and basal metabolic rates were established half the individuals were allocated to one of two feeding rations; 10% of wet weight/day (excess ration) or 3% of wet weight/day (maintenance ration), for 14 days, after which time resting metabolic rates and personality scores were determined. There was evidence that more active individuals had faster resting metabolic rates. However, resting metabolic rates were not correlated with the level of boldness. Individuals on excess food rations changed their resting metabolic rate, with the change a function of initial metabolic rate. Individuals did alter their personality after altered rations. There was limited evidence to support the differences in boldness among individuals as a function of food availability.

November 12, 1015-1030 [52]

Recent advances in exploring of trans-epithelial proton and ammonia transport pathways in cephalopods

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Cephalopods have evolved complex sensory systems and an active lifestyle to compete with fish for similar resources in the marine environment. Their highly active lifestyle and their extensive protein metabolism has led to substantial acid-base regulatory abilities enabling these organisms to cope with CO₂ induced acid-base disturbances. In convergence to teleost, cephalopods possess an ontogeny-dependent shift in ion-regulatory epithelia with epidermal ionocytes being the major site of embryonic acid-base regulation and ammonia excretion, while gill epithelia take these functions in adults. Although the basic morphology and excretory function of gill epithelia in cephalopods were outlined almost half a century ago, immunohistological and molecular techniques are bringing new insights to the mechanistic basis of acid-base regulation and excretion of nitrogenous waste products (e.g. NH₃/NH₄⁺) across ion regulatory epithelia of cephalopods. Our further studies using perfusion techniques in cephalopod also proved that besides renal appendages, gill epithelia were major sites of acid-base regulation and ammonia transport.

Using cephalopods as an invertebrate model, recent findings reveal partly conserved mechanisms but also novel aspects of acid-base regulation and nitrogen excretion in these exclusively marine animals. Comparative studies using a range of marine invertebrates will create a novel and exciting research direction addressing the evolution of pH regulatory and excretory systems.

November 12, 1030-1045 [53]

Exploring neurohypophysial sepiatocin functions in cuttlefish epithelium

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Cephalopods were proved to process epithelial acid-base regulatory machinery; however, its evo-devo bases for extracellular pH homeostasis are still poorly understood. In this study, we used embryos (stage 28) of cuttlefish, *Sepia pharaonis*, to integrative examine expressions of the epidermal morphogenesis marker ($\Delta Np63$), neurohypophysial hormone (sepiatocin) and neurohypophysial hormone receptor (sepiatocin receptor 2, *str2*) under CO₂-induced acidification. Intact resting respiration data showed that *S. pharaonis* embryos, whose gills were well developed in stage 28, were capable of surviving under CO₂ perturbations as low as to pH7.0. In addition, RNA *in situ* hybridization images indicated that both $\Delta Np63$ and *str2* were expressed in embryonic epithelium and adult gills, the dominant sites for acid-base regulation; in contrast, sepiatocin and *str2* were found to be upregulated accompanied with those stimulated genes for epithelial acid-base regulation (e.g. VHA, NBC, NHE3, RhP and NKA) in CO₂-acidified condition. In conclusion, the present work inferred that the promptly activations of sepiatocin and *str2* might be involved in operating epidermal ion fluxes; accordingly, in order to cope with acid-base disturbances during their oviparous development, cephalopod embryos have evolved sophisticated evolution pathway regarding epithelium differentiation and neurohypophysial hormones regulation.

November 12, 1045-1100 [54]

Oxidative stress in coastal and oceanic cephalopod from the Bay of Biscay in relation to their metal accumulation and detoxication capacities

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Cephalopods display in their tissues high concentrations of anthropogenic contaminants, especially, the trace elements. Nevertheless, little is known on their physiological response to these bioaccumulated metals and the ecotoxicological consequences. Metals are known for their ability to enhance the production of reactive oxygen species (ROS) but it is still difficult yet to correlate the oxidative stress with metal levels recorded in these organisms. Here, we apply a multi-species approach, investigating a set of antioxidant enzymes activities in oceanic and coastal cephalopod with contrasting habitats, feeding ecology and metal concentrations. The concentrations of Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, V and Zn have been measured in the digestive gland of squid *Loligo vulgaris*, *L. forbesi*, *Todarodes sagittatus*, *Teuthowenia megalops* and *Histioteuthis reversa*, the sepiidae *Sepia officinalis* and *Rossia macrosoma*, and the octopus *Eledone cirrhosa*, and *Octopus vulgaris*, all sampled in the Bay of Biscay. Additionally, the activities of the catalase - CAT, superoxide dismutase - SOD and glutathione peroxidase - GPx have been determined in the same tissue. Variations of the oxidative stress recorded in these species have been discussed as a function of their metals levels, their detoxication capacities (expressed as metallothioneins concentrations) and their physiology and ecology.

November 12, 1100-1115 [55]

A Next-Generation approach to profiling the *Octopus bimaculoides* optic gland

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Since the mid-20th century, octopuses have been known to possess a complex hormone regulatory system. The optic gland, a coleoid endocrine organ, is thought to control gonad development, sexual maturation, and death through hormone signaling[1]. Despite the important roles it plays throughout development, the optic gland remains understudied. To explore the molecular mechanisms of optic gland hormone signaling, we carried out Next Generation RNA sequencing on male and female *Octopus bimaculoides* optic glands. These transcriptomes were compared with those of central brain, optic lobe, viscera, posterior salivary gland and the ovaries to probe the molecular underpinnings of the optic gland. Using differential expression analysis, we identified collections of genes with highly specific expression profiles. We found genes with elevated expression in the optic gland and neural tissues and, strikingly, genes that are specifically enriched in either the female or male optic gland. Interestingly, most of these sex-specific transcripts are specific to the female optic gland, suggesting that there may be extensive regulatory networks dedicated to female reproductive behaviors such as egg-laying, brooding and senescence.

[1] Wodinsky, J. (1977). Hormonal inhibition of feeding and death in octopus: control by optic gland secretion. *Science*, 198(4320), 948–951. <http://doi.org/10.1126/science.198.4320.948>

November 12, 1115-1130 [56]

Statocyst sensory epithelia ultrastructural analysis of cephalopods exposed to noise

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Many anthropogenic noise sources are nowadays contributing to the general noise budget of the oceans. The extent to which sound in the sea impacts and affects marine life is a topic of considerable current interest both to the scientific community and to the general public. Cephalopods potentially represent a group of species whose ecology may be influenced by artificial noise that would have a direct consequence on the functionality and sensitivity of their sensory organs, the statocysts. These are responsible for their equilibrium and movements in the water column. Controlled Exposure Experiments, including the use of a 50-400Hz sweep (RL = 157±5 dB re 1 µPa with peak levels up to SPL = 175 dB re 1 µPa) revealed lesions in the statocysts of four cephalopod species of the Mediterranean Sea, when exposed to low frequency sounds: (n=76) of *Sepia officinalis*, (n=4) *Octopus vulgaris*, (n=5) *Loligo vulgaris* and (n=2) *Illex condictii*. The analysis was performed through scanning (SEM) and transmission (TEM) electron microscopical techniques of the whole inner structure of the cephalopods' statocyst, especially on the *macula* and *crista*. All exposed individuals presented the same lesions and the same incremental effects over time, consistent with a massive acoustic trauma observed in other species that have been exposed to much higher intensities of sound: Immediately after exposure, the damage was observed in the *macula statica princeps (msp)* and in the *crista* sensory epithelium. Kinocilia on hair cells were either missing or were bent or flaccid. A number of hair cells showed protruding apical poles and ruptured lateral plasma membranes, most probably resulting from the extrusion of cytoplasmic material. Hair cells were also partially ejected from the sensory epithelium, and spherical holes corresponding to missing hair cells were visible in the epithelium. The cytoplasmic content of the damaged hair cells showed obvious changes, including the presence of numerous vacuoles and electron dense inclusions not seen in the control animals. The lesions described here are new to cephalopod pathology. Given that low-frequency noise levels in the ocean are increasing (e.g. shipping, offshore industry, and naval maneuvers), that the role of cephalopods in marine ecosystems is only now beginning to be understood, and that reliable bioacoustic data on invertebrates are scarce, the present study and future investigations will bring an important contribution to the sustainable use of the marine environment.

November 12, 1130-1145 [57]

Preliminary study of cuttlefish seedlings (*Sepia lycidas* and *Sepia pharaonis*) cultivation technology in China

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Sepia lycidas and *Sepia pharaonis* are both warm-water cuttlefish species, which are widely distributed in the South China Sea, with their characteristics of large size, fast growth rate. To solve the key techniques of cuttlefish seedlings culturing in artificial breeding, the studies on reproductive biology of *S. lycidas* and *S. pharaonis* were carried out from 2007. The results showed as follows: 1) the mature production technical route. Followed by parent spawning, oosperm collecting, incubation and larval stage I (body length 5-10 mm), larval stage II (body length 10-15 mm), seedlings stage I (body length 1.5-3 cm, mantle length above 1.0 cm), seedlings stage II (body length above 3 cm, mantle length above 2.0 cm), feed domestication, finally, seedlings for sale; 2) intermediate rearing. The survival rate of cuttlefish (seedlings stage I) can be improved by rearing them in a shrimp culture pond, and they would also grow faster. After 10-15 days of incubation period, the body length of cuttlefish will be 3-4 cm, mantle length 2-3 cm, weighing about 3 g; 3) feed domestication. Firstly, optimized initial feed of cuttlefish larvae, by comparing and analyzing their nutritional composition, then domesticated them static feed to lay a foundation for artificial culture seedlings in the shrimp culture pond. Moreover, the first discovered and established the best food conversion period of seedlings is when their body length are 3-4 cm (mantle length 2-3 cm); 4) cultivation pattern. Intermediate rearing cuttlefish seedlings in earthen ponds would cost 30% lower than the cement pond.

Session: Life history and roles in marine ecosystems

November 13, 0830-0900 [58], Keynote talk

Early life-history of cephalopods: an ecological perspective on critical events

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Cephalopod early life stages are wonderfully diverse showing adaptive specializations for survival in a variety of marine environments. Indeed, the study of cephalopod early stages is necessarily multidisciplinary as they are transitory stages that must face developmental and ecological demands to function successfully either as planktonic or benthic organisms prior to transition to the adult realm. These development modes, planktonic and benthic, shown many contrasting morphological, behavioural and ecological features as a result of adaptive environmental pressures. It is well acknowledge that the early life history of cephalopods is a period of intense mortality, nevertheless, very little information exists on the major presumed causes of early mortality: first feeding, starvation and predation. Estimates of cephalopods early mortality require accurate identification of early stages, accurate measures of their abundance, their feeding and growth rates, as well as their main predators and prey. These topics have fundamental gaps on our knowledge and represent the main challenges for future studies as they have the potential for generating important new insights into ecology, population dynamics and evolution of cephalopods life-history. I will emphasize critical events during early development and discuss patterns associated with habitat and morphology in search of commonalities and gaps in the realm of cephalopods early life development.

November 13, 0900-0915 [59]

How to measure ecological importance in the marine systems (example: cephalopods)

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New method of assessing ecological importance and trophic advantage of a distinct group of marine animals over other groups is proposed. Crucial for the present approach is the concept of “predation window” (PW; known from ecological literature) defined as the ratio of prey length to predator length (in some instances weight and other suitable indicators may be used). Used formula was PW as the range PY/PR min to PY/PR max. It is argued that for opportunistic predators, predation window is one of the main determinants of predation spectrum (S). The latter is defined as the ratio of a number of dominant species (80% weight) being constantly eaten by the given predator (n) to the total number of edible and accessible species within predator’s distribution range and within his predation window (m), listed and recorded for this predator ($S=n/m$). The last proposed ratio is the energetic value of most frequently taken prey (nv) to the prey with the highest energetic value (hv) from his food list ever recorded ($V=nv/hv$). It is proposed that these indicators are combined as a feeding strength, $FS1=PW_{min}SxV$, $FS2=PW_{max}SxV$. Predator’s signature (PS) is defined as an index comprising the following probabilities: of encountering any prey (a), level of tolerance for that prey (b), eating that prey (c), and high/low energetic gain from eating that prey (d). $PS = abcd$. Relations between FS and PS are also explored. When analysing food relations, directions in a network are important, and must be taken into consideration. Notation used for this is as follows: species as prey, “-“; species as predator, “+“; both predator and prey, “PP“, no interaction, “0“.

The comparison, based upon these indicators, suggests considerable ecological advantage squids have over marine mammals.

November 13, 0915-0930 [60]

Beak development in *Illex argentinus* paralarvae and its possible relation to the feeding mode of rhynchoteuthions

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Ommastrephids form important fisheries resources worldwide. Their paralarvae are one of the smallest cephalopod hatchlings (1 mm mantle length (ML)) and possess a peculiar and transient feature, the tentacles fused into a proboscis. The feeding mode with fused tentacles is still poorly understood. Moreover, little work has been directed towards understanding morphological changes of key feeding structures, such as the beak, which can provide essential information for the understanding of the feeding strategy of rhynchoteuthions. A description is presented for the morphology and morphometry of beak development in *I. argentinus* paralarvae from 1.2 to 15 mm ML, a size range that includes the transition of feeding mode with and without the proboscis. The upper jaw (UJ) of the beak was very fragile and rudimentary in small individuals, displaying a conspicuous groove between the lateral walls. This groove progressively disappears and gives place to the formation of the hood and rostrum in paralarvae of 3-4 mm ML, a size range when the proboscis already started division, evidencing a synchronism between UJ development and proboscis division. A discontinuity in growth was observed for both the UJ and lower jaw. In the former, it occurred at 3.8 mm ML, which is also the size after which hard parts of copepods have been observed in the paralarval digestive tract; and in the latter, it was found in larger individuals (9.7 mm ML). The implications of these findings are presented and discussed.

November 13, 0930-0945 [61]

Ontogenetic and stock variation of beak stable isotopes ^{13}C and ^{15}N of neon flying squid *Ommastrephes bartramii* (Cephalopoda: Oegopsida) in the North Pacific Ocean

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Stable isotopes (^{13}C and ^{15}N) are useful tools for understanding the migration patterns and dietary shifts of marine animals. In this study, we performed isotopic analysis on *Ommastrephes bartramii* beaks to examine differences in geographic range, migration patterns, and diet composition between the eastern and western stocks in the North Pacific Ocean. Isotopic values of the upper beak (UB) and the lower beak (LB) were also compared. A generalized additive model (GAM) was used to select variables that explain ontogenetic variation. Isotopic signatures (^{13}C and ^{15}N) were significantly different between the two stocks whereas there was no difference in C/N ratios. All isotopic values were significantly different between the UB and LB. The ^{13}C signature increased with latitude and mantle length (ML) with greater variability in the eastern stock. None of the variables could explain the variation in ^{13}C values for the western stock. The ^{15}N signature increased rapidly with ML in the eastern stock, whereas ^{15}N gradually increased with latitude and ML in the western stock. The isotopic variations might indicate different migration patterns and feeding behaviors of the two stocks. The ^{13}C and ^{15}N values of the UB were lower than those of the LB, possibly due to variable chitin/protein ratios at different developmental stages. Future studies should account for a greater sample size and should consider the functionality of the UB.

November 13, 0945-1000 [62]

Observations on the spawning behavior, egg masses and hatchlings of the Japanese flying squid *Todarodes pacificus* in a mesocosm tank

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The spawning behavior of ommastrephid squids has never been observed under natural conditions. Previous laboratory observations of Japanese flying squid (*Todarodes pacificus*) suggest that pre-spawning females might rest on the continental shelf or slope before they ascend above the pycnocline to spawn, and that the egg masses might settle in the pycnocline. Here, two mesocosm experiments were conducted in 6-m-deep, 300 m³ tank to investigate this hypothesis. In the first experiment, a 2.5–3.5 m thermocline was created in the tank (22°C above and 17°C below the thermocline). In the second experiment, the temperature was uniform (22°C) at all depths. Prior to spawning, females did not rest on the tank floor. In the stratified water column, egg masses remained suspended in the thermocline, but in unstratified water column, they settled on the tank bottom, collapsed, and were infested by microbes resulting in abnormal or nonviable embryos. Eleven females spawned a total of eighteen egg masses (17- to 80-cm diameter), indicating that females can spawn more than once. Paralarvae hatched at stage 30/31, and survived for up to 10 days, allowing us to observe the most advanced stage of paralarvae in captivity. Paralarvae survived after consumption of the inner yolk, suggesting they might have fed in the tank.

November 13, 1000-1015 [63]

Exploring methods to directly age cuttlefish via statolith increments

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Cephalopods are economically valuable in fisheries worldwide; however, biological information essential for fisheries management remains poorly known for many species. Age-based population parameters are important for stock assessments and management of cephalopod fisheries. Statoliths have been a focus of research in the past few decades with the daily deposition of the growth increments validated for some species but not others. Statoliths have proven to be useful for squid populations, yet the growth increments remain unreadable in octopuses and most cuttlefishes, with increments observed in only two cuttlefish species. This study investigated the use of statoliths as an aging tool for three small cuttlefish species, *Sepia opipara*, *S. plangon* and *S. rozeella*, in subtropical waters off northern New South Wales, Australia. Several techniques were trialled to view the growth increments within the statoliths, which included thin sections viewed with light microscopy, staining methods and Scanning Electron Microscopy. The relative success of these methods including possible future research directions will be discussed. This research will help inform future aging studies and further develop direct aging techniques for cuttlefish populations worldwide.

November 13, 1015-1030 [64]

Hatching sites and migratory routes of the swordtip squid *Uroteuthis edulis* inferred from statolith analysis

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Despite the commercial importance of swordtip squid *Uroteuthis edulis* in coastal areas of the southwestern Japan, data on its hatching sites and migratory routes are lacking. To infer this information, we first revealed a significant negative correlation between ambient water temperatures and strontium:calcium (Sr:Ca) ratios in statoliths through tank experiments. Next, we measured the Sr:Ca ratios from the nucleus to the edge of each statolith of the squid in spring, summer and autumn migratory groups, before counting statolith microincrements to elucidate their hatching dates, and then found ontogenetic variation of Sr:Ca ratios unique to each seasonal group, which is characterized by shape and maturity of individuals. In the results, it was inferred, using a climate modeling and downscaling subsystem around Japan, that hatching sites of all three groups would be located in the southern East China Sea. Although the migratory route of the spring group would be almost same as that of the summer group because of constant northeastward currents in the East China Sea, individuals in the spring group would be affected by cold waters in the northern East China Sea in winter and spring, which could make the male's body grow longer before reaching Japan. On the other hand, individuals in the autumn group would grow in the southern Sea of Japan during summer after moving from the East China Sea with the currents. They would stay in water depth zones whose water temperatures are 20-22°C until the vertical mixing of thermoclines in autumn, remaining immature.

November 13, 1100-1115 [65]

Morphological characteristics of paralarvae of cephalopods found in Thai waters

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Paralarvae of nine species of cephalopods, such as the bobtail squid (*Euprymna hyllebergi*), sharp-tail pygmy squid (*Idiosepius pygmaeus*), bigfin reef squid (*Sepioteuthis lessoniana*), needle cuttlefish (*Sepia aculeata*), spineless cuttlefish (*Sepiella inermis*), Pharaoh cuttlefish (*Sepia pharaonis*), marble octopus (*Amphioctopus aegina*), lesser blue-ringed octopus (*Hapalochaena maculosa*), and muddy argonaut (*Argonauta hians*) were collected from broodstock that had been cultured in the laboratories of the Department of Marine Science, Faculty of Fisheries of Kasetsart University in Bangkok, Thailand. The external morphological characteristics of the paralarva of such species were observed and recorded, such as the number and pattern of chromatophores, weight, length, and length index of the mantle. The compiled information would be useful reference for the identification of cephalopods, especially at species level.

November 13, 1115-1130 [66]

A preliminary study on life history of Opisthoteuthid octopuses in Suruga Bay, Japan

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Opisthoteuthid octopuses, men-dako in Japanese, are by far the most popular and well known deep sea invertebrate in Japan, an icon of the abyss among the pop culture scene. Public aquariums throughout the country are trying to exhibit them alive. It is an ideal organism to attract attention of the mass toward the abyss and marine environment. Despite their popularity, little has been studied on the biology of Opisthoteuthids in Japan. We have collected Opisthoteuthids from Suruga Bay and Hokkaido, and what new findings came out of our study is presented here.

Opisthoteuthids were collected among the catch of commercial deep sea bottom trawler operating in Suruga Bay. Additional specimens were obtained from Rausu, Hokkaido. Opisthoteuthids were bagged individually and frozen. Measurements were made immediately after thawing. Weights of whole body, viscera and gonads were measured. As a criteria for body size, projected area of each individual were measured as well as length of each tentacle. Tissue samples were taken for DNA analysis.

Three species of Opisthoteuthids were caught. *Opisthoteuthis depressa*, and *O. japonica* from Suruga Bay and *O. californiana* from Rausu, Hokkaido. *O. depressa* was the dominant species in Suruga Bay. All female *O. depressa* weighing more than 100 g were mature with egg in the oviduct throughout the year, indicating year round reproduction. Results of DNA analysis identified small immature specimens as *O. depressa*, indicating year round settlement of the species in Suruga Bay. All *O. californiana* caught were males.

Session: Climate change

November 13, 1130-1145 [67]

Ocean warming, altered food webs and the global proliferation of cephalopods?

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Depletion of commercial fish stocks and climate change have caused significant change to the world's oceans in recent decades. Owing to their unique set of biological traits, it has been speculated that cephalopods may respond positively to these changing oceanic conditions relative to other marine taxa. We assembled a global time-series dataset of cephalopod abundance (catch data standardised to fishing or sampling effort) to ascertain whether cephalopod populations have increased. We obtained sixty-seven time-series, from both fisheries-dependent (54%) and fisheries-independent (46%) sources, which ranged from 10 to 61 years in length and spanned the time period from 1953 to 2013. All major oceanic regions were represented (69% northern hemisphere, 31% southern hemisphere), along with key taxa (52% squid, 31% octopus, 17% cuttlefish and sepiolids). We used generalised additive mixed models (GAMMs) to investigate temporal trends in cephalopod abundance by life history group (demersal, 'bipartite' and pelagic), oceanic region and data type. Our results indicate that cephalopod populations have increased over the last six decades, a trend that was remarkably consistent across life history groups and regions. We suggest that global-scale, directional processes, common to a range of coastal and oceanic environments, have facilitated this increase; specifically, ocean warming and the gradual reduction in fish biomass from marine systems. We quantitatively assessed the former mechanism and show that cephalopod abundance trends can be explained, in part, by concurrent temperature increase.

November 13, 1145-1200 [68]

Recent cephalopod fauna of the western part of the Arctic under the pressure of ongoing climate changes

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Under the pressure of recent climate changes, especially due to average temperature increase in the Atlantic water masses, which move to the Arctic, structural changes in the Arctic ecosystems were observed (Walther et al., 2002; Boitsov et al., 2012). Fauna of cephalopods there also have been changing since 2004 and until now. The range expansion in the eastern direction has been established for *Gonatus fabricii*, the only squid species constantly inhabiting the Arctic. The appearance of boreal-subtropical species, which normally don't inhabit the Arctic, has been observed. *Todaropsis eblanae* has been sampled almost annually since 2006. The new northernmost points of distribution were recorded for *Teuthowenia megalops* (2009) and *Sepietta oweniana* (2013). *Todarodes sagittatus* was recorded in the Arctic twice (2010, 2014) since 1983. It's well-known, that after foraging in the Arctic this squid migrate back to the Northern Atlantic. Thus, the expansion of boreo-subtropical cephalopods into the Arctic during the last decade can be divided into two groups: (1) a range expansion due to the recent Arctic warming; (2) a foraging migration happening relatively regularly (Sabirov et al., 2012; Golikov et al., 2013, 2014).

November 13, 1200-1215 [69]

Swimming behavior of paralarval squid, *Doryteuthis pealeii*, raised under ocean acidification recorded with a novel 3D tracking system

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Ocean acidification (OA) has emerged as a major ecological concern with oceanic pH decreasing at geologically unparalleled rates. Previous work has demonstrated impacts of OA exposure during embryonic development on the aragonitic statolith (the balance and orientation organ) of paralarval squid, *Doryteuthis pealeii*. This study sought to examine the potential effects of this OA-induced impairment on hatchling swimming behavior by developing a novel, simple, and affordable method for 3D tracking and analysis of larval movement. Squid eggs were raised in CO₂ concentrations ranging from 400 to 2200 ppm (eight CO₂ levels in the 2D trials; four in the 3D). 2D trials in 2013 showed paralarvae in higher CO₂ levels spent less time at the surface, while 3D trials in 2014 demonstrated no effect of CO₂ on swimming depth. In 3D trials, multiple swimming metrics, including average and peak velocities, volume transited, and average turning angle, displayed decreasing trends as CO₂ levels increased suggesting some impairments to swimming ability. These differences were not significant, likely due to the pronounced natural variability in paralarval response within trials conducted across the study's seasonal timeframe. OA impacts to both statoliths and mantle length demonstrated notable cohort and interannual variability, which may account for the variable responses in swimming behavior seen across experiments. Effects of OA on squid paralarval swimming require more data and analysis to be fully understood, but the newly developed 3D tracking system provides a powerful and accessible method for future studies.

November 13, 1215-1230 [70]

Combined effects of ocean acidification and warming in the early ontogeny of the common octopus (*Octopus vulgaris*)

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In the present study we investigated a comprehensive set of biological responses to ocean warming (+ 3°C) and acidification ($\Delta\text{pH}0.4$) during the early ontogeny (embryogenesis) of the common octopus *Octopus vulgaris*. Ocean acidification and warming led to a significant drop in the survival rates of octopus embryos ($P<0.05$). The embryonic period was also shortened by increasing temperature in both pH treatments ($P<0.05$). Embryo growth rates increased significantly with temperature under present-day scenarios, but there was a significant trend reversal under future summer warming conditions ($P<0.05$). Besides pronounced premature hatching, a higher percentage of abnormalities was found in summer embryos exposed to future warming and lower pH ($P<0.05$). Under the hypercapnic scenario, oxygen consumption rates decreased significantly in late embryos and newly hatched paralarvae ($P<0.05$). Upper thermal tolerance limits were positively influenced by acclimation temperature, and such thresholds were significantly higher in late embryos than in hatchlings under present-day conditions ($P<0.05$). In contrast, the upper thermal tolerance limits under hypercapnia were higher in hatchlings than in embryos. Here we show that the stressful abiotic conditions inside the embryo's capsules will be exacerbated under near-future ocean acidification and summer warming scenarios and is expected to negatively affect the survival success of octopus early life stages.

November 13, 1400-1415 [71]

The South African ‘chokka’ squid (*Loligo reynaudi*): which environmental variables are important under a changing climate?

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Squid are extremely fast growing with a rapid population turnover and physiologically able to respond to environmental changes, with temperature identified as the key driver. This is not necessarily the case for *Loligo reynaudi*. Effects of environmental (temperature, dissolved oxygen, turbidity, depth), time (time of day, season), location (longitude, region) and stock (adult and juvenile) were tested against the distribution and abundance of adult and juvenile squid *Loligo reynaudi* on the Agulhas Bank, southern Africa. Generalized additive models (GAMs) were used to test the effect of these covariates on data collected from routine research trawl surveys. Results showed mean *Loligo* catches were highest in autumn inshore and lowest in autumn offshore. For all years, depth, total trawl catch, and most importantly, turbidity were significant covariates affecting adult and juvenile squid catches. Region was important but explained very little of the variation, while location in terms of oceanographic province was important for all squid and adults but not juveniles. Temperature was a significant covariate for adult squid but not juveniles and oxygen was a significant covariate for juveniles but not adult squid. The final model showed that *Loligo* catches were highest between depths of 60 to 120m, with bottom turbidity of <2.0NTUs ($0.035\text{ml}^{-1} \text{PMC}_{\text{turb}}$) in locations between 20 and 23°E in the western to central Agulhas Bank when total trawl catches did not exceed 1 metric ton. The usefulness and implications of these results in better managing this important resource and fishery, under a changing climate, are discussed.

November 13, 1415-1430 [72]

Essential embryo habitat of the market squid, *Doryteuthis opalescens*, including the first direct and continuous measurements of pH and O₂

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We provide data to define essential embryo habitat for the market squid, *Doryteuthis opalescens*, and, for the first time, report baseline environmental data within embryo habitat. Previously, environmental conditions had to be inferred from short-term temperature measurements. Methods combined video surveys taken at La Jolla and Monterey, USA using SCUBA (N=139), tow camera (N=11), remotely-operated vehicles (N=18) and use of remote sensors (tidbit temperature loggers (N=8), SeaBird CTDs (N=15), and a sea pH_{Ox} = SeaBird CTD, durafet pH sensor and Aanderaa O₂ optode (N=4)). Essential embryo habitat was defined by observed depth (10-100 m depth), embryo density (0.1-350.1 capsules•m⁻²), area (0.15-7.32•10⁶ m²), by repeated use, and by association with high [O₂] and pH (and low pCO₂). Embryo habitats include several bedform types but commonly occurred on sand and submarine canyon walls. ROV observations of embryos recorded [O₂] from 70-280 μM, pH from 7.65-8.10, T from 9.8-18.1 °C, and S from 33.3-33.9 PSU. Evidence is consistent with spawning adults selecting sites that are exposed to relatively higher [O₂] and pH with relatively fewer predators (e.g. sandy bedforms). Laboratory experiments showed that some embryos exposed to seawater with low [O₂] and pH could develop but most developed poorly. To some extent, *D. opalescens* can mitigate harsh environmental conditions through spawning site selection and, by a lesser extent, through embryogenesis.

November 13, 1430-1445 [73]

A hypothetical model of the effect of changing sea temperature on the migration of the Japanese flying squid *Todarodes pacificus*

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The Japanese flying squid, *Todarodes pacificus*, is the most commercially important cephalopod species in Japan. It migrates broadly, and changes in the migration pattern greatly affect local fishing conditions. In Hokkaido, since 2001, annual catches have decreased in the Sea of Japan and in the Pacific off South Hokkaido, and increased in the Pacific off East Hokkaido and in the Sea of Okhotsk. Since 2010, the increase in the Sea of Okhotsk and decrease in the Pacific off South Hokkaido have been drastic. Annual research in the northern Sea of Japan in late May has shown that both density and mantle lengths have decreased. The hatching season of individuals from the northernmost sampling station might have been delayed. To define changes of the spawning migration season in autumn to winter, a mean catch date (MCD) was determined based on squid-jigging catch data from Hakodate (Pacific Ocean) and Okushiri Island (Sea of Japan). The MCD has delayed gradually since 2001 in Hakodate and delayed rapidly since 2010 at Okushiri. Since 2010, sea surface temperatures around Hokkaido have been below average during January-June and remarkably high during July-December. A hypothetical model shows that high temperatures in summer and autumn cause the feeding migration route to extend northward, causing an increased influx into the Sea of Okhotsk and decreased catches in Southern Hokkaido. Further, high temperatures in autumn delay the start of the southward spawning migration, the hatching season, and the beginning of the feeding migration of the next year class.

Session: Fisheries, stock assessment and management

November 13, 1445-1500 [74]

Distribution and abundance of cephalopods in UK waters: long-term trends and environmental relationships

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As part of a project which aimed to evaluate the feasibility of developing indicators of marine ecosystem status based on cephalopods, we analysed spatiotemporal variation in abundance, and environmental relationships, using trawl survey catch data for cephalopods in UK waters (1980-2013) from Cefas and Marine Scotland Science databases. These data presented some challenges, notably the use of several different trawl gears, variable tow durations, and varying levels of taxonomic resolution. Accounting for gear type and tow duration, data were analysed separately for each cephalopod family and season to account for different phases of the life cycles being present at different times of year. The families investigated were Loliginidae, Octopodidae, Ommastrephidae, Sepiidae and Sepiolidae.

A GAM framework was used to summarise spatiotemporal variation in abundance at family level and the relationships of spatial and long-term temporal variation with environmental variables, including depth, substrate (available for inshore waters) and several oceanographic variables (e.g., SST, chl signals), also considering fishing pressure. Long-term trends for each family varied between areas and seasons, although this may reflect the presence of several species within families. In Scotland, where *Loligo vulgaris* is rare and *L. forbesii* is normally distinguished from *Alloteuthis spp.*, survey data suggested a peak in abundance of this species around 1990 and a generally increasing trend since the mid-1990s. Spatial patterns in distribution in all families were related to both physiographic and oceanographic features. As expected substrate type had most effect on those families in which eggs are attached to objects on the seabed.

November 13, 1500-1515 [75]

English Channel Loliginid squid stocks: surplus production models used to estimate population biomass and stock status in a context of variable environment

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Squid resources in European Atlantic Waters are dominated by Common Squids : *Loligo forbesii* and *Loligo vulgaris*. In the English Channel where both species occur and are exploited by French and English fishers these stocks are the highest in value for demersal trawlers although they are still unmanaged. Under the EU Marine Strategy Framework Directive (MSFD) a list of descriptors have been defined in order for European Marine Ecosystems to reach (or be maintained at) a Good Environmental Status (GES). In the case of commercially exploited fish or shellfish populations should be within safe biological limits. However, the definition of a healthy stock is still a challenge in English Channel squids. Several stock assessment methods were tried in previous exercises (depletion methods, monthly bases Virtual Population Analysis) but because of data availability they could not be updated annually. These exercises have underlined the high recruitment variability and the subsequent rapid changes in exploitation rates.

The present work makes use of surplus production models to estimate cohort biomass and to derive reference points and exploitation diagnostics for these populations. Abundance indices are derived via delta-GLM methods from France Otter Bottom Trawl LPUE. Abundance is combined with France and UK catches to fit non-equilibrium production model based on the Schaefer (logistic) population growth model. Squid recruitment is known to be influenced by environmental variations therefore, a modification of the model was introduced (using climatic variables in the pre-recruitment period). Results about stock status suggest that management measures should not rely only on long term averages but should rather take into account short term environmental variation. Model outputs include biomass estimates which are useful beyond fishery management and could help to better understand the role of Loliginid squids in the English Channel food web and ecosystem.

November 13, 1515-1530 [76]

Ecological risk assessment methods relevant to cephalopods taken as byproduct in multi-species fisheries in New South Wales, Australia

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Faced with insufficient data to meet the needs of ecosystem-based fisheries management, agencies are increasingly relying on ecological risk assessment methods to assess and rank ecological components or species with respect to their likelihood of becoming unsustainable or overfished in response management strategies. Two factors are used to assess this risk: (1) the fishing pressure exerted on each component or species by the fishery and (2) the capacity of a component or species to respond to this fishing pressure. The latter typically includes life history attributes that are known to influence their capacity derived from ecological literature, such as longevity, age-at-maturity, growth rates, fecundity and maximum size. Given their typically short lifespans and high growth rates, the criteria to assess these attributes are often inherently biased against cephalopods and other short-lived invertebrates, especially when compared against much longer-lived teleosts and elasmobranchs in multi-species fisheries. This result contributes to a general perception among stakeholders that cephalopods are at little risk of overfishing because of their high productivity. In this paper, we revise standard ecological risk assessment methods to incorporate other criteria relevant to cephalopods, such as recruitment variability, age structure and reproductive strategy, to better reflect their potential risk of becoming overfished or unsustainable when taken as byproduct in multi-species fisheries. The method will be demonstrated using examples from New South Wales commercial fisheries.

November 13, 1530-1545 [77]

Environmental effects on squid availability to a seasonal inshore fishery: Untangling drivers, mechanisms and scales.

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A seasonal fishery for longfin inshore squid (*Doryteuthis pealeii*) occurs in the shallow waters of Nantucket and Vineyard Sounds (northeast USA). Inter-annual variability in landings presents challenges for fishers and managers due to the ‘boom-or-bust’ nature of the fishery. Data from an inshore spring trawl survey (1978-2012) were analyzed to test the relative influence of environmental variables on variability of biomass indices at multiple scales. To assess linkages between stock-wide distributional shifts and inshore abundance, the annual mean along- and cross-shelf location of the *D. pealeii* population was estimated from offshore spring trawl surveys conducted on the continental shelf. Annual inshore biomass indices and mean locations on the continental shelf were tested for temporal patterns and relationships with indices related to ocean temperatures and circulation patterns, including the North Atlantic Oscillation (NAO), Atlantic Multidecadal Oscillation (AMO), and the Gulf Stream North Wall Index (GSNWI). At a fine scale, seawater temperature was most correlated with squid catch at each station relative to date or year, while survey date and year were most correlated with annual mean biomass indices. At a larger spatial scale, NAO, GSNWI and cross-shelf center of population abundance were most correlated with inshore biomass indices. Multivariate analyses indicated that the relationships among variables changed during the time series. A multi-scale approach is required to model the relative effects of local environmental variables and ocean basin-scale processes on measures of squid abundance, distribution, and availability to surveys and fisheries.

November 13, 1545-1600 [78]

Operational prediction of the habitat suitability index (HSI) distribution for neon flying squid in central North Pacific by 4D-VAR ocean data assimilation system “SKUIDS”

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The neon flying squid (*Ommastrephes bartramii*) has a wide-spread distribution in subtropical and temperate waters in the North Pacific, which plays an important role in the pelagic ecosystem and is one of the major targets in Japanese squid fisheries. We have developed a new 4D-VAR (4-dimensional variational) ocean data assimilation system for predicting ocean environmental changes in the central North Pacific (35-45N, around the date line), the main fishing grounds for Japanese commercial vessels in summer. We call this system “SKUIDS” (Scalable Kit of Under-sea Information Delivery System). SKUIDS can provide realistic prediction fields of 3-dimensional ocean circulation and environmental structures including meso-scale eddies in the potential fishing area of neon flying squid. By using these prediction fields of temperature, salinity, sea surface height, horizontal current velocity, etc., we produced daily habitat suitability index (HSI) maps of the neon flying squid, and provided them to the Japanese commercial vessels in operation. Squid fishermen can access the web site for delivering the information of ocean environments in the fishing ground by using Inmarsat satellite communication on board, and show the latest fields of satellite-derived sea surface temperature, chlorophyll-a concentration, sea surface height, eddy kinetic energy, and predicted fields of subsurface temperatures and HSI. The real-time operation was started from May, 2015. In this study, we present the details of SKUIDS and the web-delivery system for squid fishery, and some preliminary results of the operational prediction in this summer.

November 13, 1630-1645 [79]

Relating purple back flying squid (*Sthenoteuthis oualaniensis*) abundance to environmental parameters using GIS and GAM in South eastern Arabian Sea

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Purple- back flying squid (*Sthenoteuthis oualaniensis*) is a short-lived, fast growing ommastriphide cephalopod found in high abundance in the Arabian Sea. The present work aims to study the distribution pattern of Purple-back flying squid and the relationship between abundance and environmental variables in the Southeastern Arabian Sea were analyzed using geographical information system (GIS) propped statistical modelling (GAM-Generalized Additive Models) predictions were made and oceanic squid abundance in relation to physical and environmental conditions. The GIS maps showed that highest abundance were observed mainly with in 14° N, 72°E. Temperature was the most important variable, which showed strong positive association with *S. oualaniensis* abundance with optimum temperature between 28.03 to 28.80 °C. Salinity (32.70 to 35.08 ppt), Dissolved Oxygen (6.02 to 6.27 mg/ litre) and pH (7.75 to 8.30) also showed positive association with abundance. The *S. oualaniensis* abundance was negatively related to Chlorophyll, Zooplankton and Mixed layer depth values. GAM analysis indicated that environmental conditions influence the abundance and also provided a predictive model. The GAM model demonstrates that the relationship between latitude and abundance is highly significant, even though longitude and pH were also significantly related. Model selections were carried out based on the AIC criteria. The model (**Abundance ~ s (Lat) + s (Lon) + s (SST) + s (pH) + s (Sal) + s (Chl) + s (DO) + s (Z.plankton) + s (MLD) + s (D20)**) explained almost 85 % of the variance in purple- back flying squid abundance.

November 13, 1645-1700 [80]

Morphology and life history strategies of *Illex argentinus* in the north extreme of species distribution

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The present study aimed to differentiate local populations of *Illex argentinus* from the winter migratory group in Brazilian waters based on geometric morphometrics. Ontogenetic morphological variations were found for both sexes. In spite of the migratory group was composed of larger individuals (with centroid size larger than 0.4), large animals of both groups had similar morphology. These results suggested that the change in shape was related to an adaptation to the environment. On the other hand, the comparison of the reproductive attributes of adult squids, removing the size effect, showed different relationships for total and gonad weight and especially for gutted body weight in advanced maturity stages for males and females of these groups. Residuals of gutted body weight demonstrate that muscular thickness of the mantle of migratory group was thinner. Additionally, the reconstruction of size distributions for 60 days before the capture using daily growth increments deposited in gladius of squids captured in a scientific cruise with short hauls (~30min) allowed the identification of two patterns: (i) growth depensation, in which large individuals of a population tend to get even larger, compared to smaller individuals. This process is enhanced by intra-specific competition when the amount of available food is scarce and non-concentrated; and (ii) the migratory group was negatively skewed distributed, probably induced by the selective removal of smaller squid by cannibalism, which associated to the consumption of mantle would provide the energy needed to fuel the migration into less productive oligotrophic waters.

November 13, 1700-1715 [81]

Habitat selection by *Octopus vulgaris* in the Cíes Islands archipelago (NW Spain)

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The selection of spawning habitat of a population of *Octopus vulgaris* that is subject to a small-scale exploitation was studied in the Cíes Islands within the National Park of the Atlantic Islands of Galicia (NW Spain). The technique used was visual censuses by scuba diving. We conducted 93 visual censuses from April 2012 to April 2014. The total swept area was 123.69 ha. Habitat features (season, depth, zone, bottom temperature, swept area, bottom substrate type, and creels fishing impact) were evaluated as predictors of the presence/absence of spawning dens using GAM models. *O. vulgaris* has a noteworthy preference for spawning in areas with hard bottom substrate and moderate depth (approximately 20 m). The higher density of spawning dens (1.08 ha⁻¹) was found in a surveyed area of 50.14 ha located in the northeastern part of the northern Cíes Island. We propose to protect the area comprised from Punta Escodelo to Punta Ferreiro between 5 and 30 m depth. This area has a surface of 158 ha equivalent to 5.98% of the total marine area of the Cíes islands. The strengths and weaknesses of a management strategy based on the protection of the species' spawning habitat are discussed. Moreover, censuses targeting *Octopus vulgaris* living in dens on sandy bottoms showed that there were no significant differences between octopus presence in dens during open and closed fishing seasons. Depth had a significant negative relationship with occupancy. The average number of dens per 1000 m² was 3.84±0.84 in June and 3.89 in October. The area per den was 260 m². Den number estimations varied between 1586 and 2057. The largest number of dens (76.5%) was found between 5 and 10 m depth. Den distribution was clumped. No significant differences were found between octopus size classes (small, medium and large) and den diameter. Associate dens were observed. There were no significant differences in den diameter and shell types found around the middens. Many dens could be "permanent". The surveyed area had around 1100 individuals, mainly small specimens. No significant differences were found between octopus size and depth. Substrate, den type and food abundance and availability (especially razors *Ensis arcuatus*) seem to be the main factors influencing dens and octopus density and distribution. Den availability does not appear to be a limiting factor in this case. Temperature, den availability, predators and fishing pressure influencing density and distribution are discussed. Rodas inlet may be a preferential habitat for *O. vulgaris* individuals ranging from 200 to 2000 g, but especially small specimens (≤1000 g).

November 13, 1715-1730 [82]

Acoustical quantitative estimation of the Japanese common squid *Todarodes pacificus* distribution in a jigging fishery using LED panels and metal-halide lamps

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The Japanese squid jigging fishery usually uses metal-halide (MH) lamps as fishing lights. However, the use of light emitting diode (LED) panels as new fishing lights is on a rise in order to save energy and decrease costs and carbon dioxide emissions by this fishery. Here, we investigated differences in the performance of these two light sources by acoustical density measurements using a quantitative echo sounder. Surveys in the Sea of Japan were conducted during autumn in 2014. We used two squid jigging vessels that were equipped with both LED panels and MH lamps. Two combinations of light intensity and spread of the different light sources between two vessels were made almost the same so as to operate under similar lighting conditions. Both light sources on each vessel were alternately used on a daily basis in an overnight operation. Acoustic measurements were conducted in both vessels using SIMRAD EK60 split-beam echo sounders during fishing operations. Acoustic data were collected for 10 min every hour during overnight operations when jigging machines had stopped so as to prevent any potential influence on acoustic scattering from fishing lines and squid lures. Maximum and average acoustic densities for each operation ranged from 0 to 0.10 squid/m³ and from 0 to 0.05 squid/m³, respectively. The results were not significantly different between LED panels and MH lamps, which indicate that the performance of LED panels as source of fishing lights was similar to MH lamps.

November 10, 1730-1745 [83]

A hypothesis of fishing lights function in capture process of squid jigging fisheries targeting Japanese flying squid *Todarodes pacificus*

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In squid jigging fisheries targeting Japanese flying squid *Todarodes pacificus*, many strong metal halide lamps requiring large amounts of electric power are used as fishing lights. Operation methods of fishing lights have been developed over a long time by trial and error. Therefore most of the capture processes remain unknown, and this lack of comprehension is an obstacle for implementing technologies for improving the energy efficiency of this fishery.

In this study, we discuss the capture process during squid jigging by experiments on the behavior of squid to light, and by observations of fishing lights using underwater camera.

Experimental results indicated that squid respond to the light direction rather than the light intensity. Squid oriented to light from a horizontal direction, and moved toward the light source and remained in the environment where light was coming from above them.

Underwater observations showed that far from the boat, the light from metal halide lamps reached the camera from a horizontal direction, while nearby the boat, the light reached the camera almost vertically.

From these results, we consider that the fishing lights from squid jigging boats have two functions. The first is to lead squid toward the boat from a wide area, and the second is to retain the squid under the boat. When converting fishing lights to an energy saving light source like LEDs, the arrangement of lamps should be considered so as not to impair the function of conventional fishing lights.

November 13, 1745-1800 [84]

Stress marks of two fishing methods in octopus beaks

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The day of capture from the wild is an event that is coded as a mark in cephalopod beaks, as recently shown in Perales-Raya *et al.* (2014) for the common octopus (*Octopus vulgaris*). In order to compare whether different fishing methods may induce a stress mark in the animals, here we examined beaks from 65 octopuses caught in the Bay of Naples (Mediterranean Sea, Italy) by two different methods, jigging and traps. After capture, the animals were maintained in the laboratory under controlled conditions during different periods of time (2 to 55 days) until death. The age of the octopuses was estimated by counting daily rings in the lateral wall surface of upper beaks. They ranged between 132 and 255 days old (jigging group: 172 - 561g Body Weight; N = 33) and between 153 and 396 days old (trap group: 180g - 660g BW; N = 32). Microstructure of beak sections was analyzed in all the individuals and the stress mark after capture compared for both groups. We found a high degree of coincidence between the day of capture and a stress mark in the beak, although 6 octopuses showed no stress mark the day of capture, all of them caught by traps. The mean differences between elapsed days and number of rings after capture mark are ± 0.9 days for jigging and ± 1.5 days for traps, which seems to indicate a higher coincidence between day of capture and 'stress mark' in octopuses caught by jigging.

November 13, 1800-1815 [85]

**Can those easy killed octopus die easy in Portuguese and Italian fisheries?
- Ways to die and numbers killed in small scale fisheries in both countries**

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In the North Atlantic Ocean, Portugal is the country with the largest *Octopus vulgaris* fishery, while in the Mediterranean, Italy has a significant stake in octopod fisheries including both *Eledone cirrhosa* and *Octopus vulgaris*. Both countries rely heavily on small scale fisheries when extracting *Octopus vulgaris* from the wild for human consumption. Unlike large commercial vessels, small scale fisheries provide a “personalized predator-prey relationship” between human predator and octopod prey, in such a way that killing is largely an individual matter. Hunting of underwater prey which are out of sight of the human predator remains impersonal in small scale fisheries, conducted against a mass of unspecified composition and dimensions, much like industrial fisheries. Killing, on the other hand, is done one to one, face to face, and the choice of weapons matters to both. Here we describe killing methods and practice in both countries, provide a short history of extraction and killing methods employed, compare the putative consequences of different methodologies under the existing knowledge on pain, suffering and distress, as provide advice on the preferred methods. We also provide estimates of the impact of small scale fisheries in octopod killing in both countries by estimating numbers of animals killed from landings and known size structure.

November 13, 1815-1830 [86]

Re-evaluating the ecological status of squid paralarvae by molecular tools in a seasonal upwelling system

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Squids represent an important economic resource in NW Spain (Galicia) and studies of squid paralarvae and their response to oceanographic and climatic variation are scarce in comparison with other invertebrate larvae and fishes in this region. Consequently, in this study we investigate the evolution of Loliginids paralarvae in Galician coastal waters from 2000 to date. Paralarvae collected until 2012 were identified using morphometric and meristic parameters and reference collection of cephalopod paralarvae. This task is difficult and unreliable due to paralarvae alteration during trawl and conservation that could hinder their identification to species level. Based on their similarity and because the main fishery in Galicia is represented by *Loligo vulgaris*, the paralarvae were identified as this species. In 2012 the introduction of molecular tools to identify paralarvae, revealed that a high percentage of the squid paralarvae studied belong not only to the main fished squid species, *Loligo vulgaris*, but also other Loliginids species such as *Alloteuthis subulata* and *Alloteuthis media*. It has to be taken into account that to that date, all paralarvae studies in this area had been considered as *Loligo vulgaris*, which clearly bias accurate ecological studies and the subsequent management measurements. These results have changed the ecological scenario, and suggest that previous assumptions about Loliginids abundance evolution during the last fifteen years must be reviewed.

Session: Systematics and Biogeography

November 14, 0830-0845 [87]

Taxonomists' work is never done: Updates in oegopsid systematics and zoogeography from New Zealand

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New Zealand possesses the fourth largest Exclusive Economic Zone (EEZ) in the world, and appears to be a relative hotspot of cephalopod biodiversity, with 107 species presently reported, from 71 genera, in 35 families. The AUT Lab for Cephalopod Ecology and Systematics (ALCES) is a research group whose outputs have largely aimed to improve understanding of New Zealand's local teuthofauna (primarily of deep-sea squids), extending out to global revisions of certain groups where possible, and addressing issues commonly faced by cephalopod taxonomists. During these projects, it has become apparent that most thorough reviews of locally occurring cephalopod groups substantially revise our understanding of the true local diversity, often resulting in the description of new species and/or the recognition of new distribution records for NZ waters. In particular, undescribed taxa appear to exist in the Onychoteuthidae, Brachioteuthidae, and lepidoteuthid families; recent collection efforts have also revealed the presence of more than a dozen species (known to science but previously unreported in NZ waters) from eight families. Additionally, recognising the importance of practicing integrative taxonomy, in the course of these projects we are also building a substantial library of cephalopod tissue samples to support this work, and to better enable comparison of the NZ fauna with worldwide material.

November 14, 0845-0900 [88]

Early mode of life and hatchling size in cephalopods: influence on the species distributional ranges

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Cephalopods have direct development and display two major developmental modes: planktonic or benthic. Planktonic hatchlings are small and go through some degree of morphological changes during the planktonic period, which can last from days to months, with ocean currents enhancing their dispersal capacity. Benthic hatchlings are large, miniature-like adults and comparatively have reduced dispersal potential. We examined the relationship between early developmental mode, hatchling size and species latitudinal distribution range of 105 species hatched in the laboratory, which represent 12% of the total number of live cephalopod species described to date. Results showed that species with planktonic hatchlings reach broader distributional ranges in comparison with species having benthic hatchlings. In addition, squids and octopods follow an inverse relationship between hatchling size and species latitudinal distribution. In both groups, species with smaller hatchlings have broader latitudinal distribution ranges, thus, squid and octopod species with larger hatchlings have latitudinal distributions of comparatively minor extension. This pattern also have emerged when all species are grouped by genus (n=40), but was not detected for Sepiids, a group composed mainly by species with large and benthic hatchlings. Hatchling size and associated developmental mode and dispersal potential seem to be one of the main influential factors to determine the distributional range of cephalopod species.

November 14, 0900-0915 [89]

Biogeography of the Sepiolinae (Cephalopoda: Sepiolidae)

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The Sepiolinae, a subfamily of the Sepiolidae, presently comprises five genera, namely *Euprymna*, *Iniotheuthis*, *Rondeletiola*, *Sepietta* and *Sepiola*, and 32 definite species. The members of these genera are distributed from the eastern Atlantic (including the Mediterranean), through the Indian, to the western Pacific Ocean. No sepioline species was ever found along either coast of the Americas. *Euprymna* includes 11 established species, plus a few others either unresolved or undescribed, occurring throughout the Indo-West Pacific. Both species of *Iniotheuthis* live in the Indo-West Pacific. *Rondeletiola* accounts for an Atlantic-Mediterranean well defined specific entity and a South African one in need of confirmation. *Sepietta*, with three species, is limited to the North Atlantic-Mediterranean area. Lastly, *Sepiola* is the most speciose genus: it includes 15 ascertained, 1 unresolved and 1 questionable species. Moreover, this is the only genus represented in both the Atlantic-Mediterranean (12 species) and the Indo-Pacific (3 species) regions, which fact, in combination with morphologic considerations, contributes to cast doubts upon its monophyly. The species of *Euprymna* and *Sepiola* tend to have restricted distributions, whereas members of *Sepietta* and *Rondeletiola* are widely distributed. This fact depends upon the interaction of the preferred habitat with the modes of life and reproduction. It appears that the primary center of origin of the Sepiolinae is the Indo-Pacific, whereas the Atlantic-Mediterranean represents a secondary center of origin. The ancestors of the two groups seemingly became separated by the vicariant event of the closure of the Tethyan Sea.

November 14, 0915-0930 [90]

Cephalopod fauna of Antarctic waters: combining new information from predators, nets and habitat suitability prediction models

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Cephalopods play an important ecological role in the Southern Ocean, being the main prey group of numerous top predators. However, their basic ecology and biogeography is still poorly known. We assessed the habitats and distributions of Southern Ocean cephalopods, using net-catch data and predators diets to develop habitat suitability models for 15 of the commonest pelagic squid in the Southern Ocean. The individual habitat suitability models were overlaid to generate a “hotspot” index of species richness. The “hotspot” regions in the distribution of cephalopods from the Southern Ocean are related to oceanic waters, across various oceanic fronts. Complementary to this work, we provide new information on Antarctic cephalopods, using Antipodean and Gibson’s albatrosses (*Diomedea antipodensis antipodensis* and *D. antipodensis gibsoni*, respectively) as biological samplers, known to cover huge areas of the Southern Ocean. A total of 9111 cephalopod beaks, from 41 cephalopod taxa, were identified from their diets, with the families Histioteuthidae and Onychoteuthidae, as the most important cephalopods numerically and by reconstructed mass, respectively. Combining both datasets (i.e. habitat suitability precaution models and predator diets), we provide evidence from predators of the circumpolar distribution of numerous key cephalopod species in the Southern Ocean, and provide new information on poorly known cephalopods. Our work emphasize a need for future work to focus on projecting these distributions under scenarios of climate change and their effects on the distributions of Antarctic squid from the Southern Ocean, using habitat suitability prediction models.

November 14, 0930-0945 [91]

A review of pygmy squid, *Idiosepius* (Cephalopoda: Idiosepiidae), with descriptions of a new species, and placement of southern Australian endemic *I. notooides* in a new genus

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A new species of pygmy squid, *Idiosepius*, is recognised from eastern Australia. It differs from *I. notooides* Berry, 1921; and *I. pygmaeus* Steenstrup, 1881 (also found in Australian waters) in a number of traits, including the number of club sucker rows, shape of the funnel-mantle locking cartilage and the modification of the hectocotylus, as well as its molecular ‘signature’ (12S, 16S and COI). In addition, for the first time, gill lamellae count has been shown to be a useful trait in determining species boundaries among idiosepiids. The new species is found in shallow water from Sabina Point, Shoalwater Bay, 22°23′45″S, 150°18′13″E to Coila Lake, 36°02′00″S, 150°07′30″E and it is sympatric with *I. notooides* in the southern part of its range. This species has been misidentified as *I. paradoxus* (Ortmann, 1888), but *I. paradoxus* does not appear to occur in Australian waters, despite some earlier reports. *Idiosepius* sp. nov. is compared with all nominal *Idiosepius* and a current summary of *Idiosepius* systematics is provided. In addition, the southern Australian endemic, *I. notooides*, is elevated to its own new genus based on molecular and morphological characters.

November 14, 0945-1000 [92]

Morphometry and morphological phylogeny of *Sepia pharaonis* Ehrenberg, 1831 complex (Cephalopoda: Sepiida) in Thai Waters

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Three hundred fifty eight specimens of pharaoh cuttlefish, *Sepia pharaonis*, Ehrenberg, 1831 were collected from 30 localities in Thai Waters, Gulf of Thailand (Pacific Ocean) and the Andaman Sea (Indian Ocean). Specimens were grouped according to sex, habitats and 4 categorized types of colour patterns on dorsal mantle. The “type 1” pattern is with large stripes along the outer side of the mantle and large stripes in the middle part of the mantle. The “type 2” pattern is similar to the type 1 along the outer side, but with small stripes in the middle part of mantle. The “type 3” and “type 4” patterns are with small stripes along the outer side, but the “type 3” is with large stripes in the middle part and the “type 4” with small ones. Morphometry of 75 characters from 5 characters sets of external, cuttlebone, digestive system, reproductive system and hectocotylus morphology were compared. The male and female cuttlefish were significantly different in 39 characters. Four types of males and females were significantly different in 29 and 18 characters. In overall, differences of colour patterns were more prominent in males than in females and in the Andaman Sea than in the Gulf of Thailand. Phylogenetic analyses of seven high weighted characters revealed 4 likely clades of populations, corresponding to 4 types of colour patterns on dorsal mantle.

November 14, 1000-1015 [93]

Allopatric variation of *Sepia pharaonis* Ehrenberg, 1831 complex (Cephalopoda: Sepiida) in Two Oceans of Thai Waters Based on Morphology and Mitochondrial DNA Sequences

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Allopatric variation the pharaoh cuttlefish, *Sepia pharaonis* Ehrenberg, 1831, in Thai Waters is hypothesized to be partly initiated by a geographic barrier, the peninsular Thailand, which separates the Pacific Ocean (the Gulf of Thailand) and the Indian Ocean (the Andaman Sea). The variations of the cuttlefish are recognized as different colour patterns on dorsum. Specimens of pharaoh cuttlefish were collected from 30 localities for qualitative and quantitative morphological determination and partial sequencing of mitochondrial DNA. The phylogenetic trees revealed two likely major clades, one in the Andaman Sea and another one in the Gulf of Thailand. The cuttlefish in the Andaman clade are with mixed colour patterns but what in the Gulf of Thailand clades are mostly with one colour pattern. Mixing of colour patterns mostly in the Andaman clades indicate the current occurrence of gene-flow and, on the other hand, suggesting the origin of *Sepia pharaonis* in the Pacific and subsequent dispersion during the Cenozoic into the Indian Ocean.

November 14, 1015-1030 [94]

Cephalopods of the Sargasso Sea: distribution patterns in relation to hydrographic conditions

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Here we report on a comprehensive collection of mainly early life cephalopods that were sampled during two research cruises to the Sargasso Sea with the FRV Walther Herwig III in April 2014, and the RV Maria S Merian in April 2015. In 2014, 714 specimens were collected by a pelagic midwater trawl, and further 1,349 specimens by an Isaac Kidd midwater trawl. A total of 2,487 cephalopods were caught during the cruise in 2015. They belonged to 36 species (20 families). The most abundant family was represented by the flying squids (Ommastrephidae). Identification of cephalopods was supported by DNA barcoding of the COI locus. The subtropical convergence zone (STCZ) was found approximately around 27 °N. This frontal system was characterised by a sharp near-surface temperature gradient and divided the Sargasso Sea into a northern and a southern area. This distinction was also reflected in the cephalopod community composition. For example, the cranchiid *Leachia lemur* prevailed in the northern part, and the cirrate octopod, *Japetella diaphana* was mainly distributed in the southern part of the study area. PCA and RDA analyses detected a significant correlation between species occurrence and sea surface temperature. Ordination analysis (MDS) showed significant differences in the cephalopod assemblages between day and night with midwater forms (Enoploteuthidae, Pyroteuthidae) dominating the night catches, probably due to their upward migration into the top 200m during the night.

November 14, 1100-1115 [95]

New records and undescribed taxa of Triassic ammonoid *Eutomoceras* (Ammonoidea: Ceratitida: Ceratitidae) and belemnoid cephalopods (Coleoidea: Belemnitida: Duvaliidae) in Thailand

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Specimens of ceratitid ammonoids *Eutomoceras* Hyatt, 1877 (Cephalopoda: Ammonoidea: Ceratitida: Ceratitidae) were collected from the Lower Triassic Strata in Changwat Phatthalung, eastern part of the peninsular Thailand. Six taxa of *Eutomoceras* Hyatt, 1877 comprise two species, *Eutomoceras* aff. *laubei* Meek, 1877 and *Eutomoceras dunni* Smith, 1904 and four undescribed ones, *Eutomoceras* sp. A, *Eutomoceras* sp. B, *Eutomoceras* sp. C and *Eutomoceras* sp. D. Two forms of damaged asymmetrical rostrums of duvaliid belemnoids were also collected from the same locality and identified as *Duvalia* sp. indet. I and *Duvalia* sp. indet. II

November 14, 1115-1130 [96]

Shallow water octopuses of the southern Caribbean, Tayrona sector

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Our knowledge on tropical octopuses is lacking. Due to their high diversity and abundance of cryptic species, they are considered to be understudied. A tropical bay, Bahía de Taganga, in the southern Caribbean Tayrona sector, was sampled to see how many octopus species were present. This is an unusual tropical bay since oceanographic conditions cause no coral reefs to be present. The results show that at least ten species from four genera live in the area. Hard bottom preference is clear as seven different species live on it and only three on soft substrate (gravel to mud). All catches were made manually in less than 10 m depth. Trapping or trawling was not suitable for the area because of fishery regulations. All species are small to medium in size with very small octopuses (pygmy octopuses) only reported rather than captured. Two species are dominant and all the rest are scarce among them *Amphioctopus burryi* and *Callistoctopus* sp. More research is needed to understand the composition of this very diverse cephalopod community and compare it to a coral reef community.

November 14, 1130-1145 [97]

What have we learned about the cephalopod fauna of the Gulf of Mexico? Initial results of the NOAA Offshore Nekton Sampling and Analysis Program

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The Deepwater Horizon Oil Spill (DWHOS) necessitated a whole-water-column approach for assessment that included the epipelagic (0-200m), mesopelagic (200-1000m) and bathypelagic (>1000m) biomes. The latter two biomes collectively form the largest integrated habitat in the Gulf of Mexico (GoM). This habitat received the initial oil/methane discharge, plus millions of liters of dispersant, and contained persistent deep (~1100m) plumes of oil, methane, and dispersant, demonstrating that DWHOS had an extensive deep-pelagic component. As part of the Natural Resource Damage Assessment process, the NOAA Offshore Nekton Sampling and Analysis Program (ONSAP), was implemented to evaluate impacts from the spill and enhance the basic knowledge of the biodiversity, abundance, and distribution of deep-pelagic GoM fauna. Here we provide the initial results of a field campaign in 2011 that includes two cruise series with different midwater trawl types (large otter-type and MOC-10) in which the pelagic fauna was sampled from 0-1500 m. During this campaign, over 10,000 cephalopods were collected. Prior to this work, 93 species of cephalopods were known from the GoM. At least 67 cephalopod species were sampled by ONSAP and ongoing analyses will certainly increase this number, as hard-to-identify taxa are resolved. Of these species, 4 are previously unknown in the GoM, including previously undescribed species. Cephalopod vertical distribution patterns in the GOM will be highlighted from the MOC-10 sampling.

November 14, 1145-1200 [98]

Recent advances in the family Cranchiidae from New Zealand and adjacent waters

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Representatives of the squid family Cranchiidae Prosch, 1849, are present in almost all oceans. Despite their global abundance, little is known about the ecological role this family plays in both epipelagic and deep-sea trophic systems and cranchiid systematics remain relatively unstable. The family currently contains 13 accepted genera, and is thought to contain about 60 species; however, this figure remains speculative. Although all currently accepted genera have been preliminarily described, recent research has shown that some cranchiid squid go through several distinct morphological growth stages before reaching maturation, making their identification more complicated. This presentation will provide a brief overview of the genera in this family, including key morphological traits used for identification, and aims to explain some of the recent advances of our knowledge of Cranchiidae in the waters around New Zealand, including new morphological features, changes in distribution, and the identification of potential new species.

November 14, 1200-1215 [99]

The genus *Ommastrephes* d'Orbigny, 1834: a single species or more than one hidden behind a single name?

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The genus *Ommastrephes* d'Orbigny, 1834 in 1834–1847 is currently accepted to be formed only by a widely distributed species: *O. bartramii* (Lesueur, 1821). According to its known antitropical distribution, the species occur in temperate waters of the Atlantic, Pacific and Indian Oceans. Current taxonomic status proposed that *O. bartramii* is formed by three different populations/subspecies distributed in the North Atlantic, Southern Hemisphere and in the North Pacific, respectively. Here, all the available published cytochrome oxidase I information for ommastrephid squids plus new sequences are integrated, reanalyzed and the reliability for the use of DNA barcoding in the whole family is evaluated. Among ommastrephids, intraspecific *p*-distances range from 0-2 % and the interspecific from 3.2-23.4 % suggesting the presence of a barcoding gap. The available information for *Ommastrephes* from both the North and South Pacific waters was compared with recently obtained Atlantic specimens, suggesting the existence of more than one species within this oceanic genus.

November 14, 1215-1230 [100]

Japanese source materials and knowledge used by Férussac & d'Orbigny to describe Japanese cephalopods in the 19th century

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Férussac and d'Orbigny (1834-1848) published the monograph, *Histoire naturelle générale et particulière des Céphalopodes Acétabulifères vivants et fossiles*, in which Japanese Cephalopods were described for the first time in scientific literature. Among the Japanese species, *Amphioctopus fangsiao* (d'Orbigny, 1839-41) is still valid today. To describe Japanese cephalopods, they used two kinds of source materials; the one is images drawn by Tilesius in the Krusenstern's Atlas which was published after the Russian's first round-the-world expedition (1803-1806); the other is Japanese literatures, *Wakan sansai zue* (Japanese Encyclopedia) and *Umi no sachi* (a haiku anthology book of wood engravings in colour), which had been stored in Paris at the end of 18th century. On behalf of biological specimens, these literature sources, both text and images, were used as scientific materials by French scholars. Sasaki (1929) and Gleadall and Naggs (1991) have already pointed out these Japanese literature sources and their validity, however, they could not find out their provenances. This paper evaluates Japanese natural history drawings and literature in the late 18th century which worked as media of natural history objects to facilitate scientific classification in the West. This paper also illustrates historical backgrounds, provenances of the Japanese source materials and Western scientific perspectives of Japan under the closed-door policy to produce scientific knowledge of Japanese species in the context of the East-West exchanges in the late 18th century.

November 14, 1400-1415 [101]

Systematics of the whip-lash squid (Cephalopoda: Mastigoteuthidae)

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Squids in the family Mastigoteuthidae Verrill, 1881 are ecologically important, being prey to many apex predators, yet the diversity and systematics of the family remain poorly understood. Mastigoteuthid taxonomy has been controversial and unstable because species in this family are rarely caught and often damaged during capture due to their delicate nature. Out of 21 named species, recent reviews have accepted eight to 17 species. The higher taxonomy in this family has been reclassified using integrative taxonomy to distinguish five genera: *Mastigoteuthis* (Mt.) Verrill, 1881, *Idioteuthis* Sasaki, 1929, *Mastigopsis* (Mp.) Grimpe, 1922, *Echinoteuthis* Joubin, 1933, and *Magnoteuthis* (Mg.) Salcedo-Vargas & Okutani, 1994. An additional genus, *Mastigotragus* (Mr.) Young, Vecchione, & Braid 2014, has also been recently erected for the species *Mr. pyrodes*. A complete taxonomic review of the New Zealand mastigoteuthids was undertaken for the first time to identify and describe locally occurring species. Six species have been identified from New Zealand waters: *Mt. agassizii* Hoyle, 1904, *Mt. psychrophila* Nesis, 1977, *I. cordiformis* (Chun, 1910), *Mp. hjorti* (Chun, 1913), *Mg. osbeai* sp. nov., and *E. famelica* (Berry, 1909). A full review of this family is still required; an integrative taxonomic approach will be essential because there is often low interspecific and high intraspecific morphological variation.

November 14, 1415-1430 [102]

Systematics of the Octopoteuthidae Berry, 1912 (Cephalopoda: Oegopsida)

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Octopoteuthids have been collected from every ocean except the Arctic and are an ecologically important group, being both key prey for apex marine predators as well as active predators themselves. However, owing to the family's taxonomic disarray and the difficulty of identifying them to species, they have remained poorly understood. Five genera, twenty-one species, and two subspecies have historically been attributed to the Octopoteuthidae, although recent accounts place the number of valid taxa at two genera and between six and eight species. A global review of the family undertaken over the last five years is nearing completion, resulting in the recognition of eleven species, with nine species accommodated in *Octopoteuthis* and two in *Taningia*. Of the eleven species, five are undescribed and two of these have never before been reported. Morphologically, the family is distinctive but very conservative, with species of *Octopoteuthis* being nearly morphometrically indistinct. Photophore patterning and arm hook morphology are considered the most valuable characters, although additional characters (some newly recognised) are required for identification to species, particularly within *Octopoteuthis*. Most octopoteuthid species appear to have geographic ranges limited to a single ocean basin or a part thereof, with the exception of two cosmopolitan, antipolar species and two temperate southern hemisphere species.

Session: Culture and welfare

November 14, 1430-1445 [103]

Recent progress in culturing the East Asian common octopus

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The East Asian common octopus, like its Atlantic and Mediterranean counterpart *Octopus vulgaris*, is a fast-growing, high-value, over-fished species. There are therefore a number of factors driving attempts to raise this species commercially using aquaculture techniques. The research project reported here consists of (1) a laboratory-based closed-circulation facility focussed on the raising of paralarvae, 3D spacing of young octopuses, and the use of sea cucumbers as detritivores and of seaweeds for processing nitrogen products; (2) a small-scale open-circulation pilot commercial facility focussed on the raising of paralarvae, feed experimentation, and growth throughout the life cycle; (3) a laboratory-based open-circulation facility focussed on raising paralarvae, development of artificial feed recipes, and 3D spacing of adult octopuses; and (4) a laboratory focussed on detailed chemical analysis of octopus and octopus feed composition. This presentation will provide a summary of the major findings of this co-operative research group, which is currently in its third year.

November 14, 1445-1500 [104]

The COST Action FA1301: A Network for Improvement of Cephalopod Welfare and husbandry in Research, Aquaculture and Fisheries - Ceph*sIn*Action

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⁵ Presentation on behalf of 43 Members of the Management Committee of Ceph*sIn*Action

Cephalopods molluscs are listed in Article 1 of Directive 2010/63/EU making them the first and sole invertebrates considered in the European legislation regulating the use of animals for scientific and educational purposes. The diversity of living forms, physiological adaptations, extraordinary genome and behavioral complexities make cephalopods a challenge for the scientific community. Only about tens species are currently utilized as “Laboratory Animals”, and a few others are exploited for their potential in aquaculture. Compliance with the Directive 2010/63/EU and the increased concern for animals’ welfare face scientists and regulators with added challenges. These that provide unprecedented advantages prompting questions for research, education, bioethics and social dimension.

Ceph*sIn*Action has this ambitious aim: fostering an interdisciplinary network of experts aimed to promote sharing of tools, training and to increase scientific knowledge to improve approaches required for studying cephalopods in different contexts. Ceph*sIn*Action operates to facilitate multi-disciplinary scientific exchanges to integrate knowledge on welfare practices, to promote cephalopod research, conservation and public awareness. The COST Action FA1301 is strongly committed to provide to early career investigators a framework of excellence and inclusiveness aimed to foster a modern view for cephalopods and increasing their interest to ask key questions in research and innovation with these animals. Bringing new generations facing with ‘different models’ where new ideas can be tested will support international cooperation and the exploration of new research frontiers in the European Research Area, an open space for knowledge and growth.

Currently, Ceph*sIn*Action (www.cephsinaction.org) includes 19 countries and involves more than 180 researchers.

Poster presentations

Session: Behavior

P1

Cephalopod intelligence, sociality and communication III) Individual recognition and sympathy in oval squid (*Sepioteuthis lessoniana*)

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To discriminate other conspecific (individual recognition) is help to choice adaptive performance in social animals. In addition, to recognize other's emotion is also adaptive for survival, which has been recently documented in human and in other animal like rodents as well. Among cephalopods, teuthoid squids form organized school which suggests their advanced society. Oval squid *Sepioteuthis lessoniana* can form well-organized, defensive and offensive school, which would indicate some aspects of their sociality. We can therefore assume oval squid posse some social recognizing abilities by which they can keep their social system. The aim of this study is to investigate experimentally the ability of individual recognition and emotional contagion of oval squid. First, we applied habituation-dishabituation paradigm to examine individual recognition. Two squid, namely, focal squid and displayed squid were selected. They could directly see to each other through the transparent tank wall. The focal squid were getting to exhibit body pattern 'clear', as if it habituated to the neighboring displayed squid. However, the focal squid exhibited different body pattern if the displayed squid was exchanged with new one, which showed dishabituation occurred. Second, we examined emotional contagion of oval squid. A squid (demonstrator) was exposed with some stimuli such as a prey, a predator and a conspecific. And the other squid (observer) was able to see how the demonstrator behaved. The observer got close to the demonstrator when the demonstrator attacked a prey, while the observer inked when the demonstrator inked by danger.

P2

Being influenced from, and learning by conspecifics: the case of cephalopods

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Social learning is classically conceived as a behavioral trait shown by gregarious and long-lived animals. The question whether social phenomena occur even in solitary animals has been long debated. Recent data support the view that it may also occur in solitary species. Octopuses and their allies, cuttlefishes and squids, are invertebrates well-known for the extreme richness of behavioral repertoire and plasticity. Their mimicry, communicative skills, and tool-use capabilities further support this view. I will review the most recent available knowledge on cephalopod learning capabilities, focusing on forms of social influence including learning by observation of conspecifics. I will also provide recent evidences on neural modulation involved in inter-individual interaction and recognition, and discuss the constraints and peculiarities of social 'learning' capabilities of these animals, and their potential evolutionary meanings.

P3

Ontogeny of schooling behavior in the squid *Sepioteuthis lessoniana* (Kuwa-ika type)Yuki Yonezawa¹, Chikatoshi Sugimoto¹, Tetsuya Umino² and Yuzuru **Ikeda**¹¹ Faculty of Science, University of the Ryukyus, Okinawa, Japan. E-mail: ikeda@sci.u-ryukyu.ac.jp² Graduate School of Biosphere Science, Hiroshima University, Hiroshima, Japan

Sepioteuthis lessoniana in southwest Japan is a species complex consisting of “Aka-ika”, “Shiro-ika”, and “Kuwa-ika” types that exhibit phenotypic and genotypic differences. Among these three *Sepioteuthis*, Kuwa-ika type is the smallest and is locally distributed at the coastal waters of Ryukyu Archipelago, Japan. Kuwa-ika type is sometimes consumed as fishery resource but this is locally limited, which have masked their natural habitat and life history. In the present study, we investigated the ontogeny of schooling behavior in *Sepioteuthis lessoniana* (Kuwa-ika type) reared from hatching to about three months of age. Just after hatching, individuals swam in random direction, then, they became to swim parallel up to the two months of development. The distance and angle between nearest-neighbor individuals decreased along with these post-hatching durations. In addition, the swimming distance of individuals in experimental duration decreased with increasing synchronized hovering of individuals. These observations clearly suggest that Kuwa-ika type swim randomly during early phase of post-hatching, then, form a school one month after hatching. These features are similar to ontogeny of schooling behavior of *Sepioteuthis lessoniana* (Shiro-ika type), which we have previously investigated. In Kuwa-ika type, exhibition of some body patterns were synchronized after they formed school. Diversity of schooling behavior among teuthoid squids will be discussed.

P4

Cephalopod intelligence, sociality and communication VII) Operant conditioning in tropical octopus (*Abdopus aculeatus*)

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Since “J. Z. Young School” documented based on psychological studies for various aspects of learning and memory in octopus, we have recognized octopus as the most intelligent invertebrate. The abilities that octopus possess are, for example, discrimination of shape, size and direction, foraging mapping and observational learning that is difficult even in chimpanzee. However, most of these intellectual abilities were investigated for single species, namely, *Octopus vulgaris* that inhabit temperate waters. Among over 200 species of octopuses, there might be diversity for intelligence as well as life histories. With the aim of obtaining fundamental knowledge for intelligence of other octopuses, we therefore have studied learning and memory of tropical octopus *Abdopus aculeatus* that inhabit coral reef of Ryukyu Archipelago, Okinawa. *A. aculeatus* of Okinawa-jima Island origin were trained by operant conditioning to touch 3 dimensional objects (e.g., sphere). Then, they were trained to touch 2 dimensional objects (e.g., circle). Finally, they were trained to touch an electric monitor in which the object they have learnt was appeared. Throughout these trials, *A. aculeatus* successfully learnt to touch the object and discriminated its particular shape. However, not all individuals could pass the task. Also, there was difference among individuals how fast they learnt the task. These results would open the further possibility to test by operant conditioning learning and memory of this tropical octopus and other octopuses as well.

P5

Prey selection and handling in North Pacific giant octopus (*Enteroctopus dofleini*) from Hokkaido**Masaki Kitaoka¹ and John R. Bower²**¹ Graduate School of Fisheries Sciences, Hokkaido University, Hakodate, Japan

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The North Pacific giant octopus (*Enteroctopus dofleini*) occurs in the North Pacific Ocean from Japan to Baja California, Mexico. It feeds on a wide diversity of prey comprising mainly of bivalve and gastropod molluscs and decapod crustaceans. Several methods are used to feed on shelled prey, including pulling apart the shells, breaking the shells, and drilling. The present study was conducted to determine the preferred prey and prey-handling methods of *E. dofleini* in Hokkaido. Four octopuses weighing 4.5-6.4 kg were used in captive feeding experiments. Each was kept individually in a circular, 2-m-diameter tank. Several types of shelled prey were placed in each tank, and shells and uneaten prey were removed 48 hours later. The prey comprised five bivalve species (Chinese surf clam (*Macra chinensis*), northern great tellin (*Megangulus venulosa*), Japanese scallop (*Mizuhopeten yessoensis*), short-neck clam (*Ruditapes philippinarum*), and purplish bifurcate mussel (*Septifer virgatus*)) and one echinoderm (Japanese purple sea urchin (*Anthocardia crassispina*)). The preferred prey included the Chinese surf clam, northern great tellin, Japanese scallop, and short-neck clam. Four types of prey-handling behavior were recorded to open the shells: pulling apart, breaking, chipping and drilling. Regardless of prey species and size, the most common method was pulling apart. These results suggest drilling behavior might be less common in Hokkaido than in the Northeast Pacific.

P6

Complex visual ecology of the southern pygmy squid - good eyesight for docking, poor eyesight for hunting

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The southern pygmy squid, *Idiosepius notoides*, lives in seagrass meadows, where, aside from swimming for finding food and mates, they spend much of their time attached to seagrass fronds or seaweed. In natural and captive environments, pygmy squid show preference for locating close to dark objects that are used as a stabilizing docking site as well as presumably an object to hide behind or camouflage against. Their visual ecology remains largely unknown and here we describe the discovery of a non-hemispherical eye in this species and the variable levels of image quality this delivers and their potential adaptation for different visual tasks are discussed. Firstly, the pygmy squid is the second cephalopod to possess a retinal bump where a large portion of dorso-temporal retina forms a severely hyperopic zone. Coupled with vertical head bobbing behavior and the resulting focus differential, a unique range finding mechanism for foraging and seeking an attaching site, results. Given an artificial black stripe in the aquarium, pygmy squid come close to the potential attachment site (ca. 3 mantle length) using head-first or tail-first movement. Our behavioural results show that tail-first movement is both more time-efficient and accurate in reaching the attaching site compared to head-first locomotion. This suggests that the anterior retina and its corresponding good eyesight are usually used to direct this attaching behaviour.

P7

Ethogram of reproductive body patterns in the oval squid *Sepioteuthis lessoniana* (Cephalopoda: Teuthida) from northeastern Taiwan

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Cephalopods use dynamic body patterns and postures for camouflage and communication. This complex visual communication also plays an important role in their reproductive behavior. In the oval squid *Sepioteuthis lessoniana*, males of different body sizes use distinct mating body patterns and postures to interact with females, which results in different spermatophore planting sites in females and fertilization success. However, little is known about the significance of these body patterns and postures in courtship behavior, and the evolution of these mating types in reproductive behavior. To study visual communication and reproductive strategy in *S. lessoniana* from northeastern Taiwan, we examined body patterns of males and females during courtship (i.e., ethogram of distinct chromatic component expression) at spawning grounds in wild and in captivity. We characterized total of 39 body pattern components (27 chromatic, 5 postural, and 7 locomotor) of males and females. Analyses of temporal sequences of these chromatic component expression during male-male and male-female interactions revealed that some components are specific to male competition and female choice, and they are predictable in mating success. Studying dynamic body patterning of oval squids during courtship behavior thus provides a unique window to examine visual communication in animal reproduction.

P8

Behavior of Japanese flying squid *Todarodes pacificus* to Light Emitting Plasma illumination

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To effectively introduce light emitting plasma (LEP) lights in the Japanese flying squid *Todarodes pacificus* fishery, information is needed on how the lights affect behavior of the squid. Here, we investigated how *T. pacificus* react to LEP lamp and self-ballasted mercury vapor lamps (SB-MVL) whose light emitting characteristics are similar to metal halide (MH) lamps mainly used in current squid fisheries. We kept *T. pacificus* in a large experimental tank and irradiated light sources. For light source, we used LEP and SB-MVL. As irradiance of LEP was higher than SB-MVL, we used neutral density (ND) filter to adjust irradiance of LEP same as maximum irradiance of SB-MVL. We irradiated light sources either laterally to the other side of wall or vertically to the bottom from center of the tank. When ND-filtered LEP or SB-MVL was irradiated laterally, squid stayed mainly in shadowed area. They turned their body to lighted area and the degree of body axis was sharp (Approx. 40-56 °). On the other hand, when light source was irradiated vertically, squid stayed both shadowed and lighted area and the degree of body axis was gentler (Approx. 22-30 degree °). From our data it is assumed that the squid can react to both LEP and MH lamps similarly and can change their behavior depending on the direction in which a light sources shines.

P9

Behavioral response of spear squid *Heterololigo bleekeri* to fishing lightHajime **Matsui**¹, Go Takayama², Kazuhito Kashiwaya³, Hisashi Kaga⁴ and Yasunori Sakurai¹¹ Graduate school of Fisheries Sciences, Hokkaido University, Hakodate, Japan. E-mail: mtihjm.silverlining@fish.hokudai.ac.jp² National Research Institute of Fisheries Engineering, Fisheries Research Agency, Kamisu, Japan³ Niko-Denki Corporation, Hakodate Japan⁴ Hakodate Regional Industry Promotion Organization, Hakodate, Japan

In the spear squid *Heterololigo bleekeri* fishery, fishers use fishing lights to attract squid. To control behavior of the squids effectively, information is needed on how the lights affect their behavior. Here, we investigated how *H. bleekeri* react to Light emitting Plasma (LEP) lamp, red lamp and self-ballasted mercury vapor lamps (SB-MVL) whose light emitting characteristics are similar to metal halide (MH) lamps mainly used in current squid fisheries. We kept *H. bleekeri* in a large experimental tank and irradiated light sources. We irradiated LEP and SB-MVL sources either laterally to the side of wall or vertically to the bottom from center of the tank. When LEP or SB-MVL was irradiated, *H. bleekeri* reacted similarly by staying in shadowed area. There was no difference in behavior depend on the direction in which a light sources shines. On the other hand, when the light source was switched to red light, squid changed their behavior and began to stay both shadowed and lighted area. Spectral compositions of both LEP and SB-MVL lights have wavelengths close to the visual pigment sensitivity peak (VPSP) of *H. bleekeri* (494 nm). However, spectral composition of red light was far from its VPSP. It is assumed that the squids avoid light which have wavelength close to their VPSP regardless of the direction in which a light sources shines and respond less sensitively to light which have wavelength far from their VPSP.

P10

An ethogram of chromatic, postural and locomotor behaviors of the pharaoh cuttlefish, *Sepia pharaonis* from Okinawa Island, Ryukyu Archipelago, Japan

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Coleoid Cephalopods, such as cuttlefish, squids and octopuses have an ability to produce diverse repertoires of visual behaviors that include chromatic, postural and locomotor components, most of which are neurally controlled. It is vital to create an accurate and extensive catalogue (ethogram) of such behaviors. Such ethogram can be used as a tool for behavioral monitoring and quantitative analysis as well as specie identification of morphologically identical sub-species. In this study, an ethogram was produced for the pharaoh cuttlefish, *Sepia pharaonis* from the coastal waters of Okinawa Island, Ryukyu Archipelago, Japan. The data was collected from animals that are aqua cultured from egg mass sampled around the island and hatched in aquaria during 2010, 2011, 2012 and 2014. During this study, 65 chromatic components, 3 textural components, 14 postural components and 18 locomotor components were identified and described in detail. Currently, there is no comprehensive ethogram of *Sepia pharaonis* and many components described in this study are reported for the first time.

P11

Cephalopod intelligence, sociality and communication IV) Group dynamics of cuttlefish (*Sepia pharaonis*)

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Social animals regulate probability engaged with survival by making group. Cuttlefish make the group in breeding season, but they have been thought they spent alone almost of the life history. However, there is still possibility that cuttlefish have interaction with other individual even at non-breeding season. The recent finding for schooling behavior of broadclub cuttlefish (*Sepia latimanus*) would support this assumption. We therefore examined existence of group dynamics for pharaoh cuttlefish (*S. pharaonis*) at offensive or defensive situations. Cuttlefish were put on two situations, namely, grouped (5 individuals) and alone. Predator (defense) or dietary organism (offence) was presented to the cuttlefish, and some of their behaviors were recorded. In the offensive situation, both cuttlefish in grouped and alone similarly noticed the dietary organism. However, cuttlefish in grouped fed more number of diet compared to the solitary cuttlefish. This clearly indicates that motivation for offence can be increased by individual numbers of cuttlefish. In the defensive situation, cuttlefish in the group noticed the predator earlier than solitary cuttlefish. On the other hand, body patterning involving alarm and camouflage were more frequently exhibited in solitary cuttlefish. Furthermore, angle to the predator of the grouped cuttlefish was higher than that of solitary cuttlefish, which suggests that each cuttlefish in the group did not pay much attention to the predator. These findings suggest that behavior and action of conspecifics operate in favor of survival in cuttlefish.

P12

Testing personality in cuttlefish (*Sepia officinalis*)

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In recent years research on personality has expanded to encompass a wide variety of taxa and species, including a variety of invertebrates. Individual animals differ consistently in their behavioral response in multiple contexts. Cephalopods, which have by far the most complex and the most centralized of all invertebrate brains, represent an important and interesting and unique model for personality research.

However, there are only a few studies, with conflicting results, on personality in cephalopods. In the otherwise widely studied *Sepia officinalis* there is only one preliminary report. Such studies in cuttlefish (*Sepia officinalis*) and dumpling squid (*Euprymna tasmanica*) indicate that there are consistent differences in behavior between individuals in 'feeding' and 'threat' contexts.

Our study aims provide a more detailed look at cuttlefish (*S. officinalis*) personality. To achieve this we repeatedly exposed juvenile cuttlefish to four different testing conditions: Hunting, threat, interaction with conspecific, and interaction with novel objects. By expanding the testing contexts we show that animal personality traits influence a large variety of contexts.

P13

Analysis of *Sthenoteuthis oualaniensis* flying behaviour from recorded video

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Flying squid events have been witnessed since 1892, but a lack of research exists due to rare records. Past studies suggested that the main purposes of squid flight behaviour are predator avoidance and migratory energy cost reduction. In this study, the first video analysis of squid flight gives a better understanding of the behaviour. *Sthenoteuthis oualaniensis* flight distances were obtained from the video in pixels, and then converted to metres. Overall flight duration, distance, velocity, and acceleration were estimated for the jet launching phase and the total flight. After a comparison with data from previous studies for other squid species the results suggest that *Sthenoteuthis oualaniensis* may be a more optimal species for this flight behaviour. The squid flight behaviour also appeared organized and dynamic. Instantaneous acceleration and velocity changes during the jet launching and gliding phases suggest that squid individuals adjust their speeds to fly within the group. These speed adjustments were possibly achieved using bursts of water, fin movements/angling, and/or other methods. In conclusion, this study fills a gap in squid flight research, as acceleration was not previously analyzed. It also improves general understanding of the behaviour, thus, could be applicable for the maintenance of healthy squid stocks and fisheries.

P14

Cephalopod intelligence, sociality and communication VI) Gradual exhibition of the defensive body patterns in cuttlefish (*Sepia pharaonis*), inferred by model and animated predatorsKohei **Okamoto**¹, Akira Mori¹ and Yuzuru Ikeda²¹Department of Zoology, Graduate School of Science, Kyoto University, Kyoto, Japan.
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Coleoid cephalopods, which have the most sophisticated color changing ability in the animal kingdom, dramatically change their body pattern when they encounter predators. Cephalopods give us unique opportunities for understanding the function and evolution of animal colorations because cephalopods are expected to express adaptive body patterns according to situations. However, the functions of the body patterns that cephalopods use for avoiding predation are still not fully understood because they change their body patterns in a highly complex manner in response to the movement of predators. Therefore, we firstly used a model fish as a stimulus to control the movement of a potential threat. We presented a model fish moving in three different trajectories against cuttlefish, *Sepia pharaonis*. Cuttlefish expressed body patterns with seven categories (uniform light, disruptive, center circle, dark square, vertical stripe, all dark and eyespots) in response to the movement of the model. These body patterns were used according to the distance and trajectory of the model fish. Secondly, we made a three dimensional computer graphics of fish, by which we can present more complex and controlled predator stimuli. We presented animated fish against *S. pharaonis* and found that cuttlefish show biologically appropriate responses against the animated predator along with its movement. We confirmed that animations are effective methods for examining the behaviors of cephalopods.

P15

Individual identification using body pattern in cuttlefish (*Sepia pharaonis*)Kohei **Okamoto**¹, Akira Mori¹ and Yuzuru Ikeda²¹Department of Zoology, Graduate School of Science, Kyoto University, Kyoto, Japan.

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In some species of coleoid cephalopods (squids, cuttlefish and octopuses), individuals behave quite differently from each other, which may reflect their personalities. Therefore, we can look deeper inside of their behaviors if it is able to identify individuals in single species. There are two main methods for identifying individuals: using artificial tags and using natural markings. Because artificial tags could cause abnormal behaviors, using natural markings is more suitable method for studying cephalopods behaviors, such by difference of chromatic pattern of individuals. However, because coleoid cephalopods are able to change their body pattern dynamically, identifying individuals by their appearance is still difficult. The purpose of this study is to show that a pharaoh cuttlefish, *Sepia pharaonis*, could be individually identified by using its chromatic component of body pattern. We focused on the black spots that are distributed in the center its mantle. For a natural marking to be useful for individual identification, there must be sufficient polymorphism among individuals and also the marking must be consistent over time. We firstly examined whether there are individual differences in the distribution of the black spots of cuttlefish. The number and the location of the black spots differed between individuals. Secondly, we examined whether the number and the location of the black spots are consistent in each individual of cuttlefish. The number and the relative location of the black spots did not change over one month. These results suggest that black spots in the mantle are useful for discriminating individuals in *S. pharaonis*.

P16

Behavioural patterns of recently settled octopuses under climate change

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Projected near-future ocean conditions are expected to alter behavioural patterns in marine organisms. However, little information is available regarding climate change impacts on cephalopod behaviour, especially after settling, a transition from the pelagic lifestyle of a paralarvae to the benthic life of a future adult. The aim of the present study was to assess, for the first time, if future ocean conditions, warming (+3 °C) and acidification ($\Delta 0.4$ in pH) modify activity patterns and play abilities of recently settled *Octopus vulgaris*. We verified that higher temperatures increased the activity rates and line crossings of octopuses, whereas lower pH decreased both parameters. Octopuses under higher temperature conditions presented higher curiosity regarding the objects given to them, with touching the pieces and exploring them extensively when compared to present-day conditions and lower pH scenarios. The higher activity rates can have negative impacts on this sensitive life stage, where they have to learn how to live like an adult, since they are more responsive and playful. Thus, increased activity can take a toll on energy expenditure rates and increase their visibility to predators when comparing to more discreetly behaving octopuses.

P17

The hunting efficiency of Japanese flying squid affects their prey's schooling behavior

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Observations were conducted on predator-prey interactions between Japanese flying squid *Todarodes pacificus* and Japanese anchovy *Engraulis japonicus* in a large experimental aquarium. Sometimes when the squids actively foraged, the anchovy schools remained far away from the squid and descended to avoid predation. Other times the anchovies swam freely among the squid and were able to avoid predation. Response strategies and survival rates of anchovy can be explained by the hunting ability of the squid. Factors affecting their hunting efficiency included time of introduction into the aquarium, age, and light conditions. For example, on the day of introduction, squids display fierce hunting behaviors evidenced by the attack and foraging of single anchovy by multiple squids.

P18

Effect of water temperature on seasonal migration of North Pacific giant octopus *Enteroctopus dofleini* in Soya/La Pérouse Strait

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The effect of water temperature on seasonal migration of the North Pacific giant octopus *Enteroctopus dofleini* was investigated using a mark-and-recapture method employing temperature-logger tags in the Soya/La Pérouse Strait. A total of 77 and 85 tagged octopuses were released in waters approximately 2-m deep around three ports near Cape Soya in December 2013 and June 2014, respectively. Sixteen individuals were recaptured in the Soya/La Pérouse Strait from April to November 2014. The water temperatures recorded by the tags retrieved from the recaptured octopuses ranged from -1.4 to 21.0°C. The water temperatures recorded by the tags of octopuses caught at depths of < 2 m near ports from April to June in 2014 ranged between -1.4 and 10.0°C, which was similar to the water temperatures measured inside the port. A regression tree analysis of water temperatures recorded by the logger tags retrieved from octopuses that were recaptured at depths of 15 to 51 m from June to November 2014 revealed that the octopuses migrated from the areas around ports to deeper waters from 25 June to 26 July 2014. The water temperatures recorded before migration were in the range 9.6 to 20.4°C, implying that octopuses do not migrate at water temperatures of <9.6°C; rather, octopus migration in the Soya/La Pérouse Strait appears to occur when water temperatures are 9.6°C. Octopuses in areas where the water is around 2-m deep and temperatures are 21.0°C escape to cooler waters.

P19

Genetic background for sociality of the oval squid (*Sepioteuthis lessoniana*)

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In oval squid (*Sepioteuthis lessoniana*) school, we can see some functional formations and role-like behaviors of a particular schoolmate. In addition, there are dominance hierarchy and complicated social network among the schoolmates. So far, some genetic background of the individual variation of behaviors, namely, personality has been revealed for some animals (e.g., mammals, birds). Based on the idea that there is also personality in the schoolmates of the oval squid, we surveyed genetic differences for school of different origin. In addition, we also tested behavioral diversities among the schoolmates by observing the dominance hierarchy and social network. In the genetic study, SNP survey for *COI* region of mitochondrial DNA and fragment analysis for 11 microsatellite loci were analyzed. Four haplotypes were found in mtDNA and average 11 alleles per loci were found in microsatellites. Clusters in phylogenetic tree constructed from the microsatellite result were corresponding to haplotypes of mtDNA. Some clusters contained many number of schoolmates that had high rank on dominance hierarchy and hub position on their social network. Furthermore, the cluster differentiation was corresponding to the life span of each individual.

P20

Ontogeny of visual ability in the oval squid (*Sepioteuthis lessoniana*)Chikatoshi **Sugimoto**¹, Shuhan Lei², Toru Ao¹, Xiumei Zhang² and Yuzuru Ikeda¹¹Department of Marine and Environmental Sciences, Graduate School of Engineering and Science, University of the Ryukyus, Okinawa, Japan. E-mail: ikeda@sci.u-ryukyu.ac.jp²The Key Laboratory of Mariculture, Ocean University of China, Qingdao, People's Republic of China

Since coleoid cephalopods are highly visual animals that possess human-like lens eyes, details of their visual ability can provide useful information to understand how they behave in nature and evolution of visual system in animal kingdom. In the present study, we surveyed ontogenetic process of the visual ability of the oval squid (*Sepioteuthis lessoniana*) by use of two months 1) anatomy of eyes and 2) behavioral tests. According to the anatomical observation, it revealed that the retina of hatchling differentiated completely, being formed by visual cells and nerve fibers. The depth of rhabdom can be interpreted as proportional to sensitivity, and the density of visual cell nucleus as visual acuity. Based on these criteria, the visual sensitivity and acuity of oval squid developed as squid grew. Since inclined eye position toward arm of hatchling changed to parallel position to body axis as squid grew, visual field became wider. Behavioral tests by use of reaction of squid against live prey organisms showed that squid found prey at further location as they grew, which shows gradual development of visual acuity up to two months old. This is consistent with the retinal development. The visual field of squid got nearly almost all directions as squid grew up to two weeks old.

P21

Head withdrawal reflex in squid paralarvae: A review and appraisal

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Many squid paralarvae are known to retract their head, arms, and tentacles (or proboscis) into the mantle cavity when disturbed. This withdrawal reflex is thought to be a defensive behavior. In this study we review this withdrawal behavior in different squid species. Different stimuli, such as mechanical stimuli, chemical stimuli, and light are known to initiate this reflex. It may help paralarvae escape from predators in different ways. In the early paralarval stages, the head is completely withdrawn into the mantle cavity and the animal assumes a spherical posture with expanded orange chromatophores. This might represent an aposematic adaptation to imitate unpalatable prey. As the paralarvae grow and increase in length, the head is partially withdrawn into the mantle cavity resulting in deimatic displays that might distract visual predators, thus giving the paralarva an opportunity to escape.

P22

Movement patterns of giant Pacific octopus *Enteroctopus dofleini* in a shallow coast

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We studied the movement patterns of giant Pacific octopus, *Enteroctopus dofleini* along a shallow coast (depth: 1-22 m) located in northern Japan. Three individuals were tracked for 36 days by attaching ultrasonic transmitters. We recorded area use, daily activity patterns and spatial scale of movements of all individuals. Local convex hull (LoCoH) kernel method estimated their utilization distribution (UD) was limited to 20% area for 95% of their time within the minimum convex polygon (MCP) area. Although all were released at ~5m depth at same spot their core UD's were at depths ~20 m, and non-overlapping between individuals within the study area. Our results indicate the movement and distribution range of octopus was influenced by depth and presence of competent conspecifics.

P23

Number sense and prey capture strategy in pharaoh cuttlefish *Sepia pharaonis* (Cephalopoda: Sepiida)

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Studies of numerical cognition have been extended from strictly humans and non-human primates to include many other vertebrates and invertebrates in last two decades. Cephalopods have a complex nervous system which is likely to equip them with a high level of cognitive ability. Since identifying the quantity of preys is an important factor in animal's feeding decision, we sought to examine whether cuttlefish (*Sepia pharaonis*) have the number sense and use this information together with their satiety state to determine their prey capture strategy. When animals were presented with a choice of 1 vs 2, 2 vs 3, 3 vs 4, and 4 vs 5 live shrimps, cuttlefish chose the large number of preys significantly. However, if the choice was between one live and two dead shrimps, cuttlefish preferred the small number of prey. More importantly, when the choice was between one large and two small live shrimps, cuttlefish chose the large one when they felt hunger, but chose the two small preys when they are satiated. These results demonstrate that cuttlefish have the capability of numerosity discrimination, and their choice of prey numbers depends on the quality of prey and their appetite state. This also suggests that cuttlefish integrate both external and internal information to decide their prey capture strategy, a cognitive ability that is crucial in their forage behavior.

P24

Cephalopod intelligence, sociality and communication V) Effect of environmental enrichment in the behavior of cuttlefish *Sepia pharaonis*

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Both abiotic and biotic factors in captive environment strongly affect the development of the neural circuit and related behavior of the pup, which is defined as environmental enrichment. In this study, we investigated the effect of environmental enrichment in the behavior of cuttlefish (*Sepia pharaonis*). First, we examined the effect of abiotic environment on ontogeny of cypsis by cuttlefish. We found that cuttlefish raised in the enriched environment (sand with objects) exhibited the appropriate body patterns according to the substrates compared with cuttlefish raised in poor (without sand and objects) and standard (sand) environments. Second, we examined the effect of abiotic and biotic environments on ontogeny of cognition of cuttlefish. We applied “prawn-in-the-tube” paradigm to test cognitive abilities, namely, learning and memory of cuttlefish. Cuttlefish raised in various environments could learn the task. However, only cuttlefish raised in enriched environment retained for 24 hours what they have learnt. In addition, the depth perception (inferred from success of hunting behavior) of cuttlefish raised in poor and isolated (i.e. asocial condition) environments did not develop well compared with cuttlefish raised in standard and enriched environments. Third, we examined the effect of architectural lighting design on ontogeny of cognition of cuttlefish. All cuttlefish that were raised in even (evenly illuminated), diffuse (indirectly illuminated), focal (directly illuminated) or video projected (the focal tank with videos of coral landscape) environments were able to learn and remembered the task. Meanwhile, cuttlefish raised in focal and video projected environments got the highest score for depth perception.

P25

Ontogeny of learning and memory in the cuttlefish *Sepia pharaonis*

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European cuttlefish (*Sepia officinalis*) is a well-known subject for learning and memory, which has been investigated the unique paradigm, namely, “prawn-in-the-tube” by Martin Wells (1958). On the other hand, we have few knowledge for ontogeny of learning and memory in other cuttlefish. In this study, we therefore investigated ontogeny of learning and memory for pharaoh cuttlefish (*Sepia pharaonis*) by use of “prawn-in-the-tube”. The cuttlefish was first presented living mysids in a transparent tube for 18 minutes (training session). After number of strike against the tube by cuttlefish decreased for enough low number, particular intervals (5 and 60 minutes) was set. Then, the tube with mysids was presented again to the cuttlefish (retention session). The cuttlefish of 1 month old were able to learn the task, and remembered what they learnt for 5 minutes. However, they were not able to retain this memory for 60 minutes. Cuttlefish of 2 month old were able to remember the task for 60 minutes. We also observed histologically the ontogeny of the central nervous system (CNS) of the cuttlefish. The whole volume of the CNS increased, as cuttlefish got older. The volume of vertical lobe, a part being involved in the learning and memory, increased dramatically from hatching to 1 month old, then it kept gradually increasing up to 2 month old.

Session: Biodiversity and conservation

P26

IUCN red list assessment of the conservation status of cephalopod species

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On behalf of the *International Union for Conservation of Nature (IUCN)* we have assessed the conservation status of 735 species of cephalopods. Published assessments (available at <http://www.iucnredlist.org>) include 242 squid species (orders Oegopsida, Bathyteuthida, Idiosepiida), eight bottletail squids, 118 cuttlefishes, 62 bobtail squids, 63 pelagic octopuses and *Spirula spirula*. Of these 494 assessments, 291 are Data Deficient, 197 are Least Concern and six fall into threatened categories. Five of the threatened species are cirrate octopuses: two are considered Vulnerable, two Endangered and one Critically Endangered. These five species have suffered population declines because of heavy fishing on deep-sea finfish. The giant cuttlefish, *Sepia apama*, is assessed as Near Threatened due to population declines associated with fishing on mating aggregations and the potential consequences of pollution.

Draft assessments include 47 squids (order Myopsida), 193 benthic octopuses, and *Vampyroteuthis infernalis*. Of these, 119 are Data Deficient, 118 are Least Concern and three fall into threatened categories. *Uroteuthis edulis* meets the Vulnerable category due to an estimated 30% population decline. *Heterololigo bleekeri* meets the Endangered category due to an estimated 50% population decline. Both declines are associated with fishing pressure. *Octopus kaboroa* is Endangered because of declines associated with its vulnerability to the New Zealand scampi fisheries. Taxonomic issues have delayed some important assessments, notably nautilus species and *Octopus minor*. The large percentage of Data Deficient species (56%) emphasises the need for cephalopod research. Researchers are invited to contribute to draft assessments and to provide updates on published assessments to keep them relevant.

P27

Seasonal variation in species composition of cuttlefish in Yilan Bay, northeast TaiwanZih-Heng You, Jing-Yu **Chen** and Chih-Shin Chen

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Global production of cephalopods is increase in the recent four decades. Understanding life-history traits and population structure of exploited stock of the exploited species was critical to form strategy for effective fisheries management. Most of the current studies was focused on commercially important target species (particularly squids), yet less studies for cuttlefish. The Yilan Bay is one of the important fishing grounds for the trawl fishery around North Taiwan. In this study, seasonal variation in species composition of cuttlefish and life-history traits of the dominant cuttlefish species were studied. The samples of cuttlefish were collected from a trawl vessel monthly from Dasi fishing port (Yilan County) from January 2011 to December 2013. A total of 1903 individuals (207.4 kg) of cuttlefishes were collected during the studied period which encompassed ten species. Production and species diversity of cuttlefish was higher in cold-water season (from November to March). The dominant species varied by season, *S. esculenta* dominated in cold-water season, while *S. madokai* dominated in warm-water season (from May to September). The maximum ML for female and male *S. esculenta* was estimated at 180 mm with a lifespan of ca. 27 months. Higher GSI values of female and male for *S. esculenta* occurred in November and January which suggesting they might spawn in spring. ML-at-maturity for females (114 mm) was higher than males (109 mm). Seasonal variation in species composition of cuttlefish in Yilan Bay could be influenced by oceanographic conditions which dominated by different water mass seasonally.

P28

A new octopus species, *Octopus longitubus* (Cephalopoda: Octopodidae) from Taiwan

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Octopus TW1 (Lu, 1998) is a commercial species of octopus which is found in the sea off the west coast of Taiwan on muddy to sandy substrates. This is a small to moderate sized, elongate and narrow-armed species, pale purple brown coloured on the dorsal surface and cream on lateral and ventral surfaces. The arms are subequal in length, decreasing from the dorsal arm pair (1>2>3>4). The hectocotylized arm has approximately 65 suckers and with a large copulatory organ occupying approximately 8-12% of arm length. Eggs are very large, approximately 12 mm in length and around 20-25% of mantle length. The most distinct morphological characters of Octopus TW1 are the relatively long funnel (70-86.5%ML) and the unique shape of the funnel organ (¥ Λ /). Based on the partial mtCOI sequence, Octopus TW1 shows the species status and the affinity to Callistoctopus and Octopus minor group. Octopus TW1 is the only octopus that has been found in estuary of Taiwan, indicating its ability to tolerate low salinity environment.

P29

Cephalopod biodiversity in the vicinity of Bear Seamount, western North Atlantic

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Seamounts provide complex habitat for deep sea fauna, but our understanding of seamount-associated communities is poor compared to other marine ecosystems and fundamental questions about biodiversity, population abundance, colonization, community structure and function remain largely undescribed.

In 2000, the National Systematics Laboratory (NOAA/NMFS) began a program of exploratory trawling to document the biodiversity of Bear Seamount (39° 55'N 67° 30'W), the most inshore of the New England Seamounts, located within the U.S. Exclusive Economic Zone (EEZ) south of Georges Bank. Bear Seamount (BSM) rises from the continental slope at depths of 2000-3000 m to a generally flat summit at 1100 m depth. Here we synthesize the sampling plans and preliminary results from 8 cruises that took place between 2000 – 2014 to describe the cephalopod biodiversity and assemblage structure at BSM. One hundred and seventy four midwater and 57 bottom tows were completed over the 8 cruises. All 5,091 cephalopods were identified, dorsal mantle lengths were measured and representative photographs, tissue samples and voucher specimens were taken at the time of capture. Seventy four species were collected in the midwater and 28 from the benthic tows. In 2012, beaks were collected from 26 species for stable carbon and nitrogen isotopes analysis.

These specimens and the associated environmental data comprise the most comprehensive collection of cephalopods from the New England Seamounts and serve as a baseline of biodiversity in an area of increasing fishing pressure.

P30

Trace element concentrations in the top predator jumbo squid (*Dosidicus gigas*) from the Gulf of CaliforniaRui **Rosa**¹, Carlos Vale² and Joana Raimundo²

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Jumbo (or Humboldt) squid, *Dosidicus gigas*, is a large jet-propelled top oceanic predator off the Eastern Pacific. The present study reports, for the first time, concentrations of V, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Cd and Pb in gills, mantle and digestive gland of this powerful pelagic squid in the Gulf of California. Zinc and Cu were the most abundant elements. All elements, with the exception of As, were largely stored in digestive gland; particularly Cd that reached concentrations between 57 and 509 mgg⁻¹. Significant relationships between tissues were found for Co (digestive gland–gills), As (gills–mantle) and Cd (digestive gland–mantle). Proportionality of Cd concentrations between mantle and digestive gland suggested that detoxification capacity by digestive gland was insufficient to avoid the transfer of this element to mantle and other tissues. Nonetheless, Cd concentrations in the mantle were always below the regulatory limit and, therefore lack of constraints for human consumption. On the basis of the fishery landings, one may estimate that up to 1 t of Cd can be annually removed by jumbo squid fisheries.

P31

Cuttlefish capsule: An effective shield against contaminants in the wild

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Increasing anthropogenic pressures in estuaries are responsible for the rise of contaminants in several compartments of these ecosystems. Species that benefit from the nursery services provided by estuaries are exposed to such contaminants (*e.g.* metals and metalloids). It is therefore relevant to understand if marine invertebrates that use these areas as spawning grounds accumulate contaminants in their tissues throughout embryogenesis. This study aimed to quantify As, Co, Cr, Cu, Mn, Ni, Se, Pb, V and Zn concentrations in both capsule and embryos of the common cuttlefish (*Sepia officinalis*) in Sado Estuary (Portugal). Moreover, embryos at their initial, intermediate and final stage of development were collected in sites subjected to different anthropogenic pressures. In general, the capsule accumulated higher element concentration throughout embryogenesis which indicates that the capsule acts as an effective barrier against contaminants uptake by the embryo. Although the capsule becomes thinner throughout embryogenesis, embryo's protection does not seem to be compromised at later development stages. Additionally, the higher concentrations of As, Cu, Se and Zn in the embryo in comparison to the capsule suggests important biological roles during the embryogenesis of this cephalopod mollusc.

P32

Taxonomic list of species of cephalopods in the Tamaulipas coast in the Gulf of Mexico, Mexico

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Information derived from four fishing cruises during October 2012, January and April, 2013 and February 2014 along the Tamaulipas coast in the Gulf of Mexico is presented. All cephalopods found in samples from 76 shrimp trawls, which 1 h lasted in three depth intervals were identified. Morphological identification was performed using the original descriptions of organisms and guidance based support in the form of hectocotylus (in the case of males). A total of 12 species of adult cephalopods squids and octopuses were identified for the area; these include three orders, one suborder, three families, six genera, eight species and three subgenera: *Rossia bullisi* (Voss, 1956); *Doryteuthis* (Amerigo) *pealeii* (Lesueur, 1821); *Doryteuthis* (*Doryteuthis*) *plei* (Blainville, 1823); *Lolliguncula* (*Lolliguncula*) *brevis* (Blainville, 1823); *Benthoctopus januarii* (Hoyle, 1885); *Octopus macropus*; *Octopus* cf *vulgaris*; *Pteroctopus tetracirrhus* (Chiaie, 1830) and *Octopus* (*Macrotritopus*) *defilippi*. Because of their presence and abundance in the area, two main species of squid have been identified: *Doryteuthis plei* and *Doryteuthis pealeii*, this one being with better characteristics to propose a commercial fishery. It notes that in Mexico, there is not a fishery on these species of squid, which remain as a part of shrimp fishery's bycatch in the Gulf of Mexico.

Session: Climate change

P33

Effects of increased CO₂ on squid metabolism and hypoxia tolerance

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By the end of this century, the oceans are expected to decrease in pH by a mean of 0.3 units and have expanding areas of hypoxia such as oxygen minimum zones and coastal environments. Both of these environmental changes can be stressors on organisms. Loliginid and ommastrephid squids have been proposed to be particularly sensitive to climate change due to their high metabolic demands and highly pH sensitive blood O₂-binding pigment. We present manipulative studies on the effect of increased CO₂ on squid hypoxia tolerance. Humboldt squid, *Dosidicus gigas*, is a diel vertical migrator that naturally experiences high CO₂ in deep, cold oxygen minimum zones during the day and experiences warm oxygenated surface waters at night. Its metabolic rate is high even among squids, however, and it has not historically experienced high CO₂ in warm surface waters. *Doryteuthis pealeii* is an active loliginid that often encounters increased CO₂ in coastal bays. Squid were exposed to high CO₂ and the critical partial pressure of O₂ (P_{crit}) was assessed using intermittent respirometry. CO₂ increases from ambient atmospheric levels (400 µatm) to levels anticipated by the end of the century (1000 µatm) led to decreases in hypoxia tolerance in *Dosidicus gigas* at surface water temperatures. The implications of these stressors on squid and their metabolism and biogeography will be discussed.

P34

Embryonic development of tropical octopus (*Octopus maya*) under ocean warming

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In 100 years an increment of between 2-3°C is expected to occur in superficial sea water (0 a 100 m depth) of northern hemisphere which could change the ecology and in consequence, the fisheries of many tropical species. The present study was designed to test the effects of temperature in embryos that were maintained at 24°C (optimum) or that were exposed to an increasing temperature ramp from 24 to 30°C (1°C every 5 d) in attempt to evaluate if *O. maya* embryos are capable to tolerate thermal stress recreating a scenario of short winter with ocean warming. To test those effects, we made evaluations of growth of embryos, respiratory metabolism and antioxidant defense in order to define the limits of temperature that embryos can tolerate when temperature is increased at a constant rate. Results demonstrated that, *O. maya* embryos are not capable to compensate temperatures beyond 27°C. Although embryos in the temperature ramp developed at the same rate than the ones maintained at 24°C, there were marked differences within the morphological development of the ones that were exposed to the temperature ramp suggesting that high temperature affected the embryos differently. When temperature reached 30°C, few embryos survived as a consequence of a metabolic impairment and the incapacity of the embryos to maintain redox balance that resulted in oxidative stress. Such results demonstrate that the embryological development of *O. maya* has a limit around 27°C above which physiological distress occurs.

P35

Functional evolution of branchial acid-base regulation in squid (*Sepioteuthis lessoniana*) for sensitivity in times of CO₂-induced seawater acidificationYing-Jey **Guh**¹, Marian Y. Hu², Jay-Ron Lee³, Yu-Chi Chen⁴, Po-Hsuan Sung⁵, Chang-Jen Huang¹ and Yung-Che Tseng⁴¹ Institute of Biological Chemistry, Academia Sinica, Taipei City, Taiwan² Institute of Physiology, University of Kiel, Kiel, Germany³ Institute of Cellular and Organismic Biology, Academia Sinica, Taipei City, Taiwan⁴ Department of Life Science, National Taiwan University, Taipei City, Taiwan. E-mail: yct@ntnu.edu.tw⁵ Department of Life Science, National Taiwan University, Taipei City, Taiwan

Cephalopods have evolved strong acid-base regulatory abilities to cope with CO₂ induced pH fluctuations in their extracellular compartments to protect gas transport via highly pH sensitive hemocyanins. The present work used adult squid *Sepioteuthis lessoniana* to investigate the effects of acute (few hours) to long-term (up to 168 h) seawater acidification on pelagic squids and the mechanistic basis of branchial acid-base regulation. Routine metabolic rates were significantly depressed by 40% after exposure to pH 7.3 conditions (0.4 kPa *p*CO₂) for 168h. Animals fully restored extracellular pH accompanied by an increase in blood HCO₃⁻ levels within 20 hours. This compensation reaction was accompanied by increased transcript abundance of branchial acid-base transporters including V-type H⁺-ATPase (VHA), Rhesus protein (RhP), Na⁺/HCO₃⁻ cotransporter (NBC) and cytosolic carbonic anhydrase (CAc). Immunocytochemistry demonstrated the sub-cellular localization of Na⁺/K⁺-ATPase (NKA), VHA in basolateral and Na⁺/H⁺-exchanger 3 (NHE3) and RhP in apical membranes of the transport active branchial epithelium. Branchial VHA and RhP responded with increased mRNA and protein levels in response to acidified conditions indicating the importance of active NH₄⁺ transport to regulate acid-base balance in cephalopods. Therefore we concluded that adult pelagic squids have a well-developed branchial acid-base regulatory machinery. However, squids that evolved a lifestyle at the edge of energetic limits are probably more sensitive to long-term exposure to acidified conditions compared to their more sluggish relatives including cuttlefish and octopods.

P36

Impact of climate change on the distribution and the site of fishing ground of the Japanese common squid *Todarodes pacificus* in the Sea of Japan

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Lots of studies concerning with the impact of the climate change on fisheries have been reported in many countries and regions. The precise prediction of the impact of the climate change on the fisheries is important anywhere for a proper adaptation. In this study, we examined the impact of climate change on the squid fisheries in the Sea of Japan targeted for Japanese common squid. Japanese common squid is one of the most commercially important cephalopod species in the Sea of Japan, and has been caught by Japanese, South Korean, North Korean, Russian and Chinese fisheries, although reliable catch statistics by Chinese and North Korean fisheries are not obtained. Because fishing grounds and fishing seasons are differed in these fisheries, shifts in the distribution and migration pattern of Japanese common squid change the availability of these fisheries. So, we tried to predict the impact of climate change on the distribution and the site of fishing ground of the Japanese common squid for a proper use and manage. The distribution and CPUE of the Japanese common squid were modeled with water temperatures based on GAM, and predict the shifts in the site of fishing grounds and duration of fishing seasons. Our results showed that the impact of the climate change is most serious in the coastal areas along with the Japanese Islands. In these areas, distribution of Japanese common squid is predicted to be very low during summer and autumn at the end of this century, and the duration of fishing season shorten to one or two month.

P37

Amino and fatty acid dynamics of octopus (*Octopus vulgaris*) early life stages under ocean warming

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The oceans are becoming warmer, and the higher temperatures are expected to have a major impact on marine life, especially at the most vulnerable early life stages. The aim of the present study was to evaluate, for the first time, the amino acid (AA) and fatty acid (FA) dynamics during the early ontogeny of the common octopus, *Octopus vulgaris*, under an ocean warming scenario (+3 °C). The main essential amino acids found in octopus embryos were arginine, leucine and lysine; while aspartic and glutamic acids, and taurine were the main non-essential amino acids. Palmitic, eicosapentaenoic and docosahexaenoic acids were the main FAs found in octopus tissues. Relevant ontogenetic changes were observed, namely a steep decrease in the content of many AAs, and a selective retention of FAs, thus evidencing the protein-based metabolism of these cephalopods. Temperature *per se* did not elicit significant changes in the overall FA composition, but was responsible for a significant decrease in the content of several AAs, indicating increased embryonic consumption.

P38

Physiology of recently-settled octopuses in a changing oceanVanessa M. Lopes, Ana R. Lopes, Matilde Costa, José R. Paula and Rui Rosa

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Little is still known about octopuses' early life stages, namely *Octopus vulgaris*, regarding the transition from the pelagic paralarvae to the recently-settled juveniles. Their bodies undergo major shifts during the settling and require optimal physiological conditions. Climate change scenarios have been proven to have negative impacts on cephalopod early life stages, however only during embryogenesis and shortly after hatching. The aim of the present study was to investigate, for the first time, the impact of warming (+3 °C) and acidification ($\Delta 0.4$ in pH) on several biological and physiological parameters of recently settled *Octopus vulgaris*. Here we determined octopus' metabolic, ventilation, survival and specific growth rates and feed intake. Antioxidant enzyme activities, namely glutathione-s-transferase (GST), catalase (CAT), superoxide dismutase (SOD) were also analysed, as well as malondialdehyde (MDA), a product of lipid peroxidation and indicative of cellular damage. Our results show an increase in metabolic rates with temperature, along with specific growth rates and feed intake, but decreasing in the combined scenario (warming and acidification). MDA, GST, CAT and SOD increased with temperature but not with acidification. Thus, here we showed that recently settled octopuses, still adapting to life in the benthos can be negatively impacted by future conditions.

P39

Relationship between regime shifts and the spawning pattern of *Watasenia scintillans* (Cephalopoda: Oegopsida), southwestern Sea of JapanNobuaki **Nanjo** and Akira Kozuka

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Fluctuations of fish and squid stocks have been correlated with regime shifts. The stock size of the common squid, *Todarodes pacificus*, spawning from the southwestern Sea of Japan to the East China Sea, also fluctuated with the change of the spawning pattern related to the effects of regime shifts. The firefly squid, *Watasenia scintillans*, is distributed in the western North Pacific with the main spawning season in spring when females aggregate around the 200 m isobath. The spawning of *W. scintillans* mainly occurs in the southwestern Sea of Japan. The present study investigated the change of spawning pattern of *W. scintillans*, which spatially overlaps with that of *T. pacificus*, in relation to regime shifts. The present analysis was based on the Egg and Larval Survey Database, which comprises plankton net samples collected in March, April, and May in the southwestern Sea of Japan from 1980 to 2009. The mean density of eggs in the investigated months was higher in the 1990s and 2000s, a warm regime period, than the 1980s with a cool regime. The horizontal distribution of eggs also changed in the 1990s and 2000s. Eggs were hardly collected in March during the 1980s but many eggs were mainly collected in the western area in March during the 1990s and 2000s. On the other hand in April and May high densities occurred in all investigated area during the warm regime. The present study indicates that the change of the spawning pattern of *W. scintillans* corresponds with regime shifts.

P40

Temperature as a plausible determinant of variability in Indian squid, *Uroteuthis (Photololigo) duvaucelii* stocksGeetha Sasikumar, K. S. Mohamed, K. G. Mini and K. K. Sajikumar¹

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Indian squid, *Uroteuthis (Photololigo) duvaucelii* (Loliginidae) constitute an important component of the cephalopod resources along eastern Arabian Sea. Role of environmental variables in the regulation of year-class strength gains significance in the context of changing oceanic conditions due to climate change. The growth rates, population turnover and reproduction of these fast-growing short-lived inshore squids are likely to respond quickly to environmental changes. The response in squid abundance in relation to the variability in sea surface temperature (SST) – which is the key parameter to measure climatic change – are examined in this study. As the region experiences a predictable, seasonally reversing monsoon on a semi-annual basis, the influence of rainfall is also considered. Monthly catch and catch per fishing hour of squids in commercial trawl during 1985-2011 are used as abundance indices. Trends in catch and catch rates of *U (P) duvaucelii* showed a positive relation (Pearson's correlation) with SST. Significant negative correlation was observed between the squid catch and total rainfall, as well as with the number of rainy days. The reduction in temperature associated with rainfall tends to have a negative influence on abundance. Percentage of mature females in fishing ground showed positive correlation with the SST. Given that the squid life-history is a function of their environment, with temperature often considered as one of the main driving factors, this result gains relevance as these groups may endure the global sea surface temperatures rise by 0.4–1.1°C in 2025.

P41

Variability of habitat suitability of jumbo flying squid (*Dosidicus gigas*) in the Southeast Pacific Ocean off Peru in relation to climate changeWei **Yu**^{1,4}, Qian Yi^{1,2,3,4}, Xinjun Chen^{1,2,3,4} and Yong Chen^{5,1}¹ College of Marine Sciences, Shanghai Ocean University, Shanghai, China. E-mail: xjchen@shou.edu.cn² National Engineering Research Center for Oceanic Fisheries, Shanghai Ocean University, Shanghai, China³ Key Laboratory of Sustainable Exploitation of Oceanic Fisheries Resources, Ministry of Education, Shanghai Ocean University, Shanghai, China⁴ Collaborative Innovation Center for Distant-water Fisheries, Shanghai, China⁵ School of Marine Sciences, University of Maine, Orono, Maine, USA

We developed a habitat suitability index (HSI) model to explore the impacts of ENSO phenomena on the habitat for *Dosidicus gigas* in the Southeast Pacific Ocean off Peru over 2004-2013. Fishery data sourced from the Chinese squid-jigging vessels were used for analyses, as well as the remote sensing environmental data including sea surface temperature (SST), chlorophyll-a (Chla) and sea surface height anomaly (SSHA). Due to better model performances, the arithmetic mean HSI model (AMM) was applied to predict the HSI values and identify the suitable habitat for *D. gigas* during 2004-2012. Fishery and environmental data in 2013 was used to validate the model. Results suggested that the monthly fishing effort showed a similar latitudinal distribution with the location of suitable habitat. The cross-correlation analysis found a significantly negative relationship between the average HSI values on the fishing ground and the Niño 3.4 index with a time lag of -1-3 months, implying a quick response of the squid habitat to the climate variability. Both suitable habitat areas and catches of *D. gigas* increased in La Niña periods and decreased in El Niño periods. The La Niña conditions tended to result in strengthened upwelling coupling with cool and nutrient-enhanced waters, which yielded favorable habitat condition and high catches; whereas the El Niño conditions led to weaken upwelling coupling with warm and nutrient-depleted waters, which were unfavorable for the squid habitat and reduced the catches. Our findings indicated that the large-scale climate variability had strong impacts on the squid habitat suitability.

Session: Culture and Welfare

P42

**Guidelines for the Care and Welfare of Cephalopods in Research
A consensus based on an initiative by CephRes, FELASA and the Boyd
Group**

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³ presentation on behalf of 19 co-authors and 25 contributors to the “Guidelines”, and of 43 Members of the Management Committee of the COST Action FA1301- *CephsInAction*

The Guidelines for the Care and Welfare of Cephalopods in Research represents the result of an internationally coordinated initiative, and the first attempt to develop guidelines for the use of cephalopods (i.e. nautilus, cuttlefish, squid and octopus) following the inclusion of this class of 700 known living invertebrate species in Directive 2010/63/EU. The “Guidelines” provide a response to an urgent request from the European Commission for the harmonization of the application of Directive 2010/63 in Member States, due to the lack in the Directive of any specific recommendation for the use of cephalopods in research and education. This work provide reference information for investigators, animal care committees, facility managers, animal care staff, veterinarians and regulators, and will assist in improving both the care given to cephalopods and the manner in which experimental procedures are carried out, increasing the standards and extending best-practice.

I will illustrate the approach we followed in generating the information and data contained and in coordinating the work-flow. I will also present briefly Guidelines and recommendations provided herein.

The presentation will also aim to open discussion on future directions on biology, physiology and neuroscience due to recent important initiatives (including policies) involving cephalopods, and the challenges due to the application of concepts such as “welfare” and 3Rs to cephalopods. Finally, I will illustrate knowledge gaps to prompt research to enhance the knowledge on these animals and facilitate their “use” in the future for demanding research avenues.

P43

Beak microstructure analysis as a tool to identify potential rearing stress for *Octopus vulgaris* paralarvae

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Octopus vulgaris is a viable candidate for commercial aquaculture, but rearing procedures might stress individuals and result in diminished growth and survival. This study investigated the relationship between possible stress sources (tank transposition and siphoning) when rearing *O. vulgaris* paralarvae and the deposition pattern of growth increments in their beak microstructure. Light intensity at the facility was heterogeneous, and accounted for with an experimental design consisting of blocks without replicates. Growth and survival were estimated and possible effects of handling were tested for both parameters. Increments and stress marks were counted in 120 paralarval upper jaws (UJ), and the number of UJs with a mark on the day of stress application (day 8) was quantified. Differences in light intensity, diet quantity and total number of marks in the UJ were also compared between treatments. Growth and survival were statistically similar between treatments, although the control treatment showed a tendency for higher survival rates. Age at first increment deposition coincided with day 1 of experiment, and a 1 increment.day⁻¹ deposition rate was validated for the experiment duration. The number of stress marks was significantly different between the control and other treatments, indicating that handling might cause stress and that marks can be used as a biomarker for stress, although the occurrence of stress marks on day 8 was not significantly different. Light intensity and diet might have also been relevant stressors and confounded the results. The results herein presented are important for improving rearing conditions for *O. vulgaris* paralarvae.

P44

Fatty acids composition and age estimation of wild *Octopus vulgaris* paralarvae

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Under culture conditions, lipid composition and specifically, the fatty acid profile of reared paralarvae is significantly different from hatchlings. Therefore, comparing wild paralarvae and reared paralarvae of similar age would allow us to elucidate if the changes in fatty acid profile are related with a non-optimal prey composition or are caused by the normal development. The aim of this study was analyze, for the very first time, the fatty acid profile of wild *Octopus vulgaris* paralarvae individually, determining for each paralarvae their age through deposition of daily increments on lateral hood surface of the beaks. The main goal is to clear up lipid requirements of paralarvae and optimise the diet of reared paralarvae.

P45

Intestinal flora of wild *Octopus vulgaris* paralarvae and their zooplankton prey: a metagenomic search of “probiotics” for use in aquacultureAlvaro **Roura**, Steve Doyle, Nathan Hall and Jan Strugnell

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The life cycle of *Octopus vulgaris* is closed in captivity but survival rates are extremely low precluding its commercialization. All research carried out thus far has centered in resolving the bottleneck of aquaculture: paralarval survival during the planktonic stage (~50 to 60 days). A plethora of experiments has been done to overcome this through the testing of different tank designs, physical properties, biochemistry of paralarvae and prey, and especially different diets (mainly based on *Artemia* complemented with crustacean larvae) and enrichment methods. Despite these efforts, this bottleneck persists and the solution is a goal pursued by many research groups. Apart from the research done in captivity, few studies have been done to understand the development of *O. vulgaris* paralarvae in the wild, mainly owing to their scarcity in plankton samples. Fortunately, early stages of octopus (n=5, <12 days old) and their natural prey (n=13) were collected in the Ría de Vigo (NW Spain) to identify their microbial biota using 16S metagenomics, in order to discover microbes potentially used as probiotics in aquaculture. The two extraction methods revealed different microbial assemblages: averages of 201 and 148 genera were identified in octopus and their prey through the use of Qiagen kits (662.484 reads), compared to 78 and 63 genus with the mouse tail extraction methods (808.067 reads). We identified potential “probiotic” genus candidates (i.e. shared by octopus and prey but higher percentages in prey) with both kits.

P46

Studies on the large scale artificial rearing of octopods in Chinese waters

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Octopus minor, *O. vulgaris* and *Amphioctopus fangsiao* are all widely distributed along the coastal waters of China, Korean Peninsula, as well as Japan. As important economic octopods, artificial culture has been attempted in the past 10 years. This paper presents an overall research effort on the current status and future challenges.

The embryonic development of *O. minor* lasts for 72–89 days before hatching under the conditions of a seawater temperature of 21–25 °C and a salinity of 28–31psu. The mantle length and total length of new hatchlings range from 8.5 to 11.5mm and from 25 to 31mm, respectively. Hatchlings are benthic. Under indoor culture conditions, the average weights of males and females at 250 days are 122.9 and 197.1 g, respectively. More than 100,000 juveniles have been released into the Moon Lake National Aquatic Resources Conservation Area of China, located in Rongcheng (Shandong Province), for the past 4 years.

O. vulgaris is reared and bred indoors. The mature eggs are (2.4±0.2) mm in length and (1.2±0.1) mm in width. The development of fertilized eggs require 25-35d before hatching at 20.4-23.6°C. The average length of newly hatchlings is about 3.08 mm. The paralarval stage keeps 28-33d at the hatching temperature of 25.2-22.8°C. At least 1,280,000 juveniles with 59mm in mean total length were obtained by pond culture in 2010.

The biological zero(C), effective accumulated temperature (K) and temperature coefficient (Q10) for the embryonic development of *Amphioctopus fangsiao* and the correlation between the embryonic stages(Ts) and effective accumulated temperature(K) are studied. The main results show that C for the embryonic development is 5.15°C, K at which the zygotes develop into early juveniles is 589.45°C•d, and the optimum hatching temperature ranges from 19.46°C to 23.06°C. the optimum temperature of larvae is 18-25°C, the most is 21°C and the optimum salinity is 28-30. 23,000 juveniles reach an average weight of 50 g in 5 months under indoor culture conditions, which corresponds to the commercial marketable size and therefore can be harvested for marketing.

Session: Fisheries, stock assessment and management

P47

The use of landmark-based geometric morphometrics to study bodyshape variations in *Doryteuthis sanpaulensis* (Cephalopoda: Loliginidae) from Brazilian and Argentinean waters.Augusto C. Crespi-Abril^{1,2,3}, Nicolás Ortiz^{1,4}, Ramiro Braga⁵ and Mara **Bainy**⁶¹ Centro Nacional Patagónico (CENPAT) – Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Puerto Madryn, Argentina. E-mail: crespi@cenpat-conicet.gob.ar² Universidad Nacional del Comahue, Escuela Superior de Ciencias Marinas, San Antonio Oeste, Rio Negro, Argentina.³ Universidad Nacional de la Patagonia San Juan Bosco, Puerto Madryn, Chubut, Argentina.⁴ Universidad Tecnológica Nacional, Facultad Regional Chubut, Puerto Madryn, Chubut, Argentina.⁵ Universidad Centro de Altos Estudios en Ciencias Exactas, Buenos Aires, Argentina.⁶ Programa de Pós-Graduação em Ciências Fisiológicas - Fisiologia Animal Comparada, Instituto de Ciências Biológicas, Universidade Federal do Rio Grande, Rio Grande, Brasil.

Doryteuthis sanpaulensis is a neritic species that is distributed in coastal waters of the Atlantic Ocean, from Brazil to Argentina. The aim of this work was to study bodyshape variations of individuals collected in three locations: Nuevo Gulf, Argentina (N=65), Rawson, Argentina (N=33), and waters near São Sebastião, Brazil (N=22). To analyze bodyshape variation, we performed 2 networks of 9 (9L) and 25 (25L) landmarks respectively. PCA analysis showed that individuals from Rawson and São Sebastião were similar, and individuals from Nuevo Gulf presented two distinct morphological groups. One of these groups, presented high similarities compared with individuals from Rawson and São Sebastião, while the other group presented a bodyshape typical from the gulf. The total variation of shape was 80.5% (CP1: 61% y CP2: 19.5%) for 9L and 75.4% for 25L (CP1: 54.8% y CP2: 21.6%), therefore, the best landmark configurations correspond to 9L. The similarities in bodyshape of individuals from the three locations, suggest the existence of a high connectivity between *D. sanpaulensis* population from Argentina to Brazil. In addition, the group of individuals from Nuevo Gulf presented a typical shape that was only observed in this particular location, suggesting the presence of a individuals that inhabits inside the gulf with a reduced connectivity with the main group of the species observed in shelf waters of the Atlantic Ocean.

P48

Species composition and discard rates of cephalopods from the commercial fishery in the south of Portugal (Algarve)

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Commercial cephalopod species are largely caught as by-catch of trawl fisheries and their consumption is traditionally important in Portugal. Knowledge of discarding practices and discard rates is of considerable importance for stock assessment and management of fisheries. Estimation of fishing mortality rates is fundamental for stock assessment and should take into consideration discards. This study was based on three types of fisheries (metiers) in the south of Portugal: crustacean trawl, fish trawl and purse seine. None of these metiers fish cephalopods as target species, however, the quantities caught as by-catch and discarded are significant.

A total of 21 cephalopod species, of which 12 are commercial, were identified in the fisheries discards of the three metiers. The main reasons for rejection are legal and administrative, including being below the minimum landing size, and of poor quality due to damages. The other 9 species are non-commercial and are therefore always discarded. Of the total cephalopod catch the commercial portion was much higher than the discarded one for the fish trawl and purse seine. However, for the crustacean trawl the proportion of discarded cephalopods reached 80% of the total cephalopod catch. This is due to the fact that fish trawlers and purse seines operate at shallower depths where the high commercial value species live. At greater depths there are fewer commercial species and therefore the majority of the species are discarded.

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The behaviour of the common octopus (*Octopus vulgaris*) towards fishing traps

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The octopus fishing gears mostly used in the south of Portugal are the “*alcatruç*” (a shelter pot traditionally made of clay with an amphora shape but recently replaced by plastic cylindrical shape) and “*covos*” (a baited metal cage trap covered by a plastic net, which traps the animal).

The traditional *alcatruç* made of clay has been replaced by cylindrical plastic pots with great success, due to its resistance to brake. In an attempt to continue to fish with the traditional shape, a new type of plastic pot was built in association with fishermen, with the same shape as the traditional clay pot – amphora.

To study the behaviour of the octopus towards the three types of fishing traps (traditional amphora clay pot, cylindrical plastic pot and the new amphora plastic pot), experiments were performed in tanks to answer three main questions, concerning preferences of the common octopus towards the pots: 1) What kind of material: plastic or clay (traditional); 2) What shape: cylindrical or amphora (traditional); 3) What colour: white, black or redbrick (traditional). Results show no particular preference towards material; strong preference for the traditional amphora shape; and strong preference for the black colour.

Due to a controversial discussion on the impact of baited traps *vs* shelter pots on the octopus populations, a behaviour study was performed in tanks to observe the possible exit of octopus from the trap fishing gear. Results showed that octopus come in and go out through the mesh and/or the main entrance, whenever they want.

P50

A review of the fishery for the North Pacific giant octopus (*Enteroctopus dofleini*) in Japan

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In Japan, *Enteroctopus dofleini* is fished mainly in Hokkaido and the Tohoku district, and is an important coastal fishery. Much information has been published about this species and its fishery in Japanese. The purpose of this study is to give a comprehensive review of these studies in English.

Growth rates differ widely among individuals, but do not differ between males and females. The estimated maximum lifespan is 5 years in females and four and 4.5 years in males. In Tsugaru Strait (between Honshu and Hokkaido), mature males are present in November- May. Mature females are present in December –May, and mated females are present in November –April. Based on the occurrence of mature males and females, mating is thought to occur during November-May. Spawning and spawned eggs have not been observed in Tsugaru Strait, but spawning is presumed to begin in March and last until about June. Paralarvae are thought to occur from January to March, with first settling occurring in February and active movement on the sea floor beginning in late March. We plan to focus future research on the poorly studied and rarely collected paralarval stage. Information about the fishery will also be presented.

P51

Potential role of recreational fishery on the dynamic population of the European squidMiguel **Cabanellas-Reboredo**¹ and Ángel Guerra²¹Instituto Mediterráneo de Estudios Avanzados (CSIC/UIB), Balearic Islands, Spain. E-mail: miguel.cabanellas@uib.es²Instituto de Investigaciones Marinas (CSIC), Vigo, Spain

Although in some cephalopods stocks have been highlighted the potential role of the recreational fishery, the harvest from this sector has never been estimated. This challenge (estimation of recreational harvest) could mean the key for an integrative and more reliable assessment of the mixed fisheries (commercial and recreational). Accordingly, this 5-years study related some features of the squid life-history with the recreational fishing activity with the final goal to highlight the potential importance of the activity of this sector on population dynamic of *Loligo vulgaris* in Balearic Islands (NW Mediterranean).

Ecological aspects such as the spatio-temporal movement patterns at day-scale (more active during night-time) and seasonal-scale (reaching shallow waters during cold months) has been linked with recreational fishing effort and captures (i.e., recreational fishing effort is seasonally focused during squid spawning at shallow waters and daily when squid shift to a predation behavior). With this socio-ecological approach, we propose a framework combining model-based estimates of effort (varying in space and time) with model-based estimates of catches per unit effort (varying in time), taking into account the catch variability caused by angler heterogeneity (based on its skills).

As a result, the recreational squid harvest in Palma Bay was estimated in 20.5 tonnes during 2010, representing the 34% of the total squid landings by the entire commercial fleet of Mallorca Island (59.5 tonnes). The knowledge provided certainly should constitute a baseline for a long-term monitoring program, and it demonstrates that stock assessment should incorporate the role of the recreational fishery.

P52

Spatial dynamics of two *Ommastrephes bartramii* cohorts in the North Pacific

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Neon flying squid *Ommastrephes bartramii* is a pelagic squid species and widely distributes in the temperate and sub-tropical waters. In the North Pacific, neon flying squid plays an important role in the pelagic marine ecosystem and is a commercially important resource for the international fleets from China, Japan, and Taiwan with an annual production at ca. 100,000 tons. The variability of abundance of neon flying squid has been showed could be explained by environmental factors, while the interactions between two stocks are not well known. In this study, we applied generalized additive model (GAM) to explore the abundance variation of two neon flying squid stocks using temporal, spatial and environmental variables using Taiwanese squid fishery data. The optimal model for inter-annual variation of CPUE (1986 to 2006) involved all variables, and can explain 15.7% variance of NE stock and 19.3% NW stock. The abundance of two stocks showed a contrast trend with NE stock increasing 1995 afterward and NW stock decreasing at the same time. The latitudinal variation could be explained by north-south migration route during summer and autumn. The sea surface salinity could explain higher variation of squid abundance than other environmental factors which suggested that the habitat conditions of squid aggregation might be indexed by surface salinity.

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Spatiotemporal variation of the Humboldt squid (*Dosidicus gigas*) stock in the East PacificChih-Shin **Chen** and Jing-Yu Chen

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The jumbo (or Humboldt) squid *Dosidicus gigas* is a large pelagic ommastrephid squid which distributed in the Eastern Pacific Ocean (EPO). The jumbo squid is an important component of its ecosystem in the EPO and is the main target of squid fisheries in this region. This species is a target species for regional fisheries management organization (SPRFMO) that enforced since 2012. It is essential to understand its population dynamics, environmental influences and fisheries management. In this study, spatiotemporal variation of the *D. gigas* stock in the East Pacific was studied using Taiwanese squid fishery data. The distribution of *D. gigas* abundance was higher in the coastal waters off northern Peru. The size composition harvested by the Taiwanese fleet was dominated by large-size group in recent years. The results of GAM suggested that variation of squid abundance could be explained by the temporal and spatial variables to a degree. This may result from a long-distant migration pattern for the jumbo squid and plasticity in life-history traits of squid populations. A decreasing trend of squid abundance index since 2005 has also been noted. The annual spatial patterns of fishery in recent years were different from the earlier years (before 2007) which may imply a change in migration pattern for the jumbo squid, or a change in the environmental conditions of habitat.

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Stock assessment of swordtip squid (*Uroteuthis edulis*) in the southern East China SeaYu-Jie Hsu¹, Cheng-Hsin Liao¹ and Chih-Shin **Chen**²¹ Department of Environmental Biology and Fisheries Science, National Taiwan Ocean University, Keelung 20224, Taiwan² Institute of Marine Affairs and Resource Management, National Taiwan Ocean University, Keelung 20224, Taiwan

Swordtip squid *Uroteuthis edulis* is a neritic squid distributed in subtropical and tropical coastal water in the Indo-western Pacific. This species plays a critical role in marine ecosystems and also an important fisheries resource. The southern East China Sea (SECS) is an important fishing ground for the neritic squid fishery of Taiwan, however, production of the neritic squid was decreasing in recent years. In this study, catch data of the neritic squid fleet (mainly torch-light net fleet) from the SECS from 2002 to 2013 are analyzed. Annual number of sample vessels range from 9 to 32 with a total of 19,007 records. The fishing efforts of individual vessel are standardized by comparing relative CPUE of each paired-vessel in the same day and location. Proportion of the standardized vessels ranges from 44% (in 2004) to 90% (in 2010). The abundance trends are similar for the nominal and the adjusted CPUE: increased from 2002 to 2005, decreased from 2005 to 2008, and increased again after 2009. Annual squid abundance is estimated by the depletion method. The annual fishing mortality (F) ranges from 1.50 (in 2009) to 15.75 (in 2011). The annual proportional escapement, assuming weekly natural mortality at 0.03, ranges from 36% (in 2006) to 96% (in 2008) with an average of 75%. These results suggest that the abundance of swordtip squid in the SECS might be stable in the recent decade, although limited numbers of sample vessels could hinder actual situation of squid abundance.

P55

First estimation of biomass and abundance of *Rossia palpebrosa* (Sepiolida) and *Gonatus fabricii* (Teuthida) in the Barents SeaAlexey V. **Golikov**¹, Rushan M. Sabirov¹ and Pavel A. Lubin²¹ Kazan Federal University, Kazan, Russia. E-mail: golikov_ksu@mail.ru² Polar Research Institute of Marine Fisheries and Oceanography, Murmansk, Russia

The studies of cephalopods quantitative distribution in the Arctic are scarce (Wiborg et al., 1982; Bjørke, 1995), and they are completely absent for the Barents Sea. The most abundant species of the arctic cephalopods are *Rossia palpebrosa* and *Gonatus fabricii*. Their biomass and abundance estimated for the first time in the Barents Sea. The maximum biomass of *R. palpebrosa* was more than 6 thousand tonnes with abundance more than 500 million specimens, the minimum values were about 4 thousand tonnes and 250 million specimens. High densities of biomass recorded in the northeastern parts of the Barents Sea every year and in some years also in the Central Basin. The maximum biomass of *G. fabricii* was more than 24.5 thousand tonnes, abundance was more than 1.7 billion specimens, minimum values were about 6 thousand tonnes and 1.4 billion specimens. Low densities of biomass (about 10 kg/km²) and abundance (about 1 thousand specimens/km²) were usual for the shallow parts of the Barents Sea. Immature and early maturing specimens live here. Their mean mantle length is 47±1,9 mm in the western part and 83±4,4 mm in the eastern part. High densities of biomass (more than 100 kg/km²) and abundance (more than 10 thousands specimens/km²) recorded in the deep-water troughs near the borders of the Barents Sea. Bigger and more mature specimens live here. Thus, it all confirms the suggestion that the Barents Sea is a foraging area in *G. fabricii* range, and the squids leave it as they mature (Golikov et al., 2013).

P56

***Octopus vulgaris* paralarvae vertical distribution in a fluctuating upwelling-downwelling system**Lorena Olmos Pérez* and Ángel **González**

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The Ría of Vigo (Galicia, Spain) is an estuarine system modulated by fluctuating alongshore southward and northward winds (associated with downwelling and upwelling events respectively). This lead to a very productive ecosystem that supports an important artisanal fishery of *Octopus vulgaris*. Additionally, the Islas Cíes, an important spawning site for *O. vulgaris*, connect the Ría de Vigo with the ocean through two mouths (north and south) that determinate the inflow and outflow (depending on upwelling-downwelling events). Several researches have attempt to understand the ecology of *O. vulgaris* early stages (i.e. paralarvae) in the Ría, but there is still little knowledge about the vertical behavior of Octopus paralarvae, which may be modulated by this oceanographic variability. This work studies the distribution of the paralarvae at both mouths and inside the Ría in different upwelling and downwelling events. For this, we undertook 3 oceanographic cruises at night in early autumn in 2013: One cruise to characterize distributions during an upwelling event, another during a downwelling event and a 24 hours cruise during a strong upwelling event to determinate day-night variation. Sampling strategy consisted on two transects intersecting the inflow and outflow at both mouths, and one transect following the water fluxes moving along the Ría. Sampling was performed with a multinet trawl gear from the bottom to the surface every 5 meters. Results show different patterns according to variation of oceanographic conditions.

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Relationship between stock levels of the autumn-spawning cohort of the neon flying squid (*Ommastrephes bartramii*) and oceanic environmental parameters in the spawning area in North PacificYoshiki **Kato**¹, Mitsuo Sakai¹, Toshie Wakabayashi² and Taro Ichii³¹ Tohoku National Research Institute, Hachinohe, Japan. E-mail: kyoshiki@affrc.go.jp² National Fisheries University, Shimonoseki, Japan³ National Research Institute of Far Seas Fisheries, Yokohama, Japan

The neon flying squid, *Ommastrephes bartramii*, is widespread in subtropical and temperate regions. Field observations have revealed that *O. bartramii* has a one-year lifespan and that it migrates between its spawning grounds (30-35°N) and feeding grounds (40-45°N). The north Pacific population comprises two spawning cohorts: the autumn cohort and the winter-spring cohort. Interestingly, despite their apparently contiguous hatching periods, there is a marked difference in the mantle length of each cohort. Stock levels of the autumn-spawning cohort, which is economically more important given its larger size, were low during the period of large-scale driftnet fishing (1979-1992). After an international moratorium on all large-scale pelagic drift net fishing at the end of 1992, squid stocks increased rapidly. The main fishing season runs from May to August near the international date line.

The stock levels of the autumn cohort were investigated using more than 25 yr of survey driftnet data (175.5 degrees E transect between 37 and 44.5 degrees N). Specifically, the relationship between stock levels and environmental parameters in the spawning area was analyzed. The results revealed that the chl-a density and sea surface height in the spawning area exhibited a strong relationship with the stock levels until 2007; however, no such relationship was observed from 2008 onward. This breakdown in the relationship may have been attributed to large scale fluctuations in sea surface water temperature, as indicated by the Pacific Decadal Oscillation index, which may have resulted in a shifting of the spawning area.

P58

Catch Distribution and Biological Characteristics of the Diamondback Squid *Thysanoteuthis rhombus* in the Northeast Cebu Island, Central Philippines

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Diamondback squid is considered as one of the seafood products of high commercial value in the Visayas region. It is of high demand both in local and foreign markets, but its catch distribution and biological characteristics are not yet fully examined, thus. This study was geared towards this purpose. This study conducted northeast portion of the Camotes Sea off Carmen, Catmon and Sogod, Cebu. The study site was located between 10° 35'N and between 120° 12' 30"E and 120° 10"E and was plotted using a nautical chart (NAMRA # 4427). In terms of catch distribution, results show that the town of Carmen has the highest catch record compared to the other area. Catch distribution in every area differs significantly at 1% level ($F=36.73$; $F_t=18$). In terms of biological characteristics results indicate that the total length of the squid was directly proportional to its weight, mantle length, fins width and tentacle head. The total weights were found to be larger in the month of July than in February and March. In terms of sex ratio, it was found out that in the months of February to June of the catch were females while males were found to be dominant in the month of July. Gonad was highest in the month of February and declined in the preceding months. Results indicate that the reproductive stage of this species started during the month of February as the physical environment became favorable for spawning.

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Identification of the firefly squid *Watasenia scintillans* by the quantitative echosounder in the southwestern Japan Sea

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The firefly squid, *Watasenia scintillans*, is one of the important fishery targets in the Sea of Japan. *W. scintillans* migrate to off the coast of Hyogo Prefecture, the southwestern Japan Sea, from March to May, are caught by the trawl net. For efficient fishing, it is necessary to obtain the wide-area information where *W. scintillans* are distributed. In this study, the acoustic surveys were conducted with the quantitative echosounder, in January, March, and April 2015. To obtain the information about when *W. scintillans* migrate to the survey area, and where they are distributed. During the survey, target trawl was conducted to collect the biological samples. The portions corresponding to *W. scintillans* in the acoustic data were identified, to utilize the difference in sound scattering intensity by the relationship between the frequency of echosounder and the target. The results showed that the amount of *W. scintillans* was little in January, and increased in March is the beginning of the fishing season. *W. scintillans* was distributed near the seabed in the depth range of about 200-240 m. The distribution patterns of *W. scintillans* were similar to the results of trawl collection.

P60

Jumbo squid *Dosidicus gigas*: A new fishery in Ecuador

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Given the relevance of *Dosidicus gigas* in the eastern Pacific and the incentives and possible profits obtained for harvest of jumbo squid. During 2014, the Ecuador Government decided that *D. gigas* must be a new fishery. So, in this paper, the first steps to fishery management and the future directions for jumbo squid fishery in Ecuador are documented. Several recommendations were identified in order to improve the jumbo squid fishery management such as: spatial and temporal distribution, scientific data collection, stock assessment, management strategy, and proposal of fishery management in the Galapagos Islands. This review identified as priority a fishery management plan for jumbo squid in the region.

P61

Localized depletion of an important forage species (*Doryteuthis pealeii*): perceptions, processes, and policies

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Longfin inshore squid (*Doryteuthis pealeii*) occur seasonally in the waters around Nantucket Island (northeast USA), where they are targeted by fisheries and considered an important prey species for several species of recreationally and commercially harvested fishes. Fishermen have expressed concern regarding an apparent recent decline in squid abundance and a related shift in predator distribution (e.g. striped bass, *Morone saxatilis*) away from nearshore waters. Several hypotheses have been posed by fishermen for the causes of apparent changes in fish and squid abundance and distribution, including increased commercial fishing pressure on squid and environmental effects. Following targeted outreach to the fishing community, a review of fishery regulations was conducted and regional squid and fish landings data were compiled. The above information was used to formulate testable hypotheses regarding the relative effects of fishing effort, environmental variability, and trophic dynamics on the local abundance of *D. pealeii*. These hypotheses are placed in a fisheries management context to provide a mechanism for science-based evaluation of proposed regulatory actions.

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The current status of world cephalopod fisheryChingis **Nigmatullin**

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Between 1950-2013 cephalopod catches increased dramatically from 0.58 to 4.3 million t per year. The growth and development of cephalopod fisheries took place by expansion of the fishery grounds, targeting new species, increasing the total fishing effort and industrialization. Along with industrial fisheries there has been a pronounced positive trend for artisanal fishery that developed mainly in Latin America and North-West African countries. In the last decade, annual world cephalopod catch (AWC) ranged from 3.48 to 4.3 million t with the bulk of catch (up to 35-50%) consisting of three squid species, *Todarodes pacificus*, *Illex argentinus* and *Dosidicus gigas*. AWC has strong interannual variability depending on fluctuations in stock abundance of these squid. World cephalopod fishery uses mainly the inshore stocks (97-99% of AWC). By 1990-2000s nearly all inshore stocks in the World Ocean were revealed and fully exploited by the fishery. The recent level of AWC based on inshore cephalopod resources nearly reached its upper limit. The maximum AWC of inshore cephalopods may attain 5-7 million t during favourable periods of high abundances of several main stocks, but the probability of this event is very low. At the same time there is considerable unexploited resource of oceanic squid (mainly *Ommastrephes*, *Sthenoteuthis*), that could give additional potential catch of nearly 2-4 million t. However, there is a complex of scientific, technological, organizational and financial obstacles currently preventing exploitation of oceanic squid resources that needs to be overtaken to develop a large-scale fishery in perspective.

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The long-term dynamics of abundance and biomass of the Argentine squid *Illex argentinus* in the Southwest AtlanticChingis Nigmatullin

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The Argentine squid (AS) *Illex argentinus* is the important commercial cephalopod with annual catches up to 0.7-1.15 million t. AS is a keystone predator, accelerator of ecosystem processes, integrator of local ecosystems and typical r -strategists with one-year life cycle and considerable fluctuations of abundance by the order of magnitude and more. Based on fisheries statistic data the AS abundance dynamics in 1982-2013 was described. There were three quasi-seven years' cycles of abundance dynamics and its breach in 2009-2012. Years with high and average stock abundance were predominant but in 1986, 1994-1995, 2004-2005 and 2009-2012 a sharp decreasing in abundance was observed. Each subsequent decline in abundance was deeper and longer. For the period of developed fisheries with quasi-stabilized fishing effort (1986-2013) values of annual catches were ranked on five grades that correspond to graduation of AS abundance scale. An expert assessment of possible values of cumulative annual AS biomass was made under the assumption that the annual catch consists of 10-30% species biomass. In the years of very low abundance the AS biomass was estimated at 0.6-1 million t (daily food consumption 30-50 thousand t), in the years of very high ones – 7-10 million t (350-500 thousand t), and the values of potential annual catch with no account taken of fishery profitability could be 0.2-0.4 and 1.5-2.0 million t respectively. Thus in the years with different AS abundance its fishery potential and functional role in ecosystems considerably varies.

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Historic periods and current status of *Illex argentinus* fishery, one of the world largest squid resources

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The Argentine squid *Illex argentinus* is the most abundant cephalopod in the Southwest Atlantic. It represents a typical 'straddling' stock, migrating through the EEZs of several countries, from spawning grounds over the continental shelf of southern Brazil and Uruguay to the feeding grounds on the Patagonian Shelf. *I. argentinus* fishery is one of the largest international squid fisheries in the world. There are two main periods in its >70 years fishing history. Between 1930 and 1977, *I. argentinus* was mainly caught as bycatch (50-8,300 t per year) during inshore hake fishery in Argentina and Uruguay. Since 1978, large concentrations were revealed on the outer shelf and slope of the Patagonian Shelf that induced the targeted fishery, first by trawlers and then soon followed by jiggers. Squid resources were exploited in three main fishing grounds; Argentine EEZ, Falkland Conservation Zones and high seas international waters of 45-47°S with annual catch ranging from 30-190 to 546-1,153 thousand t. Since 1993, *I. argentinus* has been exploited to full capacity, with population abundance fluctuating two orders of magnitude in different years making these fisheries much more of a 'boom or bust' activity. Conservation and economic risks are high, and these risks are exacerbated by the relative lack of co-operation among industry participants. Effective management and conservation of this important resource would benefit from some kind of RFMO that should include all countries involved in its exploitation.

P65

Difference in catchability of the winter-spawning stock of Japanese flying squid *Todarodes pacificus* among the Japanese coastal fishing groundsSuguru **Okamoto**¹, Norio Yamashita¹ and Toshiki Kaga¹¹Hokkaido National Fisheries Research Institute, Fisheries Research Agency, Kushiro, Japan.
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We estimated the catchability of the winter-spawning stock of Japanese flying squid, *Todarodes pacificus*, by squid jigging fishery among the Japanese coastal fishing grounds from 1979 to 2013. Monthly catch-and-effort data in each fishing port were collected under the projects of 'Assessment of fisheries stocks in the waters around Japan' (n = 1157). We modeled log(CPUE) using generalized linear mixed model with normal distribution. Explanatory variables in the model candidates were fishing port, year, month and interactions. The year effect has reflected the interannual variability in the squid stock. The best available model was selected by BIC. Explanatory variables in the selected model were port, year, month and interactions of port*year and port*month (interactions were mixed effects). Using the model, we calculated the least squares mean and extracted only port effect (i.e. catchability in each fishing ground). The catchabilities were high in the region off east Hokkaido (Doutou: 1.55, Urakawa: 1.58) and low in the region off Sanriku (Iwate: 0.61, Miyagi: 0.48). This difference among the fishing grounds may be attributed to the fishing ground formation or fishing operation (number of jigging or total operation time).

P66

Octopus do not yield under growing pressure. Octopus fisheries in Portugal in the 20th century and what they can yield in the 21st

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Octopus fisheries in Portugal are largely monospecific for the common octopus *Octopus vulgaris*, catches of *Eledone cirrhosa* being residual and landings relatively modern. Landing statistics time-series can therefore be considered to represent the common octopus, and they cover the species nationally dating back to 1927. Prior to 1927, statistics were maintained by region, taking into consideration the flag species of each port. Cephalopods, including the common octopus, were then not always of national importance, but they were represented in the statistics of important ports. Statistics also cover effort in different ways, depending on the period and the regions represented. Here we present several octopus LPUE time-series, from reconstructed statistics dating back to 1900. From these we produce estimates of the abundance of the species in this relatively long time period, taking into consideration the impact of technological and practical innovation where evidence provides support of changing catchability. We discuss these results and lastly provide alternatives for the possible optimization of fisheries yield from this species, based on previous results obtained from interchange of ideas with the small scale fishing sector in participatory stakeholder meetings, as well as from biological data collection from landings.

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Spatio-temporal variability in growth of *Illex argentinus* in relation to the environmental gradients experienced in the north extreme of distributionRafael **Schroeder**¹ and José Angel Alvarez Perez²

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In the north extreme of *Illex argentinus* distribution spawning and spent individuals are found along throughout the year. In the study area the oceanography regime is seasonally dynamic and changes in spatial and temporal scales. This study analyzed the spatio-temporal variability in growth as influenced by the environmental gradients by analyzing gladius microstructure of adult squid captured off southern Brazil between October of 2001 and September of 2002. Spatial growth variability was analyzed from the series of growth increments reconstructed from individuals captured within a 15 days interval in each month. Growth curves were confronted with mean bottom temperature profiles obtained in research cruises. In the period analyzed growth conditions increased from spring time achieving elevated values in summer, decreasing significantly in autumn. Significant differences in spatial variability were observed between October and March. The highest growth condition was observed during the winter between July and August when bottom temperature drop to its minimum. Growth conditions during winter were associated with low bottom temperatures of the south extreme. Temporal variability observed between summer and spring could be associated to the picks of maximum production in subsurface due to ascension of the South Atlantic Central Water to satisfactory levels induced by cyclonic vortices between October and March, being higher during the summer. In the winter, growth conditions were not in accordance with local productivity patterns. In this case, growth conditions experienced by adult squids increase at low temperatures in deep waters, probably compensated by decreasing metabolism costs.

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The artisanal fishery for *Octopus cyanea* in Cabo Delgado, North Mozambique (Indian Ocean)Luis **Silva**¹, Sérgio Marcelino José², Afonso Munduce², Arone Salença² and Guadalupe Martín³¹ Spanish Institute of Oceanography, Oceanographic Center of Cádiz, Spain. E-mail: luis.silva@cd.ieo.es² Fisheries Research Institute of Mozambique, Pemba, Mozambique.³Technology Center of the Sea, CETMAR Foundation, Vigo, Spain.

The octopus *Octopus cyanea* Grey (1847) is a shallow water species widely distributed in the Indian and Pacific oceans, being especially abundant in coral reefs of North Mozambique. Its artisanal fishery constitutes a subsistence activity for local communities, which have been traditionally carried out by women and children by using metal spears to catch the animals while walking on the coral reefs during low spring tides. In recent years, men have become more involved in this fishery by snorkelling, due to the higher market demand. In order to know the main biological and fishery aspects of this species, a first study was carried out in Cabo Delgado, North Mozambique. Main fishing areas in the mainland and in the Quirimbas archipelago were visited in order to collect relevant information on the fishery through interviews with fishermen and biological samplings. The octopus population presented a wide size range, being bigger individuals in the northern sampled area. The estimated sizes at first maturity (L_{50}) were 8.6 and 14.3 cm, for males and females respectively, corresponding to average weights of 360 and 1260 g. The species presented a long reproductive period, extended throughout the year, with a spawning peak between April and August. Abundances showed a clear spatial and temporal pattern, with lowest yields in the southern area, probably in relation to the higher fishing pressure. The estimation of average annual catches was around 2.000 tonnes, value that indicate the need to implement management measures for the sustainable exploitation and conservation of this resource.

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Cephalopod discard estimates from the hydraulic dredges fleet operating in the Gulf of Cádiz (SW Spain)Luis Silva, Ana Juárez and Miguel CojanSpanish Institute of Oceanography, Oceanographic Center of Cádiz, Spain. E-mail: luis.silva@cd.ieo.es

The fishery of the striped venus (*Chamelea gallina*) in the Spanish waters of the Gulf of Cadiz is carried out by a hydraulic dredges fleet of around 100 vessels, with artisanal characteristics. Annual landings of this only target species are around 3500 tonnes, discarding the rest of caught species. The fishing grounds are located at depths between 4 and 12 m on sandy-muddy shallow waters, along 100 km of coast, in the western of study area. Discard composition was analyzed through a monitoring consisting on monthly samplings onboard hydraulic dredges carried out between April 2008 and May 2009. The estimated discarded biomass reported 23% of total biomass caught by trip (266 kg/trip on average), the rest being biomass of the target species. A total of 92 discarded species were identified, weighed and quantified, corresponding 46 species to molluscs, 26 species to fish, 14 species to crustaceans and 6 species belonging to other invertebrate groups. The molluscs reported 66% in weight, where the three cephalopods species identified contributed with 1.2%, equivalent to 1.9 kg per trip, on average. *Sepia officinalis* was caught in all months, with 1.6 kg/trip, followed by *Octopus vulgaris* and *Eledone moschata*, with 0.23 kg/trip and 0.07 kg/trip, respectively. The distribution sizes for *S. officinalis* showed a wide range between 2 and 28 cm for all period, with average size estimated of 9 cm. The total estimated discard by fleet and year was 25.5 tonnes, which represented 13% of the total landed by the artisanal fleet in the Gulf of Cádiz.

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Assessment of octopus (*Octopus vulgaris*) in the Gulf of Cadiz using environmental parameters

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The Gulf of Cádiz is defined as the area included in the ICES Division IXa. The Spanish South Atlantic Region (Gulf of Cadiz) covers the area from Portugal to Gibraltar strait in atlantics waters. An important artisanal and trawler fishery of cephalopods has been developed mainly focused on the octopus (*Octopus vulgaris*).

Octopus vulgaris is a short life cycle (about 1 year), with a peak of spawn in these area between May and August (Silva et al, 2001) and recruitment period between October-November. The abundance of octopus in this area is related with environmental parameter. Has been showed a relationship between the rain in the previous year and the landing (Sobrin

o et al. 2001), with great variability in the recruitment. The present study we realized a first approach of assessment of Octopus in the Gulf of Cadiz. We used a Biomass Dynamic Models (BDM) (Punt et Hilborn, 1996) and Catch-MSY method (Martell et Froese, 2013). Also we modify these BDM, including data of rain as environmental parameter that affect the abundance.

The data used was total landing and effort for trawl fishery and survey data. Since 1993 the Spanish Oceanographic Institute (Instituto Español de Oceanografía, IEO) has been carrying out two series (spring and autumn) of annual bottom trawl surveys (ARSA) in the Gulf of Cadiz (SW Spain). These surveys are aimed at the assessment of the most important demersal resources found in the area.

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Relationship between sea surface temperature and catch fluctuations of the North Pacific giant octopus (*Enteroctopus dofleini*) off Wakkanai in northern Hokkaido, Japan

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This study was conducted to determine if sea surface temperature (SST) can be used to forecast future catch amounts of the North Pacific giant octopus *Enteroctopus dofleini* off northern Hokkaido. The relationship between SST and fluctuations in catches was examined from 1994 to 2014 using regression analysis. Annual catches ranged between 11,942 t (2012) and 21,648t (2003) in Hokkaido, and between 1622 t (2005) and 4256 t (2003) in Wakkanai (a city in northern Hokkaido). In Wakkanai, the fishing season runs from April through December, with most catches occurring during April-July and September-December (during summer, the octopuses are thought to move offshore to deeper, cooler water). A positive correlation was found between catch amounts during April-July and September-December of the same year (0.537, $p < 0.01$). A positive correlation was also found between SST during the spawning season and catch amounts four years later. Warmer temperatures during the spawning and planktonic period tended to be associated with higher catches four years later, but when temperatures were above 10°C, the catches four years later tended to decrease. These results suggest early season catch amounts can be used to forecast late-season catches, and that temperature during spawning and planktonic period can be used in long-term forecasts. Further study is needed to understand the mechanisms linking temperature and future catch amounts.

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A comparison of metal halide and LED lamps with Japanese common squid *Todarodes pacificus* catches in jigging fisheries

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The squid jigging fishery spends a lot of fuel using conventionally illuminated metal halide lamps (MH), leading to reduce profits in the industry due to fluctuations in fuel prices. Light emitting diode (LED) lamps have been proposed as a way to achieve energy savings in jigging fisheries. In this report, we compare the catch performance and fuel consumption of squid jigging boats using MH and LED lamps. Catch performance is evaluated by CPUE (catch per unit effort in numbers caught per line per hour). For the survey, two squid jigging boats were equipped with both MH and LED fishing lamps. One of the boats was lighted with only MHs and the other was lighted with only LEDs during the overnight operation. The illuminants of each boat were alternated daily. The power and layout of each illuminant was determined in advance of the experiment as follows. The combined irradiance of a point 50 m from the center of the boat was equalized between MHs and LEDs. In addition, LEDs were then reconfigured to conform to the distribution of MH irradiance on the surface around the boat. The CPUE using LEDs was estimated to be approximately 6% less than the CPUE using MHs in a generalized linear mixed model (GLMM) analysis. On the other hand, fuel consumption was 28% less while using LEDs than it was with MHs. In consequence, LEDs show potential to be used in the sustainable management of the squid jigging fishery.

P73

Geographical distribution and spawning depth of *Sepioteuthis* sp. 1 and sp. 2 in southwest Japan

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Although the big-fin reef squid, *Sepioteuthis lessoniana* Férussac, 1831 in Lesson (1830–1831) is distributed over a broad geographical range throughout the Indo-Pacific region, some studies have shown the existence of three reproductively-isolated species which includes the “Red squid” as *Sepioteuthis* sp. 1, “White squid” as *Sepioteuthis* sp. 2 and “Quacking squid” as *Sepioteuthis* sp. 3 in Japan.

In order to clarify the geographical range between *Sepioteuthis* sp. 1 and sp. 2, we identified about 1,000 squid samples from 19 sites in the coast of Japan using a microsatellite loci and partial nucleotide sequences of COI of the mtDNA. Also, we suggested spawning depth of *Sepioteuthis* sp. 1 and sp. 2 insights from species identification of eggs collected from various depths. Results from DNA-base identification for squid samples showed that *Sepioteuthis* sp.2 was dominant in mainland Japan except Tanegashima Island. From Tanegashima Island, *Sepioteuthis* sp.1 was found from 2 to 50 m depth; in contrast, *Sepioteuthis* sp. 2 was caught from 2 to 30 m depth. Moreover, egg samples from 40-50 m were identified as *Sepioteuthis* sp. 1, although eggs at 30 m depth were identified as *Sepioteuthis* sp. 2. Results of the present study contribute not only to the ecological and biological framework, but also to the conservation and management of a commercially important of *Sepioteuthis* sp. 1 and sp.2.

P74

***Dosidicus gigas* fishery and the environmental variability in the Humboldt Current System**Carmen Yamashiro¹, Marco Espino^{1,2} and Octavio Morón¹¹Instituto del Mar del Perú, Callao, Perú. E-mail: cyamashiro@imarpe.gob.pe²Minister of Production, Lima, Perú

The jumbo squid *Dosidicus gigas* supports an important fishery in the Eastern Pacific Ocean, and more than 50% of the total landings came from the artisanal fleet of Peru and Chile on the last 10 years. It is analyzed the environmental changes and their effects on *D. gigas* fishery in the Humboldt Current System during 1950-2014, using the data series of the Pacific Decadal Oscillation (PDO), Southern Oscillation Index (SOI), Multivariate ENSO Index (MEI) and Niño 1+2 Anomalies, and their relationships to *D. gigas* landings. Also, the evolution of the fishery, biomass and CPUE of jumbo squid in Peru is described related to the environmental changes. Landings increase moderately toward the end of a warm period in 1990-1995, were low in the interface during drastic environmental changes due to La Niña 1996 and El Niño 1997-1998, and were higher during cold conditions since 1999 to 2014. The largest catches, biomass and CPUE were recorded between small to moderate environmental variations. On the other hand, the oxygen concentration was higher in the surface layer of 50 m in the coastal areas since 1999 that enabled the high availability of jumbo squid for the artisanal fleet, that reached high catches in the years 2002-2014 with a maximum of 680 thousand tons in 2008. The high environmental variability that is characteristic of the Humboldt Current System affects the availability and abundance of *D. gigas*, so a close monitoring system will contribute for an appropriate squid fishery management.

P75

Species identification of squids caught by bottom trawl in the Northwest Pacific Seamount on July 2014

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The Northwest Pacific Seamounts area is good fishing ground for bottom fisheries. Many squids were caught by bottom trawl on July 2014. The morphological structure of caught squid is similar with that of Japanese flying squid. But there is no record that they distributed in the Northwest Pacific Seamounts area. Therefore, in this study, species identification of these squids was conducted by DNA barcoding.

Mantel lengths (mm) of these 30 squids were measured in laboratory. About 1g of muscle for DNA analysis is taken from each sample, they were reserved in ethanol. DNA extractions from their muscles were conducted by Quick Gene (Wako Co.). Mitochondrial 16S and COI regions were amplified using universal primers by PCR method. Blast analysis was conducted using these sequences, and we conducted species identification.

Sequences of mitochondrial 16S and COI regions on unidentified squids were almost same as those of Japanese flying squid *Todarodes pacificus* from the results of Blast analysis. It is first report that Japanese flying squid *Todarodes pacificus* were distributed in the Northwest Pacific Seamounts area.

These mantel sizes were ranged 132.4 and 182.4mm, and average size was 156.0mm. Judging from the size, it was considered that these Japanese flying squids were born in winter. It is thought that biomass of Japanese flying squids born in winter 2014 was very large and the flow quantity of Kuroshio current in 2014 was strong, therefore it was considered that they migrated to the Northwest Pacific Seamounts area.

Session: Genetics and evolution

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Comparative visual ecology of cephalopod from different habitats

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Investigations of vision and visual pigment evolution in aquatic visual predators focus on fish and crustacean, whereas cephalopods have been largely ignored. Since the first cephalopod opsin was sequenced in late 80's, a growing number of sequenced cephalopod opsins have become available. However a large portion of these sequenced cephalopod opsins do not examine the spectral absorbance (λ_{max}) and cephalopod opsin evolutionary history and functional change remain largely unknown. Here we document that 8 coastal cephalopods are monochromatic using photoreceptor microspectrophotometry (MSP). A combination of current MSP results, the λ_{max} values previously characterised using cephalopod retinal extracts and the corresponding phylogenetic opsin tree were used for systematic comparisons, examining the adaptations of cephalopod visual pigments to different light environments. An important finding is that spectral tuning shifts in response to different modes of life and different light conditions. Identification of seven amino acid substitution sites across diverse species suggests that these residue replacements are likely responsible in determining the direction and the magnitude of spectral shifts.

P77

Phylogenetics and new species of the lepidoteuthid families (Lepidoteuthidae, Octopoteuthidae, Pholidoteuthidae)Jesse Tyler Kelly and Heather E. Braid

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The lepidoteuthid families are a clade comprised of three deep-sea oegopsid squid families, Lepidoteuthidae, Octopoteuthidae, and Pholidoteuthidae. Although morphologically these families appear dissimilar, marked similarity of certain structures has historically suggested relatedness which has been supported by recent genetic studies. The clade is generally considered to comprise four genera and nine to eleven species, with the majority of these within the stable though unorganised Octopoteuthidae. However, the distribution of taxa within and between the Pholidoteuthidae and Lepidoteuthidae has been considerably debated, with six different classifications having been proposed for their taxa over the last century. The purpose of this study is to resolve the taxonomic confusion of these two families, and further clarify their relationship to the Octopoteuthidae. Initial sequencing has focussed on the mitochondrial genes cytochrome *c* oxidase subunit I (COI) and 16S rRNA, on tissue obtained from active collecting programs in New Zealand and from colleagues around the world. Supplementary sequences have also been mined from GenBank. Full coverage of all recognised taxa within the lepidoteuthid families has been achieved, with most represented by at least two individuals. Preliminary COI results have uncovered that *Pholidoteuthis* c.f. *massyae* comprises three cryptic species, and that a second species of *Lepidoteuthis* occurs in the Pacific Ocean. The construction of multigene trees, with additional genes to be sequenced in the future (e.g., Histone 3, and the nuclear genes 28S and Rhodopsin), is expected to shed light on the relationships between new and already recognised taxa.

P78

The evolution and morphological diversification of bioluminescence in oegopsid squids

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Bioluminescence is prevalent in the dim waters of the deep ocean and serves a host of signaling, camouflage, and searchlight functions, yet the evolution of this trait is poorly understood. Bioluminescent taxa are often closely related to nonbioluminescent ones with no clear patterns in the gains and losses of photophores among species. Oegopsid squids are an interesting example of this, with bioluminescent and nonbioluminescent species occurring in the same genus, as in *Mastigoteuthis*, or in adjacent clades, as in the ommastrephids. Luminescent oegopsids also display an impressive diversity of bioluminescent body patterns. Photophore numbers, shapes, and distributions on squids can be as diverse as the species that possess them, but at a basic level photophores can be found on the eyes, eyelids, dorsal or ventral integument, tentacles, and viscera in varying combinations. Here we use a published cephalopod phylogeny to examine ecological factors that may be correlated with the evolution of bioluminescence in oegopsid squids and the morphological diversification of photophore patterning in this group.

P79

Exploring the global population of the giant squid, *Architeuthis dux* (Steenstrup, 1957), using RADseq genetic markers

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The giant squid, *Architeuthis dux* (Steenstrup, 1957), is one of the largest molluscs in existence, and a highly popular figure in myths and popular culture, yet its biology has remained mysterious. We published an earlier population genetic study on the giant squid using 44 mitochondrial genomes in 2013. The results of this study were intriguing, but they left many remaining questions to be answered regarding the structure and dynamics of the population. Here we performed a follow up study on the same sample set, using several thousand markers obtained by a RADseq approach, in an attempt to increase phylogenetic resolution. We analysed 32 individuals of giant squid with a world-wide distribution using the GBS pipeline available at the Genomic Diversity Facility at Cornell University. We then used the ANGSD software to obtain a set of roughly five thousand independent nuclear genetic markers, which were used to look for possible large scale population structure and, as most of the samples had accompanying information on sex of the individual, we additionally experimented with a search for sex determination loci.

P80

Multiplex COI haplotype-specific PCR for rapid molecular identification of *Sepioteuthis* cf. *lessoniana* and its application to population structure analysis in TaiwanKang-Ning Shen¹, Jeng-Jia **Ye**¹, Ya-Hsien Wang² and Chia-Hui Wang²¹ Center of Excellence for the Oceans, National Taiwan Ocean University, Taiwan.² Department of Environmental Biology and Fisheries Science, National Taiwan Ocean University, Taiwan. E-mail: chwang99@mail.ntou.edu.tw.

The bigfin reef squid, *Sepioteuthis* cf. *lessoniana* is widely distributed in the Indo-western Pacific Ocean, and is one of the high economically important squid species in Taiwan. According to other population genetic structure study, the existence of three cryptic lineages of *S. cf. lessoniana* around the Indo-Pacific Ocean had been reported. In order to rapidly identify three lineages in a short time, multiplex COI haplotype-specific PCR (MHS-PCR) is one of the methods developed for species identification. This method depend on the DNA barcode of bigfin reef squid for lineage specific primer design, it can identify the different lineage rapidly based on the size of their PCR products. By using this method, nearly 200 *S. cf. lessoniana* individuals collected throughout Taiwan were analysed in order to reveal the phylogentic relationship and proportion of three lineages within *S. cf. lessoniana* in Taiwan. We used mitochondria gene (cytochrome oxidase subunit I, COI) to examine . Phylogenetic analyses using maximum likelihood methods indicated that three lineages within *S. cf. lessoniana* were found, and the highest proportion was lineage B (57.2%), followed by lineage C (35.8%) and lineage A (7%). Lineage A were the dominant in tropical southern Taiwan, while lineage B and C distributed mainly in other regions of Taiwan. In future, we can use it on identification of three lineages, and supply more applications with other studies.

P81

What the complete mitochondrial genomes can do in the identification and evolution studies on octopods?Rubin Cheng^{1,2}, Xiaodong **Zheng**^{1,3} *, Yuanyuan Ma¹, Xiaoying Zhang¹ and Qi Li¹¹ Fisheries College, Ocean University of China, Qingdao 266003, China. E-mail: xdzheng@mail.ouc.edu.cn² College of Pharmaceutical Science, Zhejiang Chinese Medical University, Hangzhou 310053, China³ Institute of Evolution and Marine Biodiversity, Ocean University of China, Qingdao, China

Octopodidae is the largest family in Cephalopoda, which includes at least 374 nominal species with two eyes and four pairs of arms. Although octopods display a wide diversity in skin coloration, behavior and life strategies, the hard structures for specimens are insufficient. This caused difficulties to investigate the phylogenetic relationships only through morphological characteristics. In addition, the higher-level systematic relationships within the octopod group remain unclear and are difficult to establish based on only morphological data. Besides barely octopods fossil record, higher-level systematic relationships within the octopod group species limits and identification are difficult to establish.

While metazoan mitochondrial (mt) genomes extensively used for studying phylogenetic and evolutionary relationships among marine species. Here, we determined the complete mtDNA sequences of seven octopods by PCR primer walking, including *Octopus minor*, *O. conispadiceus*, *O. nanhaiensis*, *O. cyanea*, *Cistopus chinensis*, *C. taiwanicus* and *Callistoctopus luteus*. Results clearly indicated that Mt genomes were circular duplex molecules ranging in size from 15,706 to 16,027bp, encoding 37 genes. All octopuses shared highly consistent gene re-arrangement. The phylogenetic analyses based on 13mt protein-coding genes revealed that the currently sequenced octopus could be divided into three groups: Group I long-arm Octopuses (*Octopus minor*, *O. nanhaiensis*, *Callistoctopus luteus*); Group II mid-arm Octopuses (*Octopus cyanea*, *O. fangsiao*, *O. vulgaris*, *Cistopus chinensis*, *C. taiwanicus*, *Amphioctopus aegina* and *A. fangsiao*) and Group III (*O. conispadiceus*). Furthermore, based on the mtDNA sequence data, we also found that the species in Genus *Octopus* were classified incorrectly, which were previously identified only by morphological characters. It is necessary to re-establish the phylogenetic relationships of Octopoda based on the mtDNA genomes, compare the common and different morphological structures of octopods, and finally determine the most conserved and reliable morphological data for the identification and classification. Furthermore, the morphological data evolution history of octopus could be also studied via phylogenetic analysis.

Overall, our data will be significant important in refining the phylogenetic relationships within Octopodiformes, and enriched the resource of markers for systematic, population genetic and evolutionary biological studies of Cephalopoda.

Session: Life history

P82

Effect of population density and water temperature on growth and maturation in squid *Illex argentinus*

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It is well known that an increase in population abundance and ambient temperature decreases growth rates and size-at-maturation in long lived marine animals. However, density dependent growth and effect of temperature on size-at-maturation are poorly studied in short lived species such as squid. An abundant squid *Illex argentinus* (Ommastrephidae) was chosen for the present study. This squid is one of the main commercial marine resources exploited by international fleets on the Patagonian Shelf (Southwest Atlantic). It is highly migratory and consists of several cohorts hatching at different seasons. Sizes and maturity were studied separately for both sexes in the most abundant winter-spawning South Patagonian stock of *I. argentinus* in March, i.e. in the middle of their feeding periods in waters around the Falkland Islands, between 1989 and 2015. Standardized catch per unit effort (CPUE) was used as an index of relative squid abundance and sea surface temperatures (SST) were provided by NOAA. It has been found that the mean size of *I. argentinus* is negatively correlated with population density (=density-dependent effect). Size-at-maturity significantly decreases and percentage of mature animals significantly increases when water temperature increases. Abiotic and biotic impacts on growth and maturation of short lived animals are discussed with their possible implications to sustainable exploitation of squid resources.

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Variation in life history traits and statolith trace elements of seasonal cohorts of *Sepioteuthis lessoniana* around North TaiwanTzu-Yun **Ching**¹, Chia-Hui Wang¹ and Chih-Shin Chen²¹ Department of Environmental Biology and Fisheries Science, National Taiwan Ocean University, Keelung 20224, Taiwan² Institute of Marine Affairs and Resource Management, National Taiwan Ocean University, Keelung 20224, Taiwan

The bigfin reef squid *Sepioteuthis lessoniana* is a neritic species widely distributed in coastal waters in the Indo-Pacific regions and is interest to fisheries for its highly commercial value. Population structure of *S. lessoniana* has been studied in Japan and Australia, while relevant study is scarce around Taiwan. In order to understand population structuring of the bigfin reef squid around North Taiwan, squid samples were collected from two locations, Ho-ping Island, Keelung (KL) and Makung, Penghu (PH), around North Taiwan from October 2012 to September 2013. A total of 949 squids were examined with mantle length (ML) ranged from 30 to 399 mm and body weight (BW) ranged from 4 to 2565 g. A subsample of 620 squids was aged by statolith microstructure. The age of the squids in KL (55 to 183 d) is similar to those in PH (77 to 186 d). The squids in KL almost hatched year-round, except January and December, while no apparent pattern can be concluded for those in PH. Two seasonal cohorts were identified, spring cohort (hatched from March to May) and autumn cohort (hatched from July to October). Concentration of nine trace elements in the statoliths were measured using solution based inductivity couple plasma mass spectrometry (ICP-MS). A significant difference in the concentration ratios of three elements (Fe, Cu, and Sr) was found for the squids between two locations, while a significant difference in the concentration ratio of two elements (Sr and Ba) was noted for the squids between two cohorts. The results of PCA and cluster analyses showed that greater variation in life-history traits was found for the squids between two locations, while greater variation in the statolith element concentrations was found for the squids between two cohorts. These results implied that *S. lessoniana* in KL and PH might undergo different migration routes during their life history, while seasonal variability in oceanographic conditions was apparent, but likely similar for the two locations.

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Hatching glands in cephalopods – A comparative study

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Hatching of embryos from their eggs begins when unicellular glands release hatching enzymes. The enzymes weaken the chorionic membrane, which is then easily broken by the movement of the embryo. Although numerous morphological and biochemical studies exist on the hatching glands of invertebrates (such as sea urchins, insects, ascidians) and vertebrates (teleosts, amphibians, and mammals), little is known about the morphology of the hatching glands (Hoyle organ) in cephalopod hatchlings.

In this study, the internal gland structure and the external appearance of the Hoyle organ is compared among several cephalopod species (*Idiosepius pygmaeus*; *Euprymna scolopes*; *Sepia officinalis*; *Loligo gabi*; *Sepioteuthis lessoniana*; *Architeuthis* sp.; *Octopus vulgaris*; *Tremoctopus gracilis*; *Argonauta hians*). In almost all cases the glandular system is restricted to the posterior part of the dorsal mantle surface. Only *Octopus* and *Argonauta* lack a specific glandular structure in this body region and the animals apparently use other mechanisms to penetrate the egg layers.

In all decapod species (*Idiosepius*; *Euprymna*; *Sepia*; *Loligo*; *Sepioteuthis*; *Architeuthis*) as well as in *Tremoctopus* only one specific cell type is present in the Hoyle organ, which synthesizes granular material. The secretory droplets are more or less uniform in electron density in *Idiosepius*, *Euprymna* and *Tremoctopus* but exhibit translucent inclusions in the other decapods. The time of gland development, first synthesis of secretory products and later degeneration after hatching vary between the species

P85

Contrasting responses to harvesting and environment of fast and slow life history species

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Owing to their contrasting life histories, cephalopods and elasmobranches are typical examples of fast and slow strategies respectively. Cephalopods have short life spans (1.5-2 years at most) and high population growth rates; they have high production, high fecundity and high mortality rates. By contrast, elasmobranches are long-lived, slow growing and late-maturing, and have low production and low mortality rates. As a consequence of these characteristics, cephalopod and elasmobranch populations have high and low resilience respectively. In this work, we analyse the responses of fast (cephalopods) and slow (elasmobranches) life histories to fishing exploitation (VMS) and environmental conditions (SST, Cha, depth). Three of the most abundant cephalopod and elasmobranch species from the Mediterranean were selected for this study: 1) Cephalopods: *Octopus vulgaris*, *Eledone cirrhosa* and *Illex coindetii*; and 2) Elasmobranches: *Scyliorhinus canicula*, *Raja clavata* and *Galeus melastomus*. Our results confirmed the foreseen contrasting responses of fast and slow life history species to natural (environment) and anthropogenic (harvesting) influences. Even though a priori foreseen, we expected neither the clear-cut differential responses between groups nor the homogeneous sensitivity to the same factors within the two taxonomic groups. Apart from depth, which affected both groups equally, cephalopods and elasmobranches were exclusively affected by environmental conditions (namely SST) and fishing exploitation, respectively. Together with providing empirical evidence to the theoretically predicted contrasting responses of cephalopods and elasmobranches to disturbances, our study also reveals useful information for the sustainable exploitation of these resources under the current Ecosystem Approach to Fisheries Management.

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Vertical distribution of common squid (*Todarodes pacificus*) paralarvae in the northern East China SeaHwa Hyun **Lee**¹, Suam Kim¹ and Chul Park²¹Pukyong National University, Busan, R Korea. E-mail: proxima07@hanmail.net²Chungnam National University, Daejeon, R Korea

Common squid is one of the most important commercial species in The Northwestern Pacific countries. The East China Sea is a major spawning ground for several cephalopods, and the investigation on the vertical distribution of common squid paralarvae in the northern East China Sea was carried out. During April 1999 and October 2000, squid larvae were collected using a 1m² MOCNESS sampler, which divided the water column by 20m interval from the surface to 120m or the bottom. More than 90% of common squid larvae were found in the mixed layer of southeastern Jeju Island. Vertical distribution of paralarvae was influenced by the depth of the thermocline. When the thermocline formed in shallow depths (*e.g.*, October 2000), most paralarvae were located near the surface layer (0-60m). However, if there were no thermoclines in the upper 120 m (*e.g.*, April 1999), paralarvae founded to distribute in more deeper areas (20-80m). Larval size within the water column also was related to the presence/absence of the thermocline. When the ocean was stratified in the surface layer, larval size below the thermocline was statistically significantly larger than those in mixed layer: larval sizes were progressively increased from 1.74mm in the surface to 3.43mm below the mixed layer. The larval sizes through the water column, however, were not significantly different in case where there was no thermocline in the upper 120m. The mean sea water temperatures, where the most paralarvae existed, were 15.4°C and 21.4°C in April 1999 and October 2000, respectively.

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Distribution and relative abundance of common squid *Doryteuthis pealeii* (LeSueur, 1821) along the coast of Tamaulipas in the Gulf of Mexico, Mexico

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Information derived from four fishing cruises during October 2012, January and April, 2013 and February 2014 along the Tamaulipas coast in the Gulf of Mexico is presented. While the design of fishing operations integrated three different exploration methods: hydroacoustic, squid jigging machine and swept area method, all samples are from trawl fishing of one hour in duration, and in three depths (10 -20, 20-30 and 30-40 fathoms) a total of 1523 organism were obtained and analyzed. The results are consistent and show differences in the patterns of distribution and abundance in both time and space (latitudinal and depth). In general, for the coast of Tamaulipas it was observed that squid were recorded throughout the study period along the whole area and in all depths. A pattern of greater abundance in the order of maximum depth is observed, which is consistent in latitudinal order, but more intensely toward the northern portion of the study area. Catches showed greater heterogeneity between depths than latitudinal areas or time. The relative abundance is highest during the months of transition from summer to fall. Additionally a higher frequency in the size structure between 91-142 mm DML was observed, remaining above 20% relative frequency, finally we observed a greater abundance of females over males particularly in the fishing areas nearest to the coast.

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Cephalopods in Central East Atlantic: past and current studies by the IEO-Tenerife team

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Cephalopod fishery in Central-East Atlantic waters takes place on the continental shelf along the coasts of Western Sahara, Mauritania, Senegal, Gambia, Guinea Bissau and Guinea. This area is one of the richest fishing grounds in the world. Spanish bottom trawlers started operating in the sixties and target species are common octopus (*Octopus vulgaris* Cuvier, 1797), cuttlefish (*Sepia hierredda* Rang, 1835 and *Sepia officinalis* Linnaeus, 1758) and squid (*Loligo vulgaris* Lamark, 1798). Until 2012, the main landing port of this fleet was “Puerto de La Luz y Las Palmas” (Gran Canaria, Spain). In the early seventies, scientists from the Centro Oceanográfico de Canarias settled in Tenerife developed a sampling/monitoring system and started conducting exploratory trawl-fishing surveys. In 2003 the EU established the Data Collection Framework (DCF), a framework for the collection, management and use of data from the fishery sector and support for scientific advice regarding the Common Fisheries Policy (CFP). The collected data allow researchers to conduct studies on life cycles, estimation of population parameters as well as dynamics, environmental variables causing changes in abundance, feeding habits and differences between populations. Here we present, by species, the main results and achievements of the team.

P89

Distribution of paralarval squids in the Florida Current

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Distribution patterns across the continental shelf east of Florida were examined for 26 species of paralarval squids during a 5-year study. The paralarvae were collected principally during daytime with a 333 um mesh, 1 m plankton net with a rigid cod-end, a flow meter and a CTD instrument, which recorded conductivity, temperature and depth every 15 seconds. Seasonal samples were taken at intervals of two nautical miles from 2 (coastal) to 26 miles (mid-Florida Current/Gulf Stream) off shore of Ft. Pierce Florida. Discrete-depth samples were also collected at selected stations near-surface, mid-depth and near-bottom. Although most sampling was conducted during daytime, limited day/night comparisons were included. Occurrence patterns are described in relation to coastal, intermediate, and Florida Current (Gulf Stream) water masses by comparing horizontal and vertical squid distribution patterns with distribution patterns of in-situ physical-chemical characteristics. Species composition was analyzed among stations based on the four sampling methods: transect, discrete-depth, drogue-buoy, and fixed-location. Cross-shelf transects detected species associated with particular water masses. Highest species diversity occurred in the Florida Current, versus coastal and intermediate waters. In discrete-depth sampling, higher abundance and diversity occurred in the mid-depth zone. Some paralarvae migrated from daytime mid-depth and near-bottom waters to surface waters at night. Drogue and fixed-site sampling demonstrated pronounced short-term differences in abundance and composition. Three-day, fixed-site oblique sampling also found significant daily variability in total abundance.

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Comparison of growth rates between different cohorts of Little Indian Squid (*Loliolus hardwicki*) based on statolith analysis from Southwest coast of IndiaK.K. **Sajikumar**¹, Gishnu Mohan¹ P.S. Alloyscious¹, Geetha Sasikumar² and K.S. Mohamed¹¹ Central Marine Fisheries Research Institute, Kochi-682018, Kerala, India. E-mail: kksajikumar@yahoo.co.in² Central Marine Fisheries Research Institute, Mangalore Research Centre, Hoige Bazar, Mangalore, Karnataka, India 575001

The Little Indian Squid *Loliolus hardwicki* has a wide distribution, from the northern Persian Gulf, the India and Burmese coasts and throughout Indonesia but with very limited availability. Age and growth rate were studied using statoliths from 127 specimens collected from Southwest coast of India in May, October 2014 and January 2015. Age based on statolith increments revealed *L. hardwicki* has rapid growth rates and the life cycle appears to be less than six months. Estimates of age ranged between 44 to 121 days for female and 52 to 110 days for male. However by grouping the samples according to hatch months, some differences in growth pattern were found. Most of the squid captured in May had hatched between February and March and those captured in October hatched in July and August. January captured specimens had hatch date in October and November months. The monthly growth index was highest in the November hatched specimens (19.58 mm ML) and lowest in the July hatched (11.92 mm ML). All groups, showed sexual dimorphism in size (female larger than male) and daily growth rates in male is slower than females. The average fecundity of species is about 14 egg/ gm body wt in July and August hatched specimens. The October and November hatched specimens had average 23 egg/gm body wt. It is concluded that *L. hardwicki* in the Arabian Sea produce at least three alternating generations each year, which are influenced by seasonal environmental conditions.

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Bio-ecology of horned octopus *Eledone cirrhosa* (Lamarck, 1798) and Musky octopus *Eledone moschata* (Lamarck, 1798) in the Gulf of CadizIgnacio **Sobрино**¹, Luis Silva¹ and Graham J. Pierce²¹ Instituto Español de Oceanografía. 11006 Cádiz Spain. E-mail: ignacio.sobрино@cd.ieo.es² University of Aberdeen. United Kingdom / University of Aveiro, Portugal

In the Spanish South Atlantic Region (Gulf of Cadiz) an important trawler fishery for cephalopods has been developed there, mainly focused on the octopus (*Octopus vulgaris*) but also catching two species of eledonid octopus (*Eledone cirrhosa* and *E. moschata*), with average landings of 500 t in the last three years.

Since 1993 has been carrying out two series (spring and autumn) of bottom trawl surveys (ARSA). Here, we present results from these surveys, describing ecological characteristics such as spatial distribution, length distribution and maturity by season and depth and trends in abundance.

E. moschata is found in the Gulf of Cadiz in a depth range from 15 to 250 m with a maximum abundance at 50 to 100 m. while *E. cirrhosa* occurs a depth range from 50 to 700 m, with a maximum abundance between 100 and 200 m. In November, *E. cirrhosa* abundance is higher in shallower waters. Annual landings of *Eledone* spp. are correlated with overall survey abundance of both species, although the two species showed different survey abundance trends.

Both species show a clear seasonal cycle in length, being larger in March than November, and the largest animals tend to be found in the deepest part of the range. In November the majority of specimens are immature, while some *E. moschata* specimens are mature in March. *E. moschata* probably spawns at the end of March while *E. cirrhosa* spawns somewhat later. There was no trend in maturity by depth.

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Impact of habitat temperature on the lifespan of Japanese common squid *Todarodes pacificus*: The life span may shorten to a half year in a warm condition.

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The life span of cephalopods is known to be flexible by environmental conditions. Some cephalopods tend to grow faster and spawn earlier in a warmer condition. But it is seldom shown these tendencies for Ommastrephid squid, because of difficulties of their reared experiments. In this study, we reared *T. pacificus* from juvenile stage in the different temperature conditions (13oC, 17oC, 20oC and uncontrolled condition), and examined impact of habitat temperature on their lifespan. Samples were obtained from set nets in the Wakasa bay where is located southern part of the Sea of Japan. We started to rear 25, 23, 36 and 32 individuals in the temperature conditions of 13oC, 17oC, 20oC and uncontrolled condition respectively during April and June. The ranges of mantle length of them were 80-100mm (about 4-5 months after hatching) at the start time of these experiments. In these experiments, anchovy and saury were fed once or twice a day. Day and night cycles were set constant to be 12 hours. In these experiments, some males started to mature in the 17oC and 20oC conditions after 20 days, but it took 40days in the 13oC conditions. Matured females were obtained and copulation behavior was observed after 1 month in the 20oC condition. The mantle length of matured individuals ranged 130-147mm, which is very small and rarely observed in the sea. These results show that the life span of *T. pacificus* is also flexible by habitat conditions and may shorten to a half year in a specific condition.

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Is morphology related to trophic niche? : Multivariate growth allometry and diet of the paralarvae and juvenile Japanese common squid *Todarodes pacificus*Kazuhisa **Uchikawa**¹, Tsuneo Goto¹ and Hideaki Kidokoro¹¹Japan Sea National Fisheries Research Institute, Fisheries Research Agency, Niigata, Japan.
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Many animals shift their niche with growth. The transition of niche such as trophic status and habitat often coincides with the ontogenetic changes in morphology. To estimate the transition in morphology, a multi-character approach would be more appropriate than a single character approach. In this study, multivariate growth allometry and diet were examined, and compared between the transition of morphology and diet in paralarvae and juvenile *Todarodes pacificus* (1.1 to 14.6 mm in mantle length; ML) collected in the Sea of Japan. Eighteen morphological characters were measured, and the digestive tract contents were analyzed. Logarithmic values of measurements were subjected to principal component analysis using the covariate matrix. The inflection point of the PC2 scores in relation to ML was defined by a piece-wise regression. The plotting PC2 on ML showed a marked change. An inflection point was 4.8 mm ML, indicating that body form differed markedly before and after the inflection point. Seven paralarvae ranging from 4.5 to 9.1 mm ML ingested a total of 10 mesozooplankton. In most of these cases, fragments of copepod appendages such as *Calanus sinicus* were found. The smallest paralarvae with copepods was 4.5 mm ML. In all paralarvae smaller than 4.5 mm ML, the contents were empty or contained gel-like material, suggesting that a transition from suspension to raptorial feeding occurs at around this size, which is about the size of the inflection point. These suggest that the transition of feeding habit is closely associated with a shift in the allometric growth pattern.

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Atlas of neon flying squid embryonic and paralarval development

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Neon flying squid *Ommastrephes bartramii* is among the most commercially important squids in the North Pacific, but little is known about its early life stages. In this study we investigated its embryonic and paralarval development. Embryos were obtained through artificial fertilization conducted on board the research vessel *Kaiyo Maru* during a cruise around the Hawaiian Islands in December 2013. We established an atlas for the normal development of *O. bartramii* from fertilized egg to advanced stages of paralarvae. Based on morphological features, different developmental stages could be clearly distinguished under a stereomicroscope. Though morphology-based identification of paralarvae to the species level is difficult, the present illustrations can complement other tools for species identification such as photographs and genetic analysis.

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Distribution and abundance of cephalopod paralarvae in north Pacific during 2013 autumn

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Plankton samplings were conducted during a research cruise entitled “Spawning stock of neon flying squid *Ommastrephes bartramii* in the North Pacific”, conducted from October to December 2013 aboard the R/V Kaiyo Maru. Paralarvae were collected from 83 stations using a large larva net (2 m diameter, 0.526 mm mesh size) towed at the surface for 15 min. Twenty-five species were identified and these belonged to family of Sepiolida (Sepiolidae), nine families of Teuthoida (Pyroteuthidae, Enoploteuthidae, Ancistrocheiridae, Onychoteuthidae, Ctenopterygidae, Brachioteuthidae, Ommastrephidae, Thysanoteuthidae, and Cranchiidae), and three families of Octopoda (Octopodidae, Tremoctopodidae, and Argonautidae). Out of 699 specimens (83 stations), *Tremoctopus* sp. was the most abundant species (18.5%), followed by *Pterygioteuthis* sp. (15.2%) and *Ommastrephes bartramii* (13.0%). The family Ommastrephidae included three species: *Ommastrephes bartramii*, *Sthenoteuthis oualaniensis*, *Eucleoteuthis luminosa*. The distributions of these three species differed markedly from each other.

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Micro-anatomical description of the reproductive system of males *Doryteuthis sanpaulensis* (Cephalopoda: Loliginidae)

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Males of *Doryteuthis sanpaulensis* sampled from Nuevo Gulf – Chubut, Argentina (n=27) and from North Coast of São Paulo – São Paulo, Brazil (n=33), had testis, ampulla, sperm duct, spermatophoric complex, Needham's sac, penis and spermatophore removed from their reproductive system to identify cell array with histology methods. The tissues were fixed in 10% buffered formalin, dehydrated and blocked in Paraplast and were sectioned at a thickness of 5µm and stained by PAS, Alcian Blue, Mallory Trichrome and Hematoxylin-Eosin. The simple squamous epithelium is substantially the same on whole organs. In testis was found all stages of cells and Sertoli's cells but not found Leydig's cells. The ampulla shows up to be well irrigated by a blood vessel. The nucleus cells of sperm duct stay nearby at basal domain; the cytoplasm has cilia at their apical domain, probably to auxiliary at movement of spermatozooids along of duct. The spermatophoric complex is made with seven different and well defined structures that looks-like glands. These structures package the spermatozooids inside the spermatophore. The submucosae of Needham's sac is an irregular dense connective tissue layer that permits to resist of stretching and excessive strains. Do not found a muscle fibers on penis, but it has intricate arrangement of collagen fibers, which provides a significant strength and elasticity of its structure.

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Effects of temperature on embryo development and metabolism of *O. maya*

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Octopus maya is one of the most promising candidates for octopus aquaculture due to its holobenthic development. The objectives of this study were to investigate: i) whether the time required for embryonic development of this species can be reduced; ii) whether high or low temperatures affect the size and physiological characteristics of embryos; iii) whether temperature affects the time taken to reach stage XX, using thermal time; and iv) the effects of incubation temperature on hatchling performance, measured as survival after 10 d fasting.

Eggs were acclimated at 18, 22, 26 and 30 °C. Embryos incubated at 30 °C reached stage XX 50 d before embryos incubated at 18°C. A mean value of 596 degrees day-1 was obtained for embryos incubated at 22 and 26 °C where embryo development was optimum. Principal component analysis showed that arm length was the morphological characteristic that separated embryos incubated at 22 °C from the rest of the treatments. Embryos in stage XIX and incubated at 26 °C had a higher metabolic rate than embryos maintained at other experimental temperatures. The best hatchling performance was obtained with embryos incubated at 22 °C. Results indicated that the optimal temperature for *O. maya* incubation is in the range of 22 to 26 °C.

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Timing of digestion, absorption and assimilation of octopus species living in tropical (*Octopus maya*) and sub-tropical-temperate (*O. mimus*) ecosystems

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Octopus culture is at an experimental level because of a lack of knowledge regarding digestive physiology and the form in which nutrients are digested. *Octopus mimus* lives at lower temperatures (15 to 21°C) than *O. maya* (22 to 26°C). The present study aimed to determine the temporality of nutrient digestion, absorption and assimilation in two octopus species living in tropical and temperate-subtropical ecosystems. In both species, the chyme volume had two peaks along the digestive tract: one at the beginning of digestion and the second at the end of the process. These phases were observed in gastric juice enzymes, the flow of nutrients in the digestive gland (DG), the appearance of metabolites in the hemolymph, as well as muscle protein and glycogen levels. Although soluble nutrient flow through the digestive tract to the DG was similar in both species, in *O. mimus*, intracellular digestion in the DG was different than that observed in *O. maya*. It was observed that, in *O. mimus*, lipids were mobilized faster than protein, while in *O. maya*, the inverse process was observed, and suggesting that in low temperatures mobilization could be a biological priority due to its role as membrane protection. Results in *O. maya* showed that proteins have the principal roles in this tropical species, which are the base of the exponential growth rate that those animals have.

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The Toll/NF- κ B pathway: a key to beneficial bacterial symbiosis in cuttlefish reproductive glands

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The female genital apparatus of Decabrachia cephalopods contains two pairs of nidamental glands, the non-symbiotic main nidamental gland (MNG) and the symbiotic accessory nidamental gland (ANG) that participate to egg case formation. Accessory nidamental glands are widespread among cephalopods. They harbour bacterial symbionts. Although the bacterial consortium of *Sepia officinalis* ANG is described, the impact of symbiosis on *S. officinalis* innate immunity pathways is unknown. For the first time in the *Sepia officinalis* model, we evidenced that the Toll/NF- κ B pathway played a key role in invertebrate innate immunity by modulating cell responses to infection by microorganisms. Illumina sequencing of mature female ANG and MNG provided two *de novo* transcriptomes. *In silico* analysis showed the presence of MAMP-binding proteins like PGRP 2&3 that can trigger the Toll/NF- κ B phosphorylation cascade. Interestingly, PGRPs were over-expressed in the symbiotic gland compared to the non-symbiotic gland. They could be involved in ANG symbiosis, as described in *Euprymna / Vibrio* light organ symbiosis. Moreover, we identified several Toll-like receptors (TLR), 9 signal transducers and the NF- κ B transcription factor. Comparison with the non-symbiotic nidamental gland transcriptome highlighted significantly different expression levels of Toll/NF- κ B pathway components in ANG. The signal transducer TRAF 4 involved in down-regulating the NF- κ B pathway was under-expressed in ANG as compared to MNG. We thus provide first evidence that bacterial symbiosis affects the innate immunity of the host reproductive gland by modulating the expression of components of the NF- κ B pathway.

P100

3D reconstruction of the digestive system in newly hatched *Octopus vulgaris* Cuvier, 1797 paralarvae

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Cephalopods are highly carnivorous and voracious animals from the first day of life after hatching. They have a high metabolism and therefore require to feed daily. This factor could be an important aspect to explain the high mortality rate observed in in this stage under culture conditions. The digestive system functionality of cephalopods in larval stages, however, is poorly known. Among cephalopods, *Octopus vulgaris* is a species with high worldwide commercial value showing high larval mortality. It is known that many marine species have no a-complete digestive systems until birth. For a better understanding of nutrition biology of *O. vulgaris* we investigated the digestive tract of hatched paralarvae by 3D histology and present 3D reconstructions for the first time. The digestive system is U shaped similar to the adult. The descending branch has a dorsal position and is formed by buccal mass, oesophagus and crop. The ascending branch, which is located ventrally, is formed by the intestine and the anus. Between both branches are located the digestive gland, the posterior salivary glands and the internal yolk sac. In the curve of the U, a caecum and the stomach can be found. This reconstructions show us that the digestive system is already complete when paralarvae hatch, although some structures such as the digestive gland and radula, need more days to be fully functional. This 3D reconstruction represents a new tool to study the morphology and functionality of cephalopods structures and biology during their first days of live.

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Three-dimensional brain atlas of pygmy squid, *Idiosepius paradoxus*

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Cephalopods have very unique behaviors, for example camouflage and also show some intelligent behaviors such as observational learning. Although these behaviors have fascinated many biologists, the brain functions underlying these behaviors still remain unclear. To understand the mechanisms, we have started to analyze the brain circuit of pygmy squids, *Idiosepius paradoxus*, as a model, because it has the small-size brain among cephalopods and because it can be raised easily in still sea water. In addition, this smallest species has similar behaviors such as camouflage as observed in other squids.

By immunolabeling the central brain with anti-*Drosophila* SYNAPSIN and anti-*Tetrahymena* TUBULIN antibodies, we three-dimensionally reconstructed the whole brain and analyzed the pathways between lobes. We could identify one new lobe in the brain and also revealed that as has been reported on the other cephalopod species, in *I. paradoxus* the juvenile brain is much (more than 35 times) bigger than the hatchling one and the relative size of the vertical lobe, which is concerned with learning and memory of visual and tactile tasks, is four times bigger in the juvenile than in the hatchling.

We are currently developing neurotracing and calcium imaging techniques to visualize the sensory maps in a live squid.

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Effects of metabolic inhibitors on metal accumulation in juvenile of cuttlefish *Sepia officinalis*

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A set of previous works demonstrated that the early-life stages of cuttlefish, *Sepia officinalis*, highly accumulate the trace elements dissolved in seawater. Additionally, the variations of environmental factors such as increasing temperature or partial pressure of CO₂ (pCO₂) modulate the bioaccumulation efficiencies of metal such as Ag, Cd, or Zn. One hypothesis is that the cuttlefish metabolism and more particularly its acid-base regulation is challenged by seawater warming and acidification, changing the gills ionic channels activities that could contribute to the metal uptake in body tissues. Based on the combined use of a unique and powerful radiotracer technique (^{110m}Ag, ¹⁰⁹Cd, ⁶⁵Zn; γ spectrometry) and specific metabolic inhibitors (e.g. verapamil, ouabain, 2,4 dinitrophenol, N-ethylmaleimide), we aim at identifying the ionic channels involved in metal uptake and in the acid-base regulation. Ten one-month old juveniles were pre-exposed for 15 min to each chemical dissolved in seawater. Then, radiotracers were added in order to obtain concentrations of 10 kBq.l⁻¹ of ^{110m}Ag and ⁶⁵Zn and 15 kBq.l⁻¹ of ¹⁰⁹Cd. Cuttlefish were then maintained for 1 hour before being sampled, weighted and counted to determine the uptake rate coefficient. Comparisons between the uptake efficiencies obtained in controlled conditions and under metabolic inhibitors action allowed to describe the mechanisms of metal bioaccumulation in these organisms.

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Giant axon in vivo research model of cephalopod (*Heterololigo bleekeri*)

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Because of their well-developed senses and large brains, cephalopods (*Loligo bleekeri*) are considered one of the most intelligent invertebrate species. The cephalopod nervous system has proven a valuable model for studying about the brain and peripheral nerves. Moreover, the giant nerve fibers of cephalopods are larger than those of other animals and have been used in neurophysiological studies for years.

One drawback of these studies, however, is that they have all been conducted ex vivo because these animals are delicate and difficult to manipulate and maintain in the laboratory setting for extended periods of time. We developed a new method to anesthetize stellate ganglion.

Using this nociception-free procedure contributes, we developed an invasive neurophysiological study model of a cephalopod for example exposing and manipulating a whole giant axon or a stellate ganglion in vivo, instead of taking out a partial giant axon. This model of a cephalopod allows us to perform neurophysiological studies not only to test electrophysiological function of one intact giant axon in vivo, but also to observe an intact axonal transport of a giant axon continuously for example an observation of degeneration and regeneration mechanisms of neurons.

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Age and growth models of squid *Doritheutis pealeii* (Lesueur, 1821) in the Gulf of Mexico, Mexico

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The age and growth analysis of *Doryteuthis pealeii*, from samples collected off the coast of Tamaulipas on the Gulf of Mexico during 2011-2014 was performed. Using growing bands in the statoliths, *D. pealeii* age was estimated in order to describe the pattern of individual growth, assuming a daily band that has been established for several species of loliginids. Since the size structure (DML) between males and females showed significant differences (females and males $\sigma=31.7918$ $\sigma= 4.7702$; Kolmogorov -Smirnov test, $p<0.05$) samples were analyzed separately by gender. The results showed a minimum age of 114 days (3.5 months) and a maximum of 269 days (nine months). Growth models, both asymptotic and non- asymptotic type were adjusted using length and estimated age data. The model parameters were estimated by maximum likelihood method and the model with the best fit was selected using the Akaike Information Criterion (AIC). The growth model that was turned to better fit was the growth model Schnute case 3, which is a non-asymptotic model that better described the growth pattern of *D. pealeii* whit rapid growth in early stages. Males had higher lengths (210 mm DML; 258 days) than females (170 mm DML; 269 days). Both sexes showed negative allometric growth, indicating the size range and weight is not proportional.

P105

Oxygen consumption rates of several juvenile neritic cephalopod speciesSusumu **Segawa**

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The oxygen consumption rate was studied in seven species of juvenile neritic cephalopods, namely, *Sepioteuthis lessoniana*, *Loligo forbesi*, *Lolliguncula brevis*, *Sepia pharaonis*, *Idiosepius paradoxus*, *Octopus maya* and *Amphioctopus fangsiao*. Oxygen consumption rates on an individual basis (M in μl oxygen / individual / h) increased linearly with increase in wet body weight (W in g), which were expressed as $M=aW^b$. Among these species the value b was relatively constant within a range from 0.79 to 0.99, while the value a varied widely from 0.20 to 0.72 with the effects on their life style, basal metabolism and temperature. Feeding activated the metabolism and a period of increment in metabolism indicated the time required for digestion and absorption of the ingested food. In *S. lessoniana*, a certain tendency of diurnal changes in oxygen consumption was observed, that changed with growth. Group effect on oxygen consumption was observed in juvenile *S. lessoniana* which suggested the importance of visual recognition of other individuals within the group of squid.

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Comparative study of ammonia regulation in cephalopods (*Octopus vulgaris* and *Sepioteuthis lessoniana*) by using perfused gillsPo-Hsuan **Sung**^{1,4}, Marian Y. Hu², Dirk Weihrauch³, Jiun-Hong Chen¹, Pung-Pung Hwang⁴ and Yung-Che Tseng⁵¹ Department of Life Science, National Taiwan University, Taipei City, Taiwan. E-mail: r03b21016@ntu.edu.tw² Institute of Physiology, University of Kiel, Kiel, Germany³ Department of Biological Sciences, University of Manitoba, Winnipeg, Canada⁴ Institute of Cellular and Organismic Biology, Academia Sinica, Taipei City, Taiwan⁵ Department of Life Science, National Taiwan Normal University, Taipei City, Taiwan

Ammonia homeostasis is very essential for cephalopods due to their high metabolic rates. However, little is known about this physiological machinery in cephalopods. Here we developed a new technical model to study ammonia excretion pathways by using perfused gills of *Octopus vulgaris* and *Sepioteuthis lessoniana*. We reported that *O. vulgaris* gills accumulated NH_4^+ at levels lower than 300 μM and excreted NH_4^+ at blood NH_4^+ exceeding *in vivo* levels (300 μM). Compared to *O. vulgaris* gills, *S. lessoniana* gills accumulated NH_4^+ at relative lower levels and excreted NH_4^+ at blood NH_4^+ exceeding *in vivo* levels. Further simulating low pH saline as extracellular acidosis (pH 7.2) demonstrated a five-fold increase excretion of NH_4^+ compared to control conditions (pH 7.6) in *O. vulgaris* gills. These results indicated that cephalopod gills are capable of mediating NH_4^+ and responding with an increased NH_4^+ excretion rates in response to acidified conditions. In addition, different species of cephalopods revealed diverse physiological tolerant capacities to cope with NH_4^+ perturbations, suggesting this phenomenon has link to their locomotion and habituations. The present study highlighted a new method to better understand the physiological process of ammonia regulation in highly ammonotelic mollusks.

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Hypoxia-driven protein regulation in muscle mantle tissue of juvenile jumbo squidsKatja Trübenbach^{1,2}, Gonçalo Costa², Carlos Cordeiro² and Rui Rosa¹¹MARE, Laboratório Marítimo da Guia, Centro de Oceanografia, Faculdade de Ciências da Universidade de Lisboa, Cascais, Portugal. E-mail: rrosa@fc.ul.pt²Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, Lisbon, Portugal

The jumbo squid, *Dosidicus gigas*, is an oceanic top predator in the eastern tropical Pacific that undergoes diel vertical migrations into mesopelagic oxygen minimum zones (OMZs) using metabolic suppression. Yet, the underlying mechanisms are poorly understood, as the glycogen storage capacity in squids is greatly limited and their diet highly protein-dominated at well-oxygenated depths. Here, we exposed juvenile jumbo squids to oxygen levels typically found in the OMZ off the Gulf of California (1 kPa at 10°C, 3h) to identify potential energy sources and pathways using proteomic tools. Our findings indicate that glycogen seems the major energy provider under hypoxia, as key metabolic enzymes involved or closely linked to glycolysis were significantly elevated and/or even developed hypoxia-induced isoforms. Yet, the contribution of anaerobic protein degradation might increase under progressing hypoxia (> 3h) due to glycogen storage depletion. Though total protein concentration did not change under hypoxia, some proteins were significantly less abundant and our findings suggest that specific proteins might be degraded via the ubiquitin–proteasome pathway - a process initiated due to reduced protection of the heat shock protein 90 chaperon machinery. Although our data suggest even more hypoxia-regulated proteins, the current lack of database entries for cephalopods strongly limits protein identification and mapping.

P108

Unraveling the life strategies of krakens: A proteomic approachKatja **Trübenbach**^{1,2}, Carlos Cordeiro², José Xavier³, Kathrin Bolstad⁴ and Rui Rosa¹

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The colossal squid *Mesonychoteuthis hamiltoni* (maximum total length (TL): 14 m; total weight (TW): 495 kg) and the giant squid *Architeuthis dux* (maximum TL = 13 m; TW = 275 kg) are the biggest invertebrates known in ocean waters. Yet, their mysterious life-styles are almost undiscovered, as those giants inhabit the inaccessible and dark depths of the oceans. The colossal squid belongs to the family of gelatinous squids (Cranchiidae) and therefore might be an ambush sit-and wait predator, whereas the giant squid, in contrast, is more muscular (family Architeuthidae) and believed to be an active hunter. However, both species managed to adapt to the cold darkness of the deep-sea. The third biggest squid in terms of ML and TW is the jumbo or Humboldt squid *Dosidicus gigas*, a member of the flying squids (Ommastrephidae). It's a powerful oceanic top predator with one of the highest metabolic rates known in the oceans, yet managed to thrive daily in mesopelagic oxygen minimum zones. Under this context, we used proteomic tools to 1) unravel the life-strategies of the kraken squids, 2) identify the major differences in protein structure and composition of mantle and tentacle tissue and 2) shed a light on evolutionary traits and deep-sea adaptation.

Session: Processing and marketing

P109

Analysis of the catches and exports of the Argentine squid *Illex argentinus* during the 2005-2012 period

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The Argentine squid *Illex argentinus* is the most abundant cephalopod in the Southwest Atlantic and a significant fishery resource for Argentina. We analyze the evolution of the catches and exports produced by the Argentine jigger and trawler fleets during the 2005-2012 period. The direct exploitation of the resource is restricted to jiggers while trawlers are allowed to land squid up to 20% of the total catch. The number of jiggers decreased from 83 (2005) to 70 (2012), with a mean of almost 80 boats operating for the whole period. In average, they were responsible for the 83.5% of the total catches. Maximum and minimum catches were registered in 2006 and 2009, with near 292,000 and 73,000 tonnes, respectively. Mean annual catch was 157,031 tonnes. Catches showed an inverse relationship with price oscillation. Maximum export income was reached in 2005 while maximum price was observed in 2011. More than 90% of the catches were allocated in the external market, including different products such as whole squid, tubes with skin, skinless tubes, tentacles, wings and rings, among others. Most of the production of whole squid was sold to the Asian markets (51% in terms of value in 2012) while the European ones bought mainly tubes with skin (96% in terms of value in 2012). Main purchasers of squid were Spain, China, Italy and Japan. A high development of the Asian markets was observed throughout the period analyzed, from 8.6% of the total exports in 2005 to 45.8% in 2012.

P110

Change of distribution and utilization of jumbo flying squid in Japan

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The utilization of jumbo flying squid (*Dosidicus gigas*) began in the early 1990s, and since 2000 in Japan, the demand for use in squid processing has increased. To examine what proportion of squid is used in the Japanese market of squid processed foods, we analyzed the squid products in two large supermarkets and one convenience store in Yokohama in 2013. DNA analysis showed that jumbo flying squid composed 30% or more of all squid food products. This indicates jumbo flying squid has become a major raw squid material in Japanese markets. We consider the reasons why this species dominates the Japanese processed-squid markets as follows:

1. Low price
2. Sufficient and stable supply
3. High versatility in use
4. Decreasing supply of neon flying squid

Squid materials are imported to Japan through two main routes: from China and from other coastal countries (Peru, Chile, Mexico, etc.). In 2014, imports from China were estimated to be about 30,000 tons or more, and consisted mostly of end products. Imports from the other countries were estimated to be about 20,000 tons and consisted of raw materials such as fillets. Imports from China rapidly expanded in the 2000s, however, in recent years the quantity is decreasing as a result of Japanese markets' safety & security concerns and increasing price.

Session: Reproduction

P111

Salt soluble component from the oviducal gland induces chorionic expansion in the ova of the Japanese common squid *Todarodes pacificus*

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In vitro fertilization of squid requires the jelly substance found in the female oviducal gland; yet, the active component of this substance that facilitates fertilization remains unknown. Here, we used biochemical methods to separate the jelly substance of *Todarodes pacificus* (Oegopsida) and *Sepioteuthis lessoniana* (Myopsida family) into four fractions; specifically, two water soluble fractions (Molecular weight (MW) >10000 and <10000), one salt soluble fraction, and one insoluble fraction. The salt soluble fraction of *T. pacificus* induced chorionic expansion (perivitelline space formation), which precedes the normal embryonic development of ova. In contrast, the salt soluble fraction of *S. lessoniana* elicited insufficient expansion of the ova, only producing embryos with high abnormality rates. These results indicate that the salt soluble component(s) (not the jelly-like substances) in the oviducal gland induce chorionic expansion and hatching in Oegopsida, and that these components may be similar in Myopsida.

P112

Characterization of the neuropeptidome of the cuttlefish *Sepia officinalis*: identification of neuropeptides and neurohormones involved in the regulation of egg-laying

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Like many other cephalopods, the cuttlefish *Sepia officinalis* exhibits a wide variety of behaviors such as prey capture, communication, camouflage and reproduction, thanks to a complex central nervous system (CNS) divided into several functional lobes that express a wide range of neuropeptides. However, the diversity of these neuropeptides is crucial to modulate behavior and physiological mechanisms associated with the main stages of their life cycle.

This work focuses on the neuropeptidome expressed during egg-laying, the last step of reproduction. We first identified neuropeptide transcripts through *de novo* construction of the CNS transcriptome using an RNASeq approach (Illumina sequencing). Then we completed the *in silico* analysis of the transcriptome by characterizing and tissue-mapping neuropeptides by mass spectrometry. To identify neuropeptides involved in the egg-laying process, we determined (1) the neuropeptide contents of the neurohemal area, haemolymph (blood) and nerve endings in mature females, and (2) the expression levels of these peptides. Among the 38 neuropeptide families identified from 54 transcripts, 30 were described for the first time in *Sepia officinalis*, 4 were described for the first time in the animal kingdom, and 14 were strongly over-expressed in egg-laying females as compared to mature males.

Mass spectrometry screening of haemolymph and nerve ending contents allowed us to clarify the functional status of many neuropeptides: neuromodulators and/or neurohormones. Besides the data concerning egg-laying regulation in cephalopods, this work brings very new and important structural and expression data about the neuropeptidome of *S. officinalis*.

P113

Sex pheromones of the cuttlefish *Sepia officinalis*: identification, structure and functions

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The cuttlefish *Sepia officinalis* can be considered as a very relevant model to study the reproductive strategies associated with seasonal migrations. By combining transcriptomic, peptidomic and functional approaches, we identified sex pheromone peptides and polypeptides/proteins involved in the parents' breeding behavior, i.e. aggregation in coastal spawning areas, mating, and egg-laying.

Two successive transcriptomic approaches yielded 4 highly redundant transcripts from the oviduct gland, co-expressed and co-secreted with capsular proteins. We called them SP, SP', SP and SP.

Peptidomic analysis of the oviduct gland yielded some of the cleavage products predicted by the precursor structure analysis, ranging between 1 and 8 kDa (Enault et al, 2012). In addition, proteomic analysis of the oviduct gland and egg capsule evidenced high-molecular-weight polypeptides/proteins derived from SP precursors. These analytical results demonstrate the coexistence of two modes of cleavage of SP precursors that generate low-molecular-weight peptides (prohormone convertase cleavages), and also 22-26 kDa polypeptides/proteins released by the eggs into the surrounding medium.

Perfusing organs such as the penis, the oviduct or the gills with egg-bathing water induced a significant change in contractile activity. Synthetic and recombinant pheromones will allow us to carry out contractile *in vitro* tests (oviduct, nidamental glands, penis, gills, etc.) and *in vivo* behavioral tests in "open space" and "Y maze" aquaria to confirm that these sex pheromones actually attract and stimulate spawning, and cause massive aggregations of mates in the coastal mating and spawning areas of Normandy in spring.

P114

Changes in the mRNA expression of gonadotropin-releasing hormone and its receptor during sexual maturation of the swordtip squid *Uroteuthis edulis*

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In vertebrates, gonadotropin-releasing hormone (GnRH) is a key decapeptide to regulate gonadal maturation on the brain-pituitary-gonadal axis. GnRH secreted from the brain stimulates synthesis and secretion of gonadotropins in the pituitary, which yields steroidogenesis in the gonad. In invertebrates, the presence of characteristic GnRH molecules has been reported in several phyla. However, there are few studies focusing on its physiological function in cephalopods. Our recent study has shown that GnRH composed of 12 amino acids (QNYHFSNGWHPG) exists in the swordtip squid, *Uroteuthis edulis*, and it is specifically expressed in the central nervous system (CNS). In the present study we isolated GnRH receptor (GnRHR) gene in the *U. edulis* to clarify the mechanism of action and the function of cephalopod GnRH. Furthermore, mRNA expression levels of GnRH and GnRHR were quantified in the CNS and peripheral reproductive tissues in immature and mature females, respectively. GnRH mRNA was increased in the CNS of mature females. GnRHR mRNA was increased in the CNS, ovary and accessory nidamental gland of mature females, while was decreased in the oviduct and oviducal gland. These results suggest that GnRH in the *U. edulis* is a multi-functional hormone which acts directly on each peripheral reproductive tissue, besides being a neuromodulator in the CNS.

P115

Reproductive biology of the diamondback squid *Thysanoteuthis rhombus* in Camotes Sea Central Philippines

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The diamondback squid *Thysanoteuthis rhombus*, (locally known as Dalupapa), is an epipelagic inhabitant found in tropical and warm temperature waters throughout the world's oceans and is rarely abundant. The said squid is exported to Japan, Korea and other neighboring countries. Because of its high demand the resources are harvested regardless of size. This study was conducted in order to provide information of its reproductive cycle so as not to disturb its spawning process. The study was conducted in Camotes Sea, Central Philippines. The biological characteristics of the squid in terms of length-mantle weight, sex ratio and gonado-somatic index was measured. Results showed that the total length and weight was correlates proportionally with the mantle weight ($r=0.99$; $P<0.05$). In terms of sex ratio, the size indicates its sexual maturity. Results also showed that most of the catches from the month of June to February were predominantly male and only in the months of July to September that females were present. The results of gonado-somatic index revealed that it was only in the month of June that a gonad was observed in both male and female squid increasing in number until the month of August as its peak and decreases in the month of September.

P116

Cellular characteristics of the nidamental glands of the mature female pharaoh cuttlefish, *Sepia pharaonis* (Cephalopoda: Sepiidae)Mong-Fong **Lee***¹, Jing-Duan Huang² and Tsu-Yin Ching³^{1,3} Department of Aquaculture, National Penghu University of Science and Technology, Penghu, Taiwan. E-mail: mongfong@gms.npu.edu.tw² Institute of Marine Biology, National Taiwan Ocean University, Taiwan

The pharaoh cuttlefish, *Sepia pharaonis*, is one of the important cephalopod fishery species in the southeastern Asia. However, the detailed reproductive physiology of this popular species has not been documented to date. The present study is aimed at casting light on the cellular characteristics of the nidamental glands (NG) of the mature female *S. pharaonis*. Paired elliptic and white NG, each with pale brown surface in the center, are located at ventral side within the mantle of mature females. NG are composed of the outer wall with connective tissues and inner septa containing the stroma constituting the frame structures and attached by three different types of epithelial cells. Histological analysis revealed two kinds of epithelial cells within the septa : Alcian blue 8GX-stained mucosubstance-secreting cells and eosinophilic-granule-secreting cells. They may represent two different types of secreting function: cells with high electron-dense secreting granules and cells with low electron-dense secreting granules, respectively under transmission electron microscope (TEM). In addition, both cilia and micovilli are present at the apex of the third type of epithelial cells under TEM. These may represent the transport and absorption functions. We report the ultrastructural characteristics of the different cell types within the NG of the mature female *S. pharaonis* for the first time. These data serve as an important basis for future studies on the cross talk between NG and accessory nidamental glands during maturation in *S. pharaonis*.

P117

First data on female and male reproductive system structure in the Antarctic squid *Psychroteuthis glacialis* (Cephalopoda: Psychroteuthidae)

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The structure of reproductive systems in 9 immature and maturing females (130-315 mm ML) and 4 immature (131-184 mm ML) and one mature male (252 mm ML, with 50 spermatophores, SP) of the squid *Psychroteuthis glacialis* is described. The structure of female reproductive system is similar to most of oegopsids with a difference in main ovary blood vessel position. The ovary structure is simple with secondary and tertiary blood vessels only that probably resulted in low potential fecundity: 10,700-15,600 oocytes. Synchronous development of oocyte fund in the period of protoplasmatic growth changed into asynchronous development during vitellogenesis. Taking into account the tubiform structure of the oviduct, it may accumulate relatively small portions of ripe eggs for intermittent spawning. Spermatophoric complex structure is similar with most of oegopsids. SP length ranges from 34.1 to 38.4 mm. Structure of ejaculatory tube and seminal reservoir of SP differ from those of other studied groups of squids and sepioids but are close to SP of the family Histiotteuthidae. These families have also similarity in some traits of paralarvae and adult morphology. Our data on SP additionally corroborate to close phylogenetic relations between Psychroteuthidae and Histiotteuthidae.

P118

Health status of post spawning *Octopus maya* (Cephalopoda: Octopodidae) females from Yucatán Peninsula, México

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After spawning, females of incirrate octopods usually stop feeding and present maternal care of the eggs until they hatch, but little information is available about the general condition of the female octopus during this period. This study aimed to evaluate the health status of *Octopus maya* females in different days after spawning (DAS). We described a total of 25 *O. maya* females in terms of several physiological (total weight, weight of digestive gland and osmotic capacity), immunological (hemocyte counts, hemagglutination and phenoloxidase activity) and metabolic variables (proteins, glucose, hemocyanine, cholesterol and acylglycerides), and compared the values registered on 1, 10, 20, 30 and 40 DAS. Both metric and non-metric dimensional scaling in combination with a permutational MANOVA were used to analyse differences between levels DAS. On metabolic variables, significant differences only between 1 and 20 DAS ($P=0.015$) were observed. Results on immunological and physiological variables showed non-significant ($P=0.375$) and marginally non-significant ($P=0.0587$) differences, respectively, indicating that the variables were not determined for the time post spawning. However, it's clear that the female's octopus weight tend to decrease with the time post spawning demonstrating that the females use their own reserves and are capable to maintain healthy until 40 DAS at least.

Session: Roles in marine ecosystems

P119

Feeding habits of sperm whales (*Physeter macrocephalus*) in the western North Pacific in spring and summer of the years 2000-2013**Tatsuya Isoda**¹, Tsutomu Tamura¹, Kenji Konishi¹, Hiroshi Ohizumi² and Tsunemi Kubodera³

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The sperm whale is the largest toothed whale and has the capability of deep diving for feeding. It plays an important role in the ecosystem as a bio-pump from the mesopelagic layer because of its high abundance. However little is known about the feeding habits of sperm whales in the western North Pacific. This study analyzed 49 stomachs of sperm whales sampled during the surveys of the Japanese whale research program (JARPN II) from 2000 to 2013. To investigate recent feeding activity, undigested and half-digested squids with lower beak remains were identified by common systematic characteristics and beak morphology. Oceanographic features around sampling positions were characterized based on ARGO's data into Sub-arctic region (SA), Transitional domain (TD), Transition zone (TZ), and Kuroshio zone (KZ). The prey species composition and calculated undigested weight were summarized for each of these. Based on the Index of Relative Importance (IRI), *Belonella borealis* and *Histioteuthis* spp. were the dominant prey in the SA. *B. borealis* and *Galiteuthis phyllura* were the dominant prey in the TD. *B. borealis* and *Taningia danae* were the dominant prey in the northern part of the TZ, while *T. danae* and *Histioteuthis* spp. were the dominant prey in the southern part of the TZ. In the KZ, *T. danae* and *Octopoteuthis* spp. were the dominant prey. Canonical Correspondence Analysis (CCA) showed a strong relationship between prey squid species composition and latitude. These results suggest that sperm whales feed on a variety of squid species in areas with different oceanographic features.

P120

Variations of trace elements concentrations in juvenile of the cuttlefish *Sepia officinalis* along the French coasts of the Bay of Biscay

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Cephalopods, and among them, the cuttlefish are known to highly accumulate metals in their tissue as soon as their early life. During the reproduction season, adults lay their eggs that develop in coastal waters all along the French Atlantic coast. Once hatched, juveniles grow in these littoral nurseries where they find appropriate food during summer and autumn. During this period, trace element concentrations measured in these juveniles reflect the chemical quality of the environment where they live. During the autumn 2013, 156 juveniles of the common cuttlefish *Sepia officinalis* have been collected from the south of French Brittany to the Arcachon Bay. The concentrations of Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, V, and Zn have been measured in the digestive gland and mantle muscle of each sample. Metals analysis revealed that the Pertuis Charentais and the semi-closed Arcachon Bay distinguish from the northern part of the Bay of Biscay with respect to Cd and Co, Fe, Mn and Ni levels, respectively. Additionally, the Hg concentrations recorded in both tissues displayed a southward gradient, suggesting that Hg discharge is more important in the southern part of the Bay of Biscay, or that the warmer waters in the south facilitate the integration and transfer of Hg in local trophic web and thus in juveniles of cuttlefish.

P121

Cephalopods from South Georgia Antarctic waters during the Austral Winter: information from top predators

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The information cephalopod fauna in Antarctic waters is poorly known, whose knowledge is mostly obtained from predator diets. Here, we assessed the importance of cephalopods in South Georgia (54°S, 38°W) in the diets of albatrosses (black-browed *Thalassarche melanophris* and grey-headed *T. chrysostoma* albatrosses) collected shortly after adults returned to their colonies in 2009 (Sept.-Dec.). Based on stable isotopic analyses of the lower beaks, we determined the habitat and trophic level (from d13 C and d15 N, respectively) of the most important cephalopods and assessed the relative importance of scavenging in terms of the albatrosses' feeding regimes. Based on lower rostral lengths (LRLs), the main cephalopod species in the diets of both albatrosses was *Kondakovia longimana*, by frequency of occurrence (F[90 %), number (N[40 %) and mass (M[80 %). The large estimated mass of many squid, suggests that a high proportion ([80 % by mass) was scavenged, showing that scavenging is much more important during the nonbreeding season. The diversity of cephalopods consumed by nonbreeding birds in our study was similar to previous work, but included two new species [*Moroteuthis* sp. B (Imber) and ?*Mastigoteuthis* A (Clarke)]. Based on similarities in LRL, d13 C and d15 N, the squid consumed may have been from the same oceanic populations or region, with the exception of *Taonius* sp. B (Voss) and *K. longimana*, which, based on significant differences in d15 N values, suggest that they may have originated from different stocks, indicating differences in the albatrosses' feeding regimes.

P122

Cephalopod fauna of Antarctic waters: Habitat and trophic ecology from stable isotope analyses

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Although cephalopods play a critical role in marine food webs both as predators and prey, there is a limited knowledge of several basic aspects of their ecology, including their habitat and trophic level, in the Southern Ocean. In this poster, we examined the ecological role of several Southern Ocean cephalopod species by analyzing d13C and d15N values in lower cephalopod beaks obtained from diet samples of wandering albatross *Diomedea exulans* from South Georgia (Atlantic Ocean), and from Crozet and Kerguelen Islands (Indian Ocean). Beak d13C values ranged from -25.7 to -17.9 ‰, and were used to assign different cephalopod species to the subtropical, sub- Antarctic or Antarctic Zones. Beak d15N values were more variable among species, ranging from 2.4 to 13.3 ‰, a difference of ~11 ‰ that represents approx. 3 trophic levels. Differences among islands in isotope ratios in the same cephalopod species (higher d15N and lower d13C values in South Georgia) were attributed to regional oceanographic processes. Antarctic cephalopods occupy niches similar to those found in some pelagic fish, seabirds and marine mammals. As cephalopods are key components in Southern Ocean food webs, these results greatly advance our understanding of the structure, energy and carbon flows in this polar ecosystem.

P123

Distribution of short-finned squid *Illex argentinus* (Cephalopoda: Ommastrephidae) inferred from the diets of Southern Ocean albatrosses using stable isotope analyses

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Climate change can affect species distributions leading to poleward range shifts and invasion of new habitats. The diets of marine predators are a potential source of information about range shifts in their prey. For example, the short-finned squid *Illex argentinus*, a species which is commercially fished on the Patagonian Shelf in the South Atlantic, has been reported in the diet of grey-headed, *Thalassarche chrysostoma*; black-browed, *T. melanophrys*; and wandering, *Diomedea exulans*, albatrosses breeding at Bird Island, South Georgia (54°S 28°W) in the Southern Ocean. Tracking data suggests that these birds may feed on *I. argentinus* while foraging in Southern Ocean waters during their breeding season. This led to the hypothesis that *I. argentinus* may occur south the Antarctic Polar Front. To test this hypothesis, we used stable isotope analyses to assess the origin of *I. argentinus*. Our results show that *I. argentinus* beaks from the diet of albatrosses at Bird Island have $\delta^{13}\text{C}$ values in the range -18.77 to -15.28 ‰. This is consistent with $\delta^{13}\text{C}$ values for *Octopus tebuelchus*, a typical species from the Patagonian Shelf. In contrast, *Alluroteuthis antarcticus*, a Southern Ocean squid, has typically Antarctic $\delta^{13}\text{C}$ values in the range -25.46 to -18.61. This suggests that *I. argentinus* originated from warmer waters of the Patagonian Shelf region. It is more likely that the albatross species obtained *I. argentinus* by foraging in the Patagonian Shelf region than that *I. argentinus* naturally occurs south of the Antarctic Polar Front.

Session: Systematics and biogeography

P124

Larval and juvenile cephalopods from the North Atlantic

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Larval descriptions of cephalopods are poor and by using DNA barcoding to confirm identifications, there is the possibility of better understanding the role of larval and juvenile cephalopods in the ecosystem in the future. We conducted a cruise from Galway, Ireland to St John's, Newfoundland, taking plankton samples along the route, and fishing with a pelagic trawl at stations positioned across a warm core mesoscale eddy on the western part of the transect. Plankton trawls were hauled from just below the deep scattering layer (DSL) to the surface, while the trawl was fished directly in the DSL based on acoustic data. In total, we collected 60 cephalopods, most of which came from the trawls; only nine being obtained from plankton nets. The specimens ranged from larval to juvenile cephalopods, and included 16 species of squid, one sepiolid (*Heteroteuthis dispar*) and one octopod (*Eledone cirrhosa*). The 16 species of squids comprise three enoploteuthids, three histioteuthids, four cranchiids, one ommastrephid, one onychoteuthid, two octopoteuthids, one gonatid and one brachioteuthid. We amplified the Folmer region of cytochrome oxidase subunit I (COI) as a DNA barcode and compared our sequences to those already available on Genbank. In most cases we found good agreement between the morphology and comparative sequence data with specimens conforming to known species. However, several specimens were not easily identified by our initial morphological investigations nor by DNA barcoding and warrant further investigation.

P125

Ecological implications of taxonomy: *Asperoteuthis lui* Salcedo-Vargas, 1999 (Cephalopoda, Chiroteuthidae) as an important prey itemHeather E. **Braid**

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Asperoteuthis Nesis, 1980 is an enigmatic genus of meso-bathypelagic squids in the family Chiroteuthidae Gray, 1849. This genus is characterised by the presence of a secondary fin, the absence of arm and visceral photophores, and unique tentacle clubs that lack suckers on the proximal half. *Asperoteuthis* has four named species, and Clarke's (1980) '?*Mastigoteuthis* A' is currently believed to also belong in the genus. However, recent ecological studies from the Southern Ocean have continued to apply the original, incorrect, vague, and undefined classification of this species as a mastigoteuthid. This is problematic because it underestimates the importance of *Asperoteuthis* in the diet of marine predators, and affects overall estimate of consumed prey biomass. The only asperoteuthid squid known from New Zealand waters, *A. lui* Salcedo-Vargas, 1999, was described from a single, badly damaged, partial specimen taken from the stomach of a ling (*Genypterus blacodes*). The purpose of this study was to resolve the status of *A. lui*, in light of additional, recent, locally collected material. Using a combination of mitochondrial genes and morphology, *A. nesisii* Arkhipkin and Lapitkhovskiy, 2008 and '?*Mastigoteuthis* sp. A' both appear to be synonyms of *A. lui*, with *A. lui* having a circumpolar distribution in the Southern Ocean.

P126

Biodiversity assessment of Mediterranean Sepiolidae by DNA barcoding

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DNA barcoding, a method for molecular identification at species-level, has been developed and widely used in many biological disciplines. It is especially useful for taxonomically-difficult groups which need of highly-specialized methods for identification, or those that can only be specifically assigned in some phases of their vital cycle. However, these methods are constrained by the taxonomic reliability of the previous existing data. The family Sepiolidae Leach, 1817 is formed by 15 genera and more than 60 species around the world. Seven of these genera are represented in the Mediterranean Sea, where 16 bobtail squid species are known. Members of the two more diverse genera, *Sepiola* Leach, 1817 (with 8 species) and *Sepietta* Naef, 1912, could only be identified when they are fully-mature. Previous molecular works have been performed in Mediterranean sepiolids. Nevertheless, inconsistencies in the specific identity between supposedly conspecifics are yet to be resolved. Here, a fragment cytochrome oxidase I of 12 species covering all the Mediterranean sepiolid genera was obtained and their bodies were vouchered to ensure the correct identification of the specimen. We provide a correct barcode for each of these species, resolve some of the previous problematic sequences and evaluate the use of DNA barcoding in this group of organisms.

P127

Systematics of the blue-ringed octopuses (Octopodidae: *Hapalochlaena*) of Australia and the Indo-West Pacific

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The blue-ringed octopuses (genus *Hapalochlaena*) are renowned for their dramatic colour patterns, high toxicity and for causing human fatalities. Despite this high profile, knowledge of the taxonomy, distributions and biology of these distinctive small octopuses has remained surprisingly poor. Historically only two nominal species have been considered valid from Australian waters, *H. fasciata*, and *H. maculosa*, with a third (frequently attributed to *H. lunulata*) requiring resolution. Through a multi-year Australia-wide SCUBA diving survey and extensive review of museum collections and fisheries catches, over 800 blue-ringed octopus specimens have been examined. At present this study has increased the number of blue-ringed octopus species recognised as occurring in Australian waters to 12, with many extending north into the waters of the Indo-West Pacific.

P128

Assessing purported anti-tropical distributions of Australian blue-ringed octopuses (Octopodidae: *Hapalochlaena*)

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Morphological similarities between warm temperate *Hapalochlaena* species in both hemispheres have lead to previous suggestions of anti-tropical distributions, particularly for 'blue-lined octopuses' typically treated under the name *H. fasciata*. Recent examination of purported anti-tropical species in other animal groups have however indicated cryptic sister taxa rather than single species separated by intervening tropical waters. Morphological examination of two northern hemisphere *Hapalochlaena* species, has found both to be morphologically distinct from the southern hemisphere taxa to which they are regularly attributed. While the northern hemisphere 'blue-lined species' appears to be a close sister species to its southern hemisphere counterpart, the 'blue-ringed species' (regularly treated as *H. maculosa*) shares only crude similarities with the southern taxa and is more closely aligned with the 'mid-ring' blue-ringed octopuses that occupy the equatorial Pacific.

P129

Soft-bottom octopuses of the shallow-waters on Taganga Bay, Tayrona Sector, Southern Caribbean

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²

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Tropical octopuses, especially soft-bottom inhabitants, are a poorly understood taxa. Historically, little research around the world has been carried out on them. Along the Colombian coast in the southern Caribbean, few species are known for this habitat, namely: *Euaxocephalus pillsburyae*, *Amphioctopus burryi*, and *Callistocephalus macropus*. This study centers on Taganga bay. Nine specimens representing three different genera and species were collected manually in shallow waters. An uncommon species for the area is *Amphioctopus burryi*, and two new species of long-arm octopuses: *Callistocephalus* sp. 1, which confirms the presence of this genus in the Southern Caribbean, and *Macrotritopus* sp. b, another sand-dweller similar to *M. defilippi*. Diagnosis and remarks are presented for each species, updating the knowledge for the area. This result points to an unsuspected octopus diversity in a small tropical bay. More research should be done in order to complete taxonomic catalogues and, in the near future, determine their role in the ecosystem.

P130

Modelling the spatial distribution pattern of Japanese flying squid *Todarodes pacificus* during northward migration in the Pacific Ocean

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Oceanographic conditions such as the northern warm-water branches of the Kuroshio Current and warm-core ring have a large influence on spatial distribution of Japanese flying squid during northward migration in the Pacific Ocean. In this study we modeled the spatial distribution of Japanese flying squid from April to September using two kinds of datasets: (1) environmental variables from the ocean model called FRA-ROMS (Fisheries Research Agency of Japan), and (2) occurrence records (date, location) from the database of Fishery Resource Conservation System (Fisheries Research Agency of Japan) and research cruises. Tested models were Artificial Neural Network, Boosted Regression Trees, GAM, GLM, RF (Random forest). The predictive accuracy of each model was compared using cross-validation and the best predictive accuracy was obtained by RF. Important variables in RF which affected the spatial distribution were 200 m depth salinity, sea surface temperature, and sea surface height. We will discuss the relationship between spatial distribution and these environmental variables at this session.

P131

Variation of body morphometrics associated to the selection of male ventral arm transformed to hectocotylus in *Illex coindetii* from the eastern Mediterranean SeaNikolaos Fotiadis and Evgenia Lefkaditou

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The modification of one or more arms to hectocotylus in cephalopod's males may be considered as a secondary feature of sexual dimorphism, while in most cases the position and shape of hectocotylus is a robust character to identify species. In *Illex coindetii* as in all species of the genus *Illex* (Family Ommastrephidae) hectocotylization consists of modifications to either of the ventral arms. The choice of hectocotylus position might be induced by endogenous and /or exogenous factors and be associated with variation in other morphometric characters. To investigate this hypothesis, for *Illex coindetii* from the eastern Mediterranean, male individuals with 11-17 cm range in mantle length, were sampled during bottom trawl surveys carried out in summer of 2014 and 2015. The preliminary analysis presented here, concerns

body morphometric variables including dorsal mantle length, total length, shell length, maximum mantle circumference, length and width of head and fin, length of each arm excluding the ventral ones, length of tentacles and tentacular clubs. Mantle length was used as the reference length upon which indices of the above body dimensions were calculated. According to the results of a stepwise Discriminant Function Analysis, the ratios of the mantle circumference and the left tentacle length to the dorsal mantle length, 80.5% of the examined specimens were correctly classified to males with hectocotylization of left and right ventral arm respectively. Further morphometric analyses of stored hard structures, as well as demographic and molecular analyses have been planned to confirm importance of the above morphometric characters.

P132

A Phylogeny of fossil and living neocoleoid Cephalopods

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Coleoid cephalopod phylogeny is well studied via both molecular and morphological data, yet while some agreement has been reached (e.g. that extant Decapodiformes and Octopoda are monophyletic) many details remain poorly resolved. Fossil coleoids, for which much data exists, have hitherto not been incorporated into analyses. Their inclusion is highly desirable both for the support of neontological phylogenies, to better reconstruct character-state histories, and to investigate the placement of the fossil groups themselves. In this study we present and analyse a morphological data matrix including both extinct and extant taxa. Homology assumptions in our data are discussed. Our results are presented both with and without the constraint of a monophyletic Decapodiformes imposed. When analysed with this constraint our results are strikingly congruent with those from molecular phylogeny, for instance placing *Idiosepius* in a basal position within Decapodiformes, and recovering Oegopsida and Bathyteuthoidea (though as grades). Our results support an Octopodiformes clade ('vampire squid' *Vampyroteuthis* as sister to Octopoda) and an octopodiform interpretation for most fossil coleoids. They suggest the fossil sister taxon to the octopods to be Plesiot euthididae. Most fossil higher taxa are supported, although many genera, especially within suborder Teudopseina, appear para- or polyphyletic.

P133

Oceanic cephalopods collected in the eastern Atlantic Ocean by deep tows with the MOCNESSUwe Piatkowski and Holger Ossenbrügger

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Cephalopods, the majority comprising early life stages, were caught during two expeditions in the eastern Atlantic with (a) RV *Polarstern* in November 2007, and (b) RV *Meteor* in June 2013. The Multiple Opening/Closing Net and Environmental Sensing System or MOCNESS was the major plankton trawl for catching cephalopods.

During the RV *Polarstern* cruise we used a 1m² version with 335µm meshes in the cod end) as well as a large 10m² version (3 mm meshes); and during the RV *Meteor* cruise a 1m² version (2 mm meshes). During both expeditions the 1m² MOCNESS was fishing through eight layers down to a maximum depth of 1,000m; the 10m² MOCNESS went down to a maximum depth of 5,083m while fishing through four different layers. In total, we caught 155 cephalopods; 67 specimens in 2007, and 88 specimens in 2013. The majority of the cephalopods were Enoploteuthidae, Pyroteuthidae, and Cranchiidae. At about 3°30'N, 15°00'W, the 10m² MOCNESS sampled a 66 mm (ML) sized specimen of *Magnapinna atlantica* in about 1,000-2,000 m depth. Our poster will illustrate the impressive diversity of this relatively small collection and inform on the distribution patterns of the cephalopods. The RV *Polarstern* expedition contributed to CMarZ (Census of Marine Zooplankton) which has been a sub-project of the Census of Marine Life CoML); whereas the RV *Meteor* cruise was part of a long-term project to study the biogeochemistry interactions in the tropical ocean, in particular the oxygen minimum zone (OMZ) in the tropical eastern Atlantic.

P134

Cephalopod paralarvae of Northeast Atlantic (CEPAR): a barcoding project to uncover cephalopod diversity from plankton samplesAlvaro **Roura**¹, Michael Amor¹, Dirk Steinke², Angel Guerra³¹ La Trobe University, Bundoora, Melbourne, Australia. E-mail: a.rouralabiaga@latrobe.edu.au² Biodiversity Institute of Ontario, University of Guelph, Guelph, Ontario, Canada³ Instituto de Investigaciones Marinas, Vigo, Spain

The cephalopod paralarvae collected (n=377) during the surveys CAIBEX-I (NW Iberian Peninsula) and CAIBEX-III (Morocco), were identified in collaboration with the Barcoding of Life Database (BOLD) within the project CEPAR: Cephalopod paralarvae of the Northeastern Atlantic. The project collected COI data for 93% specimens (133 NW Iberian Peninsula, 244 Morocco). The paralarvae collected off the NW Iberian Peninsula included 98 octopods (*Octopus vulgaris*), 15 ommastrephids (12 *Todaropsis eblanae*, 2 *Todarodes sagittatus* and 1 *Illex coindetii*), 16 loliginids (10 *Alloteuthis subulata*, 4 *A. media* and 2 *Loligo vulgaris*), and 5 sepiolids (*Sepiola tridens*). The cephalopod assemblage collected off Morocco was more diverse with COI data for 221/244 paralarvae: 115 loliginids (107 *Alloteuthis media* and 8 *A. subulata*), 35 *Octopus vulgaris* (n = 25), 9 sepiolids (5 *Rondeletiola minor*, 2 *Heteroteuthis dispar*, 1 *Sepiola atlantica* and 1 *S. ligulata*), 18 onychoteuthids (*Ancistroteuthis lichtensteini*), 11 pyroteuthids (*Pyroteuthis margaritifera*), 28 enoploteuthids (18 *Brachioteuthis riisei*, 8 *Abraliopsis morisii* and two undefined), three cranchiids (2 *Liocranchia reinhardtii* and 1 undetermined), and one undetermined ommastrephid. Surprisingly, 11 paralarvae showed 85% similarity with *Ancistrocheirus lesueurii* the only member of the ancistrocheiridae family. A detailed investigation of the BOLD database revealed three clades within this family: the new ancistrocheirid found in this project, an undescribed species from South Africa and *A. lesueurii*. Genetic analyses revealed that all *O. vulgaris* collected belong to the same population, but they greatly differed from other “*O. vulgaris*” sequences from Japan, South Africa and Brazil suggesting the existence of cryptic species.

P135

Genus *Pyroteuthis* in Northwest Pacific (Oegopsida: Pyroteuthidae)

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Pyroteuthidae is a small-sized, warm water, epi- to mesopelagic cephalopod group, characterized by having silvery body color, various visceral photophores, pearl-like optic photophores and oval fin. At least, seven species in two genera of pyroteuthids were known from the world ocean, while its taxonomic information is scarce and very limited in Northwest Pacific. In the present study, 460 pyroteuthid specimens from Northwest Pacific were examined. As the results, two species of *Pterygioteuthis* (*P. giardi*, *P. gemmate*) and two unnamed *Pyroteuthis* were found. Two *Pyroteuthis* species were significant and separable each other and other known three congeners by 1) number and arrangement of photophores buried in tentacular stalk, 2) number of photophores on eyeball, 3) number of hooks on hectocotylized arm, 4) spermatophore length. In *Pyroteuthis* sp. A, sexual dimorphism was recognized on number and arrangement of photophores on tentacular stalk.

P136

Cephalopod fauna off southwestern Sea of Japan along San'in district, Japan

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Cephalopods collected by mainly a set net at 35 m depth and several bottom trawls at 100-300 m depth off San'in district in the southern part of the Sea of Japan during 1980-2012 were examined. More than 700 specimens were available, a total of 53 species from 18 families were identified. The registered number, photograph, catch date and locality (including depth in some cases) were given for all specimens, along with a brief note on previous records and systematic remarks for each species. The cephalopods of off San'in district were composed of many warm-water species in the coastal waters which were affected by Tsushima Warm Current. On the other hand, some cold-water squids and octopuses were found from the middle and deep seas below 200 m depth. Fifteen species (28.3% of the total) were new records from this area, and 2 species among them were first records from Sea of Japan. Cranchiidae sp. and *Octopus* spp. of them has been previously unknown or poorly understood.

Session: Other topics

P137

Variation of 210-polonium in the cephalopod community from the Bay of Biscay

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Among natural radionuclides, ²¹⁰Po is the major contributor to the radiation dose received by marine organisms (Cherry and Shannon 1974). In cephalopods, ²¹⁰Po is concentrated in the digestive gland which contains over 90% of the whole body burden of the nuclide. Although previous studies showed that ²¹⁰Po was taken up independently of ²¹⁰Pb, its parent nuclide (Smith et al. 1984), very little is known about the factors influencing its levels in cephalopods. To the best of our knowledge, no studies investigated ²¹⁰Po in many different species at the same time. In the present study, ²¹⁰Po was analysed in 62 individuals from 11 species representing a large range of feeding ecologies and habitats, including oceanic species such as *Teuthowenia megalops* and *Histioteuthis reversa*. Among species, the highest activity was measured in *Loligo vulgaris* (1475 Bq/kg) and the lowest in *T. megalops* (22 Bq/kg). However, considering the habitats (benthic vs pelagic and neritic vs oceanic), no significant differences appeared. At the species level, no differences among sexes were found so both genders were plotted together to test the size effect for the species with at least 8 individuals (i.e., *Eledone cirrhosa*, *L. vulgaris*, *L. forbesi* and *Sepia officinalis*). In the 3 former species, ²¹⁰Po levels decreased significantly with increasing size or weight but not in *S. officinalis*. In squids, this might be related to ontogenic changes of the diet that includes a large proportion of crustaceans (with high Po content) in small individuals to fish (with low Po content) in larger individuals whereas the strong dietary plasticity of *S. officinalis* at all stages of its life cycle (Chouvelon et al. 2011) might explain the lack of decrease of ²¹⁰Po with size. In comparison to the few data, from the literature, the levels of ²¹⁰Po activity in the cephalopod community of the Bay of Biscay were below than those reported in other cephalopods such as *L. vulgaris* (1700-6000 Bq/Kg), *S. officinalis* (1600-2300 Bq/Kg) and *Octopus vulgaris* (810 Bq/kg) by Heyraud and Cherry (1979), and were far below the values reported for the squid *Nototodarus gouldi* from Japan (i.e., 4800-24200 Bq/kg; Smith et al. 1984).

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P138

The Young Cephalopod Researchers (YCR) group: Origin, activities, and expectations for the future

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The Young Cephalopod Researchers (YCR) group was created during the 2012 CIAC in Florianópolis, Brazil. Its main goal is to improve communication between early career cephalopod scientists by establishing a network and collaborations between its members. It is currently composed by young scientists conducting cephalopod research in the fields of population ecology, molecular ecology, biological oceanography, physiology, modelling, fisheries, ecotoxicology, environmental pollution and climate change. YCR is a young group with yet many members to attract and strategies to define, but it has been active in a few international events. During the YOUMARES (Convention for Young Scientists and Engineers) held in Oldenburg, Germany, in September 2013, YCR organized a session entitled “Cephalopods and jellyfish as key species in marine ecosystems: a spatio-temporal multi-scale approach”. For this year’s YOUMARES in Bremen, Germany, another cephalopod session is being organized, entitled “Cephalopods and society: Scientific applications using cephalopods as models”, which will entail presentations by young scientists and keynote talks from professionals. Members of the group were also able to publicize YCR during the World Congress of Malacology in the Azores in 2013 and during the Mollusca in Mexico in 2014. Currently, YCR has 25 registered members from several countries in Latin America, Europe and from Australia, but with this presentation we hope to reach out to a greater audience and increase both the number of members and countries represented in the group, broadening also the range of research interests/skills within our network.

P139

How squid sperm determine directional changes in a pH gradient

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Squid sperm from sneaker males are known to make self aggregation by response to CO₂. This mechanism underlying this phenomenon is considered that respiratory CO₂ from sperm is hydrated by the flagellum-membrane-bound carbonic anhydrase into bicarbonate and H⁺, and CO₂ produces pH gradient. Sperm change direction when it goes for negative pH gradient. We investigated how sperm response for several pH gradients using glass pipettes in the gel pH adjusted. As the result we discovered positive directional changes against negative pH gradient. We analyzed single turn-and-run swimming episode of sperm. According to our analysis positive directional changes occur when sperm sense pH gradient. This phenomenon may be new chemotactic style.

P140

Innovation of artificial spawning zones on the seabed in Japan

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Cephalopods are an important marine resource in Japan, so a steady supply and increased production are needed. As result, resource management has been advanced, and various actions such as fishing ground maintenance have been conducted from the viewpoint of increasing production. This poster will report on examples of fishing ground maintenance technology being used in Japan to increase cephalopod production and summarize some of the results.

P141

**Notes on the longtime transportation of the Japanese pygmy squid
Idiosepius paradoxus (Cephalopoda: Idiosepiidae)**

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Pygmy squids, genus *Idiosepius*, are the smallest living cephalopods inhabiting coastal beds of seagrass and algae. They are not important for fisheries science, but ideal model animals for embryology and behavioural science because of pygmy squids are kept easily in a small container. We investigated the effect of reasonable price delivery on the Japanese pygmy squid (*Idiosepius paradoxus*) survival for conducting experiments in remote laboratory. Squids kept by various density conditions' one little oxygen packs (2.5 to 50 individuals per litter), which were transported from Aichi to Hokkaido, Japan (at about 800km distance) by delivery service on December 2007, April and July 2008. Change of water and air temperatures during delivery were measured by data-loggers. Ammonia concentration and survival number of squids were recorded after parcel arriving. We also checked the survival and water quality change by keeping in oxygen packing in the laboratory on December 2007 and April 2008 to exam how long squids survive in delivery condition. It took about 40 hours until the squids arrived at Hokkaido. Although ammonia concentration was increased with the density, all squids survived in every condition and season. Laboratory experiments showed that squids survived over 240 hours (10days) in 10 individuals per litter oxygen pack condition in winter. These results suggest that we can transport Japanese pygmy squid keeping in good condition using home delivery service.

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Mass findings of giant squid in Japanese coastal waters of the Sea of Japan during January 2014 - March 2015

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A total of 57 giant squid, *Architeuthis dux*, were found between January 2014 and March 2015 in Japanese coastal waters in the southwest Sea of Japan. Occurrences were especially high around Sado Island and in Toyama Bay. All of the squid occurred individually, and 25 were found alive. The occurrences were categorized into three groups: 1) washed ashore on a beach or found floating at the surface close to a beach (19 individuals), 2) caught by a fixed net set in coastal waters between 50 and 150 m depth (27 individuals), and 3) caught by a bottom trawl or bottom gillnet fishery several km offshore between 200 and 300 m depth (11 individuals). Two size groups were recognized - one ranging between 80 and 150 cm DML with a mode at 110 cm and another larger than 160 cm DML. The smaller group had a nearly even sex ratio and the larger group comprised all females. The relationship between occurrence and water temperature will be discussed.

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The historic study of recent cephalopod investigations: a topical and multiple-faceted problem that demands development

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There are two well-known sayings: “Study the past, if you would divine the future” (Confucius) and “Science cannot successfully develop without studying its history”. The study of cephalopods has been developing for the last 250 years, but its history is mostly absent. There are only fragmentary and local publications on this topic. We lack basic information about our outstanding researchers and, perhaps more importantly, we lack a holistic picture of the evolution of ideas in cephalopod biology. We advocate using the experience and knowledge of leading representatives of the older generation to describe this story and aspire towards the development of such a research program within the framework of CIAC. Reviews could follow two strands. (1) Stories of outstanding academic careers and the development of cephalopod research in different countries could inspire future cephalopod researchers across the globe. (2) Historical reviews of research, for example of the high quality morphology and embryology of the early 20th century, of the development of fisheries, practice, assessment and management, and of the general evolution of the paradigmatic ideas on cephalopod biology, have the potential to support more rapid progress in currently developing fields. This process began in 1972 with the translation of key works into English and has continued recently with a review of outstanding female researchers and a truly international collaboration on squid fisheries.

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Are research vessels laboratories on water? What challenges face fisheries research under animal welfare regulation?

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The new EU legislation on the protection of animals used for scientific purposes (directive 2010/63/EU) was adopted on 22 September 2010, having already been transposed to the national legislation of every EU member state. The Directive is firmly based on the principle of replacing, reducing and refining the use of animals for scientific purposes (the Three Rs). Exclusions are clearly made in the legislation, but none cover the objectives of fisheries research surveys aimed at assessing population size of the main fisheries resources, when these employ fishing methodologies. When conducting surveys in Europe, a number of cephalopod species are routinely killed without significant research benefit. Moreover, cephalopod fisheries are not significant in many EU nations, resulting in a measure of relaxation in identification of species, collection of biological data, etc., from surveys. It is therefore very arguable that most surveys are not abiding by the new legislation when they employ practices which are oblivious of the welfare of protected species and of Three Rs principles. Research vessels are floating laboratories, where the most stringent research methodologies should be applied just as much as on land. Furthermore, publication of research conducted under those circumstances must be as demanding as that resulting from land-based laboratory research. As cephalopod researchers, as reviewers and as editors, we must strive to improve research survey practice by developing methodologies that limit animal numbers, replace fishing by visual or other surveys, or that may more strongly rely on the use of fisheries dependent data, thereby reducing the need for fishing surveys.

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Recent cephalopods on postal stamps: diversity and global distribution

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Cephalopods are charismatic animals and the most sophisticated mollusks. During their evolution they have settled nearly all marine habitats. Their representatives are found in the deep-sea, in coastal lagoons, in the polar oceans, in the high seas, the shelf seas and they can even invade brackish water. They are movie stars where they mostly have to play voracious and scary monsters. They count as top athletes due to their unique physiological power; and they are quick-change artists because of their ability to change body form and colour patterns within milliseconds. Fortunately, or not, they are also an important food resource all over the planet; but some species are heavily exploited and there are stocks that show signs of overexploitation. All these attributes are responsible for their worldwide appearance on postal stamps. Here, we present a collection of postal stamps that shows cephalopods as real cosmopolitans. We will use this information to highlight their cultural importance, their systematics, their great diversity and wide-ranging distribution patterns in our seas; and we will point out some unexpected hotspots of their abundance.

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Exploring shared natural and cultural changes in coastal communities along squid migratory routes by the Tsushima Current Kaimin Network

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Pacific flying squid *Todarodes pacificus* migrate along the Tsushima current through the Japan Sea and East China Sea. The status of squid populations affects marine ecosystem structure, fishery economics and culture. Coastal communities facing these seas have historically been connected by the squid fishery. This current suffers various environmental and ecological problems - marine debris, fishery resource degradation and population decrease. Especially transboundary problems require discussion and proposals by a wide-ranging human network. Governmental and industrial organizations and committees have already been set up. However, approaching environmental and economic aspects from a consumer standpoint can potentially transcend boundaries and social sectors. Thus, academics and citizens of these areas formed a network for sharing problems and experiences, the Tsushima Current Kaimin Network. Originally “kaimin” is a Japanese term meaning sea people who live in coastal communities and cities. The Tsushima Current Kaimin Network can link interested communities in Japan from southwestern Kyushu to northern Hokkaido. West Tsushima and the north Tsugaru straits are good monitoring sites for comparative studies of the environment and squid fishery management under climate change.

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The effects and subjects with the cooperation among industry, government and universities in region– The advancement of Hakodate squid industrial cluster

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Hakodate city, a model of this research, is famous for the squid landing in Japan and the food processing industries mainly with squid are prosperous there. The amount in sales of processing industries is about 120 billion yen (for squid processing industries; about 50 billion yen). Also, the amount is pretty large in gears of squid vessels, repairs and shipbuilding industry. Now Hakodate forms the cluster of squid industries. Hakodate is trying to promote plans to become “The Conception of International Fisheries and Ocean City, Hakodate”, it is necessary to use squid for regional revitalization. Then, universities, industries and people have practiced following activities with cooperative.

The universities study on physiology, ecology, resources, fishing and process with the squid. The industrial circles promote efficiency and add-valuable for squid industries. The people participates the squid meister course to register specialists of squid. Moreover universities built the virtual Hakodate squid research institute site and practice lecture meeting on relation of estimation of squid fishing in next year. This study discusses on the effects and subjects of these cooperation activities.

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The Sasaki cephalopod collection at the US National Museum of Natural History

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The Sasaki collection at the National Museum of Natural History (USNM) comprises cephalopods examined by Madoka Sasaki following the 1906 expedition in the northwest Pacific Ocean of the American research vessel *Albatross*. We curated and assessed the conditions of these specimens and photographed the type specimens. This collection nominally includes 57 type specimens in 20 taxa (species or subspecies). In the type collection, there was one specimen in Very Good condition, 24 Good specimens, 19 Fair specimens, four Poor specimens, two Very Poor specimens, five Missing lots, and two lots known previously not to be extant, and zero Not Cataloged lots. Therefore, of the types, 42% are in Good or better condition, and less than a 25% are in Poor condition or worse. In addition to the type specimens, Sasaki identified 292 extant non-type specimens in 34 taxa. In the non-type collection, there were 41 Very Good specimens, 196 Good specimens, 36 Fair specimens, 10 Poor specimens, nine Very Poor specimens, three Empty Jar lots, 16 Missing lots, one Not Extant lot, and six Not Cataloged lots. Therefore, the majority of the specimens (62%) in the Sasaki non-type collection are in Good condition and less than 15% are Poor, Very Poor, Empty Jars, Missing, Not Extant, or Not Cataloged. These final categories will be explained in the poster but essentially comprise 26 non-type specimens in 14 taxa listed in Sasaki publications but not found in the USNM collections.

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Hatching cohort discrimination of swordtip squid (*Uroteuthis edulis*) off northeastern Taiwan in statolith structure and chemical signatures

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Present study analysed statolith chemical signature and microstructure to investigate monthly early life growth variation of swordtip squid (*Uroteuthis edulis*) off northeastern Taiwan, and to discriminate different hatching cohorts by using the statolith elemental composition of individuals at their early life stage. Samples were collected between July, 2011 and December, 2012 from northeastern Taiwan. Radius of natal ring (NRR) and incremental widths (IW) in statolith were measured and correlated to with sea surface temperature (SST). Natal ring radius of statoliths was significantly lower in the individuals hatched in July and August ($p < 0.05$), but no significant difference among the other months. The mean first 30 incremental width was significantly narrower in the individuals hatched in January and February but significantly wider in May ($p < 0.05$). The mean first 30 days statolith incremental width was positive corresponded to temperature, but not in the hottest June and July. A total of 65 statoliths elemental composition of *Uroteuthis edulis* at paralarval stage were qualified. The elemental signatures of statolith can discriminate the individuals from spring cohort (January to August) to autumn cohort (September to December) by using principal component analysis (PCA). Overall, 87.7% individuals can correctly be classified between spring and autumn cohorts by using discriminant function analysis.

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Seasonal and ontogenetic differences in ecological tracer of *Uroteuthis edulis* from the water off Northeastern Taiwan

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Ecological tracers bio-accumulate in consumer's tissues through ingestion of diet and absorption from water, and can biomonitor the seasonal variation of environment conditions. We analyse study trace elements and stable isotopes in the tissues of swordtip squids *Uroteuthis edulis* from northeastern Taiwan to examine temporal and ontogenetic variation in those ecological tracers. Chemical composition in tissues including mantle, digestive gland and gill and seawater were measured and C, N stable isotopes were also analysed. The result of PCA analyses showed that the elements composition in mantle and gill were similar to water, but not for digestive gland, indicating its diet originated. Concentrations of Fe, Ba and Pb were significantly higher in immature individuals than maturing and mature ones, while Cd was higher in maturing and mature individuals. Variation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratio in mantle of squid related to migration, prey composition, and to anthropogenic effect related to oil pollution event. These results implied that the composition of ecological tracers in tissues of *U. edulis* marked seasonal variation causing by the environmental conditions influenced by currents and food web.

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Application of tropical octopus to education in Japanese agricultural high school

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Okinawa Prefectural Hokubu Agricultural High School is located in the northern part of Okinawa-jima Island, Japan. Our school provides the educational curriculum including raising livestock and crops. Compared with other students who study at the general high school in Japan, our students can acquire special skills such as handling domestic animals and cultivation. However, the students in agricultural high school spend less time for studying basic biology. Consequently, many of our students meet difficulties when they attend the classes for the experiments on animals and plants, e.g., manipulation of microscope, dissection, drawing, etc. Due to this situation, it seems useful to introduce a teaching material and educational systems by which students can learn knowledge and related skills for basic biology. The aim of this study is therefore to examine a possibility for applying tropical octopuses as teaching material to education in the Japanese agriculture high school. For the first step, we made 'Biology Club' in our school. In that club, students were experienced to set by themselves a rearing aquarium of closed system. And for the second step, students tried to collect tropical octopus *Abdopus aculeatus*, and they also tried to rear this octopus with the aquarium system they had set up. For the third step, we carried out some behavioral experiments to test the learning and memory of the octopus. Through these consequent experiences, the students were able to learn fundamental process to understand the living organisms and also knew intellectual aspects of octopuses.

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Multiple paternity in *Octopus oliveri* (Berry, 1914) (Cephalopoda: Octopodidae) revealed through behavioral and genetic analysis

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This study contributes to the growing research on cephalopod mating systems and in particular shows that octopus mating dynamics may be more complex than previously thought. Through a combination of behavioral and genetic studies, multiple paternity was found to be the rule in *Octopus oliveri*, both in the field and in captivity. *Octopus oliveri* is widespread in the rocky intertidal zone of Oahu and mates readily in laboratory conditions making it an ideal candidate to study octopus reproductive behavior. Four sets of behavioral experiments were recorded wherein six females were mated with three males in varying order, for a total of 24 females and 12 males. Video analysis of mating behavior shows the duration of mating, fighting, and resting for 62 successful experimental trials. Mating behavior for this species was recorded for the first time. Mating was observed for all males regardless of mating size or order. Females were seen to remove intact sperm packets of some males, however it is probable that the males simply failed to direct the spermatophore into the oviducal gland. Multiple observations of females initiating mating occurred, possibly suggesting female choice. Five microsatellite markers were developed and used to test paternity in eleven broods. The results showed skewed paternity in most broods, suggesting that sperm competition is present in this species. The two predictive variables in determining male mating success were mate order and male size. This is the first study in an octopus that combines both behavioral and genetic information to determine fertilization success.