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Book of Abstracts

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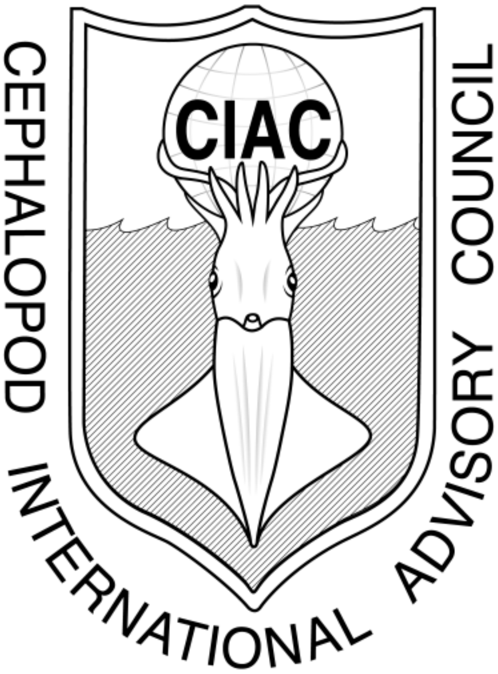

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Keynote — In memory of the Japanese great mollusk scientist, Dr. Takashi Okutani

Tsunemi Kubodera, Curator Emeritus

National Museum of Nature and Science

Abstract

On January 10, 2025, Dr. Takashi Okutani, our mentor and a leading expert in mollusk research, passed away quietly due to old age. As an honorary professor at Tokyo University of Marine Science and Technology, honorary president of the Malacological Society of Japan, and Lifetime Achievement Award (Honorary Membership) of the Cephalopod International Advisory Council, Dr. Okutani's academic achievements are widely known not only in Japan but also around the world in the fields of mollusk taxonomy and biology, as well as deep-sea organisms. I would like to express my heartfelt condolences on his death, as well as my cordial thanks for his helping guide my cephalopod studies through postgraduate to the present and encouraging my research on Japanese cephalopod fauna and deep-sea large squids, including the giant squid. We are deeply saddened by the loss of this great mollusk researcher, Dr. Takashi Okutani, and pledge to pass on the many achievements he left behind to future generations. I sincerely pray for the repose of Dr. Okutani.

Keynote — Flexible reproductive strategies of squid: from gamete to populations

Yoko Iwata, Associate Professor

Atmosphere and Ocean Research Institute, The University of Tokyo

Abstract

Flexibility is one of the most fascinating characteristics of cephalopods. These animals change their behaviors, physiology, morphology, and life history in response to environmental and social conditions. Coastal squid shows sophisticated intra-specific interactions and complex reproductive strategies, offering excellent opportunities to study how individuals select behavioral tactics and adapt their phenotypes accordingly. In loliginid squid, males exhibit distinct alternative reproductive tactics (ARTs): large males engage in a consort tactic transferring sperm to the female's oviduct, while small males conduct a sneaking tactic transferring sperm to a seminal receptacle on buccal membrane. These differing sperm transfer sites lead to distinct fertilization processes and drive divergent phenotypic adaptations in reproductive traits. Those morphological and physiological adaptations suggest that individual males typically commit to one reproductive tactic during their life history. Furthermore, the difference in sperm storage duration between the two tactics affects local population structure: more sneaker males under the condition of few mature females, as sneakers can mate with females not close to spawning. On the other hand, our recent study shows that males of pygmy squid also have ARTs with distinct sperm transfer sites, but, unlike loliginids, no morphological adaptation in reproductive traits is observed. In this species, each male conducts both ARTs flexibly depending on the female response, suggesting that an intermediate, unspecialized phenotype may be optimal. In this presentation, I would like to discuss how environmental and social factors shape the flexibility of reproductive strategies in squid.

Keynote — Antarctic octopods: time capsules and fortune tellers of global change

Jan Strugnell, Professor

James Cook University, Townsville, 4811, Qld, Australia

Abstract

Antarctic waters contain a diversity of octopods. Some of these lineages are endemic to, and only found on, the Antarctic continental shelf. Other lineages are distributed around sub-Antarctic islands and/or are known from the adjacent deep-sea. The evolution of these octopods has been shaped by physical processes that have occurred in the region across a range of time scales, including ice sheet expansion and contraction, changes in sea ice and the establishment of the Antarctic Overturning Circulation. Signatures of these past physical processes are contained within the genomes of Antarctic octopods. There is now emerging evidence that the Antarctic ice sheet, sea ice extent and Antarctic Overturning Circulation are undergoing abrupt changes as the result of Anthropogenic global warming. They may each be approaching tipping points which will have serious negative consequences for human livelihoods. This presentation will explore how Antarctic octopods have proven themselves to be both important time capsules and fortune tellers of global change.

Keynote — Ins and Outs of the Cuttlefish Camouflage System

Gilles Laurent, Director

Max Planck Institute for Brain Research

Abstract

In this talk, I will summarize our on-going efforts towards understanding the camouflage system of Sepia officinalis. The “Ins and Outs“ in the title refer not to the deep secrets that this system holds (they remain unknown to us) but to the inputs to and outputs of the remarkable brains that control camouflage. I will focus on our attempts to understand both the components of chromatophore motor control, using computer vision approaches, and the architecture of the early visual system, using serial EM connectomics together with single-nucleus and spatial transcriptomic approaches. The hope is that we can eventually understand how the statistics of a visual scene are converted into a matching skin pattern, using in vivo neural recordings in behaving animals, which we are also in the process of developing.

Keynote — Lit Partnerships: Squid, Vibrio, and the Evolution of Beneficial Mutualisms

Michele K. Nishiguchi, Professor

Department of Molecular and Cell Biology, UC Merced

Abstract

The mutualistic association between sepiolid squids (Mollusca: Cephalopoda) and their *Vibrio* symbionts is an experimentally tractable model to study the evolution of animal-bacterial associations through both wild-caught and experimentally evolved populations. Since *Vibrio* bacteria are environmentally transmitted to new hosts with every generation, it provides a unique opportunity to resolve how changing environmental conditions may affect bacterial infection, colonization, and persistence in different host species. *Vibrio* bacteria encounter potentially conflicting selective pressures, competing with one another to colonize the sepiolid light organ, but also vying for resources in the environment outside the squid. Both abiotic and biotic factors contribute to the fitness of individual strains of *Vibrio* bacteria, but which of these factors are amenable to adaptation and eventually lead to a successful beneficial association has yet to be elucidated. This seminar will cover how environmental conditions and host specificity lead to the development of symbiotically adapted *Vibrio* bacteria, generating new ideas on the evolution of beneficial associations.

Oral presentations

O1 — Deep-sea Cephalopod Observations from Schmidt Ocean Institute's First Antarctic Field Season

Kat S. R. Bolstad[1], Jeff Day[2], Patricia Esquete[3], Sasha Montelli[4], Thom Linley[5]

1 AUT Lab for Cephalopod Ecology & Systematics, Auckland University of Technology Te Wānanga Aronui o Tāmaki Makau Rau, Auckland, New Zealand
2 The Cephalopod Research Network
3 Biology Department and Centre of Marine and Environmental Studies, University of Aveiro
4 University College, London
5 Museum of New Zealand Te Papa Tongarewa

Abstract

During the austral summer season of 2024–2025, Schmidt Ocean Institute's research vessel *Falkor (Too)* conducted numerous exploratory dives in the Antarctic using ROV *SuBastian*. Cephalopods were observed on most dives, including multiple sightings of endemic oegopsids (*Alluroteuthis antarcticus*, *Moroteuthopsis longimana*, *Slosarczykovia circumantarctica*, with *Psychroteuthis glacialis* most frequently encountered) and octopods (*Megaleledone setebos*, *Muusoctopus rigbyae*, *Pareledone* spp., with *Cirroctopus glacialis* most frequently encountered). These and numerous other benthic and pelagic species were filmed in high resolution, some for the first time worldwide. The calving of a 510km2 section of the George VI ice shelf in the Bellingshausen Sea during the 'Climate Connections at the Ice–Sea Interface' voyage also provided a unique opportunity to observe thriving deep-sea communities (including cephalopods) that had been far under the ice until just days earlier. Overall, this suite of observations provides more detailed insight into the biology of these Antarctic endemics including finer resolution of their distribution (including depth ranges), as well as opportunistic insights into their live appearance, behaviour, and trophic ecology.

O2 — Population Genetics and Evolutionary History of *Uroteuthis duvaucelii* in the Central Indo-Pacific

Lorenzo Halasan[1], Hsiu-Chin Lin[1]

1 Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung, Taiwan

Abstract

The Central Indo-Pacific (CIP) is a global hotspot for coastal cephalopod diversity. *Uroteuthis duvaucelii* is one of the top commercial squid commodities in these waters, being prominently abundant in both the Pacific and Indian Ocean regions. Despite its economic relevance, the biological knowledge regarding the genetic structure, population distribution limits, and past demographic signatures of the species is non-existent. This study examined a 530 bp portion of mtDNA CO1 sequences from N=442 *U. duvaucelii* individuals collected from major fish markets throughout the CIP, alongside publicly available data entries. Haplotype clustering and maximum likelihood tree-based inferences revealed the existence of four genetic clades, with each one predominantly occupying different portions of the CIP. These clades are the (a) widespread Indo-Pacific; (b) coastal Indochina; (c) lower Western Pacific, and (d) the exclusive Indian Ocean clades. The most common haplotype was represented by n=112 individuals, diversely collected from six different collection areas. Within-clade %K2P distances were between 0.05 – 0.33%, whereas across-clade distances varied from 5.48 – 10.54% K2P. TimeTree analysis revealed that *U. duvaucelii* plausibly emerged around 7.52 MYA and the split between the Indian and Pacific Ocean clades occurred around 4.97 MYA. Historical demography suggested that the species experienced a decline in population size from 3.0 – 1.5 MYA, then followed by a rapid increase up to the present time. Our insights bridge information gaps in our total understanding of the evolutionary history of *U. duvaucelii* and other coastal decapod groups in general.

Keywords: Genetic structure, demographic history, evolution, mtDNA CO1, loliginidae

O3 — Multidecadal transport of *Illex argentinus* demonstrates optimal dispersal and recruitment potential

Aurelia Gabellini[2], Patrizio Mariani[2], Asbjørn Christensen[2], Mary Gasalla[1]

1 University of Sao Paulo, Oceanographic Institute
2 Danish Technical University

Abstract

Illex argentinus (Argentine shortfin squid) has one of the main cephalopods catches in the world. In this study, we explore conditions for recruitment of this species simulating its transport from spawning regions to putative nursery areas. Specifically, changes in transport and recruitment are assessed in terms of different larval strategies including diel vertical migration, paralarval duration, spawning time and spawning area. Those strategies were assessed using particle tracking individual-based model simulations coupled to a 3D hydrodynamic model, over a 20 years period (2000 - 2019), considering an extended spawning ground from 27oS to 38oS. Our results emphasize "spawning time" as the main factor controlling recruitment in the species.

O4 — Phylogeny of Southern Ocean Squid Using Novel Molecular Methods for Species Identification

Lucas Bastos[1,3], José Queirós[2, 5], Charlotte Havermans[3, 4], Richard Phillips[2], José Xavier[1,2]

1 Centre for Functional Ecology – Science for People & the Planet (CFE), Associate Laboratory TERRA, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, Coimbra 3000-456, Portugal
2 British Antarctic Survey, Natural Environment Research Council, Cambridge, United Kingdom
3 Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Bremerhaven, Germany
4 University of Bremen, Bremen, Germany
5 CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal

Abstract

The Southern Ocean has a high degree of species diversification and endemism, but the marine environment is changing, including warming waters in some regions and major changes in extent and duration of sea ice. An understanding of the evolutionary history of organisms through phylogenetic studies can provide insights into the capacity to adapt to present and future environmental changes.

Cephalopods are crucial in the Southern Ocean, where they serve as food resources for numerous predators, but are understudied because of sampling challenges. Presently, most studies are of beaks collected from predator stomachs. These rely on accurate species identification from beak morphology, which can only be accomplished by a few experts worldwide. DNA analysis could open up the research field by allowing species identification from beaks, but has yet to be implemented.

Here, we present the first detailed methodology for extracting DNA from cephalopod beaks using an optimised commercially available kit. Based on the COI gene sequences we successfully identified species for 77.1% of 35 beaks. Additionally, we inferred evolutionary events and past colonization patterns of squid in the SO. To infer relations among and within species, Neighbour-Joining and Maximum-Likelihood trees based on 32 COI sequences representing 11 taxa were built. This identified monophyly at the species and family levels. The p-distance analysis indicated low intraspecific divergence among Southern Ocean squid.

Our results illustrate the benefit of molecular studies for understanding evolution and facilitate future studies of taxonomy and the roles of cephalopods in marine food webs in general.

O5 — The biogeography of Mediterranean cephalopods: 20 years after

Giambattista Bello

Abstract

During the last two decades, much teuthological research has been accomplished on the Mediterranean as well as the ocean world cephalopods. Accordingly, it is now necessary to update my own "Biogeography of Mediterranean cephalopods" from 2003. First of all, the alpha-teuthodiversity of this internal sea is redefined by the inclusion of newly discovered species – either previously unknown to science (*Sepiola bursadhaesa* and *Sepiola boletzkyi*) or newly named cryptic ones (*Stoloteuthis cthulhui*, *Ommastrephes caroli* and *Ancistrocheirus alessandrinii*), whose discovery revealed the increase of endemic organisms – and alien cephalopods (*Sepia dollfusi*, *Taonius pavo*, *Taningia danae* and *Octopus cyanea*); as well as by the dismission of some taxa whose occurrence is not adequately documented (*Spirula spirula*, *Sepia pharaonis* and *Pterygioteuthis giardi*). Most importantly, a deeper perception was acquired about the overall Mediterranean cephalopod community in relation to both its geographical gradients (west-east / south-north) and depth distribution. In this respect, the current effects of both water temperature increase and fishery activities at the population level were also disclosed. The connections of the Mediterranean teuthofauna with the neighboring sea, i.e. the Atlantic Ocean through the Strait of Gibraltar passageway and the Red Sea through the recently widened Suez Canal, are also taken into consideration. Lastly, the establishment of the Mediterranean cephalopod community is reappraised within the frame of the global marine biogeography and in the light of the world ocean geological history.

O6 — Capitalising on the wealth of chemical data in lesser-known taxa: future directions for cephalopods

Zoe Doubleday[1], Jasper Willoughby[1], Minoli Dias[2], Louise Hosking[1], Natasha Leclerc[3], Shanie Brault Nikolajew[4], Melita Peharda[5], Arieli Tristão-Rézio[1], Clive Trueman[6]

1 University of South Australia
2 University of Toronto
3 Memorial University of Newfoundland and Labrador
4 University of Ottawa
5 Institute of Oceanography and Fisheries (Croatia)
6 University of Southampton

Abstract

Aquatic organisms are natural data loggers and are constantly recording chemical variations within their accretionary tissues. Chemical sclerochronology is the study of these chemical variations through time and how they can be used to understand environmental change and the physiology and ecology of species. While sclerochronological research has largely focused on bivalves, teleost fish, and hard corals, there are many other aquatic taxa rich with time-resolved chemical data. To expand focus to these ‘other’ taxa and determine the state-of-play, we compiled a database of sclero-chemical studies that span nine living Phyla and 20 Classes, including cephalopods. We then examined research trends and knowledge gaps across these taxa, as well as showcase their exciting potential to collect critical data and address pressing environmental challenges. Through comparisons with cephalopods and other taxa, we aim to promote new avenues for sclerochronological research in cephalopods.

O7 — Recent advances in the ecology and evolution of nautilus

Tajika Amane[1, 2], Landman Neil H.[2]

1 Kyoto University
2 American Museum of Natural History

Abstract

Nautilus is the only extant ectocochleate cephalopod with a chambered external shell and is often dubbed a "living fossil" for its arguably conserved conch morphology over millions of years. Foundational work in the 1970s and 1980s revealed key aspects of their ecology and functional morphology, yet many questions concerning its ecology, morphology, and evolutionary history remain unresolved. Over the past decades, population-genetic studies have greatly enhanced our understanding of its evolution and population dynamics; however, ecological and morphological aspects have received comparatively less attention. Here we present our recent multidisciplinary investigations into the ecology, morphological variation, and evolutionary dynamics of nautilus. Using stable isotopes, high-resolution X-ray computed tomography, and morphometric analyses, we (i) refine the habitat depth range of *Nautilus macromphalus* from New Caledonia, (ii) document previously unrecognized hatching size heterogeneity, (iii) precisely quantify conch morphology through ontogeny, and (iv) propose a new hypothesis for the survival of nautilus across the K-Pg mass extinction event.

O8 — Ecosystem changes recorded in squid beaks from natural history museums – retrospective stable isotope study

Alexey V. Golikov[1], José C. Xavier[2,3], Filipe R. Ceia[4], José P. Queirós[3, 5], Paco Bustamante[6], Bram Couperus[7], Gaël Guillou[6], Anna M. Larionova[8], Rushan M. Sabirov[8], Christopher J. Somes[1], Henk-Jan Hoving[1]

1 GEOMAR Helmholtz Centre for Ocean Research Kiel, Kiel, Germany
2 Centre for Functional Ecology – Science for People & the Planet (CFE), Associate Laboratory TERRA, Department of Life Sciences, University of Coimbra, Coimbra, Portugal
3 British Antarctic Survey, Natural Environment Research Council, Cambridge, UK
4 MARE – Marine and Environmental Sciences Centre/ARNET – Aquatic Research Network, Department of Life Sciences, Universidade de Coimbra, Coimbra, Portugal
5 Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Porto, Portugal
6 Littoral Environnement et Sociétés (LIENSs), UMR 7266, CNRS-La Rochelle Université, La Rochelle, France
7 Wageningen Marine Research, Wageningen University and Research, IJmuiden, The Netherlands
8 Department of Zoology, Kazan Federal University, Kazan, Russia

Abstract

Retrospective studies on climate change impacts on marine ecosystems are urgent to understand what is happening in today's oceans, particularly in polar areas, where such impacts are the most pronounced. To this end, beaks of two abundant squid species from the North Atlantic and low Arctic, *Gonatus fabricii* (dated 1882–2019) and *Todarodes sagittatus* (dated 1844–2023), were subjected to stable isotope analyses (SIA) of carbon δ13C and nitrogen δ15N. Common pitfalls of retrospective SIA studies, i.e. not accounting for the Suess effect and spatiotemporal baseline differences, were addressed using the Model of Ocean Biogeochemistry and Isotopes. Trophic position (TP) was estimated from δ15N using this model. Analysing the beaks by subsections enabled using several additional parameters, such as specialization index and ontogenetic changes in δ13C, TP and isotopic niche width. In *G. fabricii*, several parameters simultaneously showed temporal changes in the late 1990s/early 2000s, coinciding with the onset of increased climate-driven shifts in Arctic ecosystems. These parameters included an increase in diet and habitat-use generalism (= opportunistic choice rather than specialization), TP and isotopic niche width. This aligns with the increased generalization of Arctic food webs, increased primary production there, and the influx of boreal species from the North Atlantic. In *T. sagittatus*, a more generalist diet was detected in contemporary vs the XIXth century individuals. This study shows that squids (abundant opportunistic mesopredators with short life cycles) are well-suited for retrospective long-term ecology studies and play a crucial role in identifying ecosystem shifts related to environmental changes.

O9 — Triassic Nautiloids and Ammonoids from Thailand, 2015-2025

Kittichai Tongtherm[1], Jaruwat Nabhitabhata[2], Surangkana Tuanapaya[3]

1 Princess Maha Chakri Sirndhorn Natural History Museum, Prince of Songkla University, Hatyai, Thailand.
2 Centre for Marine and Coastal Studies (CEMACS), Universiti Sains Malaysia, Penang, Malaysia.
3 Reference Collection, Phuket Marine Biological Center, Phuket, Thailand.

Abstract

Triassic cephalopods were recorded from the Phukhaothong Dolomite and Chaik Limestone Members, Chaiburi Formation, Phatthalung Province in Thai peninsula. A total of sixty cephalopod fossils were collected during 2015-2025, comprise nine species of nautiloids and fifty-one species of ammonoids as new records of Thailand. The nautiloid fauna include two orthoceratids, one trigonoceratid, two syringonautilids, one aipoceratid, and three indetermined taxa. The ammonoid fauna include one ophiceratid, one flemingitid, one meekoceratid, two parananitids, two ussuriids, one noritid, one acrochordiceratid, ten ceratitids, one hungaritid, one aplococeratid, one celtitid, one hedenstroemiid, four tirolitids, seven columbitids, one nannolytoceratid, one protetragonitid, and thirteen indetermined taxa. One taxon of nautiloid and four taxa of ammonoids were new to science.

O10 — Ecological update on the common cuttlefish *(Sepia officinalis)* in the Bay of Biscay inferred from a multifaceted approach

Elodie Réveillac[1], Amélia Viricel[2], Tamas Malkocs[2], Christelle Jozet-Alves[3], Kévin Leleu[4], Vanessa Lopes[5], Emilie Roche[6], Rui Rosa[5, 7], Yohan Weiller[8], Thomas Lacoue-Labarthe[9]

1 La Rochelle University, UMR 7266 LIENSs, La Rochelle, France
2 UBO, LEMAR Plouzané, France
3 University of Caen Normandy, UMR 6552 ETHOS Caen, France
4 OFB, Parc Naturel Marin du Bassin d'Arcachon, France
5 MARE - Laboratório Marítimo da Guia, Faculdade de Ciências da Universidade de Lisboa, Portugal
6 CDPMEM Charente-Maritime, France
7 DBA FCUL Lisboa, Portugal
8 OFB, Parc Naturel Marin de la Mer des Pertuis, France
9 CNRS, UMR 7266 LIENSs, La Rochelle, France

Abstract

The common cuttlefish (*Sepia officinalis*) is one of the most exploited cephalopods in Europe. Yet, its population status in the central part of its range, the Bay of Biscay (BoB), remains poorly understood. This study aimed to update knowledge and fill gaps through three complementary approaches: (1) assessing population genomic structure across the BoB and adjacent regions using RAD-sequencing; (2) analyzing spatial and temporal trends from fisheries data; and (3) characterizing life-history traits and reproductive strategies of spawners in two major coastal areas (Pertuis Charentais and Arcachon Bay). Population genomic analyses confirmed no significant structuring between the English Channel and BoB, suggesting substantial gene flow, likely due to mixing at wintering grounds or during migration. In contrast, differentiation from the southern Iberian Peninsula was observed. Fisheries data (2010–2022) highlighted a declining catch-per-unit-effort (CPUE) trend for mature individuals, despite increasing CPUE of sub-adult individuals, but with notable local disparities such as a decline in sub-adults in Arcachon Bay. Life-history analysis confirmed the coexistence of two reproductive strategies, annual (Group I Breeders) and biennial (Group II Breeders), with spatial and phenological variation along a north-south gradient. GIIB individuals dominate early in the season and may be more vulnerable to environmental changes, particularly temperature increase. In the context of climate change, the northward shift in distribution and gradual replacement of biennial breeders by annual breeders in southern areas anticipated, could increase genetic structuring. Future research should prioritize understanding offshore wintering habitats and coastal nursery quality to inform adaptive management of the species.

O11 — Are radiogenic isotopes a viable tool for investigating cephalopod ecology?

Arieli Tristao Rezio[1], Justin Payne[1], Robert Klaebe[2], Chung Ming-Tsung[3], Zoe Doubleday[1]

1 University of South Australia
2 University of Adelaide
3 National Taiwan University

Abstract

Understanding population connectivity and structure is crucial for the sustainable management of commercially harvested species. While stable isotopes and elemental markers have revolutionised the study of animal movement, their application is limited by an insufficient understanding of how biological processes cause offsets between chemical values in animals and those in their environment, which can complicate data interpretation. On the other hand, radiogenic isotope systems, like strontium, are prized for tracking the movement and origins of organisms and biological materials, because isotopic values in biological tissues directly reflect the isotopic value in the environment. However, strontium isotopes are only suitable for terrestrial and freshwater ecosystems, and not marine systems. A promising alternative for marine species is neodymium isotopes, but research on this radiogenic isotope system is limited. We outline the incorporation and uptake of Nd in a range of animals, including cephalopods, and summarise methods for the extraction of Nd from animal tissues for isotopic analysis. We aim to broaden the application of neodymium isotopes and promote their use in tracking the movement, connectivity, and provenance of cephalopods.

O12 — Age and Growth of the colossal squid *Mesonychoteuthis hamiltoni*

Farnan Darragh[1], Queiros José P.[1, 2], Cherel Ives[3], Stevens Darren W.[4], Xavier José C.[1, 2, 5], Perales-Raya Catalina[6]

1 University of Coimbra, MARE, Marine and Environmental Sciences Centre/ARNET – Aquatic Research Network, Department of Life Sciences, Coimbra 3000-456, Portugal.
2 British Antarctic Survey, Natural Environment Research Council, Cambridge, United Kingdom.
3 Centre dÉtudes Biologiques de Chizé, UMR 7372 du CNRS – La Rochelle Université, 79360 Villiers-en-Bois, France.
4 National Institute of Water and Atmospheric Research Ltd., 301 Evans Bay Parade, Hataitai, Wellington 6021, New Zealand.
5 Centre for Functional Ecology – Science for People & the Planet (CFE), Associate Laboratory TERRA, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, Coimbra 3000-456, Portugal.
6 Spanish Institute of Oceanography (IEO, CSIC). Calle Farola del Mar, 22. Dársena Pesquera 38180. Santa Cruz de Tenerife, Spain.

Abstract

The Southern Ocean is a vital but understudied ecosystem, with cephalopods playing key ecological roles. The colossal squid *Mesonychoteuthis hamiltoni*, the heaviest known invertebrate, is a major predator and prey species in this region. This study presents the first age estimates of *M. hamiltoni* across the Indian, Atlantic, and Pacific sectors of the Antarctic. Growth increments were analysed via epi-illumination microscopy in rostrum-sagittal sections of 30 lower beaks (10 per sector) recovered from predator stomachs. A consistent pattern of growth increments was observed in the sections. They revealed that M. hamiltoni reaches estimated ages of at least 3.5 years, and based on the largest recorded beak, we estimate a lifespan of over 5.2 years. Validation of daily periodicity is currently unsuitable for *M. hamiltoni*, but similar beak increments have been cross-verified with statolith data in other oegopsids. A higher proportion of the oldest and largest individuals was found in the Pacific sector. Our results revealed a reduction in growth rate in larger adults. These findings suggest *M. hamiltoni*lives longer than the Southern Ocean endemic *Moroteuthopsis longimana* (2.2 years from beaks) and the giant squid *Architeuthis dux* (3.5 years from beaks), but not as long as the deep-sea vampire squid *Vampyroteuthis infernalis*, which may reach up to 8 years (using reproductive data). Such extended lifespans appear paradoxical given the traditionally assumed semelparity of coleoid cephalopods, although this paradigm is increasingly being challenged. Longevity in *M. hamiltoni* may reflect adaptive pressures from deep-sea dispersal and potential mating constraints

O13 — Arraial do Cabo, a center of cephalopod aggregation and mixing in southeastern Brazilian coast

Renato Dantas[1], Mariana Côrtes[2], Ana Bauer[1], Weslley Almeida[3], Carlos Ferreira[4], Tatiana Leite[1]

1 Laboratório de Métodos de Estudos Subaquáticos e Cefalópodes (LAMECE), Universidade Federal de Santa Catarina (UFSC), Florianópolis, Brazil.
2 Laboratório de Malacologia, Museu de Zoologia da Universidade de São Paulo (MZUSP), São Paulo, Brazil.
3 Reserva Extrativista Marinha do Arraial do Cabo, Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), Arraial do Cabo, Brazil.
4 Laboratório de Ecologia e Conservação de Ambientes Recifais (LECAR), Universidade Federal Fluminense (UFF), Niterói, Brazil.

Abstract

Located between tropical and subtropical regions of the Brazilian coast and within Cabo Frio Upwelling System, the Arraial do Cabo Marine Extractive Reserve presents a unique and diverse marine fauna. Considering such singularity and the lack of studies on cephalopods since the late 1980s, we evaluated the octopus and squid local diversity, habitat use and seasonality. Data was obtained from SCUBA and free diving, fisheries, lab database, citizen science and social media. A total of 14 cephalopod species belonging to 5 families were identified. Those targeted by fisheries, Octopus americanus and *Doryteuthis pleii*, were also the most observed in underwater surveys on or close to sandy bottoms, except for the squid *Sepioteuthis* aff. *sepioidea*, which occurred frequently in large schools near rocky shores. This species dominated social media records, as did the diurnal *Octopus insularis* in rocky and coral reefs. The species *Paroctopus tehuelchus*, *Eledone gaucha* and *Doryteuthis sanpaulensis* were only observed as fisheries by-catch. Species difficult to observe like *Macrotritopus* aff. *defilippi*, *Amphioctopus burryi*, *Octopus taganga*, *Paroctopus cthulu* and *Pickfordiateuthis pulchella*, were almost exclusively recorded by social media and citizen scientists. Oceanic cephalopods *Argonauta nodosus* and *Ommastrephes cylindraceus* occurred associated with cold waters of summer upwelling months and winter, respectively. Overall, Arraial do Cabo exhibited a mixed cephalopod fauna typical of biogeographic transition zones with tropical and subtropical coastal species, as well as circumtropical oceanic species. Thisstudy added three new records for the region (*O. insularis*, *A. burryi* and *P. cthulu*) and one new to Brazil (*O. taganga*).

O14 — Journey to the Depths: Taxonomic arrangement and redescription of Toyama octopus from Sea of Japan

Abhijeet Purkayastha[1]; Tomoharu Kimura[2]; Hidetaka Furuya[1]

1 Animal Morphology Lab, Department of Biological Science, Graduate School of Science, The University of Osaka, Osaka 560-0043, Japan
2 Uozu Aquarium, 1390 Sanga, Uozu, Toyama 937-0857, Japan

Abstract

Taxonomic study represents one of the foundational pillars of scientific inquiry, maintaining its critical importance from its historical origins to present day. Over centuries, this discipline has undergone remarkable evolution, transforming from purely morphological descriptions to sophisticated, integrative science that synthesizes molecular and phylogenetics, ecological dynamics, physiological adaptations, and biogeography. The present study employs such an integrative framework to address the redescription of a taxonomically ambiguous octopus species. Toyama octopus is a species of octopus from Toyama Bay described by Sasaki (1929) as *Polypus fujitai* based on the morphological description of one preserved female specimen. The rediscovery of this species in its type locality has provided an opportunity to resolve its systematic position. Molecular analysis, centered on COI gene, definitively places this species within the genus *Muusoctopus*. This molecular placement is supported by a suite of morphological characteristics that distinguish it from its congeners. The octopus species displays moderate size (83 mm mantle length) with light brown coloration and distinctive unequal slender arms. Its exceptionally large hectocotylus (compared to other species), minute ink sac (predominantly in females), prominent keels (measuring twice the membrane width relative to arm width), and dense basal chromatophores on head and arms, constitute key diagnostic features within *Muusoctopus*. Distributed primarily in the northwest Pacific, genetic analysis confirms its species status through distinctive haplotype patterns. Ecological analysis reveals significant negative allometric growth ($p<0.05$), while the presence of shallow-water morphological features in this deeper-dwelling species suggests an evolutionary transitional state, offering valuable insights into cephalopod adaptation processes.

Keywords: Redescription; Deep-sea; Biogeography; Ecology; Phylogeny

O15 — Connectivity between fishing grounds: spatio-temporal trends in Loliginid squid biomass indices and fisheries landings in the English Channel and the southern North Sea.

Anna Marcout

Université de Caen Normandie, France

Abstract

The recent increase of Loliginid squid landings from the southern North Sea is either a change in squid abundance and distribution (possibly related to climate change) or a change in fishers' behaviour (with Northern fleets targeting more and more cephalopod resources). In a preliminary step the analysis of commercial fishery statistics sheds light on the differences in seasonality of exploitation. In addition, biomass indices derived from standardised landings per unit of effort of a homogeneous fleet of trawlers provides information on squid distribution. Time series clustering is applied to look for similar patterns between fishing grounds. The successive exploitation of squid in the eastern Channel and then in the North Sea would point to a shift in the resource, which should be seen from the change in the biomass centre of gravity. These indications are discussed in the light of available survey data which are more discontinuous over time but can help to understand which one of the two *Loligo* species (*Loligo forbesii* or *L. vulgaris*) is mainly targeted in each area and what assumptions regarding connectivity should be made.

O16 — Sustainable squid fisheries in Alaska, USA

Michael Navarro

University of Alaska Southeast, Department of Natural Sciences

Abstract

Alaska remains an understudied region of the North Pacific. In recent years, fishermen report increasing squid abundance. Two species—market squid (*Doryteuthis opalescens*) and Magister squid (*Berryteuthis magister*)—have recently been proposed by fishermen for targeted commercial harvest but are not yet approved by the State of Alaska, partly due to a lack of biological assessment methods. Sustainable fishery development requires filling key knowledge gaps, including lifespan, reproductive cycles, and spawning grounds. In collaboration with fishing communities, I will present data on each species, focusing on spawning ground identification, maturity, age, and growth, providing insights to support sustainable squid fisheries and expand scientific understanding for these species to include coastal Alaska in the Northeast Pacific.

O17 — Suitability of Hard Tissues for Age Determination in *Sepia esculenta*: A Jiangsu Offshore Case Study

Yuzhe Ou[1], Bilin Liu[1]

1 Shanghai Ocean University

Abstract

Sepia esculenta is a commercially significant cephalopod in the offshore waters of China. However, due to various factors, its population is facing potential decline. Accurate age determination is essential for the sustainable management of S. esculenta resources, and the microstructural features of cephalopod hard tissues are crucial for age estimation. In this study, a total of 360 S. esculenta specimens were collected from the offshore waters of Jiangsu between September and November 2021. The hard tissues, including beaks, cuttlebones, eye crystals, and statoliths, were processed and analyzed. The results indicate that the microstructure of the rostrum sagittal section (RSS) of the upper beak of S. esculenta exhibits distinct characteristics, with alternating light and dark growth increments in a periodic pattern. The growth increments in the hood region are clearly defined, and both marker and abnormal structures were identified. The growth increments follow a "one-day round" pattern, making the upper beak the most reliable material for age determination. In contrast, the longitudinal section of the cuttlebone displays varying pigment deposition, with growth increments formed by the superposition of growth lamellae. Although the estimated age from the cuttlebone is similar to that from the beak, it does not exhibit strict daily periodicity and, thus, can serve as an auxiliary material for age determination. The eye crystals present concentric growth increments, but the distances between increments vary between the core and outer regions. The number of growth increments is highly variable, and the deposition pattern lacks daily periodicity, making eye crystals unsuitable for age determination. The pigment deposition in the statoliths is not prominent, and growth increments are generally indistinct, precluding their use in age-growth studies. Furthermore, the number of growth increments in the beak, cuttlebone, and eye crystals shows a power function relationship with mantle length, with significant correlations observed among the three structures. These findings suggest that all three hard tissues can serve as important materials for studying the growth characteristics of S. esculenta. This study provides a foundation for further research on the age and growth of *S. esculenta* and contributes to the rational management and sustainable exploitation of its resources.

O18 — Statolith Elemental Signatures Reveal Ontogenetic and Regional Variation in the Indian Squid, *Uroteuthis duvaucelii*, Across the Western Pacific

Jessica Legaspi[1], Lorenzo Halasan[2], Hsiu-Chin Lin[2], Tomoyo Okumura[3]

1 Kuroshio Science Program, Graduate School of Integrated Arts and Sciences, Kochi University, 200 Otsu, Monobe, Nankoku City, Kochi 783-8502, Japan
2 Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung 80424, Taiwan
3 Marine Core Research Institute, Kochi University, 200 Otsu, Monobe, Nankoku City, Kochi 783-8502, Japan

Abstract

The loliginid squid, *Uroteuthis duvaucelii* represents a significant commercial fishery in the Indo-Pacific region. Numerous studies on fishery biology have been conducted on this species, but only a handful have documented the statolith microchemistry of squids that reflects environmental histories and physiological changes. In this context, this study examined the trace elements in the statolith of *U. duvaucelii* caught in the waters of Taiwan, the Philippines, and Malaysia. Eight elements (23Na, 24Mg, 31P, 55Mn, 57Fe, 66Zn, 88Sr, 137Ba) were measured and quantified using the high-precision technique of laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS). The results showed that the element-to-calcium ratio in the statoliths varied by age and region. The Sr/Ca, Ba/Ca, and P/Ca ratios demonstrated a similar pattern across regions, showing higher levels in younger individuals that decrease as they age, indicating a variation in habitat characteristics and ontogenetic changes.

In contrast, the Na/Ca and Zn/Ca levels are lower in younger ages and peak in older ages, suggesting that these elements may indicate physiological changes in squids. On the other hand, no distinct pattern has been observed across ontogenetic stages for Mg/Ca, Fe/Ca, and Mn/Ca ratios in all regions. This result might suggest that various factors could influence the incorporation of these elements between areas, including endogenous and exogenous factors. Additionally, sodium, iron, and manganese varied significantly by location, indicating a spatial variation in element-to-calcium ratios.

Keywords: Loliginidae, trace elements, physiological changes, environmental histories

O19 — Octopus Intelligence Fantasy and Fact: Identifying and Allocating the Cogs of Cognition

Ian Gleadall[1]

1 AiCeph LLC

Abstract

Until recently, cognition in cephalopods was defined, analyzed and discussed carefully and objectively. Given the inherent difficulties, researchers avoided anthropomorphisms and proceeded accordingly. Recently, however, social science writers have used the Internet to cite sometimes questionable results and made exaggerated extrapolations and claims about octopus intelligence. The extrapolations away from valid scientific knowledge have now reached the realm of the absurd:

Octopuses “. . . are primed to become the dominant force on Earth should humanity die out, according to one of the world’s leading experts.” (Professor Tim Coulson at Oxford University). Coulson is recorded as stating that “[their] dexterity, curiosity, ability to communicate with each other, and supreme intelligence means they could create complex tools to build a vast Atlantis-like civilization underwater.” They “could conceivably build underwater cities and towns akin to those we recognise on land.” It is possible that “they could develop their own methods of breathing out of water . . . and hunt prey like deer, sheep and other mammals on land.”

Here, we backtrack to reality and tease out some cogs: (1) valid scientific conclusions about the presence and extent of octopus cognition and intelligence (cogs of cognition); (2) other valid uses for which octopuses require a sophisticated nervous system (cogs of camouflage, crypsis and concealment); (3) an octopus where the use of (1) and (2) could be separated and the cogs expected to adjust adaptively; and (4) appropriate scientific conclusions, drawn while recognizing false extrapolations (‘cog’ also has a meaning ‘deception’ or ‘trick’).

O20 — Towards Multi-Context Two-Way Communication Between Humans and Common Octopuses: An AI-Assisted Approach

Wolfgang Slany[1], Krishan Mohan Patel[2, 7], Aryavardhan Sharma[2], Michael J Kuba[3], Tamar Gutnick[4], Alexius Kaufmann[5], Thomas Grechenig[6]

1 Institute of Software Engineering and Artificial Intelligence, Graz University of Technology, Graz, Austria
2 Arishna IoT Solutions, Bangalore, India
3 Department of Biology, University of Naples Federico II, Naples, Italy
4 IIT, Istituto Italiano di Tecnologia, Genova, Italy
5 MareMundi Institute Krk, Punat, Croatia
6 Research Industrial Systems Engineering (RISE), Schwechat, Austria
7 University College of Teacher Education Styria, Graz, Austria

Abstract

We document and analyze two-way, multi-context communication between humans and common octopuses (*O. vulgaris*), using AI technologies and long-term behavioral observations. We show the octopus's capacity for complex, context-dependent interactions, significantly surpassing previously documented cephalopod communication abilities. Our findings challenge traditional views on invertebrate cognition and open new avenues for interspecies communication research.

O21 — Consciousness in Cephalopods

Jennifer Mather

University of Lethbridge

Abstract

Do cephalopods have consciousness? The Cambridge Declaration of Consciousness in 2012 by a group of leading animal behaviorists and neuroscientists declared that they might. But it's tricky. If consciousness is having subjective experiences, then we will never know. If it is having self-awareness, intelligence and appropriate neural organization, then yes, cephalopods have these characteristics. Hochner suggested animals, including cephalopods, should have Embodied Cognition, abilities tuned to their environment and neural controller, as well as their sensory and motor capacities (and see skin displays). Birch pointed out that animals with complex cognition would have perceptual richness, emotional richness and valence, temporality and unity (see Mather, Reviews in NeuroSci). Cephalopods can work to gain information and sensory feedback, self-monitor and control themselves across time, as well as send two messages simultaneously to different receivers. These abilities argue that they have consciousness, though perhaps their own and not our version. What will this mean for their welfare?

O22 — Communication in multispecies interactions

Eduardo Sampaio[1]

1 Max Planck Institute of Animal Behavior

Abstract

Interspecific coordination and communication provides a unique lens through which to examine the origins and evolution of signaling behaviors. Octopus–fish hunting partnerships, particularly between Octopus cyanea and fish such as coral trout (*Plectropomu*s spp.) and goatfish (*Parupeneus* spp.), exemplify such interactions. Long assumed to be unidirectional—where fish followed octopuses for opportunistic gains—recent studies show that both octopus and fish actively contribute to the coordination of the hunt through a combination of signals and cues. Fish partners can use visual signals, including referential gestures like headstands or repetitive "come-and-go" movements, to indicate prey locations. Octopuses respond flexibly by adjusting their foraging tactics depending on partner behavior. Moreover, octopuses exhibit partner-specific directed behaviors, including punitive responses (e.g., "punching") when fish act exploitatively, and fish can also displace other fish, which adds another layer to the communication network. Octopuses (and fish to a certain extent) can also provide information to other individuals through another dimension: color change. This dynamic use of signals and cues raises compelling questions about the boundary between passive and active communication, and how much each contributes to group coordination and success. Given the evolutionary distance between species and the octopus's solitary lifestyle, these interactions offer a powerful model for exploring how repeated interspecies interactions can scaffold the emergence of more complex communication systems.

O23 — Energy expenditure of cuttlefish on camouflage

Hsin-Yu Chang[1], Tzu-Yen Liu[1, 2], Pou-Long Kuan[2], Yung-Che Tseng[2], Ming-Tsung Chung[1]

1 Institute of Oceanography, National Taiwan University, Taiwan
2 Marine Research Station, Institute of Cellular and Organismic Biology, Academia Sinica, Taiwan

Abstract

The color change system of cephalopods has been assumed to be energetically expensive, potentially accounting for a certain level of metabolic scope. Yet, it has rarely examined the actual cost at the individual level. We evaluate the metabolic level of camouflage by rearing cuttlefish, *Sepia pharaonis*, in three environments with distinct background patterns: white (stable environment without camouflage requirement), fixed square size of black-and-white checkerboard (stable environment with camouflage requirement) and constantly changing square sizes of checkerboard (dynamic environment with camouflage requirement). Energy use was assessed through enzyme activities (LDH and CS) measured in gill and mantle tissues at the end of the experiment, as well as through continuous oxygen consumption measurements and δ13C values recorded in the cuttlebone over the one-month rearing period. Enzyme activities showed no significant differences among the experimental groups, and similarly, the oxygen consumption did not change significantly over time. However, δ13C values were significantly more negative in the camouflage-requiring environment, indicating higher mass-specific metabolic rates. These values showed a decreasing trend during the first half of the experiment and returned to baseline levels by the end. Our results reveal the energy expenditure associated with camouflage is dynamic and cannot be accurately captured by snapshot measurements of oxygen consumption or enzyme activity, but rather by time-averaged δ13C signals. The δ13C values further suggest a two-fold increase in energy use associated with camouflage, providing insight into cephalopod metabolic ecology.

O24 — Development of a machine learning framework for analyzing body patterns in Sepioteuthis lessoniana

Chiba Mizumo[1], Lajbner Zdenek[2], Hiroi Makoto[2], Nakajima Ryuta[2, 3], Miller Jonathan[2], Kawamura Tomohiko[1], Iwata Yoko[1]

1 Atmosphere and Ocean Research Institute, The University of Tokyo
2 Okinawa Institute of Science and Technology
3 The University of Minnesota

Abstract

Body patterns, particularly coloration and patterning, play significant roles in anti-predator defense, predation, and intraspecific communication. Cephalopods, such as squid and octopuses, possess chromatophores that enable them to change their body patterns dynamically and reversibly. Previous studies on various cephalopod species demonstrated the efficiency of classifying body patterns to understand their camouflage strategies and inter- and intra-specific communications. Classification had previously relied on direct human observation - an approach that is labor-intensive and prone to human bias, limiting large-scale quantitative analysis across many individuals. Recent advances in high-throughput imaging and machine-learning–based computer vision has made large-scale image analysis feasible. Here, we present a data-driven, machine-learning–based pipeline that quantifies squid body patterns with minimal human bias. Oval squid *Sepioteuthis lessoniana* hatchlings were reared for 92 days at the OIST Marine Science Station (Japan) and video-recorded every five days. Pose coordinates extracted with SLEAP, the animal pose estimation program, automatically cropped mantle regions from each frame, and a convolutional neural network (CNN) computed image similarity to cluster body patterns. Several distinctive clusters were identified, suggesting that this machine learning framework can support classify the body patterns of squid quantitatively for the studies of camouflage, inter- and intra-specific communication.

O25 — Exploring some reproductive traits among Mediterranean Ommastrephid squids

Blondine Agus1, 2, Pietro Battaglia3, 4, Maria Cristina Follesa1, Francesco Longo3, Emanuele Asciutto3, 4, 5, Teresa Romeo3, 6, 7, Riccardo Porceddu1, Danila Cuccu1

1 University of Cagliari, Department of Life and Environmental Science, Cagliari, Italy
2 Stazione Zoologica Anton Dohrn, Department of Integrative Marine Ecology (EMI), CRIMAC, Calabria Marine Centre, C. da Torre Spaccata, 87071 Amendolara (CS), Italy.
3 National Biodiversity Future Center (NBFC), Piazza Marina 61 90133 Palermo, Italy
4 Stazione Zoologica Anton Dohrn, Department of Integrative Marine Ecology, Sicily Marine Centre, Contrada Porticatello 29, 98167 Messina, Italy.
5 Dipartimento di Scienze della Terra e del Mare, Università di Palermo, Via Archirafi 22 90123 Palermo, Italy
6 Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Sicily Marine Centre, Villa Pace, Contrada Porticatello 29, 98167 Messina, Italy.
7 National Institute for Environmental Protection and Research (ISPRA), Via dei Mille 46, 98057 Milazzo (ME), Italy.

Abstract

Ommastrephid squids are widely distributed throughout the world's oceans and are considered among the most important and economically exploited cephalopods. Regarding the four species present in the Mediterranean *Illex coindetii*, *Todaropsis eblanae*, *Todarodes sagittatus*, and *Ommastrephes caroli* only fragmentary information has been available to explain their reproductive strategies. With the aim of updating knowledge on some reproductive aspects, we collected mature males and mated females of each species from the waters around Sardinia and Sicily, the two largest islands in the central Mediterranean. Data are presented on the number, morphology, morphometry, and histology of spermatophores and spermatangia, providing for the first time information on *T. sagittatus* and *O. caroli*. For all four species, we also examined the type and site of spermatangia attachment on the female body.

Our results revealed some differences compared to previously published data on Atlantic specimens, particularly in *T. eblanae*, for which higher values were observed in both the number and size of spermatophores, as well as in the number of seminal receptacles in mated females. We confirm the "shallow implantation strategy," in which only the base of the spermatangium is embedded in the female tissue specifically, in the buccal mass in *T. eblanae*, *T. sagittatus*, and *O. caroli*, and in the mantle cavity in *I. coindetii*. The observation of two cases of spermatangia attached in unusual sites within the mantle cavity in *T. sagittatus* and externally on the mantle in *I. coindetii* suggests the possibility of mating errors in these two species.

O26 — Squid pro quo: Female choice and the co-evolutionary dynamics of coleoid cephalopod reproduction

Theodora Mautz[1], Fernando Melendez Vazquez[1], Yoko Iwata[2], Brice Semmens[1]

1 Scripps Institution of Oceanography, University of California San Diego, USA
2 Atmosphere and Ocean Research Institute, The University of Tokyo, Japan

Abstract

Coleoid cephalopods exhibit diverse reproductive strategies, encompassing both pre- and post-copulatory mechanisms. Much of the existing research has focused on male tactics, such as male-male competition, courtship of females, mate guarding, sperm replacement, sperm allocation, and sperm quality. Meanwhile, a growing body of literature has emphasized females' roles in cephalopod sexual selection, through precopulatory displays and behaviors, postcopulatory sperm storage, and biased use of sperm for fertilization. This study combines a literature review with phylogenetic comparative analysis to explore coleoid female choice through an evolutionary lens. We first synthesize the behavioral and physiological adaptations that facilitate female control of paternity in coleoids and situate these processes within broader discourse on female choice and sexual selection. We then apply a suite of phylogenetically-informed logistic regressions and evolutionary model-fitting assessments to existing cephalopod phylogenies. Specifically, we investigate the independent gains and losses of reproductive organs across coleoids in relation to factors such as sexual dimorphism, mating system, mechanisms for female choice, and male alternative reproductive tactics (ARTs). By integrating behavioral, physiological, and evolutionary insights, we highlight the importance of female choice and the dynamic interplay between female and male traits in shaping the evolution of coleoid reproductive systems.

O27 — Polarization signals in cuttlefish mating displays: morphological adaptations for conspicuousness and potential diversity

Arata Nakayama[1], Ryosuke Ohnuki[2], Shinya Yoshioka[2], Nobuhiro Ogawa[1], Hiroki Ono[3], Mayuko Yamada[3], Toshihiko Kushihiki[4], Noriyosi Sato[5], Tomohiko Kawamura[1], Yoko Iwata[1]

1 Atmosphere and Ocean Research Institute, The University of Tokyo
2 Graduate School of Science and Technology, Tokyo University of Science
3 Marine Biological Science Section,Shimane University
4 Aquarium Asamushi
5 School of Marine Science and Technology, Tokai University

Abstract

Cephalopods possess polarization vision. In addition, specific areas of their body surface, such as the arms, reflect polarized light, which is suggested to function as a visual signal. Many cuttlefish species in the subgenus *Doratosepion* exhibit species-specific sexual ornamentations such as extensive elongation of arms or fins, raising the possibility that these traits are used for polarization signaling in reproductive communication, accompanied by morphological adaptations to enhance signal conspicuousness. Here, in one of those species Andrea cuttlefish *Sepia andreana*, we applied video polarimetry technique and discovered that male's sexually ornamented arms exhibit courtship-specific polarization signal. This signal combines horizontally and vertically polarized light, achieving maximum contrast for cephalopod polarization vision system. By integrating microstructural analysis and optical measurements of these ornamented arms, we demonstrated that this complex polarization pattern arises from a combination of polarized reflection by reflective cells (iridophores) and transmission through transparent, birefringent muscle layer, which rotates the polarization angle of light from horizontal to vertical. We further investigated courtship display in another *Doratosepion* species, *S. misakiensis*, and identified a courtship-specific polarization signal. While this display is composed solely of a horizontally polarized pattern, it is highly dynamic, with horizontally polarized stripe on his arm repeatedly turned on and off, likely contributing to the conspicuousness of the display. Our findings reveal that the conspicuous polarization signal is achieved through distinct morphological and behavioral adaptations in each species, suggesting the significance of polarized light in reproductive communication and highlighting the potential diversity of polarization signaling among cuttlefish.

O28 — Genetic evidence of mating system in pair-forming diamond squid assessed by microsatellite markers

Azad Kamrun Naher[1, 2], Kato Yoshiki[3], Ruku Rohana Akter[1], Hirohashi Noritaka[1]

1 Graduate School of Natural Science and Technology, Shimane University, Japan
2 Department of Aquaculture, Bangladesh Agricultural University, Mymensingh, Bangladesh
3 Japan Fisheries Research and Education Agency, Yokohama, Japan

Abstract

The diamondback squid *Thysanoteuthis major* is a large nektonic squid of the monotypic family Thysanoteuthidae distributed in tropical and subtropical waters worldwide. They are often observed in pairs of one male and one female with similar sizes. Their pairing begins at an immature stage and continues into adulthood, indicating behavioral monogamy. However, no compelling evidence is recorded for supporting genetic monogamy, which made us interested to study the genetic basis of these partnerships. Therefore, a study was carried out to investigate whether these squids are truly monogamous, by genotyping the stored sperm in the female's multiple seminal receptacles (SRs) which are ventrally located on the female's buccal membrane. Four highly polymorphic microsatellite markers were newly developed for genotyping the sperm. The microsatellite-based fragment length analysis (FLA) showed that one female mated with at least 2 to 5 males, indicating that they are not monogamous. Even each SR within the females also exhibited multiple paternity. Interestingly, the FLA electrograms showed multi-allelic peaks with significant similarity in peak patterns among SRs within female. This contrasts with the case of the oceanic Japanese common squid, *Todarodes pacificus*, where each SR exhibits different pattern of paternity composition. Therefore, inspite of moving in pairing, the females are not engaged only with the pair-bonded partner for mating, as suggested by the genetic evidence.

O29 — Molecular Regulation of Gonadal Differentiation in the Bigfin Reef Squid (*Sepioteuthis lessoniana*)

Guan-Chung Wu[1], Li-Lun Tang[1], Yung-Che Tseng[2], Pou-Long Kuan[2]

1 Department of Aquaculture, National Taiwan Ocean University, Keelung, Taiwan
2 Marine Research Station, Institute of Cellular and Organismic Biology, Academia Sinica, Yilan, Taiwan

Abstract

Sex determination systems in animals can involve genetic factors, environmental factors, or a combination of both. A recent study on octopuses revealed that females possess a hemizygous Z chromosome, suggesting a ZZ/ZO sex determination system, which may be conserved across cephalopods. However, there are few reports focusing on morphological changes during the gonadal differentiation process in cephalopods. Moreover, the molecular mechanisms underlying sex determination and gonadal differentiation in cephalopods remain unclear. To address this, we used the culturable bigfin reef squid as a model to investigate the mechanisms of sex determination and gonadal differentiation in cephalopods. First, we identified a Z-specific DNA marker and determined the genetic sex of the bigfin reef squid and distinguished between ZZ males and Z0 females. Histological analysis showed that the first female characteristic-meiotic oocytes-appeared at 2 days after hatching (dah), while the first male characteristic- seminiferous tubules-appeared after 24 dah. We further analyzed gene expression profiles using RNA-seq data and found that a large number of genes were significantly downregulated in females at 2 dah compared to 0 dah. In contrast, only a few genes showed significant changes between 0 and 2 dah in males. Less differences in gene expression were observed between the sexes at 0 dah. These findings demonstrate that ovarian differentiation occurs earlier than testicular differentiation at both the morphological and molecular levels. Overall, our study provides new insights into the molecular mechanisms of sex determination and gonadal differentiation in cephalopods.

O30 — Sex determination and sex ratio at birth in the firefly squid, *Watasenia scintillans*

Kinoshita Manami[1], Kusama Satoshi[2], Hirohashi Noritaka[1]

1 Shimane University Graduate School of Natural Science and Technology
2 Uozu Aquarium

Abstract

It had long been an enigma how cephalopods determine their sex. However, a recent study with high-precision, whole-genome next-generation sequencing identified Z chromosome in California two-spot octopus. Independently, we developed the microsatellite marker (Ws113) in the firefly squid, *Watasenia scintillans*. Using Ws113, we found that females are monoallelic, while males are mostly biallelic, indicating female heterogamety. Female W. scintillans are highly monogamous storing spermatangia from a single male and spawning an egg string in captivity. This allows us to identify the Ws113 genotype and therefore determine the sex of paralarvae. We examined the sex ratio using a total of 1501 hatchings from 10 females. We found that in one case, hatchlings were highly male-biased (1:13), however, the overall sex ratio was slightly female-biased (male:female=0.9:1). This is the first demonstration of the sex ratios at the hatching stage in cephalopods. Furthermore, we have developed a protocol for Ws113-based genotyping of embryos at 48 hours post-fertilization. This new protocol enables us to estimate both the primary sex ratio (at fertilization) and the secondary sex ratio (at hatching), as well as investigate the possibility of sex-linked embryonic death.

O31 — Biomimetic Models Reveal the Scaling Principles and Morphological Adaptations of Cuttlefish Suckers

Baowen Zhang[1], Brett Klaassen van Oorschot[1], Sietze Thijssen[1], Yoerick Lankhof[1], Guillermo Amador[1]

1 Wageningen University & Research

Abstract

Cuttlefish suckers, distributed along their arms and tentacles, exhibit remarkable size variations, ranging from tens of micrometers to tens of millimeters in diameter. Previous studies have found a unique power-law relationship between sucker diameter and attachment stress: smaller suckers generate disproportionately higher attachment stresses. However, the underlying mechanisms driving this relationship remain poorly understood. In this study, we developed a biomimetic model to elucidate this phenomenon. By simplifying the morphology of cuttlefish suckers, we designed and fabricated a series of bio-inspired artificial suckers with diameters ranging from 3 mm to 36 mm. The attachment performance of these suckers was systematically measured in both water and air, including pull-off force, displacement, and pressure. Our results show that the biomimetic suckers replicate the power-law relationship observed in natural cuttlefish suckers, suggesting a shared attachment mechanism. Furthermore, our model provides insights into the morphological adaptations of cuttlefish suckers, particularly asymmetric sucker designs and stiff internal tissues. By uncovering the biomechanical principles underlying cephalopod sucker attachment, this study motivates the design of next-generation artificial grippers with enhanced adaptability and robustness.

O32 — Cephalopod prey analysis of a deep-sea predator, *Omosudis lowii* from the northern Gulf of Mexico

Claire De Noyo[1], Tracey Sutton[2], Heather Judkins[1]

1 University of South Florida St. Petersburg
2 Nova Southeastern University

Abstract

Little is known about the feeding dynamics of deep-sea communities, despite ecological importance and an estimated global biomass in the order of gigatons. Mesopelagic and bathypelagic communities contribute to marine biodiversity and ecosystem stability while directly combatting climate change via carbon sequestration. The hammerjaw (*Omosudis lowii*) is an understudied, deep-sea fish found in temperate and tropical waters worldwide, including the Gulf of Mexico. Hammerjaws have distensible jaws and stomachs, allowing them to capture extremely large prey relative to their body size. This project is the first to investigate the species trophic ecology by providing baseline data in regard to diet composition, feeding selectivity, and niche breadth. A stomach content analysis study was conducted using over 500 preserved hammerjaw specimens collected by the Deep Pelagic Nekton Dynamics of the Gulf of Mexico Consortium (DEEPEND) on research cruises spanning 2011 to 2023. Squid are the primary prey item for smaller individuals while larger hammerjaws had a mix of fish and squid in their diet. Sampling location, time of day, salinity and temperature did not indicate that further analysis was warranted. Ultimately, this project contributes to the growing knowledge base of the Gulf of Mexico's complex marine food web. With increasing anthropogenic and environmental threats such as climate change, overexploitation, and petroleum production, this research is critical to the understanding of deep-sea biodiversity and stability.

O33 — Analysis of the Flight Mechanism of Flying Squid : Yaw Stability Influenced by Arm Shape

Aizawa Kei[1], Ito Fumiaki[2], Inada Yoshinobu[2]

1 Course of Mechanical Engineering, Graduate School of Engineering, Tokai University
2 Department of Aeronautics and Astronautics, School of Engineering, Tokai University

Abstract

Some marine organisms escape predators by leaping out of the water and flying. The neon flying squid is one such species, capable of agile and stable flight while turning above the ocean surface. It has a shape similar to a canard aircraft but lacks a vertical stabilizer, making its yaw control and stability mechanisms unclear. In this study, we created a 3D model of the squid's flight posture based on photographs and specimens to investigate how the arm configuration affects yaw stability. The model was designed using Blender, printed with a resin 3D printer, and tested in a wind tunnel at a wind speed of 10 m/s, approximating the squid's flight speed. By varying the yaw angle, we measured the yawing moment. The results showed that arm arrangement significantly influences yaw stability, and by configuring the arms in a more planar manner, the squid can achieve sufficient stability even without a vertical stabilizer.

O34 — Acoustic telemetry reveals spatiotemporal segregation in deep-sea predator-prey dynamics

Antoni Sánchez-Márquez[1], Ivan Masmitja[1], Maria Vigo[2], Víctor Martín-Vélez[1], Jacopo Aguzzi[1], Nixon Bahamon[1], José Antonio García[1], Bernat Hereu[3, 4], Joan Baptista Company[1], Joan Navarro[1]

1 Institut de Ciències del Mar (ICM), CSIC, Passeig Marítim de la Barceloneta 37-49, 08003 Barcelona, Spain.
2 Department of Integrative Ecology, Stazione Zoologica Anton Dohrn, Sicily Marine Centre, Lungomare Cristoforo Colombo (complesso Roosevelt), Palermo, Italy.
3, Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia, Universitat de Barcelona (UB), Barcelona, Spain.
4 Institut de Recerca de la Biodiversitat (IRBIO), Universitat de Barcelona (UB), Barcelona, Spain

Abstract

Predation risk shapes prey behavior through a landscape of fear, influencing spatial ecology by modulating predatory and anti-predatory interactions that promote species coexistence. While well-documented in terrestrial ecosystems, these dynamics remain poorly understood in aquatic habitats, particularly in remote deep-sea environments. Using acoustic telemetry, we simultaneously tracked the Norway lobster (*Nephrops norvegicus*) and two key predators—the small-spotted catshark (*Scyliorhinus canicula*) and the horned octopus (*Eledone cirrhosa*)—in a Mediterranean deep-sea no-take reserve (350–400 m depth). Diel activity analyses revealed temporal segregation: Norway lobsters displayed diurnal and cathemeral activity, small-spotted catsharks were primarily diurnal, and horned octopuses were strictly nocturnal. Spatially, catsharks occupied the largest home ranges and exhibited the highest mobility, followed by Norway lobsters and then horned octopuses. Home range overlap analysis indicated partial spatial segregation: horned octopuses overlapped with catsharks and Norway lobsters only in peripheral ranges (95% HR), whereas catsharks and lobsters exhibited progressive overlap from peripheral to core areas. These findings highlight temporal segregation as a mechanism to reduce intraguild competition and predation risk, while spatial patterns reflect Norway lobsters' predator avoidance strategies. This study advances understanding of deep-sea predator-prey dynamics, revealing how spatiotemporal partitioning facilitates coexistence in resource-limited ecosystems.

O35 — Neurons, Muscles, and Venom: Identifying Drivers of Cephalopod Predation

Kim Kirchhoff[1], Melvin Williams[1], Belkes Stambouli[1], José Ramón Pardos-Blas[2], Amy Courtney[3], Andrew L. Sugarman[4], Daniel J. Vanselow[4], Christian Carrasco[5], William R. Schafer[3, 6], Keith C. Cheng[4], Eve Seuntjens[6], Mande Holford[1, 7]

1 Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA, USA
2 Natural History Museum, Madrid, Spain
3 MRC Laboratory of Molecular Biology, Cambridge, United Kingdom
4 Department of Pathology, Pennsylvania State University College of Medicine, Hershey, PA, USA
5 New York Research and Mentoring for Postbaccalaureates at Hunter College, CUNY, NYC, NY, USA
6 Department of Biology, KU Leuven, Leuven, Belgium
7 Department of Invertebrate Zoology, The American Museum of Natural History, NYC, NY, USA

Abstract

Coleoid cephalopods—including octopuses, squids, and cuttlefish—produce venom in their posterior salivary glands (PSGs). Despite the pivotal role of venom in their evolution and ecology, the regulatory mechanisms underlying venom release remain poorly understood. To explore the potential role of neuronal control in venom secretion, we conducted multimodal histological profiling and live imaging using neuronal and muscular markers on fixed, paraffin-embedded PSG tissue sections across coleoid lineages, *Euprymna berryi*, *Doryteuthis pealeii*, *Sepia bandensis* and *Octopus bimaculoides*. Our results indicate an intimate neuromuscular network throughout the PSG and lays the foundation for deeper investigations into the signaling dynamics and regulatory mechanisms of venom release.

Specifically, we observed a branching tubular architecture that is consistent across coleoid PSGs. Micro-computed tomography, alongside hematoxylin-eosin and tetrachrome stainings, confirmed the presence of two distinct tubular types: distal secretory and proximal striated tubules, defined from the perspective of the venom duct. This differentiation in the tubular structure suggests a method for "pushing" venom components through the salivary duct to the buccal mass for dissemination. Additionally, filamentous actin staining identified muscle fibers encircling the tubules, which were closely associated with α-bungarotoxin-positive neuromuscular junctions—suggesting coordinated activity. This neuromuscular organization was further supported by the mirrored immunohistochemical signals of pre-synaptic phosphoproteins and neuronal nuclei. A pilot calcium imaging experiment in ex vivo PSGs provided first real-time insights into potential neural signaling pathway activation. Collectively, our findings support the hypothesis of neuromuscular regulation of venom secretion in cephalopods.

O36 — Activation and deformation of antagonistic muscles in Octopus vulgaris arm motion

Janina Leonie Röckner

Istituto Italiano di Tecnologia

Abstract

The arm of an octopus lacks any rigid structures analogous to human bones. Motion is performed by coordinating activation in three different muscle types. Although this allows the arm to move with infinite degrees of freedom, several of the octopus arm motions, like withdrawal and reaching, are performed following stereotypical patterns. However, the pattern of activation and specific contribution of the two main arm antagonistic muscles remain unclear. Understanding these dynamics is important in the field of soft robotics, as it may help modelling and simulating soft artificial structures. In this work, we investigate the involvement of longitudinal and transverse muscles in arm reflex responses and arm extension elicited through either mechanical or electrical stimulation. Previous studies of electromyography recordings of the aboral longitudinal muscle from an ex-vivo arm preparation, showed that a wave of muscle activation propagates from the base to the arm tip during arm extension. Here we recorded electromyography activity from the arm antagonistic muscles and synchronised it with muscle deformation data, measured using piezoelectric transducers, to quantify their activation during 3D movements. Our data show coactivation of antagonistic muscles during arm reflex responses and extension. The transverse muscle consistently exhibited a lower activation level compared to the longitudinal muscle. Both muscles showed a consistent profile of activation with a decreased activity during stretch in consecutive trials. Incorporating biological data derived from our arm muscle mechanics

O37 — Diversity and Evolution of Neuronal Cell Types in Cephalopods

David Hain[1], Simone Rencken[1], Georgi Tushev[1], Elena Ciirdaeva[1], Gilles Laurent[1]

1 Max Planck Institute for Brain Research, Frankfurt, Germany

Abstract

The independent evolution of large brains and complex behaviors in soft-bodied cephalopods represents a remarkable case of convergence, offering unique insights into neural design principles. Analyzing the cell types and circuits in cephalopod brains provides a valuable opportunity to study convergent evolution and identify shared or divergent principles underlying sophisticated computations. To understand cephalopod brain evolution at a molecular level, we profiled cell type diversity using single-nucleus RNA sequencing for the European cuttlefish, *Sepia officinalis*, and the dwarf cuttlefish, *Sepia bandensis*, complemented by spatial transcriptomics for *S. officinalis*. Integrating these data with published transcriptomic resources from other cephalopods, including *Octopus bimaculoides* (Songco-Casey et al., 2022) and *O. vulgaris* (Styfhals et al., 2022), we performed detailed cross-species comparisons between decapods and octopods. Despite about 270 million years of separate evolution, initial analyses reveal high conservation of major neural cell types. These comparisons explore both the conservation of core cell types and the emergence of novel types potentially underlying species-specific adaptations and behaviors, such as variations in social behavior and skin patterning in *S. bandensis* and *S. officinalis*. Future work will extend these analyses beyond cephalopods to include other molluscan and spiralian species, elucidating how complex cephalopod nervous systems evolved from simpler ancestral forms. Ultimately, this comparative framework seeks fundamental insights into nervous system construction and the diverse molecular pathways to cognitive complexity.

O38 — Unbiased and Targeted Spatial Transcriptomics of the Octopus chierchiae Central Brain

Alyson Hally[1], Jessica Stock[4], Chaichontat Sriworarat[2], Genevieve Stein-O'Brien[2, 5], Gül Dölen[3], Caroline Albertin[4], Loyal A. Goff[1, 2, 5]

1 McKusick-Nathans Department of Human Genetics, Johns Hopkins University School of Medicine
2 Solomon H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine
3 University of California, Berkeley
4 Woods Hole Marine Biological Laboratory, University of Chicago
5 Kavli Neurodiscovery Institute, Johns Hopkins University

Abstract

The pygmy zebra octopus (*Octopus chierchiae*) is an attractive model organism for neuroscience, owing in part to its small size, early adult behaviors, and its capacity for multi-generational breeding in culture. We have created a high-quality reference genome and annotation to enable high-throughput molecular profiling of the *O. chierchiae* central brain. Using this, we have established a cellular and spatially-resolved atlas of the cell types within the central brain. Using a flexible approach specifically adapted for cephalopod cellular physiology, we have established a compendium of single-nucleus transcriptional profiles, revealing the cellular diversity within the adult central brain of *O. chierchiae*. In parallel, we have conducted both unbiased and targeted spatial transcriptomic analysis, at cellular and subcellular resolution to accurately localize cell populations and reveal spatially-resolved patterns of gene co-regulation across anatomical features. We reveal organizing principles of cell type identity and architecture in the cephalopod central brain. We identify developmentally-associated genes with significant differential expression across major regions, establishing similarities and contrasts to established model organisms. The integration of these data provides a detailed view of the cellular and molecular organization of the octopus central brain, a critical step for unraveling the neurological underpinnings of cephalopod intelligence and behavior. The availability of this atlas enhances the utility of *O. chierchiae* for targeted functional studies, providing a valuable asset for comparative neurobiology.

O39 — A Chromosome-Scale Genome and Brain Cell Atlas of the Common Cuttlefish *Sepia officinalis*

Simone Rencken[1, 2], Georgi Tushev[1], David Hain[1, 3], Elena Ciirdaeva[1], Oleg Simakov[4], Gilles Laurent[1]

1 Max Planck Institute for Brain Research, Frankfurt am Main, Germany
2 Radboud University, Donders Institute for Brain, Cognition and Behaviour, Nijmegen, Netherlands
3 Faculty of Biological Sciences, Goethe University, Frankfurt am Main, Germany
4 Department of Neuroscience and Developmental Biology, University of Vienna, Vienna, Austria

Abstract

Coleoid cephalopods exhibit remarkable adaptations, including the largest invertebrate brains, camera-like eyes, a unique embryonic development, and sophisticated, neurally controlled camouflage. The common cuttlefish Sepia officinalis is a key model organism across diverse fields such as neurobiology, behavior, evolution, and biomechanics. More recently, it has become a model to investigate the neural mechanisms underlying cephalopod camouflage, using quantitative behavioral approaches alongside molecular techniques to characterize the identity, evolution, and development of neuronal cell types.

Despite significant interest, a high-quality annotated genome for this species has been lacking. To address this, we generated a chromosome-scale genome assembly for *S. officinalis*, spanning 5.68 billion base pairs across 47 repeat-rich chromosomes. Gene linkage analysis confirms the existence of 47 chromosomes, revealing clear homologies with related species such as *Euprymna scolopes* and *Doryteuthis pealeii*. Our work includes a comprehensive gene annotation and full-length transcript predictions that should be helpful for further evolutionary and single-cell expression studies. This genome provides a valuable resource for future research on the evolution, brain organization, information processing, development, and behavior in this important clade.

Using the genome as a reference, we are profiling the cell type diversity in the cuttlefish brain using single-nucleus RNA sequencing, in situ hybridization and spatial sequencing methods to generate a comprehensive molecular map of the cuttlefish brain.

O40 — Hierarchical processing and polarization encoding in the cephalopod visual system

Tomoyuki Mano[1], Konstantinos Tsaridis[1], Yutaka Kojima[1], Giovanni D. Masucci[1], Thi Thu Van Dinh[1], Rudi Tong[1], Vasileios Glykos[1], Lada Dolezalova[1], Keishu Asada[1], Daria Shumkova[1], Loranzie S. Rogers[2], Maxime Hamon[1], Matteo Santon[3,4], Makoto Hiroi[1], Teresa L. Iglesias[1], Nicholas W. Bellono[2], Martin J. How[3], Yukiko Goda[1], Leenoy Meshulam[5], Sam Reiter[1]

1. Okinawa Institute of Science and Technology Graduate University, Okinawa, Japan
2. Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA, USA
3. School of Biological Sciences, University of Bristol, UK
4. Hydrobiological Station "U. D'Ancona" & Department of Biology, University of Padova, Padova, Italy
5. Department of Physics, Department of Neuroscience, and Carney Institute for Brain Science, Brown University, Providence, RI, USA 1. Computational Neuroethology Unit

Abstract

Vertebrates and cephalopods have independently developed camera-type eyes — a striking example of convergent evolution. While many vertebrates detect color through wavelength-sensitive retinas, cephalopods have instead evolved retinas that detect light polarization. Polarization vision is thought to confer ecological benefits in underwater environments, such as enhanced object detection and intraspecies communication. The cephalopod visual center, the optic lobe (OL), exhibits a highly organized and distinctive structure with two parts: the cortex, which bears morphological similarity to the vertebrate retina (hence often termed the "deep retina"), and the medulla, which has a characteristic tree-like anatomical organization. However, the mechanisms by which the OL integrates luminance and polarization signals to support cephalopod behavior remains elusive, largely because in vivo neural recordings have been challenging to implement in these soft-bodied animals.

Here, we developed a novel head-fixation method to perform two-photon in vivo calcium imaging in the brain of awake juvenile squids (S. lessoniana). We recorded calcium responses from populations of OL cortex neurons while delivering visual stimuli that varied in intensity and polarization. Our data revealed distinct neuronal classes defined by spatiotemporal tuning and intensity–polarization specificity. These included (i) integrators of horizontal and vertical photoreceptor inputs, (ii) neurons selective for a single polarization orientation, and (iii) neurons that compute the degree of linear polarization (DoLP) by subtracting orthogonal signals. We also observed orientation- and direction-selective neurons in the inner granule layer, but rarely in the outer granule layer, suggesting a feed-forward architecture for progressively complex feature extraction.

We then performed in vivo electrophysiological recordings using Neuropixels probes, targeting downstream regions of the OL cortex including the medulla and peduncle lobe. We found that receptive field sizes increased with OL depth, a hallmark of hierarchical visual processing. Medulla neurons often displayed additive integration of intensity and polarization contrasts, while others showed strongly nonlinear selectivity. Anatomical experiments, including single-neuron dye filling and brain-wide retrograde tracing, further supported a model of hierarchical visual information processing through the medulla's tree-like organization. Collectively, our study provides the first in vivo physiological characterization of the cephalopod visual system, offering new insights into the neural mechanisms underlying their specialized underwater vision as well as broader features of convergent evolution in visual systems.

O41 — Stability and Dynamism in Bobtail Squid (*Euprymna berryi)* Synapse Development

Ishaan Ghosh[1], Joshua Rosenthal[2], Narayanan Kasthuri[3,4]

1 Department of Organismal Biology and Anatomy, Biological Sciences Division, University of Chicago, Chicago, Illinois, USA
2 Marine Biological Laboratory, Woods Hole, Massachusetts, USA
3 Department of Neurobiology, Biological Sciences Division, University of Chicago, Chicago, Illinois, USA
4 Biosciences Division, Argonne National Laboratory, Lemont, IL, USA.

Abstract

Cephalopods and mammals have convergently evolved the largest nervous systems in animals, albeit of significantly different organization and developmental trajectories. Post-natal mammalian neurodevelopment is characterized by dynamic, experience-driven synaptic rearrangements, followed by broad stability in synaptic organization. Aberrations in this process of synapse arrangement are linked to significant cognitive and behavioural deficits. However, whether cephalopod synapse development similarly experiences dynamism in arrangement followed by stability is unknown. Evidence indicates the process may unfold differently. For instance, cephalopods extensively use RNA editing to modulate synaptic function in response to external stimuli which is absent in mammals. To understand how synapse development unfolds in cephalopods, we utilized volume electron microscopy to investigate synapse ultrastructure across developmental time points (embryos, hatchlings, adults) in the plexiform layer of the optic lobe in *Euprymna berryi* (hummingbird bobtail squid). Important features we focused on include synapse density and size, synaptic binding-partner identity, axonal elaboration, and vesicle density. We hypothesize that like mammals, cephalopod synapses experience dynamic changes in these features at earlier life stages before exhibiting greater levels of stability as the animal matures. We further hypothesize that by similarly investigating synapse development in RNA-editing knockout models, that initial levels of dynamism will be suppressed or absent. Characterizing synaptic development in cephalopods will enrich our understanding of how big brains form and function, and provide insight into the evolution of complex nervous systems.

O42 — Embryonic development of a distributed nervous system in *Octopus vulgaris*

Ali Elagoz[1], Mark Lassnig[1], Maxence Lanoizelet[1], Diana Neacsu[1], Marie Van Dijck[1], Elke van Rooij[1], Enora Geslain[1], Eve Seuntjens[2003-02-01 00:00:00]

1 Department of Biology, KU Leuven, Leuven, Belgium
2 Leuven Brain Institute, KU Leuven, Leuven, Belgium
3 Leuven Institute of Single Cell Omics, KU Leuven, Leuven, Belgium

Abstract

The nervous system of *Octopus vulgaris* resembles mammalian nervous systems in terms of neuronal number and wiring complexity, but develops in a distributed manner and is differently organised. Coleoid cephalopods have evolved such a large nervous system independently from vertebrates. In that sense, they represent ideal animal models to investigate the molecular mechanisms essential to evolution of expanded nervous systems.

Octopus vulgaris spawns a large quantity of small eggs, and embryos develop over forty days into paralarvae that hatch out and spend an additional two months in the water column before settling and becoming benthic. The paralarval brain contains a large diversity of neural and glial cell types of which many are still maturing. Our research tries to unravel how and when these cell types are being generated in the embryo, and has shown that the brain arises from a neurogenic region around the eye placode that is spatially patterned. Disconnected from this brain neurogenic zone, we find neurogenic areas that form the arm neural cords. Using single-nuclei RNA sequencing, spatial gene expression and morphogen factor perturbation, we are trying to unravel the molecular determinants of cell type diversification during this distributed nervous system development.

O43 — Large-scale genome size variations in the octopus - insights from Vampire squid

Yoshida Masa-aki[1], Setiamarga Davin[2], Hirota Kazuki[3], Tóth Emese[4], Kon-Nanjo Koto[4], Kon Tetsuo[4], Simakov Oleg[4]

1 Shimane University
2 National Institute of Technology, Wakayama College
3 The University of Tokyo
4 University of Viena

Abstract

Animal genome sizes exhibit remarkable diversity, and understanding the factors underlying this complexity remains a major challenge in modern genomics. Cephalopod genomes, except for that of the nautilus, generally range from 2 to 5 Gb, comparable to the human genome. Much of this genome size expansion is due to the amplification of transposable elements (TEs), and the pattern of TE proliferation is thought to drive changes in gene-coding regions and chromosome structure. In cephalopods, the amplification of TEs appears to have contributed not only to large-scale genome structural reorganization but also to morphological evolution. In this study, we present a draft genome assembly of the vampire squid *Vampyroteuthis* sp., which at over 11 gigabase pairs represents the largest cephalopod genome sequenced to date. We also report a chromosome-level genome assembly of the paper nautilus Argonauta hians, which possesses a genome size of approximately 1.5 Gb—about half that of previously sequenced octopuses—revealing for the first time an eightfold difference in genome size among octopods. Investigation of genome size evolution factors showed that the *Vampyroteuthis* genome is characterized by massive amplification of LINE elements, contrasting with the predominance of SINE expansion in other octopus genomes and resembling the genome organization observed in squids. Synteny analyses suggest that this basal octopod lineage retains a chromosomal structure similar to that of decapodiforms. Comparative analysis indicates that multiple chromosomal fusions occurred after the divergence of octopuses from the squid lineage. This secondary fusion and reshuffling within the octopus genome contributed to the emergence of a more intertwined genome architecture. Our findings demonstrate that the transition from a squid-like to an octopus-specific genome organization reflects significant genomic innovation within cephalopods, highlighting the derived and specialized nature of the octopus genome.

O44 — Para-Para: Comparative Genomics of Shell Matrix Proteins in *Nautilus pompilius* Reveals the Paradox of Paralog Usage in Mollusks

Davin Setiamarga[1, 2], HIROTA Kazuki[1, 3], SASAKI Takenori[4], YOSHIDA Masa-aki[3]

1, Graduate School of Science, The University of Tokyo
2 National Institute of Technology (KOSEN), Wakayama College
3 Graduate School of Natural Science and Technology, Shimane University
4 The University Museum, The University of Tokyo

Abstract

Gene duplication is a major driver of evolutionary innovation, generating paralogs that may retain, divide, or modify ancestral functions. The calcified shell of conchiferan mollusks forms through a shell matrix protein (SMP)-guided mineralization process. SMPs are often duplicated, yet their evolutionary dynamics remain poorly understood. Unlike most cephalopods, early-diverging nautiloids such as *Nautilus pompilius* retain an external shell. *N. pompilius* exhibits direct development, and its embryonic shell mirrors the adult shell, suggesting unique genetic mechanisms for shell formation. In this study, we analyzed the expression of 85 SMPs in embryonic and adult *N. pompilius* using deep transcriptomic data from rare embryonic samples. Over 95% of adult SMPs were already expressed in early embryos. The SMPs clustered into broadly expressed (Group 1) and mantle-specific (Group 2) categories. Despite conventional phylogenies indicating lineage-specific paralog recruitment, microsynteny and subdomain phylogenies suggest conservation of orthologs, while Ka/Ks analyses revealed limited purifying selection on core domains. Twelve tandemly arranged SMP pairs, including tyrosinase and chitinase, showed functional divergence despite genomic proximity. Cross-species comparisons with the pearl oyster Pinctada fucata and the Pacific oyster *Crassostrea gigas* highlight conserved genomic linkages but divergent paralog usage. The continuous SMP expression in embryonic and adult *N. pompilius* contrasts with stage-specific patterns in bivalves, underscoring lineage-specific strategies in shell biomineralization in mollusks, besides revealing distinct evolutionary solutions to maintaining conserved morphologies.

O45 — Effects of RNA editing on transcriptome-based phylogenetic analyses of Decapodiformes

Abigail Pratt[1], Conrad Williams[1], Frank Anderson[1]

1 School of Biological Sciences, Southern Illinois University, Carbondale, Illinois, USA

Abstract

The cephalopod clade Decapodiformes consists of Spirulida (ram's horn squid), Sepiolida (bobtail squids), Sepiida (cuttlefishes), Myopsida (inshore squids), and Oegopsida (oceanic squids). Relationships among clades within Decapodiformes have remained unclear throughout the history of systematics, with different relationships recovered using different datasets and analyses, but recently, transcriptome (i.e., RNA-seq) data have shown promise for elucidating decapodiform phylogeny, due to their conserved nature and large amount of data. However, coleoid cephalopods have a high level of A-to-I RNA editing not seen in any other organisms examined to date, particularly within nervous system tissue. This editing occurs when enzymes act on RNA, changing adenosine to inosine which is then read as guanine. Due to the changes in RNA, the high level of editing seen in coleoids could have a major impact on transcriptomic phylogenetic analyses. In this study, we aimed to elucidate the effects that RNA editing has on transcriptome-based estimates of decapodiform phylogeny with two different analyses. One compared a tree built with RNA-seq data downloaded from GenBank with a tree inferred with transcriptomes in which editing sites had been recoded as missing data, and the other compared transcriptomic trees inferred from nervous tissue samples with trees inferred from non-nervous tissue samples (nervous tissue transcripts should be more heavily edited than non-nervous tissue transcripts). Both analyses resulted in trees with differing topologies between the two datasets.

O46 — Comparative analysis of diverse decapodiform genomes reveals rapid radiation at the Cretaceous-Palaeogene boundary to fill coastal niches

Gustavo Sanchez[1], Fernando Ángel Fernández-Álvarez[2], Ainhoa Bernal-Bajo[3], Elizabeth Heath-Heckman[4], Raphael Lami[5], Margaret McFall-Ngai[6], Michele Nishiguchi[7], Spencer Nyholm[8], Oleg Simakov[9], Louise Allcock[10], Daniel Rokhsar[1]

1 Molecular Genetics Unit, Okinawa Institute of Science and Technology, Onna, Okinawa, Japan.
2 Spanish Institute of Oceanography, Centro Oceanográfico de Gijón (IEO, CSIC), Gijón, Spain
3 Spanish Institute of Oceanography, Centro Oceanográfico de Baleares (IEO, CSIC), Palma, Spain
4 Department of Integrative Biology, Michigan State University, East Lansing, MI USA
5 Sorbonne Université, UPVD, CNRS, UMR8176, Laboratoire de Biodiversité et Biotechnologies Microbiennes (LBBM), Observatoire Océanologique, 66650 Banyuls-sur-Mer, France
6 Department of Biosphere Sciences and Engineering, Carnegie Institution for Science, Pasadena, CA 91125, USA
7 Department of Molecular and Cell Biology, University of California, Merced, California, USA
8 Department of Molecular and Cell Biology, University of Connecticut, Storrs, Connecticut, USA
9 Department for Neurosciences and Developmental Biology, University of Vienna, Vienna, Austria
10 School of Natural Sciences and Ryan Institute, University of Galway, University Road, Galway H91 TK33, Ireland

Abstract

Decapodiform cephalopods occupy a wide range of marine habitats, from the deep ocean to shallow coastal environments. Yet the evolutionary relationships among major lineages remain uncertain, with implications for understanding the origin of internalized shell structures (such as the gladius, cuttlebone, and coiled shell, which are derived remnants of the ancestral chambered shell), as well as the ecological shift between deep-sea and coastal habitats. To address these questions, we generated high-quality genome assemblies for two previously unsampled lineages – the ram's horn squid *Spirula spirula* (Order Spirulida) and the pygmy squid *Idiosepius pygmaeus* (Order Idiosepiida) – and for the Japanese flying squid Todarodes pacificus, expanding representation within the Oegopsida and improving overall phylogenetic resolution. We integrated these genomes with eleven other decapodiform genomes from the Aquatic Symbiosis Genomics Project and other published sequences. Phylogenomic analyses support a new topology that separates open-ocean lineages (Oegopsida and Spirulida) from the remaining coastal and shallow-water orders (Sepiida, Myopsida, Idiosepiida, and Sepiolida). Molecular clock estimates suggest a rapid radiation of modern decapodiforms around the Cretaceous–Paleogene boundary (K/Pg, ~65 Mya), more recent than previously thought. Comparative genomics reveals broadly conserved chromosome-scale synteny across decapodiforms (with the notable exception of I. pygmaeus), and highlights evolution of components of the biomineralization toolkit in several lineages. Together, our new phylogenetic topology, comparative genome analyses, and signatures of adaptive evolution support a scenario in which decapodiform cephalopods originated from deep-sea ancestors and radiated into coastal and shallow-water habitats during the K/Pg transition.

O47 — Pelagic bobtail squid harbors a degraded bioluminescent symbiont

Arno Hagenbeek[1], Gustavo Sanchez[1], Fernando Fernandez-Alvarez[2], Ryosuke Kimbara[1], Lin Zhang[1], Noriyosi Sato[3], Daniel Rokshar[1, 4, 5], Filip Husnik[1]

1 Okinawa Institute of Science and Technology
2 Institut de Ciències del Mar (ICM-CSIC)
3 Tokai University
4 University of California
5 Chan-Zuckerberg BioHub

Abstract

Shallow-water bobtail squid are well known for their bi-lobed luminescent organs, which harbor *Aliivibrio* bacteria. This squid-bacterial symbiosis provides the squid with a source of bacterial bioluminescence for nocturnal camouflage while hunting, and the bacteria with a stable environment for growth. In contrast, open-water bobtail squid inhabit deep pelagic environments and possess a rounded form of this organ. Previous microscopy and culturing experiments suggested that these squid lack bacterial bioluminescent symbionts, suggesting that the organ may generate light without symbionts. To further investigate the luminescent nature of open-water bobtail squid, we sequenced genomic DNA from the rounded organ of *Sepiolina nipponensis*, which phylogenetic analyses confirmed to be a member of the subfamily Heteroteuthinae. In our metagenomic search for the presence of symbionts, we identified a fully circularized genome closely related to *Aliivibrio*. Notably, the ~1 Mb genome size of this novel symbiont is roughly three times smaller than that of A. fischeri. Genes related to metabolism are severely degraded or lost in the open-water symbiont, indicating an inability to synthesize many essential amino acids and cofactors. Despite this extensive genome reduction, the lux operon, essential for bioluminescence, remains completely intact. Our preliminary analysis indicates that the novel symbiont is phylogenetically distinct from the A. fischeri clade. These findings suggest that *S. nipponensis* harbors a previously unknown bioluminescent *Aliivibrio* symbiont, which exhibits extensive genome reduction similar to obligate endosymbionts of other animals.

O48 — Analyzing the gill microbiome of *Lolliguncula brevis* response to environmental change to determine if these effects follow microbial community shifts seen within the water column

Leone Yisrael[1, 2, 3], Sarah Pacocha Preheim[1], Shaina Morris[1], Yue Zhang[1], Keith Arora-Williams[1], Amanda Hinton[1], Katrina Pagenkopp Lohan[2], Anand Gnanadesikan[1], James Gartland[4]

1 Johns Hopkins University
2 Smithsonian Environmental Research Center
3 Maryland Sea Grant
4 Virginia Institute of Marine Science

Abstract

Estuarine microbiome studies provide insight into the composition and richness of microbial communities found in specific marine environments or hosts. However, these environments undergo constant flux which is exacerbated by climate change. Previous studies in Chesapeake Bay have found microbial communities respond to environmental change collected from the water column. However, we do not know much about microbiomes within cephalopod species residing in this environment. Since gills are constantly interacting with the water column, it is important to determine if gill microbiomes reflect the microbial community in the water column the host inhabits or discover whether the gills link to a core microbiome, regardless of how the water column microbial community changes. Additionally, the microbiome can be potentially linked to the ability of osmoregulation, a unique ability of the study species. To fill this knowledge gap, we created a comparison study to focus on a particular cephalopod found in Chesapeake Bay, the Atlantic Brief Squid (*Lolliguncula brevis*).

To test this, we collected 70 Atlantic Brief Squid gill samples to undergo shotgun metagenomics. We sought to discover what microbial communities were present and then compared them to a previous study that used shotgun metagenomics to analyze the microbial communities found in the water column from similar fishing areas. We examined if the collective microbial communities from each group were dissimilar. Additionally, we determined whether the gill microbial communities extracted from the squid produced a core gill microbiome.

O49 — Defining epigenomic and transcriptomic profiles of the highly regenerative octopus arm

Filippo Macchi[1], Kirsten Sadler[1, 2]

1 Program in Biology, New York University Abu Dhabi, Abu Dhabi, P.O. Box 129188, United Arab Emirates
2 Center for Genomics and Systems Biology, New York University Abu Dhabi, Abu Dhabi, P.O. Box 129188, United Arab Emirates

Abstract

Octopus arms are primarily composed of muscle, neurons, skin, and the highly sensory and tactile structures, the suckers. The regeneration of the arm following amputation or injury has captured the imagination of scientists for centuries, but the molecular mechanisms underlying arm regeneration have not been described in cephalopods. As a first step to understand the basis of limb regeneration, we sought to define the molecular and cellular identity of the uninjured limb of *Octopus bimaculoides*. We used bulk RNA sequencing to define the transcriptomic identity of octopus arm tips from over 5 male and female animals and found that the most highly expressed genes are involved in protein translation and motor activity, with genes involved in chromatin remodeling also being enriched. To understand the epigenetic regulation of these genes, we adapted the CUT&Tag protocol for cephalopod tissues to profile the histone modifications that in other species represent repressive (H3K27me3 and H3K9me3) and active (H3K4me3) epigenetic marks. Collectively, these marks define the regulatory landscape of the octopus arm transcriptomic profile and serve as a foundation for understanding how this profile changes during the regeneration of this complex structure.

O50 — A search for the germline in *Doryteuthis pealeii*

Emeline Manganella[1], Julia Lucey[2], Caroline Albertin[1]

1 Marine Biological Laboratory, Woods Hole, MA, US
2 Wellesley College, Wellesley, MA, US

Abstract

Cephalopod molluscs have a suite of evolutionary novelties, including their flexible, suckerlined arms and color changing skin. They also have evolved a novel developmental program: they have lost the spiral cleavage program characteristic of other molluscs. Non-cephalopod molluscs undergo a stereotyped set of holoblastic cell cleavages that divide the embryo into quadrants that will give rise to the left, right, dorsal and ventral axes. The dorsal quadrant, termed "D" is the source of mesendoderm and the germ cells in spiralians. These quadrants appear to be lost in cephalopods, which undergo superficial, bilateral cleavages that lack overt similarities to the quadrants in spiralians. In this study, we set out to identify the germ cells in cephalopods. We identified orthologs of genes commonly involved in germ cell specification and identity (nanos, vasa, piwi and pl10) in D. pealeii and conducted in situ hybridization chain reaction experiments to determine if these markers, as well as mesoderm and endoderm markers, exist in this species and if they are consistent with the spiralian D-cell lineage. We discovered that some of these germline markers are expressed in regions where the future gonads will be located in *D. pealeii*. We also employ tracing methods to determine the lineage of these cells. Understanding the embryonic origins of the germline will aid in the future creation of transgenic and knockout lines necessary for further study of the novel cephalopod biology and development of new tools in these emerging model organisms. This work has been supported by NSF EDGE grant 2220587 and the NSF REU program.

O51 — Building the cephalopod brain: conservation, innovation, and convergence in *Doryteuthis pealeii* neurogenesis

Jessica Stock[1], Caroline Albertin[1]

1 Marine Biological Laboratory, Woods Hole, MA, United States

Abstract

Large nervous systems have evolved twice: in vertebrates and in coleoid cephalopods. With around 500 million neurons, cephalopods have the largest invertebrate nervous systems, far exceeding those of their spiralian relatives (gastropods, annelids, platyhelminths) and even rivaling those of many vertebrates. To elucidate the developmental origins of the cephalopod nervous system and its relationship to other animals, we employed lineage tracing, *in situ* hybridization chain reaction (HCR), live cell imaging, and CRISPR/Cas-9-mediated genome editing. In vertebrates, neurogenesis is heavily dependent on external patterning mechanisms, a large and diverse pool of neural progenitors, and long-range cell migration to achieve the cell type diversity and size of their nervous systems. In contrast, development of many invertebrates, including spiralians, is characterized by a stereotyped cleavage program that gives rise to highly conserved cell fates and lacks extensive cell migration. We find that while cephalopods have lost the characteristic spiral cleavage program, they still produce a stereotyped fate map. Notably, neurogenic niches are located outside of the developing brain, and extensive long-range cell migrations are required to feed newly born neurons into the growing nervous system. This work uncovers a combination of spiralian features, vertebrate-like mechanisms, and evolutionary novelties that drive the development of the cephalopod brain and sheds light on fundamental principles for building a large nervous system.

O52 — Arm-crown in the early life phases of cephalopods: a search for developmental patterns

Erica A. G. Vidal[1], Gabrielle A. R. Regmont[2]

1 Cephalopod early-life stages Laboratory. Center for Marine Studies, University of Parana - UFPR. Pontal do Paraná, PR. 83255-976, Brazil.
2 Graduate Program in Coastal and Oceanic Systems – UFPR. Pontal do Paraná, PR 83255-976, Brazil.

Abstract

The arm crown of cephalopods performs many functions beyond prey capture, demonstrating a complex functional integration. Few studies detailed the homology of the arm crown in hatchling, which is key to understanding phylogenetic relationships. This study evaluates and compares the arm-crown patterns of hatchlings (planktonic paralarvae and benthic juveniles) across different cephalopod groups. Literature review and new data were complied, focusing on factors such as degree of development, arm formula, and sucker distribution for 68 species of all Coleoidea orders. The arm crown of cephalopod hatchlings can be divided in two groups: rudimentary (where some arms are lacking or underdeveloped) or developed (where all arms are present). A high degree of similarity in the developmental pattern of the arm crown was observed in some Octopodidae and in Myopsida, Sepiida, and Sepiolida, indicating conserved patterns. In Decapodiformes, hatchlings with rudimentary arm-crowns lack differentiated tentacular clubs, whereas those with developed arm crowns exhibit them. As expected, Oegopsida hatchlings displayed high morphological diversity in the arm crown patterns aligning with their wide ecological range. However, notable similarities were found among hatchlings of Cranchiidae, Brachioteuthidae, Ommastrephidae, Onychoteuthidae and Thysanoteuthidae. Additionally, the arm crown of Spirulidae and Idiosepiidae hatchlings lack tentacles entirely and suggest a specialized evolutionary pathway unique among all Decapodiformes. This work highlights the importance of early-life morphology in providing insights into the evolutionary history of cephalopods. The arm crown's developmental patterns offer a useful framework for testing phylogenetic hypotheses and exploring adaptive diversification in cephalopods.

O53 — The octopus axial nerve cord is segmented

Cassady Olson[1], Natalie Grace Schulz[1], Clifton Ragsdale[1]

1 University of Chicago

Abstract

The prehensile arms of the cephalopod are among these animals most remarkable features, but the neural circuitry governing arm and sucker movements remains largely unknown. We studied the neuronal organization of the adult axial nerve cord (ANC) of *Octopus bimaculoides* with molecular and cellular methods. The ANCs, which lie in the center of every arm, are the largest neuronal structures in the octopus, containing four times as many neurons as found in the central brain. In transverse cross section, the cell body layer (CBL) of the ANC wraps around its neuropil (NP) with little apparent segregation of sensory and motor neurons or nerve exits. Strikingly, when studied in longitudinal sections, the ANC is segmented. ANC neuronal cell bodies form columns separated by septa, with 15 segments overlying each pair of suckers. The segments underlie a modular organization to the ANC neuropil: neuronal cell bodies within each segment send the bulk of their processes directly into the adjoining neuropil. Cellular analysis establishes that adjoining septa issue nerves with distinct fiber trajectories, which across two segments (or three septa) fully innervate the arm musculature. Sucker nerves also use the septa, setting up a nerve fiber "suckerotopy" in the sucker-side of the ANC. Comparative anatomy suggests a strong link between segmentation and flexible sucker-laden arms. In the squid *Doryteuthis pealeii*, the ANCs in the arms and the sucker-rich club of the tentacles have segments, but the sucker-poor stalk of the tentacles does not. The neural modules described here provide a new template for understanding the motor control of octopus soft tissues.

O54 — Utilizing Fluorescence Lattice Lightsheet Microscopy with Tissue Clearing and Expansion to Study Neural Development in *Sepioteuthis lessoniana* and *Sepia pharaonis*

José Jiun-Shian Wu[1], Bi-Chang Chen[1]

1 Research Center for Applied Sciences, Academia Sinica

Abstract

Seeing is believing. The development of microscopes has been closely linked to the development of biological research and the cephalopods are one of the starting point of neurobiology. Cephalopods, such as the bigfin reef squid (*Sepioteuthis lessoniana*) and the pharaoh cuttlefish (*Sepia pharaonis*), are ideal models for studying neural development due to their complex behaviors and advanced nervous systems. Traditional histological methods have provided valuable insights into cephalopod neural anatomy but are limited to two-dimensional sections, lacking the three-dimensional context necessary for understanding complex neural architectures. To overcome these limitations, this research proposal aims to utilize lattice lightsheet microscopy (LLSM) combined with tissue clearing and tissue expansion techniques. Tissue clearing methods, such as CLARITY and CUBIC, will be employed to make cephalopod tissues transparent, enabling deep penetration of light and high-resolution imaging of neural structures. Additionally, expansion microscopy (ExM) will be used to physically enlarge tissue samples, thereby increasing the effective resolution of light microscopy. For *S. lessoniana*, the study will focus on the embryonic and paralarval development of the central nervous system, particularly the optic lobes, which are crucial for its visual communication and camouflage abilities. In *S. pharaonis*, the emphasis will be on the development of the optic lobes and other brain regions involved in its sophisticated visual processing. The methodology involves collecting samples at multiple developmental stages, from early embryos to adults, and labeling specific neural markers using fluorescent probes. These labeled samples will undergo tissue clearing and/or expansion before being imaged with LLSM or confocal fluorescence microscopes to generate high-resolution three-dimensional reconstructions of the nervous system. The large-tissue clearing and super-resolution optics could show the detailed maps of neural development across different stages, and help the identification of key developmental milestones, and comparative analyses between the two species to elucidate conserved and divergent features of cephalopod neural development. This research will not only enhance our understanding of cephalopod neurobiology but also provide insights into the evolution of complex nervous systems in invertebrates. Furthermore, the optimized protocols developed for tissue clearing and ExM in cephalopods can be adapted for other model organisms, thereby broadening the applicability of these techniques in developmental neuroscience.

O55 — The neural basis of cuttlefish camouflage and social behavior

Tessa Montague[1, 2], Thomas Barlow[2], Erica Shook[2], Daniella Garcia-Rosales[2], Connor Gibbons[2], Eva Castagna[2], Larry Abbott[2], Richard Axel[1, 2]

1 Howard Hughes Medical Institute
2 Zuckerman Mind Brain Behavior Institute, Columbia University

Abstract

Cuttlefish dynamically change the color, pattern and texture of their skin to camouflage with their surroundings. Camouflage is achieved by expanding and contracting pigment-filled saccules in the skin — chromatophores — through the action of motor neurons that project from the brain. Thus, the patterning of the skin is a physical manifestation of neural activity in the brain. In addition to camouflage, cuttlefish communicate with conspecifics using a series of innate skin patterns, thereby revealing the animal's internal state. We are using cuttlefish skin behaviors to understand how the physical properties of the visual world are represented by patterns of neural activity in the brain, and how this representation is transformed into an approximation of the physical world on the skin. We have performed a series of experiments to develop the dwarf cuttlefish, *Ascarosepion bandense*, as a model to investigate the neural basis of camouflage. We have described the stages of embryonic development, sequenced the genome and neural transcriptome, completed a 3D brain atlas, developed camouflage and social behavioral paradigms, and examined dwarf cuttlefish behavior in the wild. Furthermore, we are generating transgenic cuttlefish that express genetically-encoded calcium indicators and light-activated channels, permitting the live imaging and manipulation of neural activity. These technologies should permit us to simultaneously record neural activity and measure behavior to uncover how visual information is deconstructed in the brain, and then reconstructed into an image of the physical world on the skin.

O56 — Network Analysis of the Cortical Optic Lobe of *Sepia officinalis*

Max Brodeur[1], Dominic Evans[1], Ali Karimi[1], Gilles Laurent[1]

1 Max Planck Institute for Brain Research

Abstract

Vision alone is sufficient for cuttlefish to generate background-matching camouflage patterns using a high-dimensional array of chromatophores. The cortex of the optic lobe receives direct photoreceptor input, but while many morphological cell types have been described using Golgi staining (J.Z. Young, 1962), little is known about the circuits they form to process the visual scene. Recent advances in volume electron microscopy (vEM) have made it possible to study brain circuits at a nanometer resolution. Using two vEM datasets acquired from the optic lobe cortex of *Sepia officinalis*, we obtained the first drafts of the early visual system connectome—a synaptic-level connectivity graph. In this work, we apply network analysis techniques to look at connectivity clusters and network motifs to classify neurons by connectivity defined cell-types and uncover the microcircuitry that may help explain how visual information is processed in the cuttlefish brain.

O57 — Cephalopod camouflage

Roger T. Hanlon

Abstract

Rapid Adaptive Camouflage is one of the most distinctive features of cephalopods, and the past few decades have produced a wealth of new data on its many forms and functions. I will focus on behavioral aspects of camouflage by first reviewing how cuttlefish visually sense the background to extract specific visual cues to decide on the specific camouflage pattern type to deploy (Uniform, Mottle, Disruptive). I shall also show new octopus field data illustrating how they use the tactics of background matching, disruptive coloration or masquerade on diverse “microhabitats” while foraging throughout Caribbean coral reef systems. I will demonstrate new field examples of ultrafast camouflage that is used for both primary and secondary defense. Finally, I will present a quite comprehensive list of cephalopod camouflage publications and make it readily available to CIAC to help inspire future research on key gaps in knowledge.

O58 — Cuttlefish survival and camouflage abilities under extreme flooding events

Joana Martins[1], Carlos Gonçalves[1], João Saldanha da Gama[1], Melanie Court[1,2], Rui Rosa[1,2]

[1] Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

[2] MARE - Marine and Environmental Sciences Centre & ARNET - Aquatic Research Network, Faculdade de Ciências, Universidade de Lisboa, Av. Nossa Senhora do Cabo, 939, 2750-374 Cascais, Portugal

Abstract

Extreme events are becoming increasingly frequent and intense, leading to cascading negative effects on marine ecosystems. Here, we aimed to investigate the impact of ecologically relevant extreme flooding conditions (exposure to acute salinity values of 17 and 7) and non-extreme conditions (salinity values of 35, 28, and 24), during 4 days at late embryogenesis, on the survival and camouflage abilities of the European cuttlefish (*Sepia officinalis*) hatchlings. Our findings showed that both extreme flooding conditions elicited 100% mortality. Although latencies to camouflage did not change under non-extreme conditions, lower salinities led to decreased camouflage capabilities. At salinity 24, only 38.2% of the cuttlefish were able to camouflage, and the dark uniform pattern was highly displayed. At higher salinities (28 and 35), camouflage success was greatly enhanced to 96.7% and 94.3%, respectively, with cuttlefish exhibiting either a dark uniform or disruptive patterns. Thus, here we show, for the first time, that short-term extreme flooding conditions elicit deleterious impacts on cuttlefish early ontogeny, namely at developmental and behavioural levels.

O59 — Disentangling Chromatophores Motor Units with Computer Vision

Mathieu Renard[1], Johann Ukrow[1], Margot Elmaleh[1], Dominic Evans[1], Gilles Laurent[1]

1 Max Planck Institute for Brain Research, Frankfurt, Germany
#Joint first authors

Abstract

Cephalopod chromatophores are skin pigment organs that enable unmatched camouflage through rapid and flexible patterning. Although their morphology is well known, the organization of their motor control is not entirely understood. Here, we combine high-resolution videography with a dedicated computer-vision pipeline (CHROMAS) to investigate chromatophore control and their likely innervation in *Euprymna berryi* and *Sepia officinalis*. By segmenting chromatophores into radial slices and analyzing anisotropic deformations, we applied dimensionality reduction (PCA) and source separation (ICA) to estimate the number and spatial influence of motor neurons in individual and groups of chromatophores. On average, four independent components were detected (suggesting innervation by four motoneurons), each forming contiguous petal-shaped domains rather than causing uniform expansion. Clustering thousands of components revealed motor units spanning multiple chromatophores, most involving fewer than 14 but occasionally spanning more widely. These motor units displayed a wide variety of geometries, ranging from compact local groups to elongated or fragmented structures, and they often overlapped, with repeated co-innervation of chromatophore pairs occurring far more than expected by chance. Expansion was consistently faster and more stereotyped than relaxation, consistent with active contraction (for chromatophore expansion) and passive recoil (for chromatophore contraction). Together, these results show that individual chromatophores are not singular or uniform pixels, but rather contrast elements that can be fractionated into smaller territories, themselves coordinated with those of other chromatophores. This geometry of neural control enables, among others, the generation of "virtual" chromatophores (by convergence of adjacent territories of neighbouring chromatophores) as well as that of noise in the distribution of pixel shapes.

O60 — Representing cuttlefish skin patterns

Erica Shook[1], Tessa Montague[1], Thomas Barlow[1], Daniella Rosales-Garcia[1], Richard Axel[1], Larry Abbott[1]

1 Columbia University

Abstract

Dwarf cuttlefish, like octopus, are masters of camouflage. To camouflage the cuttlefish brain must solve a hard computational problem; the brain needs to process the visual environment, compute an appropriate pattern, and then orchestrate the expansion and contraction of hundreds of thousands of pigment filled sacs to generate it. How they accomplish this feat is unknown. Here we hypothesize that the cuttlefish brain encodes a low dimensional representation of the visual world from which it generates an appropriate camouflage pattern. It's an open question what visual features the cuttlefish brain encodes. We argue the brain is encoding visual texture. Visual textures are spatially homogenous images with repeated structure and random variation. Visual texture is unique because it can be compactly and efficiently represented by a small set of summary statistics. We propose these texture statistics as the low dimensional representation used for camouflage. Using a texture synthesis algorithm we show many cuttlefish skin patterns can be well represented by texture statistics. Next, we present a wide array of visual stimuli to freely behaving cuttlefish and show that texture statistics can be used to quantify camouflage and that they capture cuttlefish pattern change better than other large scale representations. This work is a first step in uncovering the neural algorithm underlying cuttlefish camouflage given that the representation the cuttlefish brain uses to generate camouflage patterns constrains the brain's encoding of the visual world and the generation of a skin pattern.

O61 — Post-reproductive neurodegeneration and "programmed" death of cuttlefish (*Sepia officinalis*) progenitors: consequences of an Alzheimer's disease-type tauopathy?

Maureen Pred'homme

Université de Caen Normandie

Abstract

Cuttlefish (*Sepia officinalis*) are short life-span (20-22 months) cephalopods that show rapid CNS degeneration, known as post-reproductive degeneration. The immediate consequence is the abrupt cessation of egg-laying and the death of females that are still fertile. Aging manifests itself through behavioral and physiological alterations: loss of perception, inability to regulate bathymetry, motor decline and instinctual feeding disorders. While the hypotheses of neuroendocrine dysfunctioning and/or immune system failure cannot be ruled out, vertebrate-like CNS neurodegeneration is the major focus of the work carried out in this study. In this context, new CNS transcriptomes from cuttlefish at different stages of their life cycle were sequenced and subjected to analysis. They revealed 686 differentially expressed transcripts. In addition, in silico analysis revealed the expression of molecular markers associated with Alzheimer's disease, including amyloid-β peptide precursor (APP), Tau protein (involved in neurofibrillary tangles), as well as APP-cleaving enzymes (ADAM, BACE-1, PSEN). Immunolabeling with homologous antibodies showed variable distributions and abundances of some of these biomarkers in the CNS depending on the life cycle stage and correlated with post-reproductive decline. TEM observations of the synthetic Ab42 peptide in solution showed spontaneous organization into amyloid polymers similar to the organization described in vertebrates. These results support the hypothesis of a neurodegenerative mechanism shared between cuttlefish and vertebrates, involving molecular pathways analogous to those of Alzheimer's disease. The natural aggregation of Aβ peptide in cuttlefish and the spatio-temporal expression of the identified markers make it a major model for studying neurodegenerative processes.

O62 — Frequency domain characterizing ‘EEG-like’ signature in *Octopus vulgaris*

Marianna de Luca

Stazione Zoologica Anton Dohrn

Abstract

Functional analogies between cephalopod molluscs and vertebrate brain areas and complex cognitive abilities support the existence of sentience and some sort of primary consciousness in these invertebrates. Recent studies have revealed both evoked and spontaneous neural signals from areas of the octopus’ central brain, using two-photon calcium imaging for optic lobes (OLs) electrical activities, and Neuropixels probes to collect supraoesophageal mass (SEM) bioelectrical signals showing that EEG-like signatures could be collected and analysed to characterize neural dynamics in freely behaving animals. We applied a new minimally invasive method to record, for the first time, neural signals in living – sedated – *Octopus vulgaris*. Subcutaneous electrodes provided reliable recordings and allowed to collect both visually evoked and spontaneous neural signals at the level of the OLs and SEM. The averaged EEG-like amplitudes and frequency band power (1-40 Hz) were calculated and compared between light-off and light-on conditions. ‘Resting state’ patterns and oscillations are generally used to represent brain signals in absence of any specific stimuli or tasks and give a measure of the intrinsic brain activity. Spectrograms resulting from our data reveal an increase in power for all the frequency spectra during the light-on condition (compared to light-off). Power spectral density analyses highlighted that in both conditions the total power of the electrical signals was mainly distributed across low frequency bands, i.e. Delta, Theta and Alpha. Our findings contribute to the identification of hallmarks of cognitive states in octopus and deepen our understanding of neural dynamics of these invertebrates.

O63 — Inside Out: sexual dimorphism and neural plasticity shape the brain and behaviour in octopuses

Wen-Sung Chung[1], Nyoman D. Kurniawan[2], Fabio Cortesi[1]

1 School of the Environment, University of Queensland, St Lucia 4072, QLD, Australia
2 Australian Institute for Bioengineering and Nanotechnology, Centre for Advanced Imaging, The University of Queensland, St Lucia 4072, QLD, Australia.

Abstract

Sexual dimorphism is common in animals where females and males differ in body size, sex-dependent characteristics and life history. Behavioural sexual dimorphisms, like courtship display or parental care, are often influenced by differences in the nervous system (e.g., mammals and birds). To date, sexual dimorphism among octopods is characterised by the male hectocotylus arm and adult size differences, where the egg-bearing females are often significantly larger than males. Additional examples include mate guarding behaviour in the male *Abdopu*s and venom usage during copulation by the male *Hapalochlaena*. Apart from these renowned characteristics, knowledge about sexually dimorphism in the octopus brain remains largely incomplete. Leveraging advanced brain imaging developed by our team, it is now feasible to delineate the plasticity of octopus brains in both sexes throughout their lifespans. The male brain continuously grows, reaching its maximum size at the reproductive stage. Conversely, the female brain ceases to grow as the gonads develop, and then rapidly shrinks during reproduction. By assessing how the sex-biased neuroarchitecture changes in gross neuroanatomy and the associated neural networks, we shall recognise octopuses as a novel model for rethinking neural plasticity and their advanced cognitive capability.

O64 — Mining of the neuropeptide genes in the common Chinese cuttlefish *Sepiella japonica* and exploration of its' pleiotropic functions

Chang-feng Chi, Shuang Li, Xu Zhou, Li-bing Zheng

National and Provincial Joint Engineering Research Centre for Marine Germplasm Resources Exploration and Utilization, School of Marine Science and Technology, Zhejiang Ocean University, 1st Haidanan Road, Changzhi Island, Lincheng, Zhoushan 316022, China

Abstract

Cephalopods are key species in marine ecosystems and are also one of the largest and most potentially valuable protein resources in the ocean. Cephalopods hold significant positions in marine fisheries, especially in the China Blue Ocean Pastures. The common Chinese cuttlefish *Sepiella japonica* used to be one of the 'four major marine products' in the East China Sea, but its wild resources were endangered due to overfishing. Following breakthroughs in the artificial breeding technology of *S. japonica*, it is now the main promoted species for stock enhancement in the East China Sea. However, during the artificial breeding, the widespread problems such as germplasm degradation, immunocompromise caused by pathogen infection, and dependence on biological bait severely retarded the promotion of the scale of artificial culture and the restoration and conservation of wild resources. Based on this, our research group has been focusing on neuropeptides of *S. japonica* as the research perspective since 2007. Taking the systematic identification and characterization of multiple neuropeptide genes as the thread, we have carried out the investigation and verification of the pleiotropy of neuropeptide physiological functions, and initially clarified that neuropeptides are widely involved in the reproductive, immune and feeding regulation processes of S. japonica. These findings will provide valuable insights for addressing the challenges encountered in the sustainable mariculture development of *S. japonica*, and lay a theoretical foundation for promoting the restoration and conservation of wild resources of S. japonica in China.

Keywords: Cuttlefish; *Sepiella japonica*; Neuropeptide; Gene expression regulation; Reproduction; Immunity; Feeding

O65 — Octopus welfare biomarkers: a minimally invasive tool for proteomic immune response analysis

Andrea González-Alonso[1], Mónica Carrera[1], Sara Pérez-Polo[1], Manuel Pazos[1], María M. Costa[1], Sonia Dios[1], Francisco Gambón[2], Camino Gestal[1]

1 Instituto de Investigaciones Marinas (IIM), CSIC. Vigo, Spain
2 Departamento de Inmunología, Complexo Hospitalario Universitario de Vigo. Vigo, Spain

Abstract

The common octopus, *Octopus vulgaris* is highly valued for human consumption and has strong potential for aquaculture due to its rapid growth, adaptability, and high protein content. It also serves as a model species in biomedical research. Overfishing and rising demand have made sustainable aquaculture increasingly important to protect wild stocks and support local economies. Ensuring animal welfare is now a key priority, in accordance with European directives, with efforts focused on disease prevention and improving resistance to pathogens. Although early research suggested invertebrate immunity was limited to innate responses, recent studies show octopuses can develop pathogen recognition through a wide range of receptors and acquire protection following exposure to threats. This study aimed to identify, using minimally invasive methods, the molecules involved in immune responses in *O. vulgaris* and to understand their complexity. Using advanced proteomic analysis (LC-MS/MS), after extracting plasma proteins from haemolymph of octopus immunostimulated with zymosan or inactivated *Vibrio lentus*, and tagging with Tandem Mass Tag (TMT), we obtained 10,417 PSMs from 401,730 spectra, resulting in 359 unique proteins, some with peptides with potential as biomarkers. This approach may enable, through the establishment of Operational Welfare Indicators (OWI), new strategies to improve octopus health and welfare in aquaculture settings, supporting sustainable production and conservation of wild populations.

O66 — Multigenerational Culture of *Ascarosepion bandense* to Generate a Transgenic Cephalopod

Connor Gibbons[1], Thomas Mungioli[1], Tessa Montague[1, 2]

1 Columbia University
2 Howard Hughes Medical Institute

Abstract

For decades, various cuttlefish species have been successfully cultured in the lab. With recent advances in cephalopod husbandry and gene editing techniques, the creation of genetically engineered cephalopods is now a reality. However, to generate mutant and transgenic cuttlefish, it is necessary to maintain a large, stable colony with high egg output – demanding robust and reliable husbandry practices. We are in the process of generating transgenic dwarf cuttlefish (*Ascarosepion bandense*) as a model to investigate the neural basis of camouflage. To achieve this, we maintain a population of ~250 *A. bandense* at all life stages, and have generated a suite of methods to improve both fecundity and survivorship. These methods include: (1) a housing strategy that yields daily egg production at a fixed time each day; (2) a non-invasive genotyping method to determine the sex of individuals as young as 6 weeks old; (3) a robust protocol to raise injected embryos in vitro and acclimate them to aquatic systems; and (4) a simple and modular housing system made from 3D-printed pieces and acrylic, that can accommodate dozens of animals for genotyping or behavioral experiments. All of our methods are freely available via our web tool, Cuttlebase.org, and provide a high standard of welfare for the multigenerational culture of not only *A. bandense*, but also many other small cephalopod species well suited for the lab.

O67 — Cephalopod health management in aquaculture through novel disease control strategies

Mehmet Zoral[1], Zdenek Lajbner[1], Lucia Zifcakova[1], Jonathan Miller[1]

1 Okinawa Institute of Science and Technology

Abstract

Cephalopods are emerging as a promising set of organisms for research in neuroscience, behavioral sciences, omics and fisheries science. The need is growing to assess cephalopod health in natural and controlled environments, especially considering climate change-related challenges. The European Parliament expanded animal welfare legislation to include cephalopods in 2010. However, there remains a significant lack of knowledge about the pathogens affecting cephalopods in wild and artificial environments, highlighting the need for further research. We discovered new pathogen species affecting cephalopod health in aquaculture operations, including *Ikanecator primus*, *Liburna oophaga*, *Nybelinia enterika*, *Phoreiobothrium* sp. and *Vibrionaceae* species (mainly *Vibrio harveyi* and *V. chagasii*). In 2024, peracetic acid is identified as an effective treatment agent in combating *I. primus* and *L. oophaga* during incubation of squid and cuttlefish eggs in our research, and we developed new biosecurity protocols for cephalopod husbandry. Our research represents the first practical procedure for treatment and control of cephalopod pathogens in incubation period of cephalopod eggs globally. These findings contribute to improved husbandry techniques in laboratory and artificial settings, while also promoting sustainable practices.

O68 — Natural tranquilliser in cephalopod eggs: What substances work in Octopus maya?

Carlos Rosas[1], Andrea Perera[2], Celeste Tuyub[3], LuisMolina-Carrillo[4], Kurt Paschke[5],
Leonardo Rene Franco-Mezo[2], Claudia Caamal-Monsreal[1]

1 UMDI-Sisal, Fac. de Ciencias, Universidad Nacional Autónoma de México
2 Instituto Tecnológico Nacional Tizimin
3 Instituto Tecnológico Nacional de México, Campus Conkal
4 Muséum National d'Histoire Naturelle, France
5 Instituto de Acuicultura, Universidad Austral

Abstract

Previous hypotheses postulated that the perivitelline fluid of squid eggs contains a tranquilliser substance that reduces the metabolic rate of embryos before hatching, favouring the process, preventing premature hatching. ¿Is the same for octopus embryos? Previously, it was observed that in *Octopus maya* embryos, routine metabolic rate (RMR) increases with development until reaching a maximum at stage XIV-XV, after which it decreases, reaching its lowest level just before hatching. Is this behaviour provoked by the tranquillising substance reported in squid, which induces the RMR reduction in *O. maya* embryos? We evaluated the relationship between the chorion's capacity to supply oxygen and oxygen levels within perivitelline fluid to assess if oxygen availability could modulate the RMR and act as a tranquillizing factor in octopus embryos. Oxygen diffusion was evaluated by measuring the partial pressure gradient between the external (PO_2out) and internal (PO_2in) environments of the egg, which is modulated by oxygen consumption of the embryo (RMR) and the oxygen conductance of the egg (GO_2: mgO2h-1 kPa-1). These measurements were complemented by analysis of changes in the egg's effective surface area (ESA) during development. As was expected, ESA and GO2 increased throughout development, with maximum values during the growth phase. On the contrary, the thickness of the chorion and the oxygen concentration (OC) inside the egg were reduced from 20um and 20 kPa in blastulation to less than 14 um and 2 kPa at the end of the growth phase, thickness and OC, respectively. Those results suggest that although multiple interactions between growth dynamics and yolk reserves, among others, push the organisms to hatch, the decline in oxygen dissolved in the perivitelline liquid is probably the tranquillising factor that reduces the metabolic rate, favouring the hatching process, as was earlier observed in squid.

O69 — Immune cell diversity in octopus hemolymph and white body: optimizing a single cell RNA-seq protocol

María M. Costa[1], Camino Gestal[1], Isabel Ferreirós-Vidal[2], Sonia Dios[1], Andrea González-Alonso[1], Francisco Gambón[2]

1 Instituto de Investigaciones Marinas (IIM), CSIC. Vigo, Spain
2 Instituto de Investigación Sanitaria de Santiago (IDIS), Universidade de Santiago de Compostela (USC), Santiago de Compostela, Spain
3 Departamento de Immunologia, Complexo Hospitalario Univeristario de Vigo, Vigo, Spain

Abstract

Current data suggest that octopuses lack adaptive immunity but possess an effective innate immune system mediated by hemocytes. These cells perform key defense functions such as phagocytosis, infiltration, and release of cytotoxic radicals in response to pathogens or external stimuli. Although octopus immunity research is still emerging, recent studies highlight morphological and functional diversity among hemocyte subtypes and suggest a wide range of innate effector molecules, indicated by high receptor variety. Single-cell RNA sequencing (scRNA-seq) is a powerful tool to study specific cell populations. In octopus hemocytes, customized protocols ensure cell viability and integrity, crucial for single-cell transcriptomics. Challenges include cell aggregation and high-salinity needs compatible with scRNA-seq platforms. Using 10x Genomics, we profiled hemocytes from hemolymph and white body of *Octopus vulgaris*. Our optimized protocol, using Marine Antiaggregant Solution (MAS), achieved over 90% viability and preserved cell individuality. Mechanical and enzymatic dissociation enabled successful single-cell white body isolation. The protocol supported efficient GEM (Gel Beads-in-Emulsion) generation and produced high-quality libraries. Gene expression and KEGG enrichment analysis revealed five hemocyte clusters: two main cell types and three subpopulations at various developmental or activation stages. One type supports cell renewal and migration, the other handles immune response, pathogen recognition, and degradation. The white body shows a hematopoietic pattern, with precursors of circulating hemocytes, immunomodulatory and metabolically active cells.

O70 — Existence of bacterial mass on the mantle of *Todarodes pacificus* paralarvae after hatching

Kohsuke Adachi[1], Nakaya Mitsuhiro[2], 柳本 卓[3], Yamamoto Jun[4]

1 Kochi University, Nankoku, Kochi, Japan
2 Faculty of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido, Japan
3 Field Science Center for Northen Biosphere, Hokkaido University, Hakodate, Hokkaido, Japan
4 Fisheries Resources Institute, Japan Fisheries Research and Education Agency, Yokohama, Kanagawa, Japan

Abstract

The common squid *Todarodes pacificus* (Ommastrephidae) is among the predominant species comprising the epipelagic region in Japan and is commercially important. However, investigation into the early ecology of this species, including reproduction and diet is required. In our previous study, we hypothesized that the microbiome from adults was transferred to the paralarvae through egg masses (CIAC2018). In the present study, we prepared paralarvae with and without symbiotic bacteria to demonstrate the physiological significance of this transfer process. Two types of paralarvae were prepared in an aquarium: 1) paralarvae from an egg mass (PEM) and 2) paralarvae from artificial insemination (PAI), to investigate the location of target bacteria using fluorescence in situ hybridization (FISH) and scanning electron microscopy (SEM).

PAI (0 and 2 days after hatching) were obtained from an aquarium at the Hakodate Research Center Fisheries and Ocean and fixed in 70% ethanol and 2% paraformaldehyde immediately after sampling. At the same facility, PAI was fixed using the same method after artificial insemination with 25 mg/L ampicillin and streptomycin. FISH was performed using the standard protocol of sagittal sectioning, and the images were observed using a BZ-X800 (Keyence). SEM was performed using a JSM-6010LV system. In the FISH analysis, a distributed signal was detected on the cilia of the mantle in PEM, whereas no signal was detected in PAI. In the same region, a paste-like lump was observed in PEM image. Collectively, these findings, along with those from our previous study, showed that this was obtained from the parental squid through the egg mass.

O71 — A de novo transcriptome assembly to study the effects of MeHg and elevated pCO2 in Sepia officinalis juveniles.

Thomas Sol Dourdin[1], Antoine Minet[1], Paco Bustamante[1], Christelle Jozet-Alves[2], Eric Pante[3], Thomas Lacoue-Labarthe[1]

1 Littoral Environnement et Sociétés (LIENSs), UMR 7266 CNRS – La Rochelle Université, 2 rue Olympe de Gouges, 17000, La Rochelle, France.
2 Ethologie Animale et Humaine (EthoS), UMR 6552 CNRS, Université Caen Normandie, Caen, France
3 CNRS, IRD, Ifremer, UMR 6539, Laboratory of Environmental Marine Sciences (LEMAR), Université de Bretagne, Plouzané 29280, France

Abstract

Coastal ecosystems are facing multiple threats likely to stress marine organisms, such as the legacy contamination of shallow waters and the ocean acidification resulting from the increasing CO2 release into the atmosphere. Elevated seawater pCO2 and mercury (Hg), which accumulates efficiently in biota in its methylated form (MeHg), affect behaviours, cognitive performances and the central nervous systems of fish and cephalopods. The cuttlefish Sepia officinalis, an important fishery resource as well as key model to study behaviour along with nervous system development and functioning, colonizes these contaminated coastal waters to reproduce in spring. Juveniles are therefore likely to be chronically exposed to Hg and elevated pCO2 levels throughout their embryonic and juvenile life. Recently, we demonstrated that exposure to increased pCO2 and to dietary MeHg during the first month of life induced alterations in anti-predatory responses (e.g. vigilant scanning, looming responses). Thus, we used second-generation sequencing of mRNAs (RNA-seq) to elucidate molecular mechanisms underlying these alterations observed at the individual level. The mRNAs from the head of juveniles exposed to MeHg, elevated pCO2 or a combination of both were sequenced and used to assemble and annotate a transcriptome before differential expression analysis. Preliminary results revealed that only the combined stress substantially influences gene expression (315 DEGs), altering key biological processes (e.g. up-regulation of RNA processing; down-regulation of defence response). This study provides important molecular resources to promote genomic investigations on S. officinalis and gives insights on the potential impact of combined anthropogenic stressors on this coleoid cephalopod species.

O72 — Effects of cargo ship noise exposure on *Sepia officinalis* using AquaVib

Marta Solé

Universitat Politècnica de Catalunya, BarcelonaTech

Abstract

Under the SATURN EU-funded interdisciplinary research project, an experimental laboratory setup was developed: the AquaVib, a transparent acoustic chamber equipped with sound pressure, particle acceleration, dissolved oxygen, and temperature sensors, enclosed at both ends by a pair of 1 kN-electrodynamic (ED) shakers, capable to reproduce low-frequency sounds with characteristics similar to underwater radiated noise (URN). This setup provides a multimodal approach to investigate physiological, pathological, and ultrastructural effects on invertebrate species at any of their life stages. We conducted controlled shipping URN exposures on *Sepia officinalis* adults, eggs and larva stages. Different techniques were used to assess animals' responses depending on the species and life stage: i) success or delay of eggs hatching and larvae survival rate after exposure; iii) statocyst and lateral line ultrastructural analysis by Scanning Electron Microscopy and iv) analyse of larva proteome profiling. Among the different results we obtained, the following stood out after sound exposure: ultrastructural effects on the sensory epithelia of the statocyst and lateral line of Sepia officinalis larvae and adults, and effects on the survival capacity of larvae and hatching of S. officinalis eggs. Proteomic analysis on *S. officinalis* larvae shows that the significantly different expressed proteins after sound exposure were stress related proteins and cytoskeletal proteins. The sound exposure effects allowed to define a preliminary dose-response relationship for each of the two sound components (particle motion and level of pressure) of shipping URN exposures.

O73 — Thermal fingerprints in the octopus methylome reveal distinct ovarian and embryonic responses to warming seas

Sadot Ramos-Rodriguez[1], Clara Galindo-Sánchez[1], Pavel Galindo-Torres[2], Carolina Álvarez-Delgado[1], Rafael Cabral-Tena[1], Carlos Rosas[3]

1 Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), Ensenada BC, CP 22860, México
2 Centro de Investigaciones Biológicas del Noroeste (CIBNOR), La Paz, B.C.S. CP 23096, México
3 Unidad Multidisciplinaria de Docencia e Investigación (UMDI), Facultad de Ciencias UNAM, Sisal Yucatán, CP 97355, México

Abstract

Global warming represents a major stressor for marine poikilotherms, including cephalopods, whose reproduction and development are strongly influenced by temperature. Epigenetic mechanisms such as DNA methylation provide a molecular link between environmental conditions and phenotypic plasticity, with potential intergenerational effects. This study investigated temperature-induced methylation changes in the ovaries and embryos of the endemic cephalopod *Octopus maya*. Adult females were maintained at 24 °C (control) or exposed to 30 °C (stress), and embryos were incubated at both temperatures to disentangle maternal, embryonic, and combined effects. Reduced Representation Bisulfite Sequencing (RRBS) data were analyzed using MethylKit, defining differentially methylated cytosines (DMCs) with a q-value < 0.05 and an absolute methylation difference $\geq 25\%$, followed by functional annotation with the *O. maya* genome (Braker3). A few DMCs were detected in putative promoter regions, including *fa98a*, *ndk*, *aqp11*, and *tx2*. Most DMCs, however, were in intergenic regions associated with lncRNAs and miRNAs, suggesting broader regulatory consequences. The distribution of gene body methylation (GBM) revealed differential methylation in genes associated with neurodevelopment (RELN), transcriptional regulation (*rerg*, *zn596*, *zfp62*, *zn665*), and metabolism (*cide-c*, *met15*), suggesting that thermal stress alters processes critical for ovarian function. In embryos, DMCs were observed in red, aimp2, *zn420*, and *tnf10* across all contrasts. Embryos from stressed mothers showed specific methylation in *zn239*, *zn420*, and *mixl1*, consistent with maternal imprinting. Direct embryonic exposure to 30 °C affected transcriptional regulators (*znf84*, *ach10*) and metabolic/mitochondrial genes (*steap4*, *masu1*, *nduf3*). Combined maternal and embryonic stress produced additive effects, particularly in *steap4* and *syp2l.* These findings demonstrate that temperature leaves distinct methylation signatures in *O. maya*, supporting the role of DNA methylation in thermal response and functional intergenerational inheritance.

O74 — Ocean acidification alters neural processing and predatory behavior in bigfin reef squid

Garett Joseph Patrick Allen[1], Jia-Jiun Yan[2], Pou-Long Kuan[2], Oki Hayakasa[3], Kazuhiko Anraku[3], Guan-Chung Wu[4], Yung-Che Tseng[2]

1 Acadia University
2 Academi Sinica
3 Kagoshima University
4 National Taiwan Ocean University

Abstract

Ocean acidification significantly impairs the predatory behavior of bigfin reef squid (*Sepioteuthis lessoniana*) when exposed to projected conditions for the year 2100. While basic visual processing remains intact, as demonstrated through electroretinogram measurements, chronic acidification (over 90 days) induces substantial neurometabolic rewiring in the optic lobes. Transcriptomic analysis revealed that acute exposure (7 days) significantly suppresses energy metabolism and synaptic activities, whereas chronic exposure triggers compensatory upregulation of cellular maintenance pathways. Behaviorally, acidification reduced predatory willingness by up to 65% and increased hunt duration by 2.5-fold during acute exposure. These changes coincided with reduced acetylcholine receptor expression in the optic lobes and altered hemolymph HCO_3^- levels. Metabolic rates increased by 41% under chronic conditions, while ammonia excretion decreased by 52%, indicating significant physiological trade-offs. Our findings suggest that while squids maintain visual capabilities through adaptive mechanisms, the energy-intensive processes of neural integration and behavioral execution are compromised, highlighting the complex physiological adjustments that may impact predator-prey dynamics in future oceans.

O75 — Feeding habits of Japanese flying squid *Todarodes pacificus* during its population decline around Japan

Komatsubara Koki[1], Nakaya Mitsuhiro[2], Takatsu Tetsuya[2], Yamamoto Jun[3], Adachi Kohsuke[4], Yanagimoto Takashi[5], Fujioka Hidebumi[6]

1 Graduate school of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido, Japan.
2 Faculty of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido, Japan.
3 Field Science Center for Northen Biosphere, Hokkaido University, Hakodate, Hokkaido, Japan.
4 Kochi University, Nankoku, Kochi, Japan.
5 Japan Fisheries Research and Education Agency, Fisheries Resources Institute, Yokohama, Kanagawa, Japan.
6 Tottori Prefecture Department of Agriculture, Forestry and Fishery Department, Tottori, Tottori, Japan.

Abstract

Japanese flying squid, *Todarodes pacificus*, is the most commercially important cephalopod species around Japan and plays a key role in the marine food web. Since 2015, its population has sharply declined and remained at a low level, but the mechanism of population fluctuations remains unclear. Investigating its feeding habits is essential for understanding its growth and survival processes. Although several studies have reported on its feeding habits during periods of high abundance, little is known about its diet since the population decline. In this study, we investigated the feeding habits of T. pacificus during its population decline. Specimens were collected using squid jigging at night in the Sea of Japan and bottom trawl net during the daytime in the Pacific Ocean. Dietary analysis was conducted through visual observation and DNA analysis. Individuals collected by squid jigging at night fed mainly on euphausiids and fishes such as *Maurolicus japonicus* and *Scomber japonicus*, similar to feeding habits during periods of high abundance, but exhibited a lower incidence of cannibalism. In contrast, individuals collected by bottom trawl net during the daytime fed on fewer amphipods and more demersal fishes such as *Glossanodon semifasciatus*. These differences may reflect diel variations in feeding habits. Additionally, individuals collected during the daytime exhibited a relatively low vacuity index, suggesting that T. pacificus may feed more actively during the day. Further research is needed to examine daily food consumption and determine whether nocturnal or diurnal feeding plays a more significant role in this species.

O76 — Potential genomic responses to climate change in *Octopus mimus* (Cephalopoda: Octopodidae) from the Eastern Tropical Pacific

Scarlett Munguía Prieto[1], Vladimir S. De Jesús-Bonilla[1], Fausto Valenzuela-Quiñonez[1]

1 Centro de Investigaciones Biológicas del Noroeste S.C. (CIBNOR)

Abstract

Climate change is generating long-term alterations in climatic patterns, increasingly intensified by anthropogenic activities. In marine environments, impacts include rising sea temperatures, more frequent thermal anomalies, reduced dissolved oxygen and increased acidification, leading to biodiversity loss and ecological destabilization. Assessing the ecological and genomic vulnerability of marine organisms is crucial for understanding their adaptive capacity and, in turn, informing conservation strategies. *Octopus mimus*, a key fishery species in the Eastern Tropical Pacific (ETP), will be used as a model to evaluate these impacts. We will assess its ecological vulnerability by identifying potentially suitable habitat areas under current and future climate change scenarios using Representative Concentration Pathways (RCPs): RCP 4.5 (2050-2060) and RCP 8.5 (2090-2100), highlighting regions where environmental shifts may threaten population stability. A seascape genomics approach will also be applied to detect signals of local adaptation. Neutral and adaptive genetic variation will be identified using reduced-representation genomic data (ddRADseq), and genomic vulnerability will be assessed under projected environmental conditions. We hypothesize that, if rising ocean temperatures compromise adaptation in ectothermic species, *O. mimus* populations near their thermal tolerance limits will exhibit greater genomic vulnerability and a reduction in optimal habitat under extreme climate change scenarios, as previous studies suggest that environmental variables play a major role in shaping the population genetic structure of *O. mimus*. This integrative approach will help predict and mitigate the impacts of climate change on *O. mimus*, supporting its resilience and long-term sustainability in the ETP.

O77 — Do cephalopods edit mRNA in response to environmental oxygen?

Matthew A. Birk[1], Gjendine Voss[2], Brandon Dick[1], Mary Bukowski[1], Emma U. Hammarlund[3], Eli Eisenberg[4], Joshua J.C. Rosenthal[2]

1 Saint Francis University
2 Marine Biological Laboratory
3 Lund University
4 Tel Aviv University

Abstract

Coleoid cephalopods edit their mRNA in a manner that no other known organism does: they target protein coding sequences in order to replace amino acids and diversify their neural proteomes. This unique strategy is well-poised for acting as a novel mechanism for environmental acclimation in marine animals. It has recently been demonstrated that coleoids alter the amount of RNA editing that occurs in response to environmental temperature changes and that this has measurable impacts on protein function. In this study, we assess whether RNA editing is also responsive to changes in environmental oxygen levels. Brains have high demands for oxygen to fuel complex and important neural processes. We conduct experiments with the sepiolid squid, *Euprymna berryi*, to 1. determine their hypoxia tolerance, 2. expose individuals to normal or hypoxic water conditions for one week to assess RNA editing with RNASeq, and 3. determine in situ oxygen levels within squid optic lobes during changing environmental oxygen levels.

O78 — Tickled Zinc: Characterizing A-to-I RNA Edits in *O. rubescens* in response to ocean acidification

Mindlin Meg[1], Sereewit Jaydee[1], Wright Ricky[1], Onthank Kirt[1]

1 Walla Walla University

Abstract

Octopuses appear to adapt to low pH environments more readily than other marine taxa do. Does RNA editing contribute to octopus acclimation to lower pH environments? RNA editing can diversify mRNA expression. Adenosine deaminases that act on RNA (ADARs) edit single bases in mRNA transcripts from adenosine to inosine, which is then read as guanosine by ribosomes. Selectivity for this reaction is not well understood, but the role of RNA editing in adaptation to environmental conditions has been demonstrated in multiple sites in cold water octopus species, such as modifications of K+-channel, altered kinase motility, and changes in binding affinity in synaptotagmin. Transcriptome-wide RNA editing patterns of six Octopus rubescens exposed to higher acidity environments revealed extensive editing to zinc finger genes, hinting at possible mechanisms of low pH adaptation in octopuses.

O79 — Reconstructing life-time reproductive histories using steroid hormones in cephalopod beaks

Erica Durante[1], Michael Wiese[2], Ashley Meakin[2], Karina Hall[3, 4], Zoe Doubleday[1]

1 Future Industries Institute, University of South Australia, Mawson Lakes, South Australia, Australia
2 Clinical and Health Sciences, University of South Australia, Adelaide, South Australia, Australia
3 Fisheries Research, NSW Department of Primary Industries, Coffs Harbour, New South Wales, Australia
4 National Marine Science Centre, Southern Cross University, Coffs Harbour, New South Wales, Australia

Abstract

Reproductive data are vital for fisheries and conservation management. For cephalopods, reproductive data are usually obtained by analyzing gonads, which only provide data on an individual at a given time and require whole deceased specimens. We developed a novel method for extracting reproductive hormones from along the growth axis of a chitinous structure which could thus provide lifetime reproductive histories. We tested our method on two octopus species by taking small subsamples (> 2 mg) of beak tissue along the growth axis. Estra- diol and progesterone were detected in both sexes and species, but testosterone was not. Hormonal peaks were observed, likely indicating the timing of sexual maturity; however, peaks were not matched to absolute age. This is the first study to analyze hormones in the accretionary tissues of a marine invertebrate and could be used to collect vital reproductive data, such as age at maturity, on poorly understood species.

O80 — Global hotspots and ecology of cephalopod strandings

Rui Rosa[1], Elisabeth Reuter[1], Melanie Court[1], Christian M. Ibáñez[2], Henk-Jan Hoving[3], Roger Villanueva[4]

1 MARE-Laboratório Marítimo da Guia, Faculdade de Ciências, Universidade de Lisboa, Cascais, Portugal
2 One Health Institute, Facultad de Ciencias de la Vida, Universidad Andres Bello, Avenida República 440, Santiago, Chile
3 GEOMAR, Helmholtz Centre for Ocean Research Kiel, Düsternbrooker Weg 20, 24105 Kiel, Germany.
4 Institut de Ciències del Mar, CSIC, Passeig Marítim de la Barceloneta 37-49, E-08003 Barcelona, Spain

Abstract

Here, we aimed to describe, for the first time, the global hotspots, diversity trends, and underlying causes of cephalopod strandings. Following the PRISMA guidelines, a systematic literature review was performed through the Web of Science. Most of the reported strandings are restricted to few species, namely: i) the giant squid (*Architeuthis dux*), ii) the neon flying squid (*Ommastrephes bartramii*), iii) Humboldt squid (*Dosidicus gigas*), iv) European flying squid (*Todarodes sagittatus*), v) northern shortfin squid (*Illex illecebrosus*), vi) *Argonauta* spp. among other cephalopods. Ranging from single individuals to thousands of individuals, strandings have occurred throughout the continental coastlines worldwide, with a special incidence along the Eastern Pacific Rim, Japanese and European coastlines. Strandings have been hypothesized to be related to strong thermal challenges (including El Niño events), storms, acoustic injuries, harmful algal blooms, and wind and current shifts. This study expands the available knowledge from a local to a global scale, and discusses potential mitigation strategies of the underlying causes.

O81 — Convergent evolution of the shell-like eggcase in argonaut octopuses based on comprehensive microstructural observations

Kazuki Hirota[1,2], Takenori Sasaki[1], Taro Yoshimura[1], Hirosuke Hirano[3], Takeshi Toyama[4], Masa-aki Yoshida[5], Davin H.E. Setiamarga[2]

1 Graduate School of Science, The University of Tokyo, Tokyp, Japan
2 Department of Applied Chemistry and Biochemistry, National Institute of Technology, Wakayama College, Wakayama, Japan
3 Department of Civil Engineering, National Institute of Technology (KOSEN), Wakayama College, Wakayama, Japan
4 Department of Materials and Applied Chemistry, College of Science and Technology, Nihon University, Tokyp, Japan
5 Marine Biological Science Section, Education and Research Center for Biological Resources, Faculty of Life and Environmental Science, Shimane University, Shimane, Japan

Abstract

Argonautid octopods of the genus Argonauta possess a shell-like biomineralized external structure called an eggcase. Classical behavioral observations suggested that this structure is secreted by the first dorsal arm pairs rather than by the mantle tissue, as seen in typical molluscan shells, and is thus considered a product of convergent evolution. In this study, we conducted detailed microstructural analyses using scanning electron microscopy to investigate the characteristics of normal, undamaged eggcases and regions that had undergone post-damage repair. Our analysis revealed that the eggcase is composed of five components: an outermost organic membrane, an outer spherulitic-fibrous prismatic layer, a middle organic layer, an inner spherulitic-fibrous prismatic layer, and an innermost organic membrane. Both outer and inner prismatic layers exhibit bidirectional growth from the middle organic layer, a feature not observed in typical molluscan shells. Based on these observations, we propose a four-stage formation sequence: nucleation on the organic layer, radial growth of calcified structures, perpendicular growth relative to the eggcase surface, and encapsulation by an organic membrane. Interestingly, the growth pattern and crystal morphology in the eggcase resemble those of internalized shells of modern decapodiforms, stony coral skeletons, and avian eggshells, suggesting functional convergence. We also observed two types of eggcase repair: reattachment of broken fragments and regeneration through new secretion. These findings challenge previous assumptions regarding the role of the first dorsal arms in calcification and highlight the argonaut eggcase as a structurally and functionally complex extended phenotype evolved through convergence.

O82 — The Unique Mechanics of Octopus Egg Cases

Daniel G. DeMartini

Brigham Young University-Hawaii

Abstract

Octopuses produce a wide array of specialized biomaterials, each adapted to their dynamic marine environments. One striking example is the egg case threads of *Octopus bimaculoides*, which anchor egg capsules with exceptional toughness and elasticity. These threads are composed almost entirely of a single 135 kDa protein, octovafibrin, offering a rare window into the mechanical role of the ubiquitous epidermal growth factor (EGF) domain in a monolithic material. Octovafibrin contains 29 tandem EGF-like repeats, stabilized by intramolecular disulfide bonds, with N- and C-terminal C-type lecstin domains that mediate linear end-to-end self-assembly. Mechanical testing reveals that these regularly spaced disulfide bonds significantly enhance stiffness, toughness, and energy dissipation. Molecular dynamics simulations and X-ray scattering suggest that the EGF-like domains respond to force by unfolding two hidden length β-sheet structures, acting as molecular shock absorbers. These findings highlight the mechanical contributions of intramolecular crosslinking in octopus biopolymers and offer fresh insights into the broader role of EGF domains in extracellular matrix mechanics.

Poster presentations

Exploring Assessment Models for *Eledone cirrhosa*: A Step Towards Sustainable Management

Esther Abad[1], Julio Valeiras[1], Graham J. Pierce[2], Santiago Cerviño[1]

1 Spanish Institute of Oceanography
2 Institute of Marine Research

Abstract

The horned octopus (*Eledone cirrhosa*) is a small neritic cephalopod widely distributed in the Northeast Atlantic and Mediterranean Sea. In the northern Spanish shelf, it constitutes a regular bycatch in demersal otter trawl fisheries targeting finfish and crustaceans. While traditionally discarded or of low commercial value, its economic importance has increased in recent years due to higher market demand and local abundance. Despite their ecological and commercial relevance, cephalopod stocks such as *E. cirrhosa* are not routinely assessed, nor are they included in formal management frameworks in European waters. However, their short life cycle, high growth rates, and sensitivity to environmental variability make them important candidates for regular monitoring and precautionary assessment. In this study, we explore the application of three catch-based assessment models suitable for data-limited situations: (1) SPICT (a Bayesian surplus production model in continuous time), (2) OCOM (Optimized Catch-Only Model), and (3) CMSY++ (Catch-MSY in a Bayesian framework). Each model has its advantages depending on the availability of data and the specific management objectives: formal assessments when several indices are available, rapid preliminary diagnostics or estimating Fmsy and setting precautionary reference points using catch and abundance indices. The objective is to compare model outputs and diagnostic performance to identify the most suitable approach for *E. cirrhosa* and to provide a first step toward formal inclusion in scientific assessments and paving the way for better-informed decision-making.

Sex-specific responses to chronic thermal stress in reproductive *Octopus maya*

Alejandra Penelope Ayala Aguilera[1], Carlos Rosas Vásquez[2], Maite Mascaró [2]

1 Posgrado en Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México (UNAM)
2 Unidad Multidisciplinaria de Docencia e Investigación de Sisal, Facultad de Ciencias, UNAM

Abstract

Octopus maya is a thermally sensitive cephalopod endemic to the Yucatán Peninsula and a key fishery species that supports the livelihoods of numerous coastal communities. As global warming intensifies and marine temperatures rise, understanding sex-specific physiological responses is essential for anticipating population resilience. This study examines the effects of chronic non-lethal exposure to 30 °C, on reproductively mature males and females. We measured growth, aerobic metabolism and oxidative stress markers, to detect potential differences in thermal response between sexes. We hypothesize that due to higher energetic investment in egg production, females will exhibit greater physiological disruption under thermal stress, while males may show more efficient metabolic compensation. This research contributes to understanding temperature-induced physiological dimorphism in *O. maya*, revealing how ocean warming impacts key life stages. Results have relevant implications for thermal ecology, reproductive biology, and sustainable fisheries. By identifying sex-specific vulnerabilities, this work supports adaptive conservation strategies and climate-resilient management for a species vital to both marine ecosystems and the livelihoods of coastal communities in the Yucatán Peninsula.

Rhythmic gene expression in *Octopus vulgaris* paralarvae: Daily transcriptional dynamics biomarkers

Inmaculada Varó[2], Marc Ramos Llorens[2], Julia Pérez[2], Eduardo Almansa[1], Celia Báez[3], M. Carmen Hidalgo[3], M. Virginia Martín[1], Gabriel Cardenete[3], Diego Garrido[1], Amalia E. Morales[3]

1 Centro Oceanográfico de Canarias (COC-IEO), CSIC, Santa Cruz de Tenerife, Spain
2 Instituto de Acuicultura Torre de la Sal (IATS), CSIC, Castellón, Spain
3 Departamento de Zoología, Facultad de Ciencias, CEIMAR-Universidad de Granada, Granada, Spain.

Abstract

To study the temporal regulation of gene expression is fundamental to understanding the molecular rhythms that orchestrate biological processes. This study investigates the daily transcriptional dynamics in *Octopus vulgaris* paralarvae using RNA-sequencing analysis to uncover genes with sinusoidal expression patterns over a 24-hour cycle. These genes could act as biomarkers for tracking daily patterns of physiological functions, helping us to understand the biological rhythmicity of cephalopods. This may be of interest for the study of their feeding rhythms, metabolism, activity, and rest in on growing paralarvae. We employed computational modelling to analyse the RNA-sequencing data, successfully identifying 79 genes exhibiting sinusoidal expression profiles. These genes are related to periodic biological processes, highlighting their significance in the temporal molecular biology of *O. vulgaris* paralarvae. The identified candidates include genes potentially involved in metabolic cycles, neural activity, and developmental regulation, providing novel insights into the interplay between gene expression and daily rhythms in this species. This work not only enhances our understanding of transcriptional dynamics in cephalopods but also lays the foundation for future research into the chronobiology of O. vulgaris and related species. This helps us to better understand how cephalopods carry out timekeeping at the molecular level and why this is important for their environmental physiology.

Rare Argonautoidea Octopuses in the Arabian Gulf: A case study of stranded events in Abu Dhabi coastline

Alexandra Alves[1], Daniela Denk[2], Fatima Zahra[1], Emmanuel Rueda[2], Neil Wagstaffe[2], Alberta Sugianto[2], Elise Marquis[1]

1 Yas SeaWorld Research & Rescue Center, Yas Island, Abu Dhabi, United Arab Emirates
2 SeaWorld Abu Dhabi, Yas Island, Abu Dhabi, United Arab Emirates

Abstract

Argonautoidea are holopelagic octopuses found worldwide but rarely observed. Current knowledge about this superfamily comes from occasional catches, stranded individuals, and the stomach contents of top predators. Holopelagic octopus females were collected along the Abu Dhabi coastline during stranding events. The presence of two rare Argonautoidea species in the region, *Tremoctopus gracilis* and *Argonauta hians* was confirmed. Species identification was verified through a combination of morphological and genetic analyses. Phylogenetic analysis based on 16S rRNA and COI sequences revealed that the *T. gracilis* specimens clustered not only with *T. gracilis* sequences from the Arabian Sea, Japan, and India but also with Tremoctopus violaceus sequences from the Pacific Ocean (South Korea and Taiwan). Highlighting ongoing misidentifications in global databases within the genus Tremoctopus due to a lack of distinct taxonomic features. Regarding Argonauta hians, observations of maturity stages and yolked ova progression suggested that the breeding season in the Gulf includes January and February. Further research is needed to fully understand its reproductive patterns and explore the potential existence of a nursery area in the region. This study significantly contributes to the investigation of holopelagic octopuses in underexplored regions like the Arabian Gulf. To our knowledge, it provides the first recorded presence of these species in the region, indicating that they may be more abundant than previously thought. However, the scarcity of specimens limits a full understanding of their biology, ecology, and distribution.

On the complex contact mechanics of cuttlefish suckers

Guillermo Amador[1], Jan Severin te Lindert[1], Alexander Koehnsen[1], Brett Klaassen van Oorschot[1], Tabo Geelen[1], Sander Gussekloo[1]

1 Wageningen University

Abstract

Cuttlefish rely on arrays of suckers lining their arms and tentacles to capture prey. Given their importance in hunting, the suckers must be capable of providing secure attachment onto various substrates, such as exoskeletons of crustaceans and scales of teleost fish—the typical prey for cuttlefish. The rim of a sucker, which provides sealing in order to generate suction pressures, exhibits arrays of stiff microstructures (or papillae) and viscoelastic mucus. We find that the papillae enable secure attachment to rough substrates, especially when the roughness amplitude matches the geometry of the papillae. The roughness of the exoskeletons of shrimp and crab and scales of teleost fish lies within this range, hinting at a potential morphological adaptation. The mucus on the suckers was found to be highly viscous and shear thinning, with the viscosity decreasing from 40000 to 100 centipoise as shear rate increases from 0 to 2000 1/s. On the other hand, the mucus found throughout the rest of the body has a viscosity of 5-10 centipoise that does not change with shear rate, similar to the mucus on fish. These differences in viscoelastic properties hint at a possible specialization of the sucker mucus. Our findings shed light on the functional morphology of cuttlefish suckers and inform the design of versatile bio-inspired suction cups.

Finding *Dory(teuthis)*: A phylogeographic tale with a redescription of a lost species

Bianca Paiva[1], Frank Anderson[2], Isabela Pereira de Moura[1], Paul Shaw[3], Manuel Haimovici[4], Unai Markaida[5], Jasmin Granados-Amores[6], Davi de Vasconcellos Machado[4], Jonathan Ready[1], João Bráullio de L. Sales[1]

1 Federal University of Pará, Aquatic Molecular Biology Laboratory, Center for Advanced Biodiversity Studies (CEABIO), Av. Perimetral da Ciência, km 01, PCT-Guamá, Lot 11, 66075-750, Belém-PA, Brazil
2 School of Biological Sciences, Southern Illinois University, Carbondale, Illinois, USA 62901
3 Institute of Biological, Environmental and Rural Science (IBERS), Aberystwyth University, Penglais, Aberystwyth SY23 3DA, UK
4 Universidade Federal do Rio Grande (FURG), Laboratório de Recursos Demersais e Cefalópodes, Caixa Postal 474, Rio Grande/RS CEP 96201-900, Brazil
5 Línea de Pesquerías Artesanales, El Colegio de la Frontera Sur, CONACYT, Calle 10 #264, 24000 Campeche, Mexico
6 Universidad Autónoma de Nayarit-Escuela Nacional de Ingeniería Pesquera, San Blas, Nayarit, Mexico\

Abstract

Doryteuthis Naef, 1912 comprises most of the common inshore squids of the Western Hemisphere, including several species that are targets of major fisheries (e.g., *D. gahi* and *D. opalescens*) as well as important model organisms such as *D. pealeii*. Despite the scientific and commercial importance of this group, *Doryteuthis* phylogeny, historical biogeography and taxonomy remain confused. We reassessed *Doryteuthis* using morphological and morphometric comparisons and phylogenetic and biogeographic analyses of molecular data sampled from three genes. Phylogenetic analyses suggested the presence of a previously undescribed *Doryteuthis* species in eastern Pacific waters. This species was morphologically similar to (and initially identified as) Lolliguncula, and males of this species showed hectocotylization of the right arm IV. These features suggest that our "undescribed *Doryteuthis* species" is actually *Lolliguncula argus* Brakoniecki and Roper, 1985. The robust phylogenetic placement of this species within *Doryteuthis* leads us to propose that Lolliguncula argus should be reclassified as *Doryteuthis argus* (Brakoniecki and Roper, 1985). Our divergence time and biogeographic analyses suggest that *D. argus* is sister to a clade comprising *D. gahi*, *D. opalescens* and *D. sanpaulensis*. Together, these taxa constitute an east Pacific/south Atlantic clade that diverged ~35 mya from a north Atlantic lineage comprising *D. pealeii*, *D. pleii* and *D. surinamensis.*

Hunting in a tough neighbourhood: Octopus interactions with territorial and follower fish

Michaella Andrade[1], Jennifer Mather[2], Charles Morphy Santos[1]

1 Federal University of ABC
2 University of Lethbridge

Abstract

Octopuses are keystone species in shallow water marine ecosystems. Although researchers mostly focus on predator-prey interactions, there are many non-lethal but non-neutral interactions, particularly with fish. These range from possible cooperation through kleptoparasitism and scavenging to occasional octopus predation on an unwary fish. We evaluated some of these interactions from video recordings of Octopus insularis interacting with 10 fish species on Brazilian reefs, forming a repertoire of the behaviors, looking at when they were used, and tracking the consequences by Lag Sequence Analysis. We observed 101 interactions with seven non-territorial and three territorial fish. Fish territoriality had a significant impact on fish behavior and octopus behavior. The duration of interactions was significantly longer in non-territorial fish. Interactions with territorial fish involved faster approaches and higher rates of behavioral change for both animals and were characterised by fish 'Jabs'/'Swipes' and octopus Flinches and arm Slaps. Interactions with non-territorial fish were characterised by fish Follow/Circle and the octopus continuing its hunt. Lag Sequence Analysis confirmed distinct and predictable behavioral sequences for each interaction type. These results demonstrate that octopuses also need to navigate a complex set of relationships that are not always peaceful during foraging.

Stock structure and migrations of New Zealand arrow squid Nototodarus sloanii

Alexander I. Arkhipkin[1], David A.J. Middleton[2]

1 Fisheries New Zealand, Ministry of Primary Industries, Wellington, New Zealand
2 Pisces Research Ltd, Wellington, New Zealand

Abstract

New Zealand fisheries have historically targeted two species of arrow squid of the family Ommastrephidae: warm-water Nototodarus gouldi in the north and cold-water *N. sloanii* in the south. The total allowable commercial catch for the two species is the second highest in the quota management system at 82,110 t, although the average annual catch has been around 40,000 t in recent years and predominantly of *N. sloanii*. Despite being an important fishery, stock structure has been uncertain and attempts to develop stock assessments have made limited progress. Using squid length distributions from commercial bottom trawl collected by observers in the last fifteen years, it was possible to identify and trace migrations of two to three main size groups catches of *N. sloanii* around the South Island and on the Chatham Rise; however, in years when abundance of the main size group is low, a greater number of size groups are evident. The interpretation of these size groups and the population structure of *N. sloanii* could be validated by ageing their statoliths. Examination of a small number of statoliths found that growth increments are well-resolved from the nucleus to the edge of the dorsal dome, making it possible to estimate age and growth of arrow squid and confirm its annual life span. Adequately resolving the cohort structure in *N. sloanii* should allow the application of standard depletion methods of stock assessment, so providing a basis for more nuanced management of these important fisheries resources in New Zealand.

Unidentified Octopus species from Okinawa

Keishu Asada[1], Zdenek Lajbner[1], Ian Gleadall[2], Natsumi Ono[3]

1 Okinawa Institute of Science and Technology Graduate University, 1919 Tancha, Onna-son, Okinawa 904-0945, Japan.
2 AiCeph LLC, Sendai, Japan
3 University of the Ryukyus, Ryukyu University Museum

Abstract

We report an unidentified species of octopus from Okinawa Island. It is nocturnal and is found in coral reefs throughout the island. In collected specimens, total length ranges between 263-345mm with dorsal mantle length (ML) 49-63mm. Arm lengths are subequal, although the 3rd pair is the longest (3.9 × ML). Males lack abruptly enlarged suckers. This species has highly detailed body patterns and camouflage, with several distinctive features including an individually variable but distinctive, roughly triangular patch between the eyes. Phylogenetically, its closest relative is *Octopus cf. vitiensis* recorded from Hawai'i. We discuss its morphological characters and possible identity. The species successfully reproduced in captivity.

Recording from the posterior chromatophore lobe in the dwarf cuttlefish *A. bandense*

Thomas Barlow[1], Erica Shook[1], Daniella Garcia-Rosales[1], Connor Gibbons[1], Tessa Montague[1, 2], Richard Axel[1, 2]

1 Columbia University
2 Howard Hughes Medical Institute

Abstract

Cephalopod camouflage patterns are generated by the coordinated action of hundreds of thousands of neuromuscular organs in the skin called chromatophores. Camouflage is visually driven, and the motoneurons that control chromatophores project from the chromatophore lobes in the central brain. We are interested in how camouflage patterns are encoded in the brain and differentially represented across levels of processing of the camouflage circuit. To parse the relationship between skin behaviors and neural activity, we evoke skin change behaviors in awake head-fixed dwarf cuttlefish *Ascarosepion bandense* and simultaneously record activity from the posterior chromatophore lobe of the brain with high-density microelectrode arrays. We observe spiking properties and firing rates in the central brain consistent with known literature on the action of chromatophore motoneurons. To identify the latent dimensions of skin behaviors, we are developing methods to decode skin patterns from neural activity and identify latent structure in the neural ensemble. These experiments should reveal the relationship between a set of skin behaviors and brain activity, to understand the basic levels of motor control for the skin.

Current Status of Nautilus Conservation: Trends, Regulations, and Future Considerations

Greg Barord

Abstract

Over the last 15 years, population surveys of nautiluses across the Indo-Pacific were conducted to assess their current state after decades of unregulated fisheries that supported a worldwide shell trade. The surveys utilized a combination of historical market surveys and baited traps as well as modern methods of baited remote underwater video systems and radio transmitters. Non-lethal shell and tissue samples have provided information on species relatedness and isotopic analyses. The overall population data showed that nautilus population abundances and catch rates were significantly smaller in fished versus non-fished populations. These data resulted in both national and international regulations, most notably of which was the listing of Family Nautilidae into Appendix II of the Convention on International Trade in Endangered Species (CITES). While not prohibiting trade, the listing does require a CITES permit from the exporting country stating that the trade does not impact local populations negatively. More recently, all species of nautiluses have now been assessed through the International Union for Conservation of Nature (IUCN) Red List Criteria. While the conservation regulations have certainly been a positive outcome at limiting trade and increasing overall awareness, determining the next steps for nautilus research and conservation are critical to support these initiatives. There are still big knowledge gaps in our understanding of nautilus reproduction, migratory patterns, feeding habits, and number of species (to name a few), and these would be viable research areas for renewed and continued focus.

Swimming behavior and propulsive performance of longfin squid *Doryteuthis pealeii* paralarvae

Ian Bartol[1], Mel Lhuillier[1], Soraya Bartol[2], Paul Krueger[3], Joseph Thompson[4]

1 Old Dominion University
2 Virginia Wesleyan University
3 University of North Texas
4 Franklin and Marshall College

Abstract

Squids are often considered propulsively inefficient because they rely on jetting, especially during early ontogenetic stages when relative viscous forces are high. However, some recent studies on paralarvae demonstrate that they exhibit high propulsive efficiency during the jet ejection phase while station-holding and achieve impressive agility (high angular velocity) during turning. To better understand swimming behaviors and propulsive performance in young squids, high-speed video data and particle image velocimetry (PIV) data were collected simultaneously from longfin squid *Doryteuthis pealeii* paralarvae swimming in a viewing chamber. Observed behaviors included not only station-holding, but also rotational movements, spiral motions, and translational swimming, with several behavioral transitions being prominent. Behaviors were powered by short and long jet pulses of varying propulsive efficiencies, with some jet flows falling near or even below the Reynolds limit of vortex-ring-based jet propulsion reported in other jet-propelled animals. The observed diversity of swimming behaviors and jet flows in paralarvae facilitates survival during a critical ontogenetic stage.

In situ ROV-based monitoring sheds light on the biodiversity and depth distribution of cephalopod assemblage indifferent Mediterranean areas.

Pietro Battaglia[1, 2], Ludovica De Vincenzis[3], Blondine Agus[3, 4], Danila Cuccu[4], Simonepietro Canese[2, 5], Teresa Romeo[2, 6, 7,], Silvestro Greco[8]

1 Stazione Zoologica Anton Dohrn, Department of Integrative Marine Ecology, Sicily Marine Centre, Contrada Porticatello 29, 98167 Messina, Italy
2 National Biodiversity Future Center (NBFC), Palermo, Italy
3 Stazione Zoologica Anton Dohrn, Department of Integrative Marine Ecology (EMI), CRIMAC, Calabria Marine Centre, C.da Torre Spaccata, 87071 Amendolara (CS), Italy.
4 University of Cagliari, Department of Life and Environmental Science, Cagliari, Italy
5 Stazione Zoologica Anton Dohrn, Department for Research Infrastructures for marine biological resources (RIMAR), Marine Robotics Technology Implementations (ITR), Via Po' 25c 00189, Roma, Italy.
6 Stazione Zoologica Anton Dohrn, Department of Biology and Evolution of Marine Organisms, Sicily Marine Centre, Villa Pace, Contrada Porticatello 29, 98167 Messina, Italy
7 National Institute for Environmental Protection and Research, Via dei Mille 46, 98057 Milazzo (ME), Italy.
8 University of Gastronomic Sciences, Piazza Vittorio Emanuele, 9 Pollenzo, 12060 Bra, CN, Italy

Abstract

Although the technological progress in undersea imaging and exploration is improving our knowledge on distribution of deep-sea fauna, cephalopod biodiversity across Mediterranean waters are still poorly investigated. This study summarizes the results of eight oceanographic campaigns, conducted from October 2021 to November 2023 through ROV (Remotely Operated Vehicle) surveys in different Mediterranean areas (Adriatic Sea, Ionian Sea, Strait of Sicily and Sardinian Sea). In total, ROV dives were performed during both night and daylight for a total length of 555,10521 linear km and 1498 h of HD footage, exploring a depth range of 50-1265 m. During these surveys, 715 cephalopods were observed, belonging to 15 families. Overall, 409 cephalopods were identified at the species level and the most abundant were *Eledone cirrhosa* (10%), *Illex coindetii* (9%), *Alloteuthis media* (7%) and *Eledone moschata* (7%). The most abundant families were Ommastrephidae (26%), followed by Elodonidae (25%), Loliginidae (12%) and Sepiolidae (10%). Data analysis focused on depth distribution of different species and abundance per area. The ROV surveys also allowed to encounter uncommon cephalopod species, such as *Octopoteuthis sicula*, *Chiroteuthis veranyi*, *Chtenopteryx sicula*, *Opisthoteuthis calypso*, *Bathypolipus sponsalis*, and *Ancistroteuthis lichtensteinii*, providing valuable data related on in situ observations of several cephalopods. Deep-sea in situ observations collected by ROVs not only provide data on the diversity and distribution of deep-sea cephalopods, but also reveal previously unknown behavioural aspects of these animals. These surveys have contributed valuable information on species distributions in poorly explored areas and depths of the Mediterranean Sea.

Larger blue-lined octopuses have more tetrodotoxin, but toxin levels do not vary by sex or age class

Becky Williams[1], Claire Chauveau[2], Alexis Rivers[3]

1 Utah State University, Uintah Basin, Vernal, UT, USA
2 Université libre de Bruxelles, Brussels, Belgium
3 Animal Services, Tallahassee, FL, USA

Abstract

It is well-known that blue-ringed octopuses harbor tetrodotoxin (TTX), a deadly neurotoxin. However, little is known about basic variation in TTX levels across ontogeny and between sexes for the blue-lined octopus (*Hapalochlaena fasciata*) from temperate south-eastern Australia. We know that females invest TTX into their eggs in this and other species in the genus, but how TTX levels change throughout life stages has not been examined. After 120 days of collecting effort over three years (2006, 2016, and 2019), we collected 10 male and 15 female octopuses within a 30 km coastal-region of varying age classes: 11 adult, 11 juvenile, and 3 senescent. We used high performance liquid chromatography and a competitive inhibition immunoassay to quantify TTX. Individual levels of TTX were highly variable, but did not differ by year, month (Feb–May), or locality in this dataset. Hence, we pooled data for further analyses on sex and age class variation and found that: TTX levels did not vary between adult, juvenile, and senescent octopuses; TTX levels did not vary between males and females; and that while TTX concentrations did not vary with octopus mass, larger octopuses had higher total amounts of TTX.

Influence of light intensity on gonadal maturation in female *Octopus tehuelchus* assessed by non-invasive ultrasound.

Mercedes Berrueta[1], Andrea Lopez[1], Mariela Radonic[1], Bárbara Gorriti Goroso[1], E. Abud Aristizabal[1, 2]

1 Instituto Nacional de Investigación y Desarrollo Pesquero, Mar del Plata (INIDEP), Argentina.
2 Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Argentina

Abstract

This study evaluated the effect of three light intensity levels (0, 40, and 100 lux) on ovarian development in *Octopus tehuelchus* females maintained under controlled conditions with a 12L:12D photoperiod for four months at INIDEP. Atotal of 27 females were placed in a RAS. A key innovation was the use of non-invasive ultrasonography which enabled the determination of gonadal maturity stage (EM-ECO) and ovarian diameter (DO-ECO) without sacrificing animals. This allowed for individual longitudinal monitoring, reducing stress and improving animal welfare. Results showed that total darkness (0 lux) promoted faster ovarian development, with advanced maturity stages (M3F), higher final weights, and egg-laying observed as early as the third sampling. At 40 lux, egg-laying also occurred, though with slightly increased mortality. In contrast, females exposed to 100 lux exhibited limited gonadal progression, no egg-laying, and higher mortality. Statistical analyses revealed significant differences among treatments in ovarian diameter (Kruskal-Wallis $H = 17.44$, $p = 0.00016$) and final weight ($H = 23.14$, $p = 9.43 \times 10^{-6}$). The proportion of females with egg-laying was also significantly higher in 0 lux than in 100 lux (Fisher's exact test, $p = 0.0023$). These findings highlight the critical role of low light intensity in promoting reproductive success in O. tehuelchus, and support the use of ultrasonography as a valuable, non-invasive tool for reproductive monitoring in cephalopod aquaculture.

Statolith Morphochemistry as a Key to Squid Stock Structure in Europe

Bianca T. C. Bobowski[1, 2], Anne Marie Power[3], Finlay Burns[4], Pierluigi Carbonara[5, 6], Danila Cuccu[7], Marilena Donnaloia[10], Maria Cristina Follesa[7], Klaus Peter Jochum[11], Ana Moreno[8], Inna M. Sokolova[2], Maria Valls[9], Daniel Oesterwind[1]

1 Thünen Institute of Baltic Sea Fisheries, Rostock, Germany
2 Department of Marine Biology, University of Rostock, Rostock, Germany
3 Ryan Institute, School of Natural Sciences, University of Galway, Galway, Ireland
4 Marine Directorate – Scottish Government, Marine Laboratory, Aberdeen, UK
5 Fondazione COISPA ETS, Bari, Italy
6 COISPA Tecnologia & Ricerca, Bari, Italy
7 Department of Life and Environmental Science, University of Cagliari, Cagliari, Italy
8 IPMA, Instituto Português do Mar e da Atmosfera, Lisbon, Portugal
9 Oceanographic Center of the Balearic Islands (COB-IEO, CSIC), Palma, Spain
10 CNR-ISPA, National Research Council, Institute of Sciences of Food Production, Italy
11 Max Planck Institute for Chemistry, Mainz, Germany

Abstract

Illex coindetii and *Loligo forbesii* among the most economically important cephalopod species in Europe. However, they are currently not included under the European Common Fisheries Policy (CFP) and thus are not subject to coordinated fisheries management measures. This regulatory gap leads to unregulated fishing activities across European waters, raising concerns about stock sustainability and the risk of overexploitation. To support the development of future management strategies, it is crucial to identify and delineate distinct fishery stocks. In this study, shape and microchemical analysis of statoliths, combined with age determination, were applied to distinguish individuals from different fishing areas. The results revealed stock structures and the presence of distinct cohorts, providing strong evidence for potential migration patterns within and between regions. These findings not only advance our understanding of the population structure of *I. coindetii* and *L. forbesii*, but also offer an important scientific basis for the establishment of targeted, stock specific management measures. Ultimately, this work contributes to the sustainable exploitation and long-term conservation of these valuable cephalopod resources in European fisheries.

Updated squid biodiversity of the Rangitāhua (Kermadec Islands) region with a redescription of Nematolampas regalis Berry, 1913

Heather E. Braid[1], Charlotte E. Donald[1], Kat S. R. Bolstad[1]

1 AUT Lab for Cephalopod Ecology & Systematics, Auckland University of Technology Te Wānanga Aronui o Tāmaki Makau Rau, Auckland, New Zealand

Abstract

The Rangitāhua (Kermadec Islands) region is located in the most northern part of Aotearoa New Zealand's Exclusive Economic Zone and contains unique oceanographic conditions, supporting high biodiversity. Although a recent integrative taxonomic study nearly doubled the known cephalopod biodiversity of the Kermadec region, the results were limited because they were based on a single cruise. The present study comprises a thorough morphological review of all fixed squid specimens held in New Zealand national collections to provide a comprehensive review of all available material. All Kermadec squid samples in the Museum of New Zealand Te Papa Tongarewa collections were identified morphologically, including sufficient Nematolampas regalis material (65 specimens) to enable a redescription of the species including the first females. We found 23 Decapodiformes species in the present study, including five species (representing four families) that are new records for this region. Herein, *Neoteuthis thielei* and *Cycloteuthis* sp. are reported from New Zealand waters for the first time. The total biodiversity of Decapodiformes species in the Rangitāhua is currently 53 species.

Using cephalopods to monitor temporal and spatial trends of mercury for the Minamata Convention

Paco Bustamante[1], Tiphaine Chouvelon[1, 2], Thomas Lacoue-Labarthe[1]

1 CNRS-La Rochelle University
2 IFREMER

Abstract

Marine ecosystems are affected by mercury (Hg) on a global scale and are threatened as worldwide emissions are still growing despite the Minamata Convention. In addition, the general warming of oceanic water masses affects the Hg cycle, thereby increasing its bioavailability and the exposure of marine organisms. In that context, providing a large-scale and comprehensive understanding of the marine food webs contamination by Hg is essential to better apprehend the impacts of anthropogenic activities and climate change on its bioaccumulation in marine biota, and to propose mitigation measures in the frame of the Minamata Convention. Monitoring at large scales, both spatially and temporally, are relevant to such evaluation. We propose to use cephalopods as bioindicators of the environmental global contamination by Hg. Cephalopods are indeed relevant organisms to monitor Hg as 1) they reflect the contamination of the food chain on which they rely; 2) they have a wide geographic distribution and different species occupy various compartments of their ecosystems; 3) hard and soft tissues with different time integration periods can be used; 4) beaks can be collected from Museum specimens, allowing retrospective investigation of Hg time trends. In the context of the Minamata Convention, cephalopods appear to be ideal organisms to (1) monitor spatial variations of Hg in marine ecosystems at different latitudes, (2) define hotspots of Hg contamination in the different large ecosystems and highlight sensitive areas that require particular attention and protection, (3) carry long-term monitoring of Hg trends in the different parts of the world Ocean.

Life history and biological considerations for the artisanal *Octopus* fishery in the Juan Fernández Archipelago, southeast Pacific Ocean

Sergio A. Carrasco[1, 2], Miguel Espíndola-Rojas[3], Christian M. Ibáñez[4]

1 Departamento de Oceanografía, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Concepción, Chile
2 Center for Ecology and Sustainable Management of Oceanic Islands (ESMOI), Departamento de Biología Marina, UniversidadCatólica del Norte, Coquimbo, Chile
3 Centro de Investigación Ecos, Viña del Mar, Chile
4 One Health Institute, Facultad de Ciencias de la Vida, Universidad Andres Bello, Santiago, Chile

Abstract

The insular environments in the Southeast Pacific Ocean, especially those around the Juan Fernández Archipelago (~650 km from continental Chile), are characterized by significant levels of endemism. Several studies have enhanced our ecological knowledge on this biosphere reserve (UNESCO 1977); however, research on species with emerging economic importance is crucial to achieve effective improvements. In the case of coastal octopuses, two species have been historically considered as endemic and misidentified as *Octopus selkirk* and *O. crusoe*. These were recently confirmed as *O. mimus* and *O. vulgaris*, respectively. Since 2000, artisanal landings have increased consistently from 1 to 24 tons/year (freedive and trap fishery), with catches possibly corresponding to a mix of both species. Considering that the impact of the fishery remains undocumented, the focus of this ongoing study is to provide baseline information on: (i) early-life stages, (ii) morphological patterns in their natural habitats, and (iii) characterization of the artisanal fishery. Preliminary field observations revealed that egg masses of *O. vulgaris* can be found in shallow sublittoral environments, with newly hatched paralarvae evidencing distinct chromatophore patterns. Sub-adult and adult individuals present species-specific morphological characteristics, suggesting that *O. vulgaris* is the only species being caught in the trap-fishery occurring between 30 and 80 m depth. Catches are predominantly represented by males (3:1), with total average sizes of 165 mm in DML and 1700 g in TW. This information is relevant to determine priority areas for implementing management strategies, promoting genuine small-scale transformations for resource sustainability. Funding: Fondecyt 1241836 (SC).

Growth potential and optimum growth temperature of the Pacific pygmy octopus *Paroctopus digueti*

Bertha Patricia Ceballos-Vázquez[1], Diana Patricia Carreño-León[2], Armando Monge-Quevedo[2], María Guadalupe Martínez-Morales[1]

1 Instituto Politécnico Nacional, Centro Interdisciplinario de Ciencias Marinas. Av. Instituto Politécnico Nacional s/n Col. Playa Palo de Santa Rita. C.P. 23096, La Paz, BCS, México.
2 Centro de Investigaciones Biológicas del Noroeste, S.C. Av. Instituto Politécnico Nacional 195, Col. Playa Palo de Santa Rita. C.P. 23096, La Paz, BCS, México.

Abstract

Six metabolic and physiological evaluation experiments were conducted on pre-adults of the Pacific pygmy octopus *Paroctopus digueti* (24.20±4.98 mm LM). The evaluations were carried out at six temperatures (30°C, 28°C, 26°C, 24°C, 22°C and 20°C), one per day, to determine the energy expenditure of the organisms in each condition and to evaluate the free energy available for growth, known as growth potential, as well as to identify the optimum temperature for growth. The organisms used were in good health according to biometric data and the determination of their physiological condition. For each evaluation temperature, the metabolic rate, respiratory rate and growth potential were determined, finding that the optimum growth temperature is 28°C, with a growth potential or energy available for growth of 44.25±19.29 J/g/h with a metabolic rate of 0.77±0.22 mgO2/g/h and a respiratory rate of 11.40±3.34 J/g/h. It is important to mention that during the evaluations, lethargy was observed as the temperature decreased to the point that at 20°C, mortality occurred, so this evaluation was suspended. This indicates that *P. digueti* is a tropical species and makes efficient use of available oxygen, with low energy expenditure, which translates into good growth potential.

Overview of Cephalopod Species of Fishery Resources Investigation from the Northwest Pacific Ocean

Zi-yue Chen, Bi-lin Liu, Pan Liu, Hao Xu, Yuzhe Ou, Moxian Chu

1College of Marine Living Resources Science and Management, Shanghai Ocean University, Shanghai 201306, China
2 China' s Squid Fishing Technology Group, Shanghai Ocean University, Shanghai 201306, China
3 School of Marine and Atmospheric Sciences, State University of New York at Stony Brook, New York 11790, United States of America
4 Shanghai Natural History Museum (Branch of Shanghai Science & Technology Museum), Shanghai 200041, China

Abstract

From 2021 to 2024, we conducted fisheries resource investigation in the northwest Pacific Ocean aboard the 3000 tons research vessel “Song Hang” from Shanghai Ocean University, primarily using 100-200 meters mid-water trawling as the sampling method. We collected 34 cephalopod species from 19 families in the Northwest Pacific mesopelagic zone. Some species are rarely sampled and are presented here with clear photographs for the first time. This poster presents our photography summary of the specimens to highlight the regional diversity of mesopelagic cephalopods in the northwestern Pacific Ocean.

Trophic dynamics and ecological roles of cephalopods in tropical continental shelf communities

Chun-I Chiang[1], Min-Huang Xiao[1], Pei-Ling Wang[2], Ming-Tsung Chung[2], Chia-Hui Wang[1]

1 Department of Environmental Biology and Fisheries Science, National Taiwan Ocean University
2 Institute of Oceanography, National Taiwan University

Abstract

Understanding the trophic dynamics of marine predators is essential for clarifying nutritional variations and species interactions within ecosystems. This study analyzed the carbon and nitrogen stable isotopic compositions of particulate organic matter (POM), meso- and macro-zooplankton, and dominant cephalopod, fish, and shrimp species in waters around the Taiwan Banks. We aimed to (1) determine an appropriate trophic enrichment factor (TEF) for cephalopods and quantify the contributions of baseline sources; (2) assess ontogenetic and spatiotemporal variations in the trophic traits of dominant cephalopod species; and (3) evaluate the trophic niche and overlaps among cephalopods, fishes, and shrimps. Using POM and meso-zooplankton to represent primary producers and consumers, respectively, we identified a TEF of 2.9 ± 0.7 ‰ as optimal for cephalopods in tropical continental shelf ecosystems. Dominant cephalopod species exhibited trophic positions (TPs) ranging from 2.96 to 4.00, slightly lower than those of fishes (3.53–4.30). Significant size-related changes in isotopic compositions and TPs were observed only in certain benthic cephalopods, suggesting that cephalopods are not always dominant and voracious predators. Despite larger interspecific differences in TPs between neritic and benthic assemblages, cephalopods overall exhibited substantial trophic niche overlap. Feeding competition among benthic species such as *Euprymna berryi*, *Amphioctopus marginatus*, and *A. kagoshimensis* may significantly influence the availability of food resources for other species. In contrast, asymmetric feeding competition between the neritic *Uroteuthis chinensis* and *U. edulis* likely reduces their potential for long-term stable coexistence within tropical continental shelf waters.

Structuring of two size groups of swordtip squid in the southern East China Sea

Chen Chih-Shin[1], Ching Tzu-Yun[1]

1 Institute of Marine Affairs and Resource Management, National Taiwan Ocean University

Abstract

Understanding the population structure of commercially exploited species is essential for effective fisheries management. The swordtip squid (*Uroteuthis edulis*) is distributed in temperate and tropical waters across the Indo-Pacific region and is a target species for various fisheries along coastal areas. Two size groups of swordtip squid have been suggested, forming two seasonal migration groups off southern Japan, as well as two seasonal spawning groups on the southern East China Sea (ECS) shelf. However, information on the size structure of swordtip squid on the southern ECS shelf remains limited. In this study, we investigate the size groups and associated life history traits of swordtip squid in the southern ECS during 2021–2022. The squid were divided into large-sized (LS) and small-sized (SS) groups based on natural breaks in the mantle length distribution for each sex at each sexual maturity stage. The LS group hatched in winter and matured (spawned) in spring-summer, whereas the SS group hatched in summer and matured (spawned) in autumn-winter. The two size groups likely alternated between generations. Additionally, some individuals hatched in the same season but developed into different size groups, suggesting the possibility of exposure to varying environmental conditions. A hypothesis of spreading spawning (hatching) sites in the southern ECS is proposed, which may be influenced by oceanographic processes that vary seasonally and interannually. Our findings provide novel information on structuring of swordtip squid populations in the southern ECS which may facilitate conservation and management measures for the fisheries in the Northwest Pacific region.

Spatial patterns of potential habitats for Argentine shortfin squid in the Southwest Atlantic

Ching Tzu-Yun[1], Chen Chih-Shin[1]

1 Institute of Marine Affairs and Resource Management, National Taiwan Ocean University

Abstract

The Argentine shortfin squid *Illex argentinus* is widely distributed over the Patagonian Shelf in the Southwest Atlantic, with its distribution pattern influenced by environmental conditions. In this study, the suitable habitats for Argentine shortfin squid was identified using the Habitat Suitability Index (HSI) model, based on fisheries data from the Taiwanese squid-jigging fishery during 2010–2022. Six environmental variables (sea surface temperature [SST], sea surface salinity [SSS], sea surface height [SSH], chlorophyll-a [Chl-a], mixed layer depth [MLD], and photosynthetically available radiation [PAR]) were considered in this study. The Variable Importance in Projection (VIP) scores were higher for PAR, SST, SSH and MLD, and lower for SSS and Chl-a. PAR, SST, SSH and MLD were considered the critical environment variables selected in the HSI model. Two HSI models, Arithmetic Mean Model (AMM) and Geometric Mean Model (GMM), were examined by data series during 2010–2020 and validated by the data in 2021 and 2022. The results showed that the AMM-based HSI model explained better in defining the suitable habitats for the squid. The optimum habitats (HSI ≥0.6) for the squid gradually expanded from January–June. The areas of optimum habitats for the Argentine shortfin squid accounted for an average of 37.5% of the potential fishing grounds from 2010 to 2022. The findings of this study provide essential information for the development of conservation and management measures for the Argentine shortfin squid fishery in the Southwest Atlantic.

Species Composition and Spatiotemporal Patterns of Incirrata and Myopsida Paralarvae in the Surrounding Waters of Taiwan

Chuan-Wen Ho[1], Wang-Ling Tsai[1], Kuo-Wei Yen[2]

1 Department of life sciences, National Chung Hsing University, Taiwan
2 Fisheries Research Institute, MOA, Taiwan

Abstract

The coastal waters of Taiwan are important habitats for diverse cephalopods, yet knowledge of their early life stages remains limited. This study investigated the composition and distribution of cephalopod paralarvae using plankton samples collected from summer 2020 to spring 2022 as part of Taiwan's fishery environmental monitoring program. A total of 1,157 paralarvae were collected, with 148 identified as Incirrata and Myopsida. DNA barcoding revealed at least three Loliginidae, 17 Octopodidae, two Argonautidae, and one Tremoctopodidae species. Water depth and sea surface temperature were key factors shaping distribution patterns, with cluster analysis identifying two assemblages aligned along a northeast-southwest gradient. Paralarvae of *Uroteuthis edulis* were found in northern Taiwan in spring, supporting its role as a spawning ground. Additionally, benthic octopus species, including Amphioctopus marginatus and *Octopus cyanea*, were detected in Kuroshio-influenced eastern waters, suggesting possible transport via ocean currents or local spawning near Orchid and Green Islands. Sequence matches with specimens from Indonesia, Japan, Guam, and Hawaii indicate long-distance dispersal, highlighting regional and seasonal variability in cephalopod larval ecology.

Energetic responses of cuttlefish to ocean warming

Ming-Tsung Chung[1], Chia-Hui Wang[2], Li-Ling Kao[1], Pei-Ling Wang[1]

1 Institute of Oceanography, National Taiwan University, Taipei, Taiwan
2 Department of Environmental Biology and Fisheries Science, National Taiwan Ocean University, Keelung, Taiwan

Abstract

As ocean temperatures continue to rise, the ability of marine organisms to adjust their physiological traits plays a crucial role in shaping their geographic distribution and, in turn, influences the sustainable use of fishery resources. Investigating physiological traits and behaviours in natural marine environments remains challenging due to limitations in direct observation and the high cost of existing technologies. To address these challenges, this study employs a novel isotope-based approach to estimate the field metabolic rate of cephalopods. Specifically, we used stable oxygen and carbon isotope analyses of cuttlebones to reconstruct temperature histories and metabolic rates of the pharaoh cuttlefish (Sepia pharaonis), respectively. Energy use was compared across a temperature gradient between tropical and subtropical regions of Taiwan, as well as between two time periods (2009–2010 and 2022–2024). The results indicate that S. pharaonis prefers sea temperatures around 23-25°C. Within this range, the species exhibits the broadest metabolic scope, enabling flexible energy use in the natural environment. However, subtropical populations exhibit greater metabolic plasticity than their tropical counterparts. Furthermore, warming ocean conditions are pushing S. pharaonis toward its upper thermal limit. At elevated temperatures, the increased energetic demands become unfavourable for growth, potentially leading to shifts in life history traits.

Method for evaluating oxygen diffusion in octopus embryos: a study in Octopus maya.

Claudia Caamal-Monsreal[1], Celeste Tuyub[2], Kurt Paschke[3], Luis Molina-Carrillo[4], Maria del Carmen Notario[1], Carlos Rosas[1]

1 UMDI-Sisal, Fac. de Ciencias, Universidad Nacional Autónoma de México
2 Instituto Tecnológico Nacional de México, Campus Conkal
3 Instituto de Acuicultura, Universidad Austral, Chile
4 Muséum National d'Histoire Naturelle, France

Abstract

The chorion is more than a simple barrier to protect the octopus embryo. It is a complex cover involved in transporting seawater and oxygen, avoiding the introduction of contaminants and pathogens, and more. Some evidence demonstrates that the oxygen dissolved in the perivitelline fluid of *O. maya* embryos functions as a tranquillizer substance before hatching. So, the present study was directed to standardize the method to evaluate the oxygen diffusion of the chorion and possible changes in its properties during O. maya embryo development. To do that, a Ussing-type chamber was designed to know the partial pressure gradient between the egg's external (PO_2out) and internal (PO_2in) environments. Preliminary results indicated that oxygen diffusion through the chorion isindependent of the external oxygen concentration. Therefore, all oxygen diffusion measurements were done at 100% oxygen saturation. Measures were done in embryos in blastulation (VI-VII), organogenesis (IX-XIII), activation (XIV-XVI),and growth phases(XVII-XIX).Simultaneously, wecalculated changesin surface area and chorion thickness, which were used to determine oxygen conductance during embryo development.Asexpected, the surface area (mm^2),the oxygen transfer rate (mg O2/total area), and conductance (mg O2/h/kPa) all increased, reaching their highest values at the end of embryo development. This indicates that our method is effective for assessing potential changes in oxygen diffusion when embryos are subjected to high temperatures, alterations in pH, or exposure to contaminants.

Comparing the trophic niches of two Todarodes species in New Zealand using a combined approach

Samuel Clough[1], Amandine Sabadel[1, 2], Emily Fitzgerald[1], Darren Stevens[2], Sarah Bury[2], Josette Delgado[2], Heather Braid[1], Kat Bolstad[1]

1 Auckland University of Technology
2 National Institute of Water and Atmospheric Research
1, 2 National Institute of Water and Atmospheric Research/Auckland University of Technology

Abstract

Cephalopods are key trophic links from low-level consumers to apex predators and between different marine habitats. Their ecological importance will continue to rise as cephalopods fill the vacant niches left behind by the overharvesting of their main competitors. Their rising importance starkly contrasts with 90% of cephalopod species' diets remaining undescribed. This study compares the trophic ecologies of two poorly described ommastrephid species sampled from New Zealand waters: the Angolan flying (*Todarodes angolensis*) squid and the southern flying squid (*T. filippovae*). Gut content analysis via morphological prey identification and DNA barcoding of prey tissues can provide a snapshot of the squids' most recent meals, but longer-term comparative data can be obtained through stable isotope analysis. By sub-sampling the beaks and measuring their carbon and nitrogen bulk stable isotope values, we can quantify habitat and dietary changes throughout their lives. Multivariate analysis of prey data reports *T. angolensis* preferred demersal and gelatinous prey items whereas T. filippovae predated more on larger, epipelagic fish. Despite the dietary differences, there was an overlap in both SIA values and in reported dietary items. These findings demonstrate that the multiple, complementary methods can robustly model the trophic ecologies of ommastrephid species.

Cryopreservation enables long-term study of immune cells in the common octopus, *Octopus vulgaris*.

María M Costa[1], Estefania Paredes[2], Mercedes Peleteiro[3], Lucía Sánchez-Ruiloba[1], Francisco Gambón[4], Sonia Dios[1], Camino Gestal[1]

1 Instituto de Investigaciones Marinas (IIM- CSIC), Vigo, España.
2 Coastal Ecology Research Group (ECOCOST), Department of Ecology and Animal Biology, Marine Research Center, University of Vigo, Vigo, Spain.
3 CINBIO, Universidade de Vigo, Vigo, Spain
4 Department of Immunology, Vigo University Hospital Complex, Vigo, Spain

Abstract

The common octopus, *Octopus vulgaris*, has been widely used as a model in physiological and behavioral studies. Studying its defense mechanisms is key to understanding responses to external threats and ensuring animal welfare. The lack of cell cultures in molluscs limits *in vitro* assays necessary for immune cell studies, making cryopreservation a valuable tool to preserve viable and functional cells. Having access to good high-quality cells over time enables broader experimental designs, extended time courses, and avoids issues such as loss of viability and/or functionality, time constraints, or transport challenges. Additionally, high- quality cell suspensions are critical for successful applications like single-cell sequencing, where viability and functionality are the key to optimal identification. We optimized the cryopreservation protocol for octopus hemocytes, selecting the best medium, cryoprotectant, and freeze/thaw process. This study presents the first functional results of cryopreserved hemocytes. Cells cryopreserved in Marine Antiaggregant Solution (MAS) medium supplemented with Ethylene Glycol (EG) maintained viability above 80% after 15 weeks post cryopreservation, and their functional ability to phagocytize bacteria similar to fresh cells. Moreover, thawed acclimated cells exhibited a gene expression pattern comparable to fresh cells. Post-thaw acclimation was key to restoring cell function and normalizing oxidative stress gene expression. These findings support functional studies in octopus immune cells and offer tools for preserving cells in other mollusc species.

Octopus cognitive bias mirrors inner states

Mélanie Court[1], Rui Rosa[1], Catarina Caldeira-Santos[1], Océane Schott[2], Julien Bacqué-Cazenave[2], Anne Sophie Darmaillacq[2]

1 MARE - Laboratório Marítimo da Guia, Faculdade de Ciências, Universidade de Lisboa, Cascais, Portugal
2 Normandie University, UNICAEN, UMR 6552, Rennes 1-CNRS (EthoS) - Cephalopod cognitive neuroethology (NECC), 14000 Caen, France

Abstract

Cognitive bias, defined as a consistent deviation in judgment, is closely linked to emotional states in many animals. In this study, we gauged, for the first time, emotional states through decision-making in wild octopuses (*Octopus vulgaris*). After training them to link visual cues (black or white plates) with either a reward (crab) or a punishment (being chased by a net), they were presented with ambiguous stimuli with varying black and white proportions. The latency to touch the plates was used to assess cognitive biases under different environmental contexts. Our results indicate that *O. vulgaris* demonstrates emotion-sensitive cognitive biases, with individuals transferred to impoverished environments for only seven days displaying higher latencies to touch ambiguous plates, and, thus, negative expectations. These findings highlight the ability of octopuses to experience emotional states, which affect their judgments. This research enhances our understanding of cephalopod cognition and offers a valuable tool for evaluating the psychological welfare of octopuses in captivity.

Octopus artisanal fisheries and population ecology in an upwelling area off southeastern Brazilian coast

Renato Dantas[1], Carlos Ferreira[2], Tatiana Leite[1]

1 Laboratório de Métodos de Estudos Subaquáticos e Cefalópodes (LAMECE), Universidade Federal de Santa Catarina (UFSC), Florianópolis,Brazil.
2 Laboratório de Ecologia e Conservação de Ambientes Recifais (LECAR), Universidade Federal Fluminense (UFF), Niterói, Brazil.

Abstract

Artisanal octopus pot fishing is performed within the Arraial do Cabo Marine Extractive Reserve, Rio de Janeiro. During the day, three fishermen work in a medium-sized boat using five pot longlines distributed from off Cabo Frio Island to Praia Grande. This fishery supplies local businesses and presents a conflict with hookah divers, who report a decrease in catches since the introduction of octopus pots in the region. Given this context, our goal was to evaluate the population ecology of the target species Octopus americanus to provide information for future management plans. Octopus pot fishing was monitored monthly from July/2023 to March/2025 and a total of 680 specimens were analyzed. Mantle length (ML) of females ranged from 58-235 mm (mean ± standard deviation:148.3±25.5 mm), while males had a smaller size range from 90-203 mm (148.9±20.9 mm). Body weight of females varied from 166-3000 g (1166.7±444.3 g) and males varied from 244-2817 g (1412.8±467.3 g). Size at first maturity (L50) was 146.5 mm MLfor females and 126.2 mm MLfor males using an *Octopus vulgaris* macroscopic 5-stage maturity scale based on gonadal development. *O. americanus* size structure in Arraial is similar to that of commercial landings from southeastern Brazil and Santa Catarina artisanal fishing. Reproductive individuals of both sexes were observed along the year, with mature and spawning females peaking in summer (December-March) and in the transition from autumn to winter (May-July); mature males only peaked in summer. Overall, *O. americanus* reproduction seems correlated to colder upwelling (summer) and winter waters.

Nocturnal squid artisanal fisheries and population ecology in a Brazilian Marine Protected Area

Renato Dantas[1], Carlos Ferreira[2], Tatiana Leite[1]

1 Laboratório de Métodos de Estudos Subaquáticos e Cefalópodes (LAMECE), Universidade Federal de Santa Catarina (UFSC), Florianópolis,Brazil.
2 Laboratório de Ecologia e Conservação de Ambientes Recifais (LECAR), Universidade Federal Fluminense (UFF), Niterói, Brazil.

Abstract

Artisanal squid fishing is traditionally performed within the Arraial do Cabo Marine Extractive Reserve, Rio de Janeiro. During the night, with the help of light attraction, fishermen work at fixed points on the Praia Grande's rocky shore called "pesqueiros" or in small and medium-sized boats called "caícos" and "boca aberta"; fishing gear includes hand nets, squid jigging and gillnets. Given the decrease in catches over the years and the importance of this activity for the local community and market, our aim was to evaluate the population ecology of the target species *Doryteuthis pleii.* Fishing was monitored monthly from July/2023 to March/2025 and a total of 728 specimens were analyzed. Mantle length (ML) of females ranged 67.1-211 mm (mean ± standard deviation: 154.1±25.6 mm) while males had a greater size range of 87.8-363 mm (202.8±61.0 mm). Body weight (BW) of females ranged 28-133 g (71.1±19.6 g), while males ranged 27-309 g (117.7±52.4 g). Individuals of undetermined sex ranged 51-146 mm ML (104.5±19.6 mm), 4-58.1 g BW (30.2±12 g) and were mostly considered juveniles. Size at first maturity (L50) was 164.3 mm ML for females and 233.7 mm ML for males using nidamental glands and testis lengths as allometric variables. D. pleii population from Arraial has a size structure similar to that observed in coastal Florianópolis and São Sebastião Island. Mature and reproductive individuals were observed throughout the year, with peaks for both sexes in January and another in June for males, probably related to cold upwelling and winter waters, respectively.

Exploring humane slaughtering method in cephalopods

Marianna de Luca

Stazione Zoologica Anton Dohrn

Abstract

Cephalopods have been consumed as food for millennia across the world. In 2022, they accounted for 11.3% of total marine fisheries and 7% of global aquatic animal product exports. They are captured using various fishing methods and rarely killed by direct human intervention. Anoxia is the most frequent method of killing cephalopods during fishery practices, followed by ice chilling. Council Regulation (EC) 1099/2009 on the protection of animals at the time of killing establishes rules for the killing of animals as food to ensure animals are spared any avoidable pain, distress or suffering. However, these protections do not currently apply to cephalopods, and no standardized slaughtering practices exist for them. Here, we explored electrical stunning on *Sepia officinalis* and *Octopus vulgaris*, testing a combination of different electrical parameters (frequency and voltage) and using a stunning machine built for the purpose. Brain neural recordings revealed an increase in the amplitude of Visually Evoked Responses in more than 50% of the cases and a significant alteration in frequencies band power after the stunning. Stunning silenced neural traces in cuttlefish. In octopus, signs of erratic neural activity were observed (massive epileptic-like, seizure activity). Our findings suggest electrical stunning induce a temporary ‘unconsciousness-like’ state in cephalopods, aligning with regulatory requirements for minimizing animal suffering during killing.

Climate’s Effects on Wild Northwest Atlantic Squid's RNA Editing Capabilities

Bolton Jr. DeCorey[1], Grey-Avis Erin[1]

1 Grey-Avis Lab

Abstract

Coleoids, such as squid, are known for their complex morphology and their extensive phenotypic plasticity. They obtain this plasticity through the usage of A-to-I RNA editing, where new mutations are created within the nucleosides to help regulate and diversify the squid’s mRNA. RNA editing in squid is a temperature induced biological process that prominently occurs within the nervous system, with both cold and warm induced A-I editing have shown to occur within octopuses in a controlled environment; but no reports of this occurring in the wild or amongst other cephalopods. A latitudinal observational study was utilized to the differences in the rate of transcriptome changes occurring within *Doryteuthis pealeii* and *Illex illecebrosus* in relation to their environment’s temperature range.Collaboration with the Department of Marine Resources (DMR) and NOAA researchers who were both conducting their own squid oceanography research cruises allowed me to receive wild caught *D. pealeii* and *I. illecebrosus* to collect DNA and mRNA through the dissection of their brains. The goal of this study was to observe if RNA editing occurs in wild squid and to detect the differences in the amount of editing sites among wild squid that reside in varying temperatures and environments in the Northwest Atlantic.

Quantitative assessment of global DNA methylation and oxidative damage (8-OHdG) in *Octopus maya* females and embryos under thermal stress

Roman Diaz-Polo[1], Pavel Galindo-Torres[3], Claudia Ventura-Lopez[2], Fernando Diaz-Herrera[1], Carlos Rosas-Vazquez[4], Clara Elizabeth Galindo-Sanchez[1]

1 Departamento de Biotecnología Marina, Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), Ensenada, Baja California, México
2 Dirección de Impulso a la Innovación y Desarrollo, Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), Ensenada,Baja California, México
3 Laboratorio de GenéticaAcuícola, Centro de Investigaciones Biológicas del Noroeste, S. C (CIBNOR), La Paz, Baja California Sur, México
4 Unidad Multidisciplinaria de Docencia e Investigación, Facultad de Ciencias, Universidad Nacional Autónoma de México (UNAM), Sisal,Yucatán, México

Abstract

In marine invertebrates, thermal stress alters DNA methylation marks, which modifies gene expression and, in turn, affects embryonic development. It is known that cephalopods exposed to elevated temperatures increase their metabolic activity, subsequently raising oxygen demand. This process generates oxidative stress and reactive oxygen species (ROS), potentially leading to DNA damage. One of the most common lesions is the 8-hydroxy-2'-deoxyguanosine (8-OHdG) that inhibits methylation of adjacent cytosines and reduces global DNA methylation. In *Octopus maya*, exposure to thermal stress has been linked to adverse effects on embryonic development and reproductive efficiency. This has led to the hypothesis that these alterations may have an epigenetic basis. Therefore, this study investigated whether temperature modulates cellular damage (8-OHdG) and global DNA methylation (5-mC) in females and their offspring when exposed to 24°C and 30°C. The results showed significant differences in 5-mC and 8-OHdG levels, with higher levels in organisms exposed to 30°C. Additionally, real-time qPCR analysis showed variations in the expression patterns of genes related to morphogenesis and nervous system development between embryos from females exposed to 24 °C and 30 °C, as well as between embryos directly exposed to both temperatures. This evidence suggests that elevated temperatures may alter global DNA methylation in *Octopus maya*, modifying the expression of genes associated with embryonic development. These variations could be linked to the observed phenotypes, such as embryos lacking arm development and showing eye loss.

The impact of maternal thermal stress and thermal fluctuations on the physiology of *Octopus maya*

Norma Alejandra Plata Díaz[1], Carlos Rosas Vázquez[2], Maite Mascaró Miquelajauregui[2], Gabriela Rodríguez Fuentes[3]

1 Posgrado en Ciencias del Mar y Limnología, UMDI-UNAM
2 Facultad de Ciencias, UNAM
3 Facultad de Química, UNAM

Abstract

There is evidence suggesting that exposing female *O. maya* to thermal stress conditions at the final stages of sexual maturation impacts the physiology and growth of the next generation. A total of 600 juveniles of *O. maya* were used, origination from two different maternal thermal conditions (24 o 30°C), with 300 organisms from each origin. The juveniles were then randomly assigned to three different thermal treatments (24°C, 30°C, or thermal fluctuation), with 100 organisms per treatment. The exposure period lasted 50 days, with evaluations conducted every 10 days. The results demonstrated the consequences of maternal thermal stress, negatively impacting the survival and hatching weight of *O. maya* juveniles. In general, juveniles from non-stressed females consistently had higher weights than those descended from stressed females. Maternal origin had a significant effect on the respiratory metabolism and the antioxidant defense system of *O. maya* juveniles. For juveniles from thermally stressed females, an increase in metabolic rate and the value of the thermal aerobic potential was observed, along with high activity of superoxide dismutase, glutathione S-transferase and total glutathione, but low activity of catalase and cholinesterases. In contrast, the opposite was observed in juveniles from females kept at optimal temperatures. Similarly, thermal fluctuations did not favor the growth or survival of juveniles, regardless of maternal origin. Although no significant differences were found between thermal fluctuation and the control treatments (24 or 30°C), *O. maya* juveniles exposed to thermal fluctuation exhibited behaviors similar to those in the 30°C treatment.

Cellular signatures of postembryonic development and maturation in brains of *Sepioteuthis lessoniana*

Lada Dolezalova, Thi Thu Van Dinh, Yuki Goda, Sam Reiter

Okinawa Institute of Science and Technology (OIST) Graduate University, Okinawa, Japan

Abstract

Cephalopods, with their large, centralized brains and convergently evolved eyes, offer a compelling model for studying neural development. The squid Sepioteuthis lessoniana displays advanced visually guided behaviors, such as predation and camouflage, shortly after hatching. Remarkably, its brain increases nearly tenfold in size within the first two months of its life, accompanied by anatomical changes that remain largely undocumented. How the brain maintains behavioral function during this rapid growth remains an open question. We investigate post-embryonic brain development in S. lessoniana at the cellular level, with a focus on post-hatching neurogenesis. While earlier studies suggest a correlation between brain cell number and body size in cephalopods, direct evidence for proliferative populations in the brain remains limited. This study aims to: (1) quantify changes in brain cell number across post-hatching developmental stages, (2) identify proliferative and immature neuronal populations, (3) trace neuronal migratory pathways, and (4) assess the integration of new cells into existing neural circuits. Preliminary findings indicate an absence of proliferative populations within the brain itself. Instead, 5-ethynyl-2'-deoxyuridine (EdU) labeling revealed three distinct migratory pathways that appear to supply the central brain with new cells, which progressively adopt neuronal characteristics over the course of one month

Study of the *Vibrio* population present on cuttlefish skin

Dubos MP

Abstract

The cutaneous microbiome of cuttlefish evolves at the end of its life with the appearance of vibrios. To study this vibrio population, cultures were grown on different selective media. The different isolates were analyzed using 16S sequencing. The latter did not allow precise identification of the species present. Therefore, genome sequencing was carried out using long-read nanopore technology. At the same time, optimal growth temperatures were determined, as well as bacterial metabolism, using the API® ZYM System. Most of the vibrios isolated correspond to different isolates of Vibrio atlanticus with varying metabolic profiles. Other *Vibrio* species have also been identified, such as *Vibrio harveyi*, *Vibrio jasicida*, *Vibrio scophthalmi*, and *Vibrio fischeri*. The latter is best known as the symbiont associated with the light organ in *Euprymna scolopes*. In ulcerated areas observed on the skin of degenerating cuttlefish, some of these *Vibrio* strains are found in the presence of *Photobacterium swingsii*. This species has already been reported in retrobulbar lesions in *Octopus vulgaris*. This study of the *Vibrio* population present on cuttlefish skin demonstrates the presence of a diversity of species belonging to the Vibrionaceae family on the skin of aged cuttlefish, and that some of these species, through their proliferation, could be responsible for the skin ulcers observed just before the death of the cuttlefish.

Exploring cuttlefish skin and its microbiome using multi-omics approaches.

Dubos MP.

Abstract

In aquatic organisms, the skin and its mucus play an essential role in the body defense against pathogens. While the skin plays a role in physical and chemical protection, it also provides biological protection by associating with bacteria. This skin reflects the health status of the animals, particularly in cephalopods. In our study, we focused on cuttlefish skin, its mucus, and its microbiome. The composition, and particularly the proteome of the mucus, was characterized using omic approaches (transcriptomic and proteomic). The identified proteins are similar to those of fish mucus. The microbiome and its evolution were studied using metabarcoding approaches, targeting several 16S rRNA regions. Several skin and mucus samples were taken from cuttlefish before and after hatching, at one year of age before sexual maturity, from mature animals during the reproductive period, and during senescence. The skin microbiome of *S. officinalis* is mainly composed of Proteobacteria and Bacteroidetes. Some phyla, such as Patescibacteria and Tenericutes, only appear in mature adults. The data analyses illustrate a progressive development of the cuttlefish skin microbiome over the course of its life. Embryos and juveniles have a microbiome with a very small number of genera. Immature adults see the number of genera double that of early stages, and mature adults have a microbiome six times more diverse. A change in the microbiome is observed after reproduction, probably associated with dysbiosis linked to post-reproductive degeneration, illustrated by the appearance of ulcers and a larger *Vibrio* population.

Motor Primitives in the Wild: Investigating *Octopus vulgaris* Reaching and Fetching movements in their natural environment

Selina Ernst[1], Daniel Kalysch[1], Wolfgang Slany[2], Tamar Gutnick[3] and Michael J Kuba [4]

1 Carl von Ossietzky Universität Oldenburg, 26129 Oldenburg, Germany
2 TU Graz, Institute of Software Engineering and Artificial Intelligence, 8010 Graz, Austria
3 Vienna Zoo, 1130 Vienna, Austria
4 Università degli Studi di Napoli Federico II, Biologia, 80138 Naples, Italy

Abstract

Octopuses have eight highly flexible muscular hydrostat arms, with potentially infinite degrees of freedom. Their unique morphology suggests that direct central nervous control of all motor units during voluntary movement is highly unlikely. Therefore, the concept of motor primitives was proposed. They simplify the almost intractable task of purposeful control of the eight arms into a more manageable set of motor building blocks that require only limited central commands.

In the Mediterranean *Octopus vulgaris*, the motor primitives Reaching and Fetching have so far only been studied in the laboratory. The present study examines their occurrence as part of foraging behavior in the octopus' natural habitat.

Using a small observation-class remote operating vehicle (ROV), off the coast of the Croatian island of Krk, we filmed *Octopus vulgaris* (N=6) in their natural habitat. Analysis of 121 minutes of video footage showed that octopuses regularly use Reaching (N=121min; 1.17 ± 0.76 per minute) and Fetching (N=121min; 0.66 ± 0.82 per minute) movements when catching and retrieving prey. Furthermore, the previously reported stop-and-go foraging strategy was confirmed. Additionally, individual differences in hunting strategies emerged. While most octopuses predominantly used Reaching during, Crawling, and Stationary behaviours, one individual used Web-over significantly more frequently (N= 21; $p = 0.0018$).

These results demonstrate that the motor primitives described in the laboratory are also found in the natural behavior of octopuses in the wild and contributes to the understanding of arm control in complex and dynamic environments.

We would like to thank the colleges & volunteers from the Mare Mundi Krk Research Station for their help and support.

Diversity of the ‘eerie’ glass squid genus Taonius Steenstrup, 1861 (Cephalopoda: Oegopsida) in the Pacific Ocean

Aaron Evans[1], Kathrin Bolstad[1], Heather Braid[1], Tsunemi Kubodera[2]

1 AUT Lab for Cephalopod Ecology and Systematics (ALCES), Auckland University of Technology, Auckland New Zealand
2 National Museum of Nature and Science, Collection Center, 4-1-1, Amakubo, Tsukuba, Ibaraki 305-0005, Japan

Abstract

Glass’ squids (family Cranchiidae Prosch, 1849) are diverse and widely distributed, but many cranchiid genera are in need of global taxonomic revision. Although two species of *Taonius* were previously recognised from the Pacific Ocean (*T. belone* and *T. borealis*), a recent review of material from this basin has revealed the presence of at least six species, including four new to science, described here as *T. expolitus, T. notalia, T. robisoni*, and *T. tanuki*. Morphological species identities are supported by several characters, including arm sucker count, and arm and manus sucker dentition. Proposed species delimitations are further supported by differences in mitochondrial DNA (cytochrome c oxidase subunit I). Although a full systematic review of the Cranchiidae is still needed, tripling the known diversity of Taonius in the Pacific Ocean and clarifying identities of its locally occurring species represent an important step forward.

Internal models in the squid *Sepiotheutis lessoniana*

Olivier Fernandez[1], Makoto Hiroi[1], Sam Reiter[1]

1 OIST

Abstract

Internal models for sensory-motor predictions are widely distributed across neural systems. However, it remains unclear if and how internal models are used in cephalopods. The cephalopod peduncle lobe has been analogized to the vertebrate cerebellum, based on similar anatomy and behavioral effects of lesions. A common view on cerebellar computation is that it is implementing internal models for motor control. Do these facts imply that the peduncle lobe implements an internal model? To test this idea, we are conducting high-speed, markerless 3D tracking of squid (*Sepiotheutis lessionana*) during prey hunting. We seek to record the signatures of internal model-based predictions through quantitative behavioral analysis. We are combining these experiments with peduncle lobe lesions. Through profiling internal models and their basis in cephalopods we seek to discover general principles governing the implementation of sensory-motor predictions.

Investigating the cephalopod community and trophic ecology in the Santa Lucia Escarpment

Kristina Fleetwood[1], Moira Décima[1]

1 Scripps Institution of Oceanography, University of California San Diego, San Diego, CA, USA

Abstract

Deep-sea cephalopods are widespread, abundant, and critical to the connectivity of marine systems, contributing significantly to the diets of fishes, marine mammals, and seabirds. However, despite inhabiting the largest ecosystem on earth, knowledge of their diets, behavior, and overall ecology remains spatially limited. To address this paucity of trophic knowledge, we focus on cephalopods caught via midwater trawls during a recent cruise to the Santa Lucia Escarpment, located within the recently designated Chumash Heritage National Marine Sanctuary off the coast of California. High diversity of marine predators (e.g., seabirds, cetaceans) indicate a productive region supporting culturally and economically valuable species, yet to date there have been no comprehensive studies assessing pelagic community composition or trophic dynamics. Using stable isotope analysis of δ15N and δ13C of cephalopod beaks, we investigate the trophic niches of the pelagic cephalopod assemblage, while establishing a baseline of biodiversity knowledge for these ecologically important taxa. Results from this study will provide the first estimates of trophic position for pelagic cephalopods in this area, expanding our knowledge on a wide range of species including those within the Cranchiidae family, and Histioteuthis, Octopoteuthis, and Vampyroteuthis genera.

Visual Ecology of the Deep-Sea Cock-Eyed Squid

Shoshannah Fogarty[1], Fabio Cortesi[1], Wen-Sung Chung[1]

1 School of the Environment, The University of Queensland, St Lucia 4072, Queensland, Australia

Abstract

The asymmetric eyes of histioteuthid squid are renowned for their dimorphic features in size, shape, orientation of visual axis and the orbital photophore arrangement, however their visual ecology remains largely unexplored due to the challenges of sample collection and in situ observation. With anatomical examination, imaging and molecular approaches on Stigmatoteuthis hoylei, this study reveals new insights into this unique visual system. Retinal bulk-transcriptomics confirm that both eyes possess one rhodopsin tuned to the constant blue twilight zone. Furthermore, Magnetic Resonance Imaging (MRI) delineates the detailed eye structures, revealing that the large left eye is approximately twice as sensitive in catching photons as the small right eye. Given 16-17 orbital photophores surrounded the small eye, bioluminescent illumination may compensate for the sensitivity gap between dimorphic eyes. Another new finding is a group of dense and elongated ciliary muscles in the large eye that can stretch or contract to deform the eye's shape when scanning different angles of the visual scene. Furthermore, the optic lobes (OPL, visual centre) mirror the asymmetric eye design (volume of the left OPL is threefold larger than the right OPL), resulting in an extreme case of brain lateralisation in the animal kingdom. Notably, maintaining a pair of large, symmetric eyes and OPLs would be ideal for visual tasks despite high costs. Altogether, the asymmetric visual system of histioteuthid may represent a trade-off between excellent eyesight and optimal energy efficiency while residing in deep, dark environments with limited food resources.

Inferring Cephalopod Diversity of Southeast Asia from Accessible Natural History Collections

Anya Roopa Gajanur[1], Siong Kiat Tan[2], Eric Hilton[1], Zeehan Jaafar[3]

1 Virginia Institute of Marine Science, William & Mary
2 Lee Kong Chian Natural History Museum, National University of Singapore
3 National University of Singapore

Abstract

The diverse marine ecosystems of Southeast Asia host numerous cephalopod species, but comprehensive data on their diversity and distribution remain limited. This information gap can hinder conservation efforts, especially since the impacts of coastal urbanization, climate change, and fisheries on cephalopods in Southeast Asian countries, like Singapore, are still unknown. Thus, preserved specimens of cephalopods in the Zoological Reference Collection (ZRC) of the Lee Kong Chian Natural History Museum, National University of Singapore, were examined to better understand regional cephalopod biodiversity, with a focus on taxa from Singapore. A total of 1003 preserved specimens were digitized, and species identification, morphometric, and distribution data were collected; 81 species across 15 families were recorded. The ZRC consists of a diverse assemblage of well-documented and lesser-known coastal and deep-sea species. This includes some potentially new records for Singapore, such as *Inioteuthis cf. maculosa* and *Macrochlaena winkworthi*, as well as the globally largest collection (n=31) of "Octopus" *cf. favonius* collected from Singapore. This review of the LKCNHM collection will form the basis for an annotated checklist of Singaporean Cephalopoda, including representative images of specimens in the collection. This improvement in the taxonomic resolution of the collection will help update and contribute to Singaporean and regional diversity databases. The outcome of this study provides a firm foundation for future work on cephalopods while broadly contributing to the conservation of marine resources within Singapore and Southeast Asia.

Genomes under Pressure: What Octopus maya Can Teach Us about Resilience in a Warming Ocean

Clara Elizabeth Galindo-Sánchez1[1], Carlos Rosas-Vazquez[2], Oscar Juárez[3], Laura L. López-Galindo[4], Claudia Ventura-López[5], Pavel Galindo-Torres[6], Román Díaz-Polo[1], Sadot Ramos[1], Fernando Díaz-Herrera[1], Claudia Caamal-Mosnreal[2]

1 Laboratorio de Genómica Funcional, Departamento de Biotecnología Marina, Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), Ensenada, Baja California, México
2 Unidad Multidisciplinaria de Docencia e Investigación, Facultad de Ciencias, Universidad Nacional Autónoma de México, Sisal, Yucatán, México.
3 Laboratorio de Fisiología Comparada y Genómica Funcional. Programa de Acuicultura, Centro de Investigaciones Biológicas del Noroeste, S. C, La Paz, Baja California Sur, México
4 Instituto de Investigaciones Oceanológicas, Universidad Autónoma de Baja California, Ensenada, Baja California, México
5 Departamento de Biomedicina y Bionanotecnología, Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), Ensenada, Baja California, México
6 Laboratorio de Genética Acuícola, Centro de Investigaciones Biológicas del Noroeste, S. C, La Paz, Baja California Sur, México

Abstract

Octopus maya, endemic to the Yucatán Peninsula, is increasingly threatened by rising ocean temperatures due to global warming. This study investigates the molecular mechanisms underlying its reproductive resilience and vulnerability under thermal stress. Using RNA-seq and genome-wide methylation profiling, we analyzed transcriptomic and epigenetic responses in reproductive tissues and embryos exposed to elevated temperatures. We identify key genes and regulatory pathways linked to stress response, metabolic adaptation, and neural plasticity. Our findings reveal downregulation of genes associated with nervous system development, morphogenesis, and gene expression regulation in embryos under thermal stress, leading to morphological abnormalities such as malformed arms and eyes. In females, elevated temperatures affected the expression of key reproductive genes, including those encoding serotonin, dopamine, and progesterone receptors, as well as genes involved in sperm storage and egg-laying processes. These molecular alterations correlate with reduced fertilization rates and hatchling survival, indicating a compromised reproductive capacity under thermal stress. Our results underscore the importance of integrating molecular data into assessments of species' adaptive capacities to climate change, providing insights into the resilience mechanisms of tropical cephalopods.

Molecular and morphological factors contributing to the resolution of the taxonomy of *Octopus vulgaris* Type III

Gareth N. Fee[1, 2], Michael D. Amor[3], Jannes Landschoff[2, 4], Charles Griffiths[1], Alvaro Roura[5], Arsalan Emami-Khoyi[6, 7], Peter Teske[7]

1 Rondebosch, 7701, Cape Town, South Africa
2 Sea Change Trust, Oranjezicht, 8001, Cape Town, South Africa
3 Welshpool, WA 6106, Australia
4 Matieland, 7602, South Africa
5 Vigo, Spain
6 Gödöllő, Hungary
7 Auckland Park 2092, South Africa

Abstract

Many cryptic species exist in the genus *Octopus* Cuvier, 1797 due to the lack of morphological traits available to distinguish closely-related species. In recent years, the *Octopus vulgaris* species complex has received much attention with many cryptic species 'Types' being identified, most of which have now been re- or newly described as separate species. The Southern African lineage, *Octopus vulgaris* Type III was not included in a recent global morphological assessment of the complex and few specimens were included in phylogenetic studies. Through investigating both the full mitochondrial genome and multivariate analyses of 26 and 21 morphological traits for males and females respectively, we found both genetic and morphological support for the distinction of *O. vulgaris* Type III as a unique species. These results have significant implications, considering a growing octopus fishery in South Africa, as management should consider the population as distinct species from O. vulgaris ss, which is found in the Mediterranean and Northeast Atlantic.

Long-term changes of paralarvae abundance in the Southeastern Brazilian Bight

Carolina Araujo[1], Mary Gasalla[1]

1 University of Sao Paulo, Oceanographic Institute

Abstract

Cephalopods early life stages are very sensitive to environmental change which is critical to dispersal and recruitment success of populations. While seasonal and interannual variations in paralarvae abundance have been relatively well studied in association with oceanographic conditions, long-term climate fluctuations in composition and distribution are not well covered. We used a historical collection of plankton samples from 1974 to 2010 to investigate possible environmental drivers of multidecadal paralarvae assemblages' distribution in shelf areas of the South Brazil Bight (22– 25°S). Exploratory multivariate analyses of time-series indicated a well-defined segregation of neritic versus epi and mesopelagic assemblages. When biotic and abiotic variables were considered together, sea surface temperature was clearly identified as the main contributing factor. Moreover, further results from multiple factor analysis suggested salinity as the main driver for opposite responses of families within the neritic and mesopelagic assemblages (e.g. Loliginidae versus Octopodidae). The data also revealed that a temporal asynchrony between Argonautidae and Loliginidae abundances, two of the most abundant paralarvae families from the region, coincides with positive anomalies for the Southern Oscillation Index. Despite several limitations (e.g. lack of sampling continuity for some years or seasons, size and vertical distribution of paralarvae), findings show potential oceanographic drivers impacting long-term dynamics that may directly affect patterns of transport and dispersal, as well as habitat shifts of early life stages assemblages.

Quantitative macroarchitecture of the optic lobe from μCT and synchrotron imaging

Sofia Elena Georgiades[1], Yirong Xiong[1, 2, 3], Ben Gerhardt[2025-01-04 00:00:00], Ali Karimi[1], Dominic Evans[1], Gilles Laurent[1]

1 Max Planck Institute for Brain Research
1, 2, 3 HHMI Janelia/John Hopkins University
2025-01-04 00:00:00 Max Planck Institute for Brain Research

Abstract

Many cephalopods use camouflage to evade predators and blend into their surroundings. This behaviour requires animals to interpret visual scenes and rapidly match their statistics using skin pigment cells (chromatophores) controlled by the brain. In the common cuttlefish (*Sepia officinalis),* the first ganglion to receive visual information from the retina is the optic lobe, which is thought to be the main site of visual information processing and pattern extraction. Given that information processing is tightly linked to neuronal architecture, uncovering the structure of the optic lobe is key to understanding the unique visuomotor transformations of cephalopods. So far, most of our knowledge about the organisation of the optic lobe relies on the Golgi preparations of J.Z. Young (1962) and colleagues, which illustrated the presence of “cell islands” in the optic lobe’s medulla. In S. pharaonis, these “cell islands” were shown to be contiguous and to form a tree-like structure that increases in branching complexity from the optic tract region to the surface of the optic lobe (Liu et al., 2017). Here, we present the use of X-ray micro-computed tomography (μCT) to visualize and quantify the scaling laws of the tree-like medulla in S. officinalis adults and across development. We find that the network spans over 20 branching levels. We further use synchrotron imaging to achieve single-cell resolution of complete optic lobes. Ultimately, we aim to understand how branching complexity within the optic lobe informs visual processing and, thereby, camouflaging behaviour.

Use of Operational Welfare Indicators to promote sustainable aquaculture of *Octopus vulgaris*

Camino Gestal[1], Andrea González-Alonso[1], Sara Pérez-Polo[1], María M. Costa[1], Sonia Dios[1]

1 Instituto de Investigaciones Marinas (IIM), CSIC. Vigo, Spain

Abstract

Animal welfare is a growing public concern, particularly in the emerging field of octopus aquaculture. The common octopus, Octopus vulgaris, is a commercially valuable species and an excellent candidate for aquaculture diversification. Overfishing and high market demand make sustainable octopus aquaculture essential to reduce fishing pressure and conserve wild populations. Ensuring sustainability requires optimised, standardized practices that safeguard animal welfare. This includes prioritising factors such as diet, housing, environmental enrichment, behaviour, and health. This study identifies specific welfare markers, defined as biological Operational Welfare Indicators (OWIs), for use in monitoring protocols and good aquaculture practices. Here is presented the first set of biological OWIs for pathogen detection, disease diagnosis, and health monitoring in captive octopus. Daily health assessment includes behavioural observation and detection of disease signs. Non-invasive diagnostics support disease prevention and involve parasitological and microbiological analysis of tank seawater, skin, and faeces. Quantitative PCR protocols were optimised for early diagnosis and absolute quantification of the coccidia Aggregata octopiana using faecal samples and tank seawater, as well as the bacteria Vibrio spp. and V. lentus using skin mucus and tank seawater. Antibacterial and antiviral activities in skin mucus were also studied as indicators of natural defence. These biological OWIs will serve as key tools for the incipient development of octopus aquaculture.

Are there ARTs (sneakers and consorts) in *Loligo forbesii*?

Domonique Gillen[1], Leigh Barnwall[2], Jessica B. Jones[3], Anne Marie Power[1]

1 Ryan Institute, School of Natural Sciences, University of Galway, Ireland
2 Marine Directorate, Scottish Government
3 The Scottish Wildlife Trust

Abstract

Many loliginid squid show dramatic variability in size-at-maturity. In males, researchers have attributed alternative reproductive tactics (ARTs) to two different size classes at sexual maturity: ‘sneakers’, which are small at maturity, and ‘consorts’ which are larger. Interestingly, studies have suggested ARTs can be correlated to differences in the morphology of male reproductive structures. Our goal is to dive deeper into this topic by studying mature male *Loligo forbesii* to assess the morphological characteristics of their reproductive organs, specifically spermatophores and their internal structures and a possible correlation to ‘sneaker’and ‘consort’ categorization. Reproductive organs (spermatophoric complex of organs, testis, and penis) and statoliths were obtained from mature male *L. forbesii* samples collected in Irish and Scottish waters. Spermatophores were removed from the reproductive organs, counted, and the following measurements were obtained from ten spermatophores from each sample: spermatophore length (SpL), sperm mass length (SML), ejaculatory apparatus length (EAL) and cement body length (CBL). The morphology of the cement bodies inside the spermatophore was also analysed. These features will be analysed to determine whether there is any evidence to assign sneaker or consort tactics to male individuals in *L. forbesii*. This will be achieved by examining correlations between reproductive structures, mantle length as well as the age profiles of males.

Ecology of wild common octopus: the way towards a sustainable management (ECOSUMA PROJECT)

Ángel F. González[1], Jorge Hernández-Urcera[1], Lara García-Alves[1], Alvaro Roura[2], Alexandre Alonso-Fernández[3], Ana Moreno[4], Santiago Pascual[1]

1 ECOBIOMAR Research Group, Institute of Marine Research (IIM-CSIC), Vigo, Spain
2 Octolarvae, Moaña, Spain
3 INMARE Research Group, Institute of Marine Research (IIM-CSIC), Vigo, Spain
4 4 Instituto Português do Mar e da Atmosfera (IPMA), Lisbon, Portugal

Abstract

The main objective of the ECOSUMA project was to investigate some unknown aspects of the ecology of the common octopus *Octopus vulgaris* in the wild, especially during its paralarvae and juvenile stages. Ecological studies of paralarvae before they settle to the seabed suggested that they were transported to oceanic waters during their planktonic phase. This phase, as well as the ecology of subadults once they reach the fishing grounds near the coast, were virtually unknown aspects. Trophic relationships during these two phases were investigated in order to move towards an ecosystem-based approach that supports the management of this socioeconomically important resource. Comparatively, 33 prey species were identified exclusively in octopus paralarvae, which correspond to organisms present in zooplankton (copepods, hyperiid amphipods, crab larvae, ostracods, pteropods, squid larvae, cnidarians, siphonophores, and algae). Additionally, 13 species were detected exclusively in juveniles, corresponding to benthic organisms (gammarid amphipods, hermit crabs, spider crabs, fish, isopods, polychaetes, and a nematode). Only two prey species were detected in both paralarvae and newly settled individuals. Age analysis from their beaks has allowed us to estimate that their age ranged from 5 to 39 days for paralarvae, 29 to 89 days for newly settled individuals with weights between 1 and 38 g, and from 92 to 260 days for individuals weighing between 104–460 g. The study of the spatial ecology of O. vulgaris has allowed us to create an informative video explaining the activities conducted to understand the movements of adults using acoustic telemetry.

Genetic population structure of *Paroctopus digueti* (Perrier & Rochebrune, 1894) in the Gulf of California, Mexico

Mariae C. Estrada-González[1], José Francisco Domínguez-Contreras[1], Francisco Javier García-Rodríguez[1], Bertha Patricia Ceballos-Vázquez[1]

1 Instituto Politécnico Nacional, Centro Interdisciplinario de Ciencias Marinas, Av. Instituto Politécnico Nacional S/N, Col. Playa Palo de Santa Rita, 23096 La Paz, Baja California Sur, Mexico.

Abstract

The Pacific pygmy octopus, *Paroctopus digueti*, is distributed in coastal and shallow (0-20 m) lagoons in the Gulf of California. Currently, it is not commercially exploited, unlike the octopods *Octopus hubbsorum* and *O. maya*, which position Mexico as one of the principal octopus producers at the international level. However, *P. digueti* has potential as a gourmet aquaculture resource and for laboratory experimental studies because the species is adapted to captive conditions, it has a holobenthic life history, its longevity is less than one year, it is semelparous, has small spawns (50-323 eggs). Also, the capture of wild organisms is accessible. Apart from this, studies on genetic variation are a relevant component in the effective and sustainable management of marine resources. The objective is to know the genetic population structure of *P. digueti* in the Gulf of California based on the mitochondrial COI gene. It is expected that *P. digueti* presents different populations as it happens with other holobenthic octopods and is related to the geographic distance between the effective geographic distribution. A total of 144 organisms have been collected in different coastal lagoons within the gulf. Phylogenetic and genetic diversity analyses are being carried out to identify haplotypes, and the genetic population structure and isolation by distance are also being evaluated. The results obtained in *P. digueti* will be a novel approach to discussing the sustainable use of pygmy octopuses.

Optimal rearing temperature of *Octopus tetricus* paralarvae, informed by a novel micro-respirometry approach

Jeffrey Good[1], Andrew Jeffs, Quinn Fitzgibbon[3], Stefan Spreitzenbarth[1]

1 Leigh Marine Laboratory, Institute of Marine Science, University ofAuckland, 160 Goat Island Road, 0985 Leigh, New Zealand
3 Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, TAS 7005, Australia
2025-01-02 00:00:00 School of Biological Sciences, University of Auckland, 23 Symonds Street, Auckland Central, New Zealand

Abstract

In paralarval cephalopods, the internal yolk sac acts as endogenous energy source to cover metabolic demand for newly-hatched paralarvae. The rate of inner yolk sac depletion is strongly influenced by seawater temperature and has profound consequences on the early paralarval growth and survival as it dictates the speed at which sufficient energy must be obtained from exogenous sources. The gloomy octopus, *Octopus tetricus*, is native to temperate waters and their paralarvae can be hatched through artificial incubation, making them an ideal model to understand metabolism over a wide range of seawater temperatures. This research sought to use micro-respirometry to understand the metabolism of *O. tetricus* paralarvae during the transition from endogenous to exogenous energy sources and to identify an optimal rearing temperature (Topt). Eggs from multiple females were incubated in six temperature treatments between 12 and 27 °C. A total of 21 hatched paralarvae were measured for oxygen consumption rates at rest and after exercise at 0-, 2-, and 4-days post hatch (dph) at each treatment using a microplate sensor dish reader. The results show that the Topt of *O. tetricus* paralarvae lies between 17 and 21°C. Additionally, aerobic scope was shown to decrease in age within treatments, suggesting the possibility of a dynamic Topt through development. This research is the first to apply micro-respirometry methods to inform a optimal rearing temperature for octopus paralarvae and provides a model for determining the optimal rearing temperature research of other cephalopods to improve paralarval rearing for aquaculture and aquaria purposes.

Unraveling Coleoid phylogenetics through molecular diversity of mitochondrial genomes

Alyson Hally[1], Caroline Albertin[4], Loyal A. Goff[2, 3, 4]

1 Department of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD
4 Marine Biological Laboratories, University of Chicago, Woods Hole, MA
2, 3, 4 Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD

Abstract

Coleoids are a diverse clade of cephalopods characterized by their internal shell, ink sac, and arm hooks. They diverged from Nautilus around 415 million years ago (MYA) and further split into Octopodiformes and Decapodiformes approximately 245 MYA. Previous phylogenetic analyses have used morphological or molecular data, but have been limited by species coverage, genetic sequence availability, and inadequate genomic diversity, hindering accuracy and leaving significant gaps in our understanding of evolutionary relationships. In this study, we compiled mitochondrial genomic data from all coleoid species documented in the World Register of Marine Species with available primary sequence data at the National Center for Biotechnology Information. Twelve novel mitochondrial genomes are constructed de novo from available sequencing data. Phylogenetic analysis of over 160 complete mitochondrial genomes confirms established taxonomic relationships and reveals new insights. In this analysis, we examine how both assembly method biases and molecular diversity influence evolutionary relationships across coleoid cephalopods. Decapodiformes have significant gene order rearrangements, which can be utilized to trace ancestral divergence. Sequence variation within genes, particularly at variant hotspots, can explain evolutionary relationships within syntenic subgroups. Inclusion of partial mitochondrial genes expands our phylogenetic analysis to over 400 coleoid species, revealing novel evolutionary ties while solidifying ancestral connections. This study validates evolutionary relationships using quantifiable molecular diversity. Despite the large representation of species in our analysis, this represents less than half of all coleoids. As coleoid cephalopods become increasingly important in molecular biology, establishing accurate taxonomic relationships becomes critical for successful cross-species and comparative research.

Male *Octopus parvus* protect hectocotylus against unpredictable risks of arm loss

Haruki Keijiro[1], Yamate Yuta[2], Takegaki Takeshi[1]

1 Graduate School of Integrated Science and Technology, Nagasaki University
2 Integrated Science and Technology, Nagasaki University

Abstract

Predatory attacks cause not only lethal but also sub-lethal injuries to prey, and sub-lethal injuries affect the survival rate and reproductive success of prey individuals after the attacks. However, most studies of anti-predator behavior from the standpoint of the prey have focused on behaviors that avoid lethal predation, and very little is known about the behaviors and traits that reduce the effects of injury from predatory attacks. Octopuses frequently lose their arms due to predation, but it is known that male hectocotylized arm specialized for copulation is less likely to be lost than other arms. Male octopuses often curl hectocotylus tips close to the body, which is considered to be a behavior to protect it from predators, but this hypothesis has not been verified. In this study, we investigated arm loss in *Octopus parvus* in the field, and observed arm use during foraging in the aquarium, where the risk of arm loss is unpredictable. Field surveys showed that the frequency of loss of the right third arm in males (i.e., hectocotylus) was significantly lower than in females, and aquarium experiments showed that males used the right third arm to search for food in the holes less frequently than females. These results strongly suggest that male *O. parvus* protect their hectocotylus by avoiding the risk to it.

Cryptic Cephalopod Confirmation: Reinstatement of *Octopus americanus* in Southeastern Florida, USA

Colleen Nicole Hecker

Abstract

Long regarded as a cosmopolitan species, the common octopus (*Octopus vulgaris*) now represents a global cryptic species complex known as the *Octopus vulgaris* Species Complex (OVSC). The OVSC comprises closely related, geographically distant taxa, displaying subtle morphological discrepancies and genetic divergence. Recent reports indicate that the OVSC taxon in the western Atlantic Ocean, Caribbean Sea, and Gulf of Mexico (formerly known as O. vulgaris types I and II) represents the recently reinstated OVSC species, *Octopus americanus*. This reclassification remained uninvestigated in southeastern Florida, USA, a known hotspot for cephalopod biodiversity. To investigate this name change for southeastern Florida's OVSC taxon, we employed an integrative approach consisting of species-specific morphological analyses and molecular phylogenetic analyses. Morphological analyses revealed southeastern Florida's OVSC taxon to have similar gill counts and enlarged sucker placements to *O. americanus*, differing from *O. vulgaris* sensu stricto. Radular morphology closely resembled the description of *O. americanus*. Molecular phylogenetic analyses carried out for mitochondrial (16S and COI) and nuclear (rhodopsin) genes consistently clustered southeastern Florida's OVSC taxon into a monophyletic group with *O. americanus*. These results support that southeastern Florida's OVSC taxon represents *O. americanus*, and that this name change is appropriate for this species in this locality. An integrative approach to species delineation is crucial for cryptic species, especially those that play significant roles in marine food webs, ecotourism, and fisheries.

Structure, diversity and bioactivity of sex pheromones in the cephalopod *Sepia officinalis*

Joël Henry

Abstract

The common English Channel cuttlefish (*Sepia officinalis*) reproduces every year in very localized coastal spawning areas after a west-east horizontal migration of 80 to 200 kilometers. The massive arrival of spawners on the coasts of west Cotentin and the Bay of Seine is suspected to be driven by sex pheromones expressed and secreted by the genitals of sexually mature females. The present study aims to verify the existence of polypeptide pheromones of a higher molecular weight than those described above. Their size could confer them a wider range of action than that of the previously identified peptide pheromones. An experimental strategy combining transcriptomics and proteomics with functional tests and an in silico study led to the identification of a cocktail of pheromones whose molecular weights ranged between 22 and 26 kDa. Proteomic analyses combined with functional tests revealed partial pheromone release in the environment and accumulation in the outer capsule of the egg. This result suggests that the eggs act as pheromone diffusers, also able to induce stimulation of egg-laying by contact when the eggs are handled by females. The coexistence of two modes of protein precursor maturation results in the simultaneous production of peptide pheromones of 1.1 to 8 kDa and polypeptide pheromones of 22 to 28 kDa. We suspect a local role for peptide pheromones with the penis and distal oviduct as main targets, while polypeptide pheromones would be more involved in the aggregation of breeding adults in coastal spawning areas, following the model described in the genus Aplysia.

From hatchlings to subadults: Beak rings reveal the age of wild *Octopus vulgaris*

Jorge Hernández-Urcera[1], Samuel E. Soule[1], Miguel Cabanellas-Reboredo[2], Ángel F. González[1]

1 CSIC
2 CSIC

Abstract

This study was developed within the framework of the ECOSUMA project, which aimed to investigate key unknown aspects of the ecology of the common octopus (*Octopus vulgaris*) in the wild, particularly during its paralarval and juvenile stages. The paralarvae were captured during a 10-day oceanographic campaign in the Atlantic waters of Spain and Portugal using a multi-opening net. The main objective was to estimate the age of *O. vulgaris* specimens by counting the daily growth rings deposited in their upper beaks. Understanding age and growth patterns is crucial for ecological and fisheries research. We analyzed beaks from paralarvae, recently settled individuals, and subadults using an image analysis system. Method development included trials with beaks preserved by freezing and in ethanol. In paralarvae (preserved in 70% ethanol), ethanol preservation affected ring visibility, but processing allowed ring counts. Estimated ages for paralarvae ranged from 5 to 24 days. Recently settled individuals (0.7–38 g) were collected via diving, frozen, and processed; their estimated ages ranged from 29 to 89 days. Subadults (104–460 g) were analyzed after storage, selecting specimens across different weight classes (<200 g and 250–450 g). Their estimated ages ranged from 92 to 260 days. This study provides a standardized protocol for age estimation in O. vulgaris across early developmental stages, contributing valuable data for population dynamics and management strategies.

Shell games: Shelter strategies of young *Octopus vulgaris* in sandy seabeds

Jorge Hernández-Urcera[1], Samuel E. Soule[1], Miguel Cabanellas-Reboredo[2], Ángel F. González[1]

1 CSIC
2 CSIC

Abstract

Sandy seabeds present a challenging environment for juvenile *Octopus vulgaris*, where the lack of structural refuges increases exposure to predators. This study reports in-situ observations of juveniles using empty bivalve shells as improvised shelters in the Cíes Islands (NW Spain). Over eight SCUBA dives between May 2022 and June 2023, nine individuals were found inhabiting various bivalve species, selecting shells closely matching their body size. Octopuses showed strategic behaviors: orienting shells vertically for better visual monitoring, manipulating valves for closure, and blending with the substrate or shell interior to enhance camouflage. In two cases, individuals abandoned their shelters and performed bipedal locomotion while maintaining cryptic coloration, suggesting complex predator avoidance tactics. These behaviors illustrate notable behavioral flexibility and habitat-use innovation during a vulnerable life stage. The reliance on movable microhabitats like bivalve shells highlights their importance in early survival and raises questions about the cognitive and ecological drivers behind shelter choice. Understanding such adaptive strategies is essential for developing informed conservation measures, especially in coastal environments facing increasing anthropogenic pressure.

Spawning habitat of the Diamond Squid, *Thysanoteuthis rhombus*, in Dominican Republic waters

José Herrero[1], Samuel King[1], Omar Reynoso[2], Owen Nichols[3], Victor Galvan[4], Michael Navarro[4, 5]

1 Center for the Conservation and Ecodevelopment of Samaná Bay and its Surroundings, Samaná, DR
2 The National Authority for Maritime Affairs, Santo Domingo, DR
3 Center for Coastal Studies, Marine Fisheries Research Program, Provincetown, USA
4 Iberostar Group, Miami, USA
4, 5 University of Alaska Southeast, Natural Sciences Department, Juneau, USA/Iberostar Group, Miami, USA

Abstract

Formal study of *Thysanoteuthis rhombus* in the Caribbean has been sparse. In the Dominican Republic, T. rhombus has been harvested in a small-scale fishery since 2001. Working with the Los Cacaos Fishery Collective, we analyzed samples from 31 harvested squid next to Samaná Bay and only spawning *Thysanoteuthis rhombus* individuals were found in this study from November 2023 to March 2024. Over the five-month study period, all squid were large and all females had mated (indicated by attached spermatophores). Squid mass averaged between 13.5 and 15.9 kg per individual across the harvest season and were most massive during February and March. Additional fishing studies for *T. rhombus*, that include sampling from April through October, could confirm a year-round spawning population.

Newly identified spawning habitat of the Diamond Squid, *Thysanoteuthis rhombus,* in Dominican Republic waters evidenced from fishery harvest.

Herrero Laureano JA

Abstract

Formal study of *Thysanoteuthis rhombus* in the Caribbean has been sparse. In the Dominican Republic, T. rhombus has been harvested in a small-scale fishery since 2001. Working with the Los Cacaos Fishery Collective, we analyzed samples from 31 harvested squid next to Samaná Bay and only spawning *Thysanoteuthis rhombus* individuals were found in this study from November 2023 to March 2024. Over the five-month study period, all squid were large and all females had mated (indicated by attached spermatophores). Squid mass averaged between 13.5 and 15.9 kg per individual across the harvest season and were most massive during February and March. Additional fishing studies for *T. rhombus*, that include sampling from April through October, could confirm a year-round spawning population.

Phenotypic Variation in *Phyllaplysia taylori* and Trends in Seagrass Habitat

Kokoro Holcomb[1]

1 Reed College

Abstract

Phyllaplysia taylori, or Taylor's sea hare, is a marine gastropod in the Aplysiidae family (Dall, 1900). This species primarily inhabits subtidal eelgrass *Zostera marina* beds, remaining on the plant throughout its life cycle. Previous research shows significant morphological and physiological variations in individuals (Beeman, 1966; Tanner et al., 2020). This thesis aims to identify correlations between latitude, host type, and temporal changes in color morphs and dorsal stripe patterns. A secondary goal was to study habitat stability by tracking changes in *Z. marina* abundance. I used photographic data from iNaturalist, seagrass survey data from SeagrassNet, and specimens from the Natural History Museum of Los Angeles County, California Academy of Sciences, and Museum of Comparative Zoology.

I found a potential subpopulation of *P. taylori* with unique translucent pigmentation, distinct from the common green morph, and lacking dark dorsal stripes. The distribution of dorsal stripe patterns and host types seems interdependent. Phenotype changes suggest seasonally driven patterns. Survey data showed that as Z. marina abundance increases, so does the probability of *P. taylori* presence. Overall, Z. marina abundance in *P. taylori*'s range has shown negative trends over time. The discontinuity of populations and *Z. marina* patches, the infrequency of *P. taylori* on abiotic surfaces, and phenotype distribution support the idea of high genetic connectivity between populations via rafting (Tanner et al., 2025). Climate change and declining Z. marina abundance may alter *P. taylori* population structure and abundance (Macreadie et al., 2011).

Editing the arsenal: Manipulating Cephalopod venom gland development and toxin gene expression

Mandë Holford[1], Kim N. Kirchhoff[1], Belkes Stambouli[1], Amy Courtney[5], William R. Schafer[4, 5], Keith C. Cheng[3], Eve Seuntjens[4], Caroline Albertin[2], Andrew Gillis[2], Saurabh Attarde[1], James Parziale[1]

1 Harvard Universtiy
2 The Marine Biological Laboratory
3 Pennsylvania State University
4 KU Leuven
5 MRC Laboratory of Molecular Biology
4, 5 KU Leuven/MRC Laboratory of Molecular Biology

Abstract

A long-term research goal of the Holford lab is to elucidate how venoms are expressed in specialized tissue such as venom and salivary glands, and how we can manipulate their expression for the development of therapies, such as nonaddictive pain compounds, tumor-selective cancer treatment, and anticoagulants. Together with the Marine Biological Laboratory's highly successful cephalopod breeding program, we have established the foundations for tackling biological and translational questions that have remained unapproachable due to lack of appropriate venom model systems, such as, how are venom genes expressed and can we manipulate the production of specific venom compounds with a desired function, such as those targeting receptors involved in analgesic activity? This talk will investigate questions pertaining to the development of cephalopod venom glands and the expression of specific compounds in their venom arsenal. Specifically, using cuttlefish (*S. bandensis, S. officinalis*), squids (E.*berryi, D. peaelii*) we: (1) Trace the expression of already identified cephalopod venom peptides and proteins in their posterior salivary glands to produce a knowledgebase for the morphology of venom gland development from diverse taxa and reveal venom gene expression patterns that can be manipulated. (2) Knock-out specific cephalopod compounds, such as cephalotoxin (CTX), to determine how deletion of highly expressed venom arsenal components affect development of the posterior salivary glands to determine the role of dominant venom compounds in venom gland development and venom arsenal composition. We use techniques of in-situ gene expression (hybridization chain reaction), immunohistochemistry, CRISPR-Cas9 genetic engineering, and microscopy to elucidate development, differentiation, and venom gene expression.

Diversity of the squid family Brachioteuthidae in Aotearoa New Zealand and the Southern Ocean

Austin Horenkamp[2], Kathrin Bolstad[2], Heather Braid[2], Marek Lipinski[1]

1 Department of Ichthyology and Fisheries Science, Rhodes University, Makhanda, South Africa; South African Institute of Aquatic Biodiversity, Makhanda (=Grahamstown), South Africa
2 AUT Lab for Cephalopod Ecology & Systematics, School of Science, Auckland University of Technology, Auckland, New Zealand

Abstract

The deep-sea squid family Brachioteuthidae is one of the most systematically unstable cephalopod taxa in need of a global revision. The general lack of adult specimens in research collections, incomplete and inaccurate species descriptions, descriptions based on paralarvae or early juveniles only, and sexual dimorphism have all contributed to the instability of this family. As part of a global revision a local review of the New Zealand and Southern Ocean brachioteuthids is being undertaken using integrative taxonomy. While the family was founded in 1881, Brachioteuthis was only formally reported from New Zealand waters in 2019. In the meantime, four genetically distinct species from around New Zealand have been identified, some of which may represent unnamed/new species. One of these species (‘Brachioteuthis sp. Ker 2’, sensu Braid & Bolstad 2019) forms a clade with low variation with individuals from the northern Atlantic, which suggests that some brachioteuthids may have very wide geographic ranges. The key morphological characters for species-level delimitation include tentacle-club morphology including carpal (fixing) apparatus [when present], skin sculpture (useful in sexual dimorphism cases), arm modifications in males, liver morphology, eye photophore morphology and the general habitus based on shape analysis. This project represents a step forward in the systematic resolution of this complicated family. However, an integrative taxonomic approach will be required to resolve the global systematics of brachioteuthids.

Statolith analysis reveals the determination mechanism of alternative reproductive tactics in *Heterololigo bleekeri*

Hosono Shota[1], Masuda Yoshio[2], Tokioka Shun[3], Kawamura Tomohiko[1], Iwata Yoko[1]

1 Atmosphere and Ocean Research Institute, The University of Tokyo
2 Miyagi Prefecture Fisheries Technology Institute
3 Japan Fisheries Research and Education Agency

Abstract

Alternative reproductive tactics (ARTs), intrasexual phenotypic variations associated with reproduction, are observed across various taxa. In the Japanese spear squid *Heterololigo bleekeri,* males exhibit ARTs during their reproductive season (January–May), where large "consort" males compete with rivals for mating, while small "sneaker" males attempt to steal fertilization opportunities from consort males. In addition to behavioral differences, distinct morphological differences exist between tactics, and no intermediate males have been observed, suggesting that each male's tactic is determined before the reproductive season and does not change once determined. However, when and how the tactic of each male is determined remain unknown. Here, we reconstructed the individual hatch date, growth history, and experienced water temperature in early life history using statolith microstructures and trace elements. The hatch date significantly differed between consorts (early-hatched) and sneakers (late-hatched). However, no significant differences were observed in growth history up to 100 days of age. Additionally, we found no evidence that water temperature experienced until 100 days of age influenced the determination of reproductive tactics. These results suggest that ARTs of *H. bleekeri* are determined based on the hatch date, independent of the growth rates and water temperature experienced in early life history. Differences in hatch date result in variations in the length of growth period until a certain calendar date and in environmental factors — such as photoperiod — experienced at a critical life stage, which may influence the determination of ARTs in this species.

Bioluminescence as a driver of rhodopsin specialisation

Ryan B. Howard

Abstract

In the ocean, where ambient light is limited, oegopsid squids predominantly use vision to survive. In cephalopods, rhodopsin is the visual photopigment activated by light that induces phototransduction. Surprisingly, most cephalopods are colourblind, they have a single type of rhodopsin that is optimally attuned to a specific wavelength of light. The typical peak spectral absorbance of rhodopsin in deep-sea oegopsids is near 480 nm, the same wavelength of downwelling sunlight that penetrates water the deepest. However, some research indicates rhodopsin peak spectral absorbance may be depth dependent. To better understand the drivers that shape rhodopsin spectral sensitivity; we characterised the spectral sensitivity curves of rhodopsin by measuring peak absorbances and bandwidths from ten oegopsid species. Additionally, the rhodopsin gene was sequenced from 11 species and combined with known sequences from GenBank for phylogenetic analysis. Most notably, bioluminescence appears to be influential for determining the shape of rhodopsin spectral sensitivity curves.

Born to Regrow: Molecular and Cellular Pathways of Adult Plasticity and Regeneration

Pamela Imperadore

Stazione Zoologica Anton Dohrn, Department of Biology and Evolution of Marine Organisms (BEOM), Villa Comunale, 80121 Napoli, Italy

Abstract

In higher vertebrates, such as mammals, growth follows a determinate pattern—once adulthood is reached, growth halts, and individuals attain a genetically pre-defined size. In contrast, many aquatic organisms, particularly invertebrates, possess the remarkable ability to continue growing even after reaching sexual maturity. Indeterminate growth can persist throughout life, largely modulated by environmental factors. Notably, growing evidence suggests a strong link between indeterminate growth and regenerative capacity, prompting efforts to disentangle the shared and distinct molecular and cellular processes underlying both processes. Cephalopods stand out as a compelling model, combining robust regenerative abilities with continuous growth. In this study, we investigated these dual traits in the arms of *Octopus vulgaris*, with a particular focus on the distal portion—the "tip". Through in silico analyses, we identified unique biological features localized in this terminal growth zone, supported by Principal Component Analysis (PCA). PCA revealed minimal internal variance in the gene expression profile at the tip and, more notably, a distinct separation from all other tissues. This suggests a unique molecular signature that may be responsible for sustained growth. Complementary RT-qPCR experiments identified a set of genes specifically upregulated in the arm tip and/or across various stages of arm regeneration (healing, budding, re-growth). Collectively, these findings provide a foundation for further exploration of cephalopod plasticity and enhance our understanding of the fundamental biological principles underlying growth, development, and tissue regeneration.

Flexible reproductive strategies of squid: from gamete to populations

Yoko Iwata[1]

1 Atmosphere and Ocean Research Institute, The University of Tokyo

Abstract

Flexibility is one of the most fascinating characteristics of cephalopods. These animals change their behaviors, physiology, morphology, and life history in response to environmental and social conditions. Coastal squid shows sophisticated intra-specific interactions and complex reproductive strategies, offering excellent opportunities to study how individuals select behavioral tactics and adapt their phenotypes accordingly. In loliginid squid, males exhibit distinct alternative reproductive tactics (ARTs): large males engage in a consort tactic transferring sperm to the female's oviduct, while small males conduct a sneaking tactic transferring sperm to a seminal receptacle on buccal membrane. These differing sperm transfer sites lead to distinct fertilization processes and drive divergent phenotypic adaptations in reproductive traits. Those morphological and physiological adaptations suggest that males typically commit to one ART during their life history. Furthermore, the difference in sperm storage duration between the two tactics affects local population structure: more sneaker males under the condition of few mature females, as sneakers can mate with females not close to spawning. On the other hand, our recent study shows that males of pygmy squid also have ARTs with distinct sperm transfer sites, but, unlike loliginids, no morphological adaptation in reproductive traits is observed. In this species, each male conducts both ARTs flexibly depending on the female response, suggesting that an intermediate, unspecialized phenotype may be optimal. In this presentation, I would like to discuss how environmental and social factors shape the flexibility of reproductive strategies in squid.

Assessing the trophic niche of giant Australian cuttlefish (*Ascarosepion apama*) through ontogeny using carbon and nitrogen stable isotopes

Bethany Jackel[1], Lauren Meyer[1], Zoë Doubleday[2], Ryan Baring[1]

1 College of Science and Engineering, Flinders University, Adelaide, South Australia, Australia
2 Future Industries Institute, University of South Australia, Mawson Lakes, South Australia, Australia

Abstract

A sub-population of giant Australian cuttlefish (*Ascarosepion apama*, formerly *Sepia apama*) in the upper Spencer Gulf of South Australia forms the only dense cuttlefish mating aggregation known globally. Though the aggregation forms on a confined, nearshore rocky reef, much of the lifespan of these cuttlefish occurs in deeper parts of the upper Spencer Gulf. The trophic niche of giant Australian cuttlefish through this phase of rapid growth is only partially understood, and may have unrecognised connections to species of commercial, recreational and ecological significance such as blue swimmer crabs (*Portunus armatus*) and western king prawns (*Melicertus latisulcatus*). We analysed δ13C and δ15N stable isotopes within the muscle tissues of giant Australian cuttlefish and a range of small-bodied, potential prey species to uncover the trophic niche and dietary breadth of these cuttlefish throughout ontogeny. Our findings suggest the role of this giant Australian cuttlefish sub-population in the broader ecosystem, as well as the potential effects that their fluctuating population size might have on the upper Spencer Gulf food web.

Single-cell characteristics of the *Amphioctopus fangsiao* optic lobes provide insights into the coastal octopus visual system innovations

Dianhang Jiang

Abstract

Background Coleoid cephalopods (octopus, squid, and cuttlefish) have evolved a camera-like eye, the structure of which is distinct from that of other molluscs, but is similar to that of marine fishes. Elucidating the molecular characteristics underlying the adaptive innovations in coleoid eyes may deepen our understanding of visual system evolution. Here we applied single-cell transcriptomic analyses to elucidate the cellular characters of the optic lobes that is the vision center in the gold-spot octopus, *Amphioctopus fangsiao*, and performed comparative genomic analyses to investigate the genomic mechanisms that underlies the visual system innovations. Results We identify eight cell types in the octopod visual center including proliferating cells, immature neurons, dopaminergic neurons, dopaminergic + glutamatergic neurons, cholinergic neuron, GABAergic neurons, glia cells and vascular endothelial cells. The gene novelties highly related to the cephalopod neural innovation are preferentially expressed in mature neurons in the visual center. Comparative genomic analyses reveal that gene duplication and functional diversities of the existing genes may contribute to the visual system innovation in coleoids. Cephalopod-specific novel genes occupy a low fraction in the genes that specifically expressed in the eyes. In contrast, most genes that specific expressed in the eye-related tissues have orthologues in other molluscans, yet the expression domains of these genes were not restricted in the eye-related tissues. Conclusions The present study indicates that the mature neurons in the vision system might have been one of the major driving forces behind the coleoid visual system innovation, and highlights the complexity of the visual system as a combined result of several genomic mechanisms, including recruitments of novel genes, function diversities of existing gene functions and expansion of gene families.

Estimation of Fecundity in *Octopus vulgaris*: Comparison between Counting from Egg Clutches and Gonadal Oocytes.

Arturo Jimenez[1], Ignacio Sobrino[2]

1 Spanish Institute of Oceanography. Spanish National Research Council (IEO-CSIC), 11006 Cádiz, Spain.
2 Spanish Institute of Oceanography. Spanish National Research Council (IEO-CSIC), 11006 Cádiz, Spain

Abstract

The determination of fecundity in octopuses (*Octopus vulgaris*) is essential for understanding their reproductive biology and improving fisheries management strategies. Traditionally, fecundity has been estimated by counting oocytes in female gonads, although this method may overestimate the actual number of viable eggs. This study proposes an alternative approach based on counting and characterizing eggs in clutches obtained directly from octopus traps collected in the Gulf of Cádiz. Both methodologies were compared to assess their accuracy in determining actual fecundity. The results show significant differences between the two methods, highlighting that counting eggs in clutches provides a more accurate estimation of the effective reproductive capacity of *O. vulgaris*. These findings emphasize the importance of incorporating clutch-based methodologies for a better understanding of cephalopod reproduction.

Our results indicate that fecundity estimated through egg clutch counting was significantly lower than that estimated from gonadal oocytes. On average, fecundity values obtained using the first method ranged from 72,224 to 492,935 eggs. However, ovarian fecundity values ranged between 70,060 and 605,438 oocytes while effective fecundity determined from clutches was lower, suggesting that not all oocytes mature and hatch.

Variation in ontogenetic trends of stable carbon and nitrogen isotope values across accretionary structures in cephalopods: Toward better utilisation

Li-Ling Kao[1], Chun-I Chiang[2], Pei-Ling Wang[1], Chia-Hui Wang[2], Ming-Tsung Chung[1]

1 Institute of Oceanography, National Taiwan University
2 Department of Environmental Biology and Fisheries Science, National Taiwan Ocean University

Abstract

Stable isotope analysis is a widely used method for investigating the life history of cephalopods by examining various accretionary structures, such as the gladius, eye lens, and beak. However, stable isotope values can vary among these structures due to biologically controlled fractionation and metabolic processes. Therefore, we compared the ontogenetic trends of stable carbon and nitrogen isotope values between muscle tissue and accretionary structures in two cephalopod species, *Uroteuthis chinensis* and *Sepia pharaonis*. Ontogenetic trends were established through analyses of eye lenses and beaks from both species, as well as the gladius from U. chinensis and the organic matter of the cuttlebone from *S. pharaonis*. The isotope values reflect different time frames depending on the formation periodicity of each structure but consistently reveal maternal signals at the tips of the structures, which are formed during the embryonic stage. We also noticed that absolute stable isotope values varied among the accretionary structures, and that the ontogenetic ranges of both $\delta^{13}C$ and $\delta^{15}N$ values were narrower in the eye lenses compared to others. Consequently, the estimation of trophic level could differ depending on the choice of accretionary structure, highlighting the need for a comprehensive evaluation of isotopic enrichment factors in the future.

Eyes on the prize: Prey capture and avoidance by hummingbird bobtail squid

Rachael E. Kaspar[1-3], Myeka Pangilian[1-2], Kenny Vetter[1,4], Awab Magzoub[1, 3-4], & Trevor J. Wardill[1-4]

[1]University of Minnesota Twin-Cities
[2] Department of Ecology, Evolution & Behavior
[3] Department of Biological Sciences
[4] Department of Neuroscience

Abstract

Predator-prey interactions are tightly linked to habitat complexity, shaping how organisms navigate threats or exploit resources. We employ a neuroecological framework to elucidate the neurosensory pathways involved in the predator-prey interactions of the Hummingbird bobtail squid (*Euprymna berryi*). Within coral reef food webs, intricate, spatiotemporal environments drive the neuroecology of organisms—"eat lunch or be lunch"—with outcomes influenced by neurosensory inputs and environmental pressures. *E. berryi* are stealthy nocturnal hunters, yet little is known about the neural basis of their behavioral and physiological patterns during such interactions. We found that bobtails exhibit paired ocular vergence before striking prey with their tentacles, similar to cuttlefish, with lateral eye orientation restored post-capture and retraction of prey tentacles. Additionally, bobtails altered their chromatophore expression during prey tracking and before their ballistic-strike, which suggests camouflage is coordinated during approach. Our findings are the first to demonstrate that *E. berryi* hunts prey similarly to cuttlefish, utilizing eye convergence, while also modifying the visual appearance of body patterning during predatory- prey interactions. Together, this work enhances our understanding of *E. berryi* prey capture and its broader relevance to Sepiolida and Sepiida across coral reef ecosystems.

Cohort distribution and growth rates of *Berryteuthis magister* in an understudied subregion of its range, Southeast Alaska, USA

Hunter Kaufman[1], Levi Lewis[2], Richard Yamada[3], Charlotte Springer[4], Michael Navarro[1]

1 Dept. of Natural Sciences, University of Alaska Southeast
2 Dept. of Wildlife, Fish, and Conservation Biology, University of California, Davis
3 Shelter Lodge
4 College of Fisheries and Ocean Sciences, University of Alaska Fairbanks

Abstract

Magister Squid (*Berryteuthis magister)* likely play a critical role in the trophic system of Alaska's nearshore habitats as a key ecological link in benthopelagic food-webs, yet Magister Squid from the Inside Passage subregion have not been studied previously. Although thought to be abundant by local fishermen, and the focus of a recently proposed directed fishery, little is known about this species including patterns in growth rates which can affect stage-specific survival and fisheries harvest. Here, we used statolith analysis to provide new insights regarding patterns in cohort distribution and ontogenetic growth for squid harvested in 2022. Statoliths from *B. magister* harvested in April, June, July, and August of 2022 were removed, mounted, polished, and digitally imaged under a transmitted light compound microscope. ImageJ Software was used to enumerate and quantify the widths of circuli, core to edge, with circuli assumed to represent daily accretion. Statolith-based age distributions will be used to assess the presence of multiple intra-annual cohorts. Ontogenetic patterns in growth rates will be analyzed for each month squid were collected. Future application of this method will investigate inter-annual variability (2022-2025) to begin a study on low-frequency effects on growth, including growth rates of different life stages. Statolith-based data on the growth, age structure, and phenology can inform whether there should be a fishing period (limited time of year) as part of the development for a sustainable fishery management plan.

Stealthy, scaly squid: Systematics of the Pholidoteuthidae Adam, 1950 and Lepidoteuthidae Pfeffer, 1912 (Cephalopoda: Oegopsida)

Jesse T. Kelly

Abstract

The Pholidoteuthidae and Lepidoteuthidae are related families of oceanic squids characterised by unique but differing epidermal sculpture covering the external mantle surface. Both families are distributed worldwide in temperate and tropical waters, but presently only comprise a total of three species: *Pholidoteuthis massyae*, *P. adami*, and *Lepidoteuthis grimaldii*. Their distinctive morphology, combined with their rarity in collections, has likely led to a lack of critical assessment of other taxonomically significant morphologic variation, resulting in an underappreciation of species diversity (a pattern that has been recently identified in other morphologically distinct oegopsid families). Following preliminary genetic results indicating considerably greater species diversity even within single ocean basins, a global review was undertaken to more fully understand the morphology, distribution, and relatedness within the two families. Herein, eleven species are recognized - eight new to science – comprising eight pholidoteuthid species and three lepidoteuthid species. Taxonomically significant characters were found among lower beak, arm sucker, and gladii morphologies. All morphologically identified species were supported by concurrent genetic analyses of a 658 bp section of cytochrome c oxidase subunit I (COI). A third related family, the Octopoteuthidae, was included in the genetic analyses as together, the three complete a monophyletic clade. Contrary to previous work, the geographic range of most species was found be limited to a single ocean basin. The first footage of live Pholidoteuthis and Lepidoteuthis was also identified from archives during this work and is reported here.

Developmental patterning of squid tentacles regulated by Wnt signaling

Ryosuke Kimbara[1], Gustavo Sanchez[1], Lin Zhang[1], Daniel Rokhsar[2]

1 Okinawa Institute of Science and Technology Okinawa Institute of Science and Technology
2University of California, Berkeley/Chan-Zuckerberg BioHub

Abstract

Cephalopods are unique among mollusks in possessing multiple limbs that play essential roles in prey capture, locomotion, and copulation. In decapodiformes (squid, cuttlefish, and bobtail squid), a pair of these limbs have become specialized into tentacles which are adapted for predation. In tentacles, suckers are densely arranged only at the distal club, while the proximal stalk is characterized by an elongated, elastic muscular structure. This structural differentiation contrasts with the morphology of typical arms, where suckers are present throughout the arm. These differences suggest that the molecular mechanisms controlling limb compartmentalization vary between arms and tentacles in decapodiformes. To begin to characterize these mechanisms we performed transcriptome sequencing of arms and tentacles from one-day-old hatchlings of the Japanese bobtail squid *Euprymna berryi*. Each limb was dissected along the proximal-distal axis, and for each region, the oral part containing suckers and the aboral part lacking suckers were separated. We found that several genes encoding Wnt ligands such as Wnt5, Wnt7, and Wnt11 were particularly highly expressed in the tentacular club. In situ hybridization further revealed that these genes are spatially localized within specific regions of the tentacular club. Across distinct regions of the arms and tentacles, transcriptome analysis also showed that multiple Wnt genes are differentially expressed. Especially Wnt2 and Wnt4 showed high expression in the proximal arm part. These findings suggest that Wnt ligands play a central role in limb compartmentalization and that modifications in their expression patterns contribute to the morphological specialization of tentacles.

Sticking on time – investigating the biomechanics of tentacle attachment in decapod cephalopods

Alexander Koehnsen[1], Jesse Hopstaken[1], Sebastian Henrion[1], Johan L van Leeuwen[1], Remco PM Pieters[1], Guillermo J Amador[1]

1 Wageningen University and Research

Abstract

Decapod cephalopods are well known for their tentacle strikes. Their tentacles consist of a stalk capable of rapid extension and a terminal club equipped with fins and suction cups that stick to the prey. This prey capture strategy comes with several challenges. The tentacle clubs have to be extended fast enough for the prey not to escape, the position and orientation has to be correct for the suction cups to make contact, and the suction cups have to actually attach. All of this happens within under 40 milliseconds. Here, we describe the 3D kinematics of tentacle strikes of the dwarf cuttlefish Ascarosepion bandense combined with the club movements during attachment to a flat surface. This data is correlated with morphological data of the tentacle club, obtained using micro computed tomography. Tentacle strikes follow a distinct sequence, beginning with an initial positioning phase, followed by rapid extension. As the extension nears completion, the clubs unfurl lateral fins and adjust their orientation for contact, while the tips remain connected. This leads to a deceleration of the club and buckling of the stalk. Our research may provide a foundation for answering a broader spectrum of questions concerning the behavioural ecology and biomechanics of cephalopods. Additionally, understanding the deployment of flexible appendages might help us design fast and versatile bioinspired gripping devices.

Spatial Characterization of Developmental Gene Expression in the *Octopus bimaculoides* Optic Lobe

Abbigale Koenigsmark[1], M. Desmond Ramirez[1], Jeremea Songco-Casey[1], Cristopher Niell[1]

1 University of Oregon

Abstract

The large brains of octopuses grow substantially after hatching, requiring extensive neurogenesis. Immature neurons must find their proper location, obtain unique morphology, and make appropriate connections with other neurons to form functional circuits. This specificity is especially required within the optic lobe, as each of its layers contains cell types with differential molecular signatures and cellular morphology that are organized in a fashion that maintains topology in visual responses. Incorporation of immature neurons into this system requires the utilization of developmental genes, whose expression may differ between cell types and spatial location. While many genes key in the development of neural systems have been identified across the animal kingdom, there is still much to be understood about the molecular control of neural development in cephalopods. We used RNA in situ hybridization to uncover expression patterns of a suite developmental genes in the optic lobe of juvenile *Octopus bimaculoides*, including many which were previously uncharacterized. Patterns in expression were assessed across the anterioposterior, dorsoventral, and mediolateral axes. We identified multiple genes with differential expression profiles across and within the layers of the lobe, such as putative axon guidance molecules, transcription factors, receptors, and a putative calcium binding protein. Additionally, we located a deep medulla region with strong expression of multiple genes, including a known immature neuron cell type marker. This work broadens our understanding of developmental gene expression patterns in juvenile octopuses and identifies genes that may be involved in the patterning of neurons within the optic lobe.

Post-hatching development and culture methods of firefly squid *Watasenia scintillans*

Kusama Satoshi[1], Fujiwara Eiji[2], Inamura Osamu[1], Ishii Kenji[3], Hirohashi Noritaka[4]

1 Uozu Aquarium
2 Documentary Channel Co., Ltd.
3 NEC Platforms, Ltd.
4 Shimane University

Abstract

The firefly squid (*Watasenia scintillans*) is an important fishery species in Japan, well known for its various bioluminescent organs that attract scientific interest in fluorescence mechanisms. In particular, Toyama Bay, facing the Sea of Japan, becomes a spawning ground each spring as numerous female firefly squids migrate to shallow waters to lay eggs. Although these females are caught alive by local fishermen, their deep-sea pelagic lifestyle makes it challenging to keep stable culture conditions for rearing W. scintillans from hatchlings, and little is known about their developmental biology or feeding ecology. In this study, we investigated the post-hatching development of W. scintillans larvae to clarify their morphological changes and feeding behaviour, aiming to provide foundational knowledge for establishing a stable culture system. Fertilized eggs were collected from mature females in coastal Toyama Bay, and a rearing system was established for eggs and hatchlings in aquaria. Morphological observations using stereomicroscopy, fluorescence microscopy, and scanning electron microscopy revealed that arms, suckers, and buccal muscles developed by approximately five days post-hatching. Since larvae grow only with internal yolk for approximately 5–7 days, it is suggested that essential predation-related organs like arms and buccal mass developed during this period and after yolk depletoion, the larvae would start capturing prey. When provided with animal plankton (50–100 µm), one larva survived up to 14 days. Based on these results, we are now exploring appropriate prey animals, focusing primarily on similarly sized organisms such as small copepods and their nauplii.

Counterillumination and potential light shielding by skin chromatophores in *Watasenia scintillans*

Kusama Satoshi[1], Fujiwara Eiji[2], Inamura Osamu[1], Ishii Kenji[3], Hirohashi Noritaka[4]

1 Uozu Aquarium
2 Documentary Channel Co., Ltd.
3 NEC Platforms, Ltd.
4 Shimane University

Abstract

Counterillumination, which involves emitting ventral luminescence to counteract downwelling light and cloak the silhouette from predators below, has been observed in certain species that reside in the mid-water twilight zone. Since Young's groundbreaking investigation using Hawaiian luminous squids, mesopelagic squids have also been included in this group. However, there were only a few reports that followed up on Young's experiments. Here, we have successfully captured counterillumination using a super sensitive full-HD CMOS camera (NC-H100, NEC) in a Japanese firefly squid, *Watasenia scintillans*. In this species, the photophores are located on the tips of the ventral arms, the ventral rim of the eyes, and the skin. There are at least two types of skin photophores that can be distinguished by their size and the color of luminescence: blue and green. Upon illuminating the overhead dim light, both the eye and skin photophores increased their luminescence intensity, which then reversed once the light was turned off. The decay of luminescence intensity was faster in blue and eye photophores, while the green photophores exhibited a gradual decay and maintained their glow significantly in the dark. The chromatophores are positioned in a way that could potentially overlay all the photophores, making them capable of shielding light emission. To the best of our knowledge, this is the first demonstration of counterillumination in the eye photophores of cephalopods.

A literature review on the trace metal elements in cephalopod

Thomas Lacoue-Labarthe[1], Paco Bustamante[1]

1 UMR 7266 LIENSs, CNRS - La Rochelle University, La Rochelle, France

Abstract

Contamination of marine ecosystems by trace metal elements (TMEs) is a growing concern, and cephalopods have attracted attention as both bioindicators and vectors of metal within the food web. The aim of this bibliometric review is to analyse the evolution and trends in the scientific literature concerning TMEs in cephalopods. Based on more than 200 articles published over the past 75 years, the review identifies the main objectives driving this field of research. Most of the data comes from field studies and is reported in the context of seafood safety risk assessments. However, other studies focus on pollution baselines, TME metabolism and detoxication, including the assessment of metal-related effects, and on the transfer of TMEs to cephalopod predators. The majority of studied species belong to the Octopodidae and Ommastrephidae families, reflecting the importance attached to species of economic interest. Additionally, the review highlights the most commonly studied TMEs, geographic biases, and the cephalopod tissues targeted for TMEs analysis. This work identifies current research gaps and proposes future directions, with particular emphasis on the lack of knowledge regarding TME concentrations in deep-sea species and their physiological and ecological effects on this remarkable group.

Octopus changes color when exposed to ketamine

Zdenek Lajbner[1], Reuven Pnini[1], Lucia Zifcakova[1], Jessica Gordon[1], Courtney Timmons[1], Albert Murzabaev[1], Mehmet A. Zoral[1], Nana Arakaki[1], Gerald Pao[1], Eric Edsinger[1, 2], Jonathan Miller[1]

1 Okinawa Institute of Science and Technology Graduate University, 1919 Tancha, Onna-son, Okinawa 904-0945, Japan.
2 Josephine Bay Paul Center, Marine Biological Laboratory, Woods Hole, MA 02543 U.S.A.

Abstract

Many cephalopod species exhibit a remarkable intelligence and the ability to change color. Despite the enormous evolutionary distance between cephalopods and mammals, the cephalopod nervous system utilizes neurotransmitter receptors similar to those in humans, including glutamate and 5-HT receptors, which can be stimulated by ketamine in humans. We sequenced the whole genome and characterized genes encoding these receptors in *Octopus laqueus* and examined behavioral responses of *O. laqueus* and *Abdopus aculeatus* hatchlings immersed in ketamine-enriched seawater. A distinctive ketamine-concentration-dependent colorimetric response of octopuses was observed at rates significantly exceeding those detected in seawater-only controls. This effect was reversible. Similar to humans, the behavioral responses of octopuses to ketamine exhibited a large individual-to-individual variation, even when treated identically. The overall complexity of the behavioral response suggests that ketamine has a substantial effect on the central nervous system of cephalopods, and it might even induce hallucinations in octopuses.

Post-hatching brain neurogenesis in Cephalopods

Maxence Lanoizelet[1], Laenke Pepermans[1], Sruthi Loganathan[1], Lisse Mertens[1], Liam Vandekerckhove[1], Eduardo Almansa[2], Eve Seuntjens[1]

1 Department of Biology, KU Leuven, Leuven, Belgium
2 Oceanographic Centre of Canary Islands, Spanish Institute of Oceanography, CSIC, Tenerife, Spain

Abstract

Coleoid cephalopods (including octopuses, squids, and cuttlefish) are invertebrates known for remarkable cognitive abilities such as tool use, play, learning, self-recognition, and memory. These behaviors are linked to their large, complex brains, which evolved independently from those of vertebrates. Our lab recently identified a neurogenic region in late-stage *Octopus vulgaris* embryos: the lateral lips (LL), a structure surrounding the eyes that supports neural progenitor migration into the brain and regresses before hatching. However, *O. vulgaris* undergoes a nearly 100-fold increase in brain cell number from hatchling to adult, raising key questions about the mechanisms driving post-hatching neurogenesis. To study this, we developed a rearing system capable of maintaining paralarvae for up to 30 days post-hatching (dph), enabling tissue collection and in vivo experimentation. Surprisingly, using immunostaining and hybridization chain reaction (HCR), we found that the LL regrow during the paralarval stage. Consequently, optimization of ongoing experiments including CFDA cell tracking and pharmacological treatments targeting key signaling pathways, guided by genes expressed in the LL is underway. These studies aim to assess progenitor migration and investigate the molecular mechanisms involved. Our findings challenge existing assumptions by showing that LL cells undergo proliferative regrowth post-hatching, rather than neurogenesis occurring solely in the vertical lobe, where neurogenic-like cells have been recently reported in adults. Further analysis of juvenile brains will be critical to understanding the drastic post-embryonic brain growth in cephalopods.

Estimation of the natural mortality in common cuttlefish, *Sepia officinalis*: a novel approach

Laptikhovsky Vladimir[1], Allen Athena[2], Barrett Christopher[3], Barry Peter[1], Firmin Chris[1], Macleod Eleanor[4], Ourens Rosana[1], Vieira Rui[1]

1 Cefas, Pakefield Rd. Lowestoft, NR33 0HT, UK
2 Nature Bureau Ltd., Unit 2c Votec Centre, Hambridge Lane, Newbury, RG14 5TN, UK
3 Collaborative Environmental Advisers (CEA) Ltd, Mountbatten House, 1 Grosvenor Square, Southampton, Hampshire SO15 2JU UK
4 National Subsea Centre, Robert Gordon University, Aberdeen, Scotland, U.K.

Abstract

A new approach to estimate natural mortality of cuttlefish based on observed age-at-death in natural habitats was applied to the *Sepia officinalis* of the English Channel and the North Sea. A total 3,309 cuttlebones were randomly collected on beaches of the U.K., Ireland and Netherlands between May 2022 and April 2025. The total length was measured to the nearest 0.5 cm below. If broken, the cuttlebone length was estimated from the maximum width. Seabird praying marks and percentage of broken cuttlebones were recorded to assess rates of their disintegration on beaches. Each size class was allocated the mean age based on the assumed mean hatching date of 15th July and growth rates derived from length-frequencies collected from commercial fishery landings. The study suggests that a cuttlebone longevity on the shoreline is ~1-3 months. The mean natural mortality rate between the age of 3 and 24 months for the mixture of both annual and biennial spawning cohorts was preliminary estimated as M=0.2, and individual monthly values vary between seasons.

Threats and extinction risks to Cephalopoda

Sophie Ledger

Abstract

Over a fifteen year period, 759 cephalopod species have been formally assessed against IUCN Red List Criteria. The limited information on many species that have been, for example, described from just a handful of specimens and not subsequently reported in the scientific literature, led to a large number of species (422) being classified as Data Deficient. Nonetheless, the r-strategy of many cephalopods makes them apparently robust to extinction despite heavy fishing on some species: 324 cephalopod species, including all squids, are considered Least Concern. Twelve species received a threatened classification (Near Threatened, Vulnerable, Endangered, or Critically Endangered) on the basis of small or declining population sizes and small Extents of Occurrence and/or Areas of Occupancy. Of those 12 species, five are cirrate octopods and five are nautilus species. They are all primarily threatened by fishery activity to which they are particularly vulnerable because of their low fecundity. In addition, one benthic octopus and one cuttlefish were assessed as Near Threatened, both also threatened by fishing activity. As conservation actions require timely information, a cycle of re-assessments should be commenced.

In vivo imaging reveals neuronal computations underlying cephalopod vision

Tomoyuki Mano, Konstantinos Tsaridis, Yutaka Kojima, Thi Thu Van Dinh, Giovanni Masucci, Teresa L. Iglesias, Makoto Hiroi, Sam Reiter

Okinawa Institute of Science and Technology (OIST) Graduate University, Okinawa, Japan

Vertebrates and cephalopods have independently developed camera-type eyes — a striking example of convergent evolution. While many vertebrates detect color through wavelength-sensitive retinas, cephalopods lack color vision and have instead evolved retinas that detect light polarization via two distinct photoreceptor cells tuned to vertical and horizontal polarization. Polarization vision is thought to confer ecological benefits in underwater environments. The cephalopod retina consists exclusively of photoreceptor cells that send long axons to the superficial part of the optic lobe. This region, which houses neurons exhibiting a morphological resemblance to vertebrate retinal ganglion cells (RGCs), was hence named the "deep retina" by Cajal. However, the extent to which this region functionally parallels RGCs, as well as the mechanisms by which it integrates luminance and polarization signals, remain elusive. Here, using juvenile squid (S. lessoniana) as our model, we developed a novel head-fixation method to perform two-photon in vivo calcium imaging in the awake squid brain. We recorded calcium responses from hundreds of deep retinal neurons while delivering controlled stimuli that varied in luminance and polarization. Our data reveal that neurons in the deep retina can be clustered into functional cell types based on their response specificity. For instance, one class integrates excitatory inputs from both horizontal and vertical photoreceptors, while another computes the difference between horizontal and vertical signals. The gradual variation in the spatial distribution of these cell types along the depth of the deep retina indicates a sublaminar organization that corroborates previous anatomical findings. We also observed orientation-selective (OS) and direction-selective (DS) neurons that responded to moving stripe patterns with contrast defined by either luminance or degree of linear polarization (DoLP). OS and DS neurons were detected in the inner granule layer, but not in the outer granule layer, suggesting a feedforward network architecture for progressively complex feature extraction. Furthermore, the IGL exhibited more frequent spontaneous activity—often in the form of propagating waves—compared to the OGL. Collectively, our study provides the first in vivo physiological characterization of the deep retina, offering new insights not only into the neural mechanisms underlying cephalopod's specialized underwater vision but also into broader principles of convergent evolution in visual systems.

Cuttlefish survival and oxidative stress under extreme flooding events

Joana Martins[1], Carlos Gonçalves[1], João Saldanha da Gama[1], Melanie Court[1,2], Inês João Ferreira[3,4,5], Mário Diniz [4,6], Rui Rosa[1,2]

1 Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
2 MARE - Marine and Environmental Sciences Centre & ARNET - Aquatic Research Network, Faculdade de Ciências, Universidade de Lisboa, Av. Nossa Senhora do Cabo, 939, 2750-374 Cascais, Portugal
3 Associated Laboratory for Green Chemistry – Network of Chemical and Technology (LAQV-REQUIMTE), - Department of Chemistry, NOVA School of Science and Technology, Universidade Nova de Lisboa, Quinta da Torre, 2829-516 Caparica, Portugal
4 Applied Molecular Bioscience Unit (UCIBIO), Department of Chemistry, NOVA School of Science and Technology, Universidade Nova de Lisboa, Quinta da Torre, 2829-516 Caparica, Portugal
5 Marine and Environmental Sciences Centre – Setúbal Polythechnical Institute (MARE-IPS), Escola Superior de Technologia de Setúbal, Campo do IPS, Estefanilha, 2914-508 Setúbal, Portugal
6 Associated Laboratory i4HB, Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade Nova de Lisboa, Quinta da Torre, 2829-516 Caparica, Portugal

Abstract

The marine environment is undergoing major changes, mainly due to climate change, which has increased the frequency and intensity of extreme events. Here, we aimed to study the impact of ecologically relevant extreme flooding conditions (exposure to acute salinity values of 17 and 7) and non-extreme conditions (salinity values of 35, 28, and 24), during 4 days at late embryogenesis, on the survival and oxidative stress of the European cuttlefish (*Sepia officinalis*) hatchlings. The oxidative stress was evaluated by assessing biomarkers, including HSP70, DNA damage (8-OHdG) and total ubiquitin (Ubi) concentrations, as well as the activity of antioxidant enzymes, Total Antioxidant Capacity (TAC) and Ferric Reduction Antioxidant Power (FRAP). Our findings demonstrated that both extreme flooding conditions resulted in 100% mortality. Alongside, lower salinity induced lower HSP70, Ubi and TAC levels which may indicate reduced production of enzymatic substrates and proteins, i.e., metabolic suppression and decreased oxidative stress. Here we show, for the first time, that short-term extreme flooding conditions elicit harmful effects on cuttlefish early ontogeny, namely at developmental and physiological levels.

In Vivo Two-Photon Imaging of Embryonic Pharaoh Cuttlefish (*Sepia pharaonis*)

Mark Sanders[1], Isha Sehrawat[1], Yung-Che Tseng[2], Kuo-Sheng Lee[1]

1 Institute of Biomedical Sciences, Neuroscience Program of Academia Sinica, Academia Sinica, Taipei, Taiwan
2 Institute of Cellular and Organismic Biology, Academia Sinica, Taipei, Taiwan

Abstract

The Pharaoh cuttlefish (*Sepia pharaonis*) serves as a valuable model for studying cephalopod development due to its complex behaviors and accessible embryonic stages. Building upon prior characterizations of its reproductive behavior and embryogenesis, we have implemented in vivo two-photon microscopy starting at embryonic stage 20 onwards to visualize internal structures of the developing body and arms with high spatial resolution. This non-invasive imaging technique enables real-time observation of organogenesis and tissue differentiation within intact embryos, providing unprecedented insights into the morphogenetic processes underlying cephalopod development. Using this approach, we captured changes in muscle fiber organization, vascular network formation, and arm elongation in living embryos. These high-resolution datasets reveal temporal and spatial patterns of internal tissue development that were previously inaccessible, establishing a powerful framework for studying cephalopod morphogenesis at the cellular and subcellular levels. In addition, this platform sets the stage for future in vivo physiology experiments. We aim to integrate two-photon calcium imaging to monitor neural activity and muscle responses. This approach will enable the direct observation of functional maturation within the nervous system and neuromuscular circuits, providing key insights into the emergence of behaviorally relevant motor patterns in *S. pharaonis*.

A new species of *Callistoctopus* (Cephalopoda: Octopodidae) from the Penghu archipelago, Taiwan

Mong-Fong Lee[1], Shin-Yu Lee[2], Chung-Cheng Lu[3]

1 Department of Aquaculture, National Penghu University of Science and Technology, Penghu, Taiwan
2 Okinawa Institute of Science and Technology, Okinawa, Japan
3 Department of Life Sciences, National Chung Hsing University, Taichung, Taiwan

Abstract

An undescribed octopus species found in the intertidal zones off the north coast of the Penghu archipelago. This species perches on sandy substrate mixed with small reef rocks from March to July. It is a small to moderate-sized species, red-brown colored on the dorsal surface and cream on the lateral and ventral surfaces. The arms are unequal in length, dorsal pair longest, decreasing in length ventrally (arm formula 1>2>3>4). The hectocotylized arm has approximately 58-61 suckers with a sharp ligula and a calamus that is about one third of ligula length. This distinct copulatory organ occupies approximately 8-13% of arm length. Eggs are large, approximately 10 mm long and approximately 21-33% of mantle length. Gills have 8 lamellae in each demibranch. This species can burrow into the sandy bottom immediately in the wild when frightened, and hide in the sand all day in captivity. The partial mtCOI sequence (640 bp) shows the affinity of this species to Callistoctopus and Octopus minor group.

Detailed Characterization of the Male Reproductive Anatomy in the Pharaoh Cuttlefish, *Sepia pharaonis*

Mong-Fong Lee[1], Jing-Duan Huang[1], Shin-Yu Lee [2]

1 Department of Aquaculture, National Penghu University of Science and Technology, Penghu, Taiwan
2 Okinawa Institute of Science and Technology, Okinawa, Japan

Abstract

The present study is aimed at casting light on the structures of the reproductive system of the mature male pharaoh cuttlefish, Sepia pharaonis, by using histology and electron microscopy. The organs investigated included the testis, the sperm duct, the spermatophoric gland, the mucous gland, and the spermatophoric sac (Needham's sac). The main units of the testis were the seminiferous tubules within which gametes were developed. The primary and secondary spermatogonia were distributed at the inner border of the tubules, the first and second-order spermatocytes at the middle of the tubules, and then the spermatids and spermatozoa at the center region of the tubules, respectively. The cytoplasm of the spermatids was not separated during meiosis until maturation. Sperm nucleomorphogenesis was observed during spermiogenesis. Acrosomes were present at the anterior end of the spermatozoa. Sperms were aggregated along the spermatophoric gland with different secretion characteristics of epithelial cells and then packaged into a sperm rope. These secretions along the spermatophoric gland were suggested to keep the sperm alive and stable. The sheath of the spermatophore was formed when the sperm rope was processed in the mucous gland. These results provide an important basis for future studies on the endocrine regulation between the testis and the accessory reproductive glands.

Trace Element Transfer in the Muscle of Five Cephalopod Species in the High Seas of the Northwest Pacific Ocean: Implications for the Food Chain

Tiantian Leng[1], Bilin Liu[1]

1 College of Marine Living Resource Sciences and Management, Shanghai Ocean University, Shanghai

Abstract

Cephalopods exhibit a remarkable capacity for the accumulation of trace elements, which, through biomagnification in the food chain, are transferred to higher trophic level organisms. This study aimed to investigate the distribution of trace elements in the muscle tissues of five cephalopod species from the Northwest Pacific Ocean: Gonatopsis borealis, Eucleoteuthis luminosa, Ommastrephes bartramii, Onychoteuthis compacta, and Onychoteuthis borealis japonica. The concentrations of nine essential trace elements (B, Cr, Mn, Fe, Co, Cu, Zn, Se, Mo) and twelve non-essential trace elements (As, Cd, Sn, Sb, Ba, Tl, Pb, Ti, V, Ni, Sr, Al) were measured, along with carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios. Statistical analyses, including one-way analysis of variance (ANOVA), Pearson's correlation analysis, principal component analysis (PCA), and trophic magnification factor (TMF) calculations, were employed to assess species-specific differences in trace element accumulation, element correlations and sources, and trace element transfer patterns. The results revealed that Fe, Zn, Cu, and Mn were the predominant essential trace elements in the muscle tissues of the five cephalopod species, while Al, As, Cd, and Sn were the most prevalent non-essential trace elements. Significant interspecies differences in trace element accumulation were observed ($P<0.05$), with Sb and Mo showing the strongest correlation among species ($R^2=0.93$). Sr, Cd, V, Ba, Cu, and Co were primarily acquired through dietary intake, while Sb, Pb, Mo, and Tl were predominantly sourced from the surrounding water. No significant differences in food sources were found among the five cephalopod species from the Northwest Pacific ($\delta^{13}C$: -19.61‰ to -18.81‰), with a relatively narrow range of trophic levels (2.3 ~ 3.8). Biomagnification effects (TMF>1) were observed for Mn, As, and Sn within the cephalopod food chain, whereas B, Fe, Co, Sb, Ti, V, Ni, and Sr exhibited significant biomagnification reduction (TMF<1), with concentrations decreasing significantly with increasing trophic level ($P<0.05$). These findings contribute valuable scientific insights into environmental management and food safety regulation in the region.

Comparative Approaches to *Sepioteuthis lessoniana* Aquaculture in Taiwan and Okinawa: System Design, Feeding Strategies, and Broodstock Management

Giovanni Masucci[1], Chun-Yen Lin[2], Maxime Hamon[1], Teresa Iglesias[3], Ayaka Tsujimoto[3], Chen-Chi Wang[4], Sam Reiter[1]

1 Computational Neuroethology Unit, Okinawa Institute of Science and Technology (OIST) Graduate University, Okinawa, Japan.
2 Taiwan Association for Marine Environmental Education (TAMEE), Taiwan.
3 Animal Resources Section, Marine Animal Research Support (MARS) Team, Okinawa Institute of Science and Technology Graduate University, Okinawa, Japan.
4 Animal Resources Section, Okinawa Institute of Science and Technology (OIST) Graduate University, Okinawa, Japan.

Abstract

Aquaculture of the oval squid (*Sepioteuthis lessoniana*) in Taiwan and Okinawa reflects regional differences in system design, broodstock management, and feeding strategies. Taiwanese systems use open circulation and rely on seasonal wild egg mass collection. Hatchlings and juveniles are fed aquacultured *Litopenaeus vannamei* (live and defrosted), while adults receive mixed frozen diets. These systems feature low capital investment and local fishery integration but are vulnerable to environmental fluctuations and seasonal spawning. In Okinawa, the OIST Neuroethology Unit uses closed or semi-closed recirculating systems for hatchlings and juveniles combined with open water tanks for breeders. Eggs are sourced from both in-house bred and wild clutches, covering all three phenotypic forms (aka, shiro, kuwa). This dual strategy, combined with asynchronous breeding, enables near-continuous egg supply. Juveniles are fed live mysids caught from the wild; older squid receive a defrosted mix of red shrimp (*Lucensosergia lucens*), whitebait, and sardines. Water quality is tightly controlled (pH 8.1–8.2; 24–26 °C; salinity 35 ppt; $NH_3/NH_4^+ = 0$; $NO_2^- \le 0.5$ ppm; $NO_3^- \le 25$ ppm), achieving over 90% hatching and 50–85% one-month survival, depending on batch quality. OIST’s model offers higher stability and production but requires technical input and mysid access. Taiwan’s model is more accessible but less scalable. Both sites collaborate to improve juvenile diets, test artificial feeds, and explore live feed enrichment to reduce reliance on wild mysids. Disease management remains a shared challenge and research priority. These complementary strategies reflect adaptation to regional conditions and emphasize the value of international collaboration in advancing cephalopod aquaculture.

Organ-specific microbiome signatures across the digestive systems of *Sepioteuthis lessoniana* and implications for functional digestive physiology

Yu-Xuan Lin[1, 2], Garett Allen[3], Pou-Long Kuan[2], Shan-Hua Yang[1], Yung-Che Tseng[2]

1 Institute of Fisheries Science, National Taiwan University
2 Institute of Cellular and Organismic Biology, Academia Sinica
3 Department of Biology, Acadia University
1, 2 Institute of Fisheries Science, National Taiwan University/Institute of Cellular and Organismic Biology, Academia Sinica

Abstract

The bigfin reef squid (*Sepioteuthis lessoniana*), widely distributed throughout the Indo-Pacific Ocean, demonstrates exceptional metabolic efficiency essential for sustaining its predatory and migratory behaviors. Previous studies have shown functional specialization throughout the cephalopod digestive tract, with the digestive gland serving as the primary secretory and digestive center, while the cecum and cecal sac likely function as the main sites for nutrient absorption. The resident microbiota probably supports these specialized functions through enzymatic complementation, metabolite production, and pathogen defense. Over the past decade, while several studies have examined the microbiota in selected digestive organs of various cephalopod taxa, a comprehensive analysis of microbial community structure across multiple digestive organs within a single cephalopod species has not been conducted. Utilizing PacBio full-length 16S rRNA gene sequencing, we investigate the microbiome across six anatomically distinct digestive organs: the esophagus, stomach, cecum, cecal sac, digestive gland, and intestine of *S. lessoniana* specimens. Preliminary taxonomic profiling revealed differential abundance patterns predominantly featuring the Proteobacteria, Firmicutes, and Actinobacteriota phyla, with distinct organ-specific enrichments: Vibrio was abundant in the intestinal microbiome, while Mycoplasma and Cutibacterium showed significant enrichment in the digestive gland. Additionally, the stomach and cecum harbored a diverse array of Proteobacteria, including Pseudomonas and Bradyrhizobium, indicating niche specialization that aligns with organ-specific physiological functions. Ongoing investigations include transcriptomic, metabolomic, and enzymatic analyses to elucidate the functional integration of these microbial communities with host digestive physiology, potentially uncovering co-evolved symbiotic adaptations that enhance metabolic capacity in these highly active marine predators.

Hooked on fishing: How squid catch fish using sucker ring teeth

Severin te Lindert[1], Annika Berkelaar[1], Floris Biezeman[1], Karan Kohabir[1], Brett Klaassen van Oorschot[1], Sander Gussekloo[1], Guillermo Amador[1]

1 Wageningen University and Research

Abstract

Cuttlefish and squid have been observed to possess a stiff sucker ring within their suckers that, depending on the species, exhibit teeth ranging from blunt and small to large and sharp. It is unclear what advantage these sucker ring teeth might have. One might expect that teeth in fact might be detrimental for suction performance as they might interfere with forming a proper seal. Here, we studied the function of these sucker ring teeth. We hypothesize that teeth are mainly useful to avoid slippage of prey by enhancing friction. Moreover, we hypothesize that the sucker ring teeth prevent inward motion of soft materials into the sucker internal space and reducing suction force. We tested these hypotheses by combining morphological data from *Loligo forbesii* and friction and adhesion measurements with biomimetic suction cup teeth models on different substrates. Our results show that *Loligo forbesii* arm sucker teeth are highly asymmetric, suggesting that they are adapted for friction and prevent slippage of prey. Also, the suction cup models with teeth generated higher friction but not higher adhesion on soft substrates. Together, these results suggest that teeth seem to improve friction rather than adhesion on soft substrates.

Physiological and molecular insights into the symbiotic interactions between dicyemids and the cuttlefish *Sepia pharaonis*

Tsai-Ming Lu[1], Hsin-Ju Chuang[1], Shih-Han Wen[1], Yung-Che Tseng[1]

1 Institute of Cellular and Organismic Biology, Academia Sinica

Abstract

Symbiotic relationships have independently evolved across diverse animal lineages, yet the cellular and molecular adaptations that enable these associations remain elusive. Dicyemids, microscopic endosymbionts with highly simplified body plans, have been known for over a century to inhabit the renal appendages of certain cephalopod species such as octopuses and cuttlefish. However, the physiological interactions and the exact nature of this symbiosis, whether mutualistic, commensal, or parasitic remains unresolved and continues to be debated. In this study, we establish the first integrated physiological and transcriptomic symbiosis model using the cuttlefish *Sepia pharaonis* to investigate the cellular and molecular adaptations underlying the association between dicyemids and their cephalopod hosts. We directly compare cuttlefish individuals with and without dicyemid symbiosis, assessing gene expression in renal tissue. Physiological measurements, including pH values and ammonia concentrations in urine and blood, reveal distinct differences associated with the presence of dicyemids. Our findings suggest that dicyemids may exploit host metabolic products in the urine as nutritional resources, potentially influencing host ammonia metabolism and acid-base homeostasis. Furthermore, we are conducting ongoing investigations to elucidate the mechanisms that enable dicyemids to persist in the harsh renal environment. This study pioneers a systems-level view of symbiosis between dicyemids and cephalopods, offering a novel framework for investigating marine symbiotic systems. Our findings provide critical insights into how minimalist symbionts influence host physiology across biological scales, deepen our understanding of physiological integration and evolutionary innovation in symbiosis, and illuminate broader principles of host-symbiont coadaptation.

Stable isotopes of δ^{13}C and δ^{1}5N of cephalopod species in the Mexican oceans and adjacent water

Rigoberto Rosas-Luis[1, 2]

1 Tecnológico Nacional de México
2 I.T. de Chetumal

Abstract

The stable isotope analysis SIA has been applied to understand the trophic ecology of cephalopods, nevertheless, a low number of species is included. In Mexican oceans and adjacent waters, it is unknow which species have been included in SIA. In this context, the main objective of this work was to identify the species previously reported in SIA and include more species in the analysis to have a global view of their trophic ecology. Seven species of the west coast of Mexico, including the California Current US, and eight species of the east coast were included in SIA. Results of seven octopuses showed δ^{13}C values ranged between -19.16 and -10.20‰, with the most negative value reported for Argonauta spp. and the most positive for *Callistoctopus macropus*. For seven squid species, δ^{13}C values ranged between -20.1 and -14.9‰, with the most negative values reported for *Abraliopsis* spp. and the most positive for Dosidicus gigas. The δ^{1}5N values of octopuses ranged between 5.38 to 16.68‰, for squids they ranged between 6.5 and 16.9‰, with the lowest value reported for *Doryteuthis plei* and the highest for *D. gigas*. According to the results, it is possible to identify the coastal habitat of octopuses and the oceanic areas for squids. For octopuses in Mexican waters, it is also possible to identify the habitat segregation, as *Octopus briaeureus* and *Octopus insularis* showed lowest values that those of *Octopus americanus*. More species need to be included in the SIA, to better understand the ecology of these species.

Comparison of taxonomic features between African and European bathypolypodidae

Amanda Luna[1], Francisco Rocha[1], Nayely Santiago[1], Anxo Pérez[1]

1 Universidad de Vigo

Abstract

A comparison of the taxonomic characters of several species of the family Bathypolypodidae was carried out in two different regions of the North Atlantic, the Irish Sea (53°30′N, 5°00′W) and the coasts of Mauritania (18°05′09″N, 15°58′43″W). Differences in some important taxonomic characters were found, indicating intraspecific variability between the European and African populations. In this family, it is not easy to determine species on the basis of female or juvenile taxonomic characters with the literature available so far. It is urgent to determine the morphological variability in non-sexual taxonomic characters for the different world populations in order to facilitate the work of identifying the species of this family. If this morphological variability exists among the world populations, more comprehensive studies on this subject, supported by genetic studies, are needed.

The global biogeography of the sepiolidae bobtail squid

Francisco Rocha[1], Amanda Luna[1], Leonardo Mantilla[2]

1 Universidad de Vigo
2 Zoomare

Abstract

The global biogeography of the 79 nominal species of the Bobtail squid family Sepiolidae, has been reviewed. Their biogeographic patterns vary from restricted to wide transoceanic distributed species. Indo-West Pacific and Mediterranean-Atlantic Regions present the largest number of species, corresponding to 21.5% and 25.3% of the total of the species of the family, respectively. Distribution patterns of Sepiolidae species are also discussed. Several sepiolid species have transoceanic distributions, such as *Heteroteuthis dispar*, *Stoloteuthis leucoptera* and *Neorossia caroli* in the Atlantic, and Rossia pacifica in the Pacific. Bathymetric distributions related to shallow waters or pelagic distributional patterns for sepiolid species are given. Larvae distribution patterns were analyzed based on the egg size, spawning type, larva habitat and bathymetric distribution. Some considerations about the origin of the Sepiolidae and characteristics of their biogeographic patterns are presented.

Exploring Cephalopod Beak Formation through Multiomics-Based Big Data Analysis

Seto Maaya[1, 2], Hirota Kazuki[1, 2], Sasaki Takenori[3], Setiamarga Davin[2]

1 Graduate School of Science, The University of Tokyo
2 National Institute of Technology, Wakayama College
3 The University Museum, The University of Tokyo

Abstract

The cephalopod beak is a unique chitinous structure not found in other mollusks, playing a critical role in feeding and species-specific behaviors. In addition, cephalopods also possess the gladius—a chitinous internal structure considered a vestigial remnant of the ancestral molluscan shell. Both the beak and gladius are formed from extracellular matrix proteins, yet the mechanisms underlying their development remain poorly understood. To investigate the molecular basis of beak formation, we focused on the Japanese flying squid (*Todarodes pacificus*) and identified the matrix proteins of both the beak (beak matrix proteins; BeMPs) and the gladius (gladius matrix proteins; GMPs). Multiomics analysis revealed 469 BeMPs and 278 GMPs. Comparative analyses showed that a large proportion of these proteins are shared between the two structures, suggesting a common molecular toolkit. These findings imply that the cephalopod beak may have evolved using pre-existing components from ancestral shell-forming pathways.

Kraken the Code: On decoding deep-sea squids via host-associated microbiomes (Cephalopoda: Oegopsida)

Marina Maranzana[1], Syrie Hermans[2], Kevin Lee[2], Kat Bolstad[1]

1 AUT Lab for Cephalopod Ecology & Systematics, School of Science, Auckland University of Technology, NZ
2 School of Science, Auckland University of Technology, NZ

Abstract

Deep-sea squids play crucial yet poorly understood ecological roles in the largest and least explored habitat on Earth. Investigations into the microbiota of cephalopods have largely focused only on coastal and/or benthic taxa, and in particular on their digestive tracts; the findings from these studies to date suggest we have much to learn from these microbe–host interactions. In this study, we investigated the microbiota of the colossal squid (*Mesonychoteuthis hamiltoni*), using 16S rRNA amplicon sequencing, and compared it to a Ross Sea dataset found online (Accession Number: PRJNA1266853). We generated taxonomic profiles of microbial communities across different body sites, revealing varying community structures within the colossal squid and between the colossal squid and the Ross Sea dataset. Notably, we identified potential associations between specific bacterial taxa and host physiological processes such as digestion and reproduction. These findings offer new insights into the complexity and ecological significance of squid-associated microbiota, with broader implications for host health and deep-sea ecosystem dynamics.

Comparison of environmental drivers of recruitment in Loliginid squid fisheries of the English Channel and the Bay of Biscay

Anna Marcout

Université de Caen Normandie, France

Abstract

The English Channel and the Bay of Biscay are among the main fishing grounds for Loliginid squids in the Northeast Atlantic. Fishermen do not distinguish the two species *Loligo forbesii* and *Loligo vulgaris*. However differences in phenology and biological sampling suggest that in the English Channel the first peak of recruitment observed in July is the arrival of *L. forbesii* whereas the November peak concerns *L. vulgaris*. In the English Channel the influence of environmental variables on these two recruitment peaks has already been studied (Marcout et al. 2024). The objective of the present study is to compare the environmental variables that can be used to predict squid recruitment in the Bay of Biscay and in the English Channel. In the Bay of Biscay, survey data suggests that *L. vulgaris* is the widely predominant species and the seasonality of landings shows only one recruitment peak in autumn. Environmental variables were extracted for the pre-recruitment period from the Copernicus Marine Service and biomass indices were derived from standardised landings per unit of effort of French bottom trawlers. The biomass level and its spatial distribution was described by fitting a General Additive Model (GAM) to the data. Comparisons between the two fishing grounds are made on the variables that have a significant influence, on their direction of variation, and on the time lag with which they act. They contribute to indicate what patterns are specific to L. vulgaris and what may depend on the characteristics of the ecosystem.

Cephalopods as jewels

Jennifer Mather

University of Lethbridge

Abstract

Modern coleoids are known for their changeable colourful skin patterns, but some fossilized ammonoids have shimmering thin (0.5-8 mmm) interference surface layers of nacreous aragonite whose pieces are formed into a jewel name Ammolite. The shells of *Placenticeras meeki* and *P. intercalare* are discoid, rounded and compressed, usually 20-50 cm in diameter. The animals flourished in the late Cretaceous, having lived in the Western Interior Seaway 70-75 mya, and their shells are typically found in the Bearpaw formation of western North America. The iridescent surface layer is probably due to the combination of heat and pressure, as shells were buried 4 km deep, though later raised to about 120 m. Jewel quality fossils are only found in extreme south west Alberta, uncovered by and found in the banks of the St Mary's river west of Lethbridge. Open pit mining in the soft shale uncovers concretions which are split open to reveal the ammonoids. Pieces of shell are backed with shale and covered with a spinel cap for protection before being mounted as Ammolite jewels.

Annual variation in the hatching month compositions of the autumn spawning stock Japanese flying squid *Todarodes pacificus* caught in the offshore area of the Sea of Japan

Matsui Hajime[1], Miyahara Hisae[2], Wakabayashi Toshie[3], Okamoto Suguru[1]

1 Pelagic Fish Resources Division, Fisheries Resources Institute, Japan Fisheries Research and Education Agency
2 Demersal Fish Resources Division, Fisheries Resources Institute, Japan Fisheries Research and Education Agency
3 National Fisheries University, Japan Fisheries Research and Education Agency

Abstract

The autumn spawning stock Japanese flying squid *Todarodes pacificus* is mostly found in the Sea of Japan, and the main hatching months are from October to December. To clarify the hatching month compositions of the autumn spawning stock *T. pacificus*, we examined its mantle length, body weight, sex, maturity, and age in days. The squid samples were collected using squid jigging vessels from June to October 2016–2024. Additionally, a mid-surface trawl net was used on research trips to collect juvenile *T. pacificus* in April 2018, 2020, 2022, and 2023. Based on the age determination results, the hatching month compositions of the catch were estimated for each year. In 2016–2019, the main hatching months for the catch were December to February, with a peak in January. The main hatching months in 2020–2022 shifted to earlier months, from November to January, with a peak in December. Subsequently, the main hatching months in 2023 and 2024 shifted to later months, from December to March, with a peak in February. In the past, large squid presumed to have been hatched in October were caught in June and July in the Sea of Japan, but in this study, the proportion of these squid was notably low. The findings of this study raise the possibility that the main hatching month of T. pacificus has shifted to after October in recent years, which may have reduced catches of squid hatched in October.

Squid on the Street: The Art of Cephalopod Science Communication

Sarah McAnulty[1, 2]

1 Skype a Scientist
2 University of Connecticut

Abstract

Cephalopods are crucial components of many marine ecosystems, but does the average person know that? While octopuses are appreciated by the public for their beauty and intelligence, threats to cephalopods at large are often ignored. With climate change and deep sea mining threatening cephalopod populations, increasing public care for these species should be prioritized within our scientific community. The political landscape is rife with dire humanitarian and ecological crises across multiple continents. Anti-science messaging dominates many spaces (particularly online). Communicators fight uphill battles against disinformation campaigns; a situation worsening as trusted government organizations in the U.S. support these narratives. Overcoming these hurdles on behalf of our study organisms will require a creative, emotionally intelligent approach to science communication. Skype a Scientist has worked to increase awareness of squid on the streets for 8 years. We have extensive experience with multi-modal science communication approaches that incorporate public art into our projects. We are able to circumvent siloed media structures to reach people across political divides. We put science directly in the paths of people's everyday lives via murals, street art, printed materials, events, and other unconventional approaches. These projects center the communities they target, weave art into communication, and inject key messaging into the public space. In this presentation, I will cover the varied approaches we have taken in Philadelphia and share a practical toolkit that scientists can use to connect their own communities with cephalopod science.

Three-dimensional reconstruction of Deep Sea Octopus Neuroanatomy Could Reveal Unique Chemoreceptive Abilities

Tyler J. Mears

San Francisco State University

Abstract

Deep off central California, thousands of pearl octopus (*Muusoctopus robustus*) migrate through cold dark waters to hydrothermal springs near an inactive volcano to mate, nest, and die, forming the largest known aggregation of octopus on Earth. *M. robustus* exhibits a characteristic, upright reaching behaviourusing their front two arms (L1 and R1), and it has been demonstrated that they use these arms by far the least for locomotion. We aim to describe the completely unknown neuroanatomy of *M. robustus* arms to find if the L1 & R1 arms could be specialized for distance chemoreception. Fine scale Micro-CT and synchrotron scanning of fixed specimens will allow us to gain new insights into the physiology and behaviorof a deep sea octopus. Finding unique neuroanatomical features in their arms will provide insights into how this species navigates over long distance to find the hydrothermal springs they depend on and will shed light more broadly on the diversity of arm neuroanatomy that has been optimised for the many niches that extant octopods occupy.

Transcriptomic analysis of the digestive glands of the Pacific pygmy octopus *Paroctopus digueti*

María Guadalupe Martínez-Morales[1], Óscar E. Juárez[2], Dariel Tovar-Ramírez[2], Clara E. Galindo-Sánchez[3], Claudia Ventura-López[3], Bertha Patricia Ceballos-Vázquez[1]

1 National Polytechnic Institute, CICIMAR
2 CIBNOR
3 CICESE

Abstract

The Pacific pygmy octopus, *Paroctopus digueti*, shows strong aquaculture potential, highlighting the need to deepen our understanding of its biology, particularly feeding and nutrition. In the octopus, feeding starts with prey capture, followed by extracellular digestion initiated by digestive enzymes in saliva. The anterior salivary glands (ASG) and posterior salivary glands (PSG) produce saliva, which contains toxins for prey paralysis and enzymes for chyme formation. Intracellular digestion occurs in the digestive gland (DG), where various enzymes convert nutrients into energy and functional molecules. While previous studies have described these tissues, molecular-level comparisons are lacking. To address this, we used a transcriptomic approach to compare the enzymatic profiles of these three glands. Total RNA was extracted from ASG, PSG, and DG tissues dissected from three pre-adults collected in La Paz Bay, Baja California Sur, Mexico. RNA-Seq libraries were prepared and sequenced externally. Individual transcriptomes were reconstructed and merged into a non-redundant multi-tissue assembly. Differential gene expression analysis revealed distinct roles for each gland. The ASG showed high expression of genes linked to neuroendocrine regulation, including the dopamine receptor, neuropeptide prohormone-4, neuroendocrine protein 7B2, and FMRFamide neuropeptide. The PSG predominantly expressed digestive enzymes like trypsin alkaline B and C, phospholipase A2, and chymotrypsin-1. The DG showed elevated expression of probable chitinase 10, chitinase-3, acyl-CoA dehydrogenase, and the NPC intracellular cholesterol transporter 2. These findings confirm functional specialization: ASG regulates feeding behavior, PSG drives extracellular digestion, and DG metabolizes carbohydrates, peptides, and lipids.

Toward Sustainable Cuttlefish Management: Integrating Spatial and Biological Data from Portuguese SSF

Ana Moreno[1], Catarina Maia[1], David Dinis[1], Rogélia Martins[1], Ivânia Quaresma[1], Ivone Figueiredo[1, 2], Miguel B. Gaspar[1, 3], Marta Rufino[1, 2]

1 Portuguese Institute for the Sea and the Atmosphere (IPMA). Division of Modelling and Management of Fisheries Resources. Av. Dr. Alfredo Magalhães Ramalho, 6, 1495-165 Lisboa
2 Centre of Statistics and its Applications (CEAUL), Faculty of Sciences, University of Lisbon, Portugal
3 Centre of Marine Sciences (CCMAR), Universidade do Algarve

Abstract

The common cuttlefish (*Sepia officinalis*) is Portugal's fourth most valuable fishery resource and holds significant cultural importance, namely in traditional gastronomy. It is primarily harvested by polyvalent artisanal fisheries, with small-scale fisheries (SSF) using trammel nets and traps contributing over 60% of national landings. These fisheries operate mainly near lagoons and estuaries, which are critical habitats for cuttlefish during seasonal inshore migrations for reproduction. Despite its economic importance, *S. officinalis* lacks formal stock assessments and catch quotas, and key biological and population data remain limited. This study investigates the population exploited by SSF in the River Sado Estuary and adjacent coastal areas, in southwest Portugal. From 2020 to 2024, nine vessels targeting cuttlefish and sole using trammel nets were monitored using GPS tracking (30 secs temporal resolution). Combined with official landing data by size class, this allowed for spatial and temporal mapping of size-structured catch distributions. Additionally, detailed length-frequency data from approximately 400 fishing trips (2020–2022) were analysed, complemented by biological sampling for sex ratio and maturity. Results provide insight into seasonal use of estuarine habitats and size-based spatial distribution, contributing to improved monitoring and sustainable management of cuttlefish in estuarine SSF contexts.

Sharing Squid and the Sea: A Fishing Tackle Manufacturer's Work from Recreational Fishing to Conservation

Yuhei Mori[1]

1 YAMARIA Corporation

Abstract

YAMARIA Corporation, founded in 1941, began by manufacturing fishing lures for tuna longline fishing. Today, we mainly develop, manufacture, and sell "Egi," a squid fishing lure originally created in Japan. Since the 2000s, Eging—recreational fishing for bigfin reef squid (*Sepioteuthis lessoniana*) using Egi—has grown popular among anglers and is also used by some commercial fishers. In recent years, more beginners have started fishing through Eging, leading to a renewed awareness of fishing not only as fun but also as a way to connect with nature. Based on this idea, we work to balance "catching and conserving" through two main projects. The Aori Community project supports the installation of artificial spawning beds for squid with local partners across Japan. Now in its 18th year, this initiative has become more important as seaweed habitats decline due to isoyake, a coastal degradation issue. We also run Egi.com, a social platform where recreational fishers share their squid catches. The data collected from this site is now used to monitor changes in squid distribution around Japan. Through these activities, we aim to protect the value of recreational fishing as a way to interact with nature, while promoting the shared use of marine resources and continuing our efforts to live in harmony with the sea.

Expansion of occurrence of *Tremoctopus violaceus* to Southwestern Atlantic based on molecular data

Isabela Pereira Moura[1], Leilane Araujo Correa[1], Vitória Gama Alcântara[1], Cláudio Sampaio[2], Bianca Lima Paiva[1], Alan Erik Souza Rodrigues[1], Jonathan Stuart Ready[1], João Bráullio Luna Sales[1]

1 Federal University of Pará
2 Federal University of Sergipe

Abstract

Tremoctopus violaceus is an epipelagic species with unique morphological adaptations and a broad distribution across tropical and temperate oceans, playing an important role in marine ecosystems. Traditionally, individuals of *Tremoctopus violaceus* from different oceanic regions have been considered to belong to a single species. However, recent molecular studies have questioned the genetic homogeneity of this taxon, suggesting the existence of distinct evolutionary lineages within the group. In this study, we analyzed three specimens collected off the Brazilian coast (Southwestern South Atlantic) to evaluate their phylogenetic affinities. Total DNA was extracted from muscle tissue, followed by PCR amplification of the mitochondrial 16S rDNA gene under standard conditions. The resulting products were sequenced at Instituto Tecnológico Vale (ITV), and the obtained sequences were aligned and analyzed using Bayesian Inference. Our preliminary results show that Brazilian T. violaceus individuals cluster together with Mediterranean specimens (type locality), forming a well-supported clade (100% posterior probability support), while specimens from the Pacific Ocean (China, Korea, Hawaii) form a distinct lineage (100% posterior probability support). These findings demonstrate that T. violaceus does not represent a single global evolutionary lineage but instead is composed of at least two genetically distinct groups. Therefore, our study provides new insights into Tremoctopus' diversity across oceanic regions, highlighting the importance of regional molecular data for accurate biodiversity characterization and revealing the need for a taxonomic revision of this genus.

Into the Beak: Deciphering and visualizing venom expression in cephalopods

M Praveena Naidu[1], José Ramón Pardos-Blas[1], Saurabh Attarde1[1], Favour Achimba[1], Belkes Stambouli[1], Kim Kirchhoff[1], Melvin Williams[1], David Sharer[1], Afeeda Ali[1], Caroline Albertin[2], Andrew Gillis[2], Mandë Holford[1, 2]

1 City University of New York
2 Marine Biological Laboratory

Abstract

However, the composition and characterization of cephalopod venom arsenals is underexplored. There is only one reported cephalopod toxin with confirmed paralytic venom activity and an identified primary sequence: SE-CTX, which was characterized from a paralytic fraction of *Sepia esculenta* posterior salivary gland extract. Here we combine an integrated -omics dataset (genomics, transcriptomics, and proteomics) dataset to identify 23 putative se-ctx homologs across 16 squid and cuttlefish species. We group these homologs into a new venom gene family, deca-ctx representative of decapodiforms squids and cuttlefish. In half of these species, we identify the presence of two different deca-ctx genes, which are putatively the result of a gene duplication event. We visualized, for the first time, the expression of these deca-ctx transcripts in the posterior salivary glands of adult *Doryteuthis pealeii*, *Euprymna berryi*, *Sepia bandensis* and *Sepia officinalis*. We also found deca-ctx expression in the posterior salivary glands of hatchlings of *S. bandensis*, *E. berryi* and *D. pealeii*, suggesting juvenile cephalopods have the ability to express venom compounds. Deep learning predicted structures of these putative DECA-CTX proteins clustered into 20 groups and mass spectrometry validated the expression of five DECA-CTX sequences across three species: *D. pealeii*, *S. bandensis* and *E. berryi*. Together, these findings address the homology of SE-CTX expression and structural characterization across various taxa, while validating the use of cephalopods as a model system for propelling our understanding of venom evolution, development, and function.

A Squid-Be-Doo, Where are you? Situational motionless camouflage of a loliginid squid

Ryuta Nakajima

Abstract

Coleoid cephalopods have the most elaborate camouflage system in the animal kingdom. This enables them to hide from or deceive both predators and prey. Most studies have focused on benthic species of octopus and cuttlefish, while studies on squid focused mainly on the chromatophore system for communication. Motion camouflage to substrate has been recently described in one member of the semi-pelagic oval squid of *Sepioteuthis lessoniana* species complex, so called white-squid. Our current study focuses on white-squid camouflage to substrate in a stationary, motionless position. We observed disruptive, uniform, and mottled chromatic body patterns, and several arm postural components. These were also related to the squid position in the environment, either sitting directly on the substrate or hovering just few centimeters above the substrate. Several of these context-dependent body patterns have not yet been observed in *S. lessoniana* species complex or other loliginid squids. The remarkable ability of white-squid to display camouflage elements similar to those of benthic octopus and cuttlefish species might convergently evolve in relation to their native coastal habitat.

Perspectives of cephalopod farming along the Eastern Adriatic coast

Vedrana Nerlovic[1], Gorana Jelic Mrcelic[2], Svjetlana Krstulovic Sifner[1]

1 University of Split, Department of Marine Studies, Croatia
2 University of Split, Faculty of Maritime Studies, Croatia

Abstract

Cephalopods have long been recognised as promising candidates for commercial farming due to their short life cycles, rapid growth, high food conversion rates and fairly high market prices. Excessive fishing pressure, declining catches of wild populations and increasing demands for high-quality animal proteins for the growing human population are fuelling continuous efforts to establish large-scale cephalopod farming. Some challenges such as those related to food, nutrition and survival of the early stages under rearing conditions, control of reproduction, and the quality of eggs and offspring, are not fully resolved. The associated ethical issues and animal welfare also require profound discussions for finding acceptable solutions. The aim of the study was to analyse the perspectives of cephalopod farming in the Eastern Adriatic. Croatia is one of the pioneers in the Mediterranean commercial mariculture with numerous cultivation facilities for fish and bivalves, and the potential for introducing other profitable groups such as cephalopods. The National Development Plan for Aquaculture emphasises the importance of diversifying mariculture. In view of the growing market demands for cephalopods, the farming of cephalopods is becoming an increasingly interesting option for producers. There are only a few pilot cephalopod farming projects in the Eastern Adriatic so far, but global progress in this area is promising. Knowledge gained from projects, good cooperation between researchers and producers, the availability of highly qualified labour and growing market demands are a good basis for development of cephalopod farming in the Eastern Adriatic.

Intraspecific differentiation of shelf-slope and nerito-oceanic nektonic squid of the family ommastrephidae in systems of boundary currents

Ch.M. Nigmatullin

Abstract

Ecologically specific intraspecific groups (EIGs) have been identified in the squid inhabited the western boundary currents of the Southwestern Atlantic (*Illex argentinus*) and the eastern boundary currents of the Eastern Pacific (*Dosidicus gigas*) and their population structure has been studied. Low genetic heterogeneity of EIGs within one meta-population was revealed in both species. These EIGs that are present within the whole species of *I. argentinus* and in metapopulations of D. gigas inhabited the northern and southern hemispheres are of phenotypic nature. Life cycles of small-sized EIGs take place mainly in the reproductive part of the species range located in subtropical and tropical waters, whereas large-sized EIGs migrate from there to the feeding grounds located in temperate and subpolar ecosystems, returning back to warmer waters to spawn. The year-round spawning of seasonal EIGs contributes to the survival of a species with a short life cycle and monocyclic reproduction. High intrapopulation plasticity due to presence of several EIGs enables ommastrephid squid to utilize resources of several adjacent ecosystems of various productivity within their vast species ranges.

Deception in the dark—an ethogram of *Callistoctopus furvus* skin patterning and behaviour

C.E. O'Brien

Abstract

Callistoctopus furvus (the "eastern" or "sand octopus") is a relatively unstudied species of medium-large nocturnal octopus inhabiting shallow coastal areas of the tropical western Atlantic. To aid in field identification, promote further study of this species and enhance the understanding of skin patterning and behaviour in nocturnal shallow-water octopuses, we composed an ethogram of *C. furvus* from photographs and 3.11h of video collected of wild individuals in the natural environment. We catalogued two colors, 17 static chromatic components, six groups of skin patterns (divided into 16 subpatterns), three textures, 10 postures and 11 behaviours. Photographs and videos of *C. furvus* from other geographic areas could be classified using this ethogram, supporting its validity for the study of *C. furvus* throughout its geographic range. We also described eight features that can be used to differentiate *C. furvus* from co-occurring octopods through observation alone: strict nocturnality, brick red incorporated into most skin patterns and rows of regularly spaced white spots that are always at least faintly visible on the arms, a polka-dotted deimatic pattern, large "U"-shaped papillae on the mantle, six distinct mantle shapes, a shallow arm web, the execution of very few "parachute attacks" and a tendency to enter existing holes or bury itself in response to disturbance. Notable features include the use of at least one "dynamic" chromatic component, a putative masquerade as algae, and potential mimicry of trumpetfish or cornetfish. Greater observation and study of *C. furvus* and other octopods from a diversity of habitats, latitudes and temporal niches will enhance our understanding of how these factors have shaped the use of colors, body patterning and behaviour of this unique group of molluscs.

Development of squid fishery in the North East Atlantic, new resources without landing restrictions

Daniel Oesterwind[1], Erik Sulanke[2], Bianca Bobowski[1], Verena Vollmer[1], Robin Tautkus[1], Ralf Döring[2]

1 Thünen Institute of Baltic Sea Fisheries, Rostock, Germany
2 Thünen Institute of Sea Fisheries

Abstract

The International Council for the Exploration of the Sea (ICES) is an intergovernmental marine science organization in Europe. Its goal is to advance and share scientific understanding of marine ecosystems and to generate scientific advice for meeting conservation, management, and sustainability goals. Around 6000 scientists from over 700 marine institutes in our 20 member countries are involved. The work in the Atlantic Ocean also extends into the Arctic, the Mediterranean Sea, the Black Sea, and the North Pacific Ocean. One of the expert groups, named WGCEPH, focus on cephalopod life history and fisheries. The experts from different countries are working on different terms of references including aimed on improvement of our knowledge of cephalopod ecology, stock structure, socio-economic aspects of fisheries monitor the European landing trends and survey data, and work towards elaboration of stock assessment protocols. Here we will give a short overview about the development of the group, its scientific outcome and its importance in the context of EU fisheries policy including non-quota species as cephalopods are. We further describe the knowledge gaps and its potential solutions to support sustainability of cephalopod harvesting in European waters.

Genetic manipulation of the optical refractive index in living cells with *Sepioteuthis lessoniana* reflectins

Junko Ogawa, Yoko Iwata, Nina U Tonnu, Chitra Gopinath, Ling Huang, Sachihiko Itoh, Ryoko Ando, Atsushi Miyawaki, Inder M Verma, Gerald M Pao

Abstract

Reflectins are proteins unique to cephalopods which form high optical refractive index nanoparticles and are largely responsible for the structural coloration in the skin and eyes of squid, cuttlefish and octopi. We show that these proteins can be expressed in mammalian cells from transgenes in sufficient quantities to alter the optical refractive index within living cells, a property which was thought to be not manipulatable in living tissue. Expression of reflectin was shown to enhance signal in dark field and quantitative phase microscopy which demonstrates their utility as contrast agents for microscopy. Reflectins can enhance deep tissue imaging based on two principles: 1. As contrast agents and probes that can overcome/increase tissue scattering (e.g. quantitative phase contrast imaging, fluorescence and/or optical coherence tomography) and 2. Increase wide band scattering for dark field microscopy when micron size reflectin particles are generated analogous to leucophores. We selectively cloned and expressed novel reflectin genes from species living in optically complex environments and found novel reflectin genes that were greater in number, diversity and optical activity than known species. Among them we cloned multiple reflectins from *Sepioteuthis lessoniana* that unlike any other, which under certain circumstances can homogenously fill space like a liquid. Although biocompatible, long term toxicity in human cells is still a problem. We have developed an assay in mammalian cells that allows development into an in vitro evolution system that can simultaneously select for lower toxicity variants and higher refractive index.

Establishment of the non-invasive individual identification method in *Octopus sinensis* d'Orbigny, 1841 and verification of the population dynamics in the natural shallow-water environment.

Ashitani Yudai[1], Yamada Mayuko[1], Sato Noriyoshi[2], Hirohashi Noritaka[1], Ono Hiroki[1]

1 Shimane University
2 Tokai University

Abstract

Information of individual identification contributes to the behavioral and ecological study, for confirming individual behavioral range as well as estimating population size. Different from animal species having unique individual body patterns (e.g. large feline), most octopus species do not have distinctive individual body patterns. In addition to the little morphological difference among individuals, features of octopuses, frequently change their body color, skin surface texture and grow rapidly make it difficult to distinguish individuals. In order to solve these issue in the individual identification of octopuses, we developed microsatellite markers useful for individual identification in the East Asian common octopus (*Octopus sinensis*). Also, we tested seawater surrounding animals, excrements and chitinous cuticles the outer-most layer of sucker whether they are available for DNA sources without contacting animals. While enough nucleic DNA was not obtained from seawater, DNA samples extracted from excrement and a single chitinous cuticles could be used for amplifying target microsatellite fragments by PCR. Although it was difficult to obtain octopus excrement, chitinous cuticles were comparatively easily found and collectable in the natural environment. Therefore, we recruited chitinous cuticles as a DNA sources for individual identification. We applied this individual identification method for clarifying population dynamics of the East Asian common octopus in the shallow waters in Oki island, by estimating the staying duration of the nest for each individual.

Quantifying morphological variation in the Hawaiian bobtail squid using micro-CT imaging

Hannah Osland[1, 2], Yuya Nishida[3], David Scheel[1]

1 Department of Biology, Temple University
2 Institute of Culture & Environment, Alaska Pacific University
3 Department of Integrative Biology, Michigan State University

Abstract

Understanding morphological diversity provides insight into the evolutionary and ecological dynamics that shape marine organisms. This diversity can reflect phenotypic plasticity, local adaptation, and the selective pressures that organisms face within their habitats. The Hawaiian bobtail squid (*Euprymna scolopes*) has been extensively studied for its symbiosis with the bioluminescent bacterium *Vibrio fischeri*, yet little is known about its morphological and genetic diversity across its habitat range. To address this gap, we collected adult Hawaiian bobtail squid from four sites across the Hawaiian Islands of O'ahu and Moloka'i. Using micro-computed tomography (micro-CT), we visualized bobtail squid anatomy and made precise measurements of morphological characteristics, including mantle length, body volume, and surface area. We found significant variations in morphology between sites, with individuals from Maunalua Bay being notably larger than individuals from other locations. To complement these findings, we are using ddRAD-seq analyses to contextualize morphological differences within the broader population structure of this species. Together, this work contributes to our understanding of the adaptive strategies of the Hawaiian bobtail squid and broadens our knowledge of morphological diversity in marine organisms.

The bobtail squid-vibrio symbiosis in jeopardy under marine heatwave conditions

Eve Otjacques[1, 2, 3], Brandon Jatico[2], Tiago Marques[4, 5, 6], José Xavier[3, 7], Edward Ruby[2], Margaret McFall-Ngai[2], Rui Rosa[1, 4]

1 MARE – Marine and Environmental Sciences Centre/ARNET - Aquatic Research Network, Laboratório Marítimo da Guia, Faculdade de Ciências, Universidade de Lisboa, Cascais, Portugal
2 Division of Biology & Biological Engineering, Carnegie Science, California Institute of Technology, Pasadena CA, United States of America
3 MARE – Marine and Environmental Sciences Centre/ARNET – Aquatic Research Network, Department of Life Sciences, Universidade de Coimbra, Coimbra, Portugal
4 Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal
5 Centro de Estatística e Aplicações, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal
6 Center for Research into Environmental Ecological Modelling, University of St Andrews, St Andrews, Scotland
7 British Antarctic Survey, Natural Environment Research Council, High Cross, Madingley Road, Cambridge, UK

Abstract

Extreme weather events, such as marine heatwaves (MHW), are expected to intensify and become more frequent in the future, leading to adverse effects on marine life. Here, we investigated the impact of environmental warming (including MHW) on the symbiotic relationship between the Hawaiian bobtail squid (*Euprymna scolopes*) and the bioluminescent bacterium *Vibrio fischeri.* We exposed eggs of E. scolopes to three different temperatures during the embryogenesis (25°C, 27°C, and 30°C), followed by a colonisation assay under the same conditions. Decreased hatching success and reduced developmental time were observed across warmer conditions compared to 25°C. Moreover, exposure to a category IV MHW (30°C) led to a significant decrease in survival after 48 h. With increasing temperature, bobtail squids required more bacteria in the surrounding seawater to be successfully colonised. Furthermore, the capacity of the bobtail squids to maintain the symbiosis diminished significantly with temperature within 48 h. Finally, the capacity for crypt 3 formation in the bobtail squid's light organ, which is crucial for enhancing resilience under stress, also declined with warming conditions. This study showed the vulnerability of this bobtail squid species to increasing temperature (e.g., category IV MHW) and emphasises the critical need to study the dynamics of microbial symbiosis under the projected conditions for the ocean of tomorrow.

Cephalopods' digestive system structure-function in studying plastic ingestion

Cristina Pedà[1], Francesco Longo[2], Ludovica De Vincenzis[2], Marilena Sanfilippo[3], Luca Pagano[1], Emanuele Asciutto[1, 4], Federica Pizzulli[5], Pierpaolo Consoli[1], Paul Andrews[5], Giovanna Ponte[5], Teresa Romeo[6, 7], Pietro Battaglia[1]

1 Stazione Zoologica Anton Dohrn, Sicily Marine Centre, Department of Integrative Marine Ecology (EMI), Villa Pace - Contrada Porticatello 29, 98167 Messina, Italy
2 Stazione Zoologica Anton Dohrn, Sicily Marine Centre, Department of Integrative Marine Ecology (EMI), CRIMAC, Villa Pace - Contrada Porticatello 29, 98167 Messina, Italy
3 Stazione Zoologica Anton Dohrn, Sicily Marine Centre, Department of Research Infrastructures for Marine Biological Resources (RIMAR), Villa Pace - Contrada Porticatello 29, 98167 Messina, Italy
4 Dipartimento di Scienze della Terra e del Mare, Via Archirafi 22 90123 Palermo, Italia
5 Stazione Zoologica Anton Dohrn, Department of Biology and Evolution of Marine Organisms (BEOM), Villa Comunale, 80121 Napoli, Italy
6, Stazione Zoologica Anton Dohrn, Sicily Marine Centre, Department of Biology and Evolution of Marine Organisms (BEOM), Via dei Mille 46, 98057 Milazzo (ME), Italy
7 Institute for Environmental Protection and Research, ISPRA, Via dei Mille 46, 98057 Milazzo (ME), Italy

Abstract

Cephalopods are ecologically and economically important species and play a key role in marine ecosystems but are susceptible to plastic ingestion. The occurrence and distribution of plastic in the digestive system (DS) of marine organisms are related to their morphology and plastic size. The cephalopod DS is highly diverse between species, so understanding morphology is crucial to assess the fate and potential effects of plastics on this complex taxon. This study investigated plastic ingestion in three cephalopod species from the southern Tyrrhenian Sea (western Mediterranean Sea): *Sepia officinalis*, *Octopus vulgaris* and *Loligo vulgaris*, examining the distribution and occurrence of plastic in separate regions of DS, from oesophagus to anus, removed from individuals and chemically digested for plastic extraction. Plastic particles were counted and categorized by size and colour, and polymers identified by the Fourier transform infrared spectroscopy technique. This study also included an assessment of airborne contamination. Our results provide insights into potential physical and toxicological implications of plastic ingestion, shedding light on the importance of improving the investigation of the consequences of marine organisms' ingestion of plastics.

Ghosts of Cuttlebones Past: Update on the Long-Term Effects of Ocean Acidification on the Giant Australian Cuttlefish (*Sepia apama*)

James F. Peyla[1, 2, 3]*, Bronwyn M. Gillanders[2], Zoë Doubleday[1]

1 Future Industries Institute, University of South Australia
2 Department of Ecology & Evolutionary Biology, School of Biological Sciences, University of Adelaide
3 Department of Science, Mount Saint Joseph High School, Baltimore, Maryland, USA (Present Address for JFP)
*presenting author

Abstract

As atmospheric carbon dioxide has increased in recent centuries, marine organisms across the planet have had to cope with an acidifying and warming ocean. The short generation times of cephalopods allow for rapid adaptation to shifting environmental conditions, relative to their teleost competitors. Cuttlefishes, whose calcium carbonate shells have long been collected and stored in museums around the world, thus provide an excellent opportunity to document evolutionary responses to recent ocean acidification. To determine whether decreasing ocean pH has affected cuttlebone structure, we collated a timeseries spanning 158 years of Sepia apama cuttlebones from New South Wales, South Australia, and Tasmania. We then analyzed the cuttlebones' density and microstructure using standard and micro computerized tomography (CT & µCT), respectively. Our µCT analyses have shown that the thickness of the pillars connecting the septa of the cuttlebone has decreased through time, suggesting a decrease in cuttlebone density and strength as a result of acidification. Here, we present new results, particularly from our CT data, providing a deeper understanding of the long-term impacts of elevated seawater acidity on cuttlefishes. Our project, which has constructed one of the longest timeseries to date to examine the effects of ocean acidification on an animal, has the potential to inform predictions of cuttlefish populations around the world.

Investigating molecular determinants of nociception in *Octopus vulgaris* through a model hopping approach

Eleonora Maria Pieroni

Abstract

Nociception evolved to facilitate survival of organisms by triggering reflexive avoidance responses. Among invertebrate species, cephalopods represent excellent organisms in which to study complex nociception as their centralised neuroanatomy allows the elaboration of environmental stimuli into sophisticated behaviours. Clear measures of nociception have been reported in cephalopods but the molecular determinants underpinning its detection and processing are still under investigation. An in silico pipeline biased to the identification of existing Eumetazoan conserved nociceptive genes identified 99 candidates from the *Octopus vulgaris* transcriptome, including the orthologue of the classical nociceptor TRPV (OvTRPV). After in silico reconstruction of the fragmented transcript, the sequence was experimentally validated and found to be broadly expressed across neuronal and non-neuronal tissues. To investigate if OvTRPV possessed any conserved features of this noxious-triggered channel subfamily, we adopted a model hopping approach using *Caenorhabditis elegans*. The heterologous expression of OvTRPV in *C. elegans* loss of function mutants for the orthologue gene (osm-9) identified OvTRPV functioned as a polymodal receptor, being able to rescue both chemical and mechanical aversion that is lost in this strain. We are currently investigating which ligands activate OvTRPV by co-expressing it in HEK293G5A cells with an aequorin protein, allowing the detection of receptor mediated Ca2+ entry upon ligand activation. The exposure to both classical and ecologically relevant cues will help us speculate the function of this receptor. Overall, our approach allows the targeted investigation of candidate molecular nociceptors by overcoming the current challenges that limit direct genetic tractability in *O. vulgaris*.

Eight Arms, One Goal: The Transformation of Small-Scale Octopus Fisheries

Cristina Pita[1, 2], Katina Roumbedakis[2]

1CESAM - Centre for Environmental and Marine Studies, University of Aveiro, Portugal
2 Institute of Marine Research (IIM-CSIC), Vigo, Spain

Abstract

Small-scale octopus fisheries are globally important for livelihoods, food security, and local economies, particularly in developing countries due to their accessibility and low gear requirements. Meanwhile, the global demand for octopus has surged, especially in developed countries, making it a multi-billion-dollar industry. Yet, limited information exists on these fisheries, trade dynamics and value chains. This study reviews 19 small-scale octopus fisheries worldwide, examining their characteristics, supply chains, and challenges to their long-term viability and sustainability. Despite many commonalities, these fisheries can look quite different: some target domestic markets, while others supply international trade, mainly to the European Union and neighboring countries. Value addition along the chain is generally limited. Fishing methods are diverse (ranging from traps and handlines to gleaning and diving) with octopus contributing from less than 25% to over 75% of local landings, depending on the region. Management is also diverse, with some fisheries engaged in co-management or Fishery Improvement Projects (FIPs), and others transitioning from subsistence to commercial operations. Common challenges include weak monitoring and enforcement, illegal fishing, low economic returns, and inequities within value chains. However, promising opportunities exist: high international demand, digital tools for data collection and marketing, market diversification, value addition, and community organization. This analysis highlights the diversity and transformation of small-scale octopus fisheries, their integration into markets, and the need for inclusive strategies to ensure their long-term sustainability.

Mapping the Octopus Brain: Network Analysis and Evolutionary Insights

Federica Pizzulli

Stazione Zoologica Anton Dohrn, Department of Biology and Evolution of Marine Organisms (BEOM), Villa Comunale, 80121 Napoli, Italy

Abstract

The neural circuitry of Octopus vulgaris represents one of the most sophisticated examples of invertebrate brain organization. Building on the pioneering work of J.Z. Young—who provided the most authoritative anatomical description of the octopus nervous system—we applied a network-based approach to analyze its complex brain architecture. Using historical tracing data and quantitative metrics, we present the first computational network analysis of the octopus brain. Our study integrates data from classical neuronal tracing studies and recent topological modeling, mapping over 350 neural pathways connecting 32 brain regions across the supra- and sub-oesophageal masses, as well as the optic lobes. Our results reveal a highly structured "connectome" exhibiting small-world properties, multiscale modularity, hub-areas, and strong segregation-integration dynamics; all features consistent with principles found in mammalian neural systems. Similarly, a comparative analysis across 52 cephalopod species allowed us to define distinct cerebrotypes, suggesting that brain composition reflects ecological adaptations more than phylogeny alone. Hierarchical clustering and limited phylogenetic PCA suggested that brain organization in cephalopods is influenced by a combination of evolutionary history, developmental constraints, and lifestyle. This study provides novel insights into the topological and functional organization of the octopus brain and supports the hypothesis that convergent evolution has shaped similar neural architectures in disparate lineages and enhances our understanding of octopus neurobiology and offers a framework for investigating universal principles of brain organization across diverse taxa.

Cephalopod integrated analysis to identify the impacts of marine environmental pollution.

Giovanna Ponte

Stazione Zoologica Anton Dohrn, Department of Biology and Evolution of Marine Organisms (BEOM), Villa Comunale, 80121 Napoli, Italy

Abstract

The rapid increase in human activities and overexploitation of resources has significantly altered the marine environment, leading to what is now termed the "New Environmental Status" of the sea and its habitats. It is crucial to understand how marine ecosystems, communities, and species are responding and adapting to these environmental pressures in our current era, the Anthropocene. This new “Status” causes different responses in marine life, with some species acquiring new physiological and morphological characteristics, while others show changes in population dynamics and metabolic processes. Here we present a NextGenerationEU funded project aimed at exploring cephalopod fishery resources as bioindicators to investigate marine environmental pollution and its impacts on marine biota. Focusing on anthropogenic pressures in the Anthropocene , the CIAO MARE project examines the effects of marine litter and pollution on the biology, trophic ecology, physiology, morphology, and flesh quality of three cephalopods (i.e., *Loligo vulgaris*, *Octopus vulgaris*, *Sepia officinalis*), which are important target species of Mediterranean fisheries and play a key role in marine food webs. Using a multidisciplinary approach, this study evaluates how cephalopods respond to environmental changes as indicators of ocean health. It investigates the ecological effects of pollution, measures the ingestion of microplastics and their chemical transfer, and assess how pollution-related stress affects the quality of cephalopod edible tissues. The research is conducted in the southern Tyrrhenian Sea, comparing an impacted area (Gulf of Patti) with a Marine Protected Area (Capo Milazzo).

Hatching date is associated with micro cohorts in *Loligo forbesii*

Anne Marie Power

Ryan Institute, School of Natural Sciences, University of Galway, Galway, Ireland

Abstract

We hypothesised that the large variation observed in squid lifecycle traits, such as growth and size and age at maturity, is due to extended breeding throughout the year. We tested whether hatching date, specifically exposure as hatchlings to a decreasing (October to April) or increasing (May to September) temperature regime could be associated with these traits in *Loligo forbesii*, a commercially fished squid species. In agreement with predictions, hatchlings from the increasing temperature regime matured at smaller size and age and grew slower than those from the decreasing temperature regime, in both sexes. Hatchlings from the decreasing temperature regime were mature over a broad range of sizes and ages (i.e. they had high variability) but were on average significantly larger, older and faster-growing than the other group. A maturity time series in adults from Scotland showing that the length at maturity was highest in squid caught in November, and lowest in squid caught in February/ March, was consistent with the seasonal timing and age profile of hatchlings from each temperature group, termed 'micro cohorts'. In Ireland, most squid had hatched in October to April, i.e. the large/ late-maturing cohort, so only one micro cohort was dominant there, whereas, both micro cohorts were common in Scotland. The presence and abundance of micro cohort hatching has important implications for fisheries biomass and timing, and for female reproductive output. Micro cohorts may link with Alternative Reproductive Tactics (ARTs) in squid and this aspect requires further study in *L. forbesii*.

Cephalopod assemblages in bottom trawling fishing grounds from north-western Mediterranean Sea

Alberto Rico[1, 2], Marta Blanco[1, 2], Pilar Sánchez[2]

1 Institut Català de Recerca per a la Governança del Mar (ICATMAR), Passeig Marítim de la Barceloneta 37-49, 08003 Barcelona, Spain.
2 Institut de Ciències del Mar (ICM-CSIC), Passeig Marítim de la Barceloneta 37-49, 08003 Barcelona, Spain

Abstract

Under the current global change scenario, species assemblages are shifting, particularly cephalopods, which have short live cycles and constitute a significant fraction of landings in the Mediterranean Sea. Cephalopods play a crucial role in demersal communities due to their influence on ecosystem dynamics. This study characterizes the species diversity of cephalopods associated with bottom trawling fishing grounds and examines its variability in relation to depth, season and geographical area. Data were collected on board commercial trawlers, from November 2018 to December 2023, including both commercial and discarded cephalopods species. Species composition was analyzed using non-metric multidimensional scaling analysis (nMDS). Five assemblages were observed influenced by depth and geographical region. No significant seasonal effect was observed. The continental shelf is divided into three groups. The shallow Delta shelf (depth 21-50m) in the south is dominated by *Sepia officinalis* and *Alloteuthis* sp., accounting for 64% of the total biomass. The middle (depth 50-80m) and deeper shelves (depth 80-200m) are home to *Illex coindetii* (57-76% of the biomass). The slope is divided into two groups: the upper with *Illex coindetii* (43%), *Eledone cirrhosa* (21%) and *Abralia veranyi* (12 %), and the lower (deep-sea pelagic species, *Histioteuthis reversa*, 20% of the biomass). These findings suggest that cephalopod demersal communities exhibit high variability, largely driven by seabed geomorphology, even in spatially proximate areas. As fishery management policies for cephalopods in the Mediterranean are still under development, higher spatial resolution studies can provide valuable insights to support more sustainable fishery management.

Onychoteuthid squids of western Indian Ocean: insights from predators diet and molecular analysis

Evgeny Romanov[1]

1 CITEB (Centre technique de recherche et de valorisation des milieux aquatiques), Île de la Réunion, France

Abstract

Squids of the family Onychoteuthidae (Cephalopoda: Oegopsida) have been subject of numerous past and recent taxonomic studies (both morphological and molecular) across the globe (e.g. Okutani, 1981; Bonnaud et al., 1998; Bolstad, 2010, Vecchione et all., 2015; Bolstad et al., 2018; Lischka et al., 2018), however in the western Indian Ocean the family' specific composition and geographic ranges still poorly understood. Our studies during DECAPOT Project (2019-2023) mostly based on top predators (swordfish and tuna) stomach contents analysis associated with molecular methods allowed to collect new information for this family. Molecular analysis based on COI sequences demonstrate that in the southern subtropical oligotrophic gyre and in the Mozambique Channel the family represented by three genera (Onykia, *Walvisteuthis*, and *Onychoteuthis*) and by at least nine species, two of them are likely not yet formally described. We document range extension for four species: *Onychoteuthis compacta, Onykia loennbergi, Onykia robsoni, and Walvisteuthis jeremiahi*. Two clusters of COI specific signatures belonging to Onykia hasn't matched any species deposited to BOLD and GenBank. They may represent either *O. indica, O. aequatorialis* or new non-described species. Our data suggest basin-wide presence of *Onychoteuthis cf. prolata, W. jeremiahi, Onykia* sp 1, but rather restricted presence of other species inside Mozambique Channel. Onychoteuthid squids paying major role in the trophic ecology of swordfish, albacore, bigeye, and yellowfin tunas, representing second most important squid family besides Ommastrephids.

Towards Inclusive Sustainability Pathways: Insights from Stakeholder Perspectives in the Mexican Octopus Fishery

Katina Roumbedakis[1,2], Silvia Salas[2], Miguel A. Cabrera[2], Cristina Pita[1,3], Graham J. Pierce[1]

1Instituto de Investigaciones Marinas (IIM-CSIC), Vigo, Pontevedra, Spain
2Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Unidad Mérida, Mérida, Yucatán, México
3CESAM - Centre for Environmental and Marine Studies, Department of Environment and Planning, University of Aveiro, Aveiro, Portugal

Abstract

The octopus fishery in the Yucatán Peninsula, Mexico, is a vital source of livelihoods, food security, and cultural identity for local communities, while also holding global importance, ranking amongst the top octopus producers worldwide. However, the long-term viability and sustainability of the Mexican octopus fishery are increasingly threatened by overexploitation, illegal, unreported and unregulated fishing, climate change, habitat degradation, inadequate governance, among others. Understanding the complexities of this fishery and its sustainability challenges requires systematically mapping the value chain using inclusive participatory approaches. This study provides a gender-sensitive update to the octopus value chain of this fishery to identify actors, interactions between them and the factors that enable or hinder the sustainability of this important fishery. We explore how different actors envision their roles and the resources they require —such as training, financial support, or organizational strengthening— to foster a more equitable and sustainable fishery. By including voices of local key actors, such as fishers, buyers, processors, middlemen, permit holders, scientists and others, this research contributes to the development of an inclusive sustainability assessment framework to improve fisheries governance. Based on the results, we propose strategic actions to enhance the sustainability of the Mexican octopus fishery.

Diet of *Octopus vulgaris* paralarvae and juveniles off NW Iberian Peninsula

Alvaro Roura[1], Lara García-Alves[1], Andrea Ramilo[1], Elvira Abollo[1], Angel F. Gonzalez[1]

1 Instituto de Investigaciones Marinas de Vigo (IIM-CSIC)

Abstract

ECOSUMA survey was an interdisciplinary project aiming to understand the biology of early stages of the common octopus, *Octopus vulgaris*, along the NW Iberian Peninsula. Next generation sequencing was carried in the digestive tracts of 40 paralarvae (between 3 and 15 suckers per arm) and 8 juveniles (between 0,7 and 37,9 g). A total of 8,938,558 sequences were obtained, with an average of 186,220 per sample. After filtering octopus reads, an average of 3,468 sequences were detected in 44 samples (38 larvae and 6 juveniles). Contamination sequences, such as fungi, insects, or bacteria were removed (ranging between 0 and 3,388). Prey was detected in 36 samples, 30 larvae and 6 juveniles (average of 3.4 prey), belonging to 7 different phyla, 20 classes, and 48 species. The most frequent groups were copepods (57.1%), squid (47.6%), pteropods (38.1%), cnidarians (16.7%), and crustacean decapods (14.3%). Comparatively, 33 species were unique to octopus paralarvae, which corresponded to zooplankton groups (copepods, hyperiid amphipods, crab larvae, ostracods, pteropods, cnidarians, siphonophores and algae). In juveniles, 13 exclusively benthic species were detected (gammarid amphipods, hermit crabs, spider crab, fish, isopods, polychaetes and nematode). Only 2 prey items were detected in both groups (a copepod and a pteropod).

Estimating Fishing Effort in Cephalopod Small-Scale Fisheries: Sampling Required for Tracking Data

Marta Rufino[1, 2], Samuel Amaral[1, 3], Célia Fernandes[3], Paulo Ramos[3], Miguel Gaspar[1], Mafalda Rangel[4], Luis Bentes[4], Ana Moreno[1]

1 IPMA -Portuguese Institute for the Sea and the Atmosphere (IPMA). Division of Modelling and Management of Fisheries Resources. Av. Dr. Alfredo Magalhães Ramalho, 6, 1495-165 Lisboa
3 Lisbon School of Engineering (ISEL). Department of Mathematics, Rua Conselheiro Emídio Navarro, 1 1959-007 Lisboa
4 Centre of Marine Sciences (CCMAR/CIMAR LA), Campus de Gambelas, Universidade do Algarve, 8005-139 Faro, Portugal

Abstract

Small-scale fisheries (SSF, vessels under 12 meters), make up 90% of global fisheries and over 80% of the EU fleet, employing ~100,000 people. Despite their importance to coastal communities, SSF effort remains poorly quantified. Unlike large-scale fleets tracked via VMS, SSF tracking is only now emerging, but will be mandatory in the EU by 2030 (EU 2023/2842). High-resolution tracking is vital for assessing SSF effort but poses data management challenges (Rufino et al., submitted). Yet, for many countries, wide SSF tracking is not economically viable, in face of thee high number of vessels. This study estimates how many fishing trips must be tracked to reliably assess effort indicators in two cephalopod fisheries. We used GPS data (30s intervals) from 2020–2024 for 5788 trips by 9 trammel net vessels and 4670 trips by 36 pot/trap vessels targeting octopus in 2 areas off Portugal. Data were processed following Rufino et al. (2023) and Samarão et al. (2024), with effort and revenue mapped onto 500 m grids. We compared indicators from full datasets to those from random subsamples of 5–300 trips (100 iterations) to determine the minimum sampling needed when trackers aren't permanently installed. We also examined whether vessels shared fishing grounds or operated in distinct areas. Our results offer practical guidance on sampling needs for SSF monitoring, supporting better management, compliance, and marine spatial planning.

Evaluating Genetic Patterns of Octopus maya to Support Resilient and Sustainable Fisheries

Paulina Mejia Ruiz[1], Clara Elizabeth Galindo Sanchez[1], Carlos Rosas Vázquez[2]

1 Center for Scientific Research and Higher Education of Ensenada, Baja California
2 Multidisciplinary Unit for Teaching and Research, UMDI-SISAL

Abstract

Mexico has the third largest octopus fishery globally, with *Octopus maya*, an endemic species to the Yucatán Peninsula, representing 89% of national landings. This fishery generates significant economic revenue and supports over 34,000 people in the states of Yucatán and Campeche. Despite its ecological and economic importance, there is ongoing debate about the population structure of *O. maya*. Some studies suggest a single population, while others propose two genetically distinct groups shaped by differences in environmental conditions. Additionally, increasing Sea Surface Temperature (SST) has been shown to negatively impact the reproductive success and embryo viability of this species, raising concerns about its resilience under climate change. This project aims to evaluate the neutral and adaptive genomic variation of *O. maya* across key fishing areas using ddRAD-seq. Genetic diversity, gene flow directionality, and population connectivity will be assessed, along with genotype-environment associations with variables such as SST and chlorophyll. By integrating genomic and environmental data, we seek to identify management units, source and sink populations, and potential adaptive mechanisms that allow the species to persist under shifting environmental conditions. The findings will support the development of genomics-informed conservation and fishery management strategies to safeguard *O. maya*, a species under growing pressure from both fishing and ocean warming. Given the species' central role in local livelihoods and food security, this research also aims to promote social equity and long-term sustainability for the coastal communities that depend on this vital marine resource.

Lateralization of retinal structure correlated with visual lateralization in *Sepioteuthis lessoniana*

Sakurai Yuma[1], Kohtsuka Hisanori[2], Kimbara Ryosuke[3], Miura Toru[2], Tanaka Nobuaki[4]

1 Division of Biology, Department of Biological Sciences, School of Science, Hokkaido University, Japan
2 Misaki Marine Biological Station, School of Science, The University of Tokyo, Japan
3 Molecular Genetics Unit, Okinawa Institute of Science and Technology, Japan
4 Graduate School of Life Sciences, Hokkaido University, Japan/Faculty of Science, Hokkaido University, Japan/Division of Biology, Department of Biological Sciences, School of Science, Hokkaido University, Japan

Abstract

Cephalopods exhibit various unique visually guided behaviors that depend on their visual systems. As most visual fields in cephalopods are occupied by monocular vision, these animals tend to preferentially orient the left or right eye towards a visual target. Left-right differences in visual behavior (visual lateralization) are commonly observed among vertebrates and invertebrates, although studies on the neural properties correlated with lateralization are limited. To understand the neural mechanisms based on behavioral lateralization, we investigated the lateralization of retinae in the oval squid *Sepioteuthis lessoniana*, which preferentially faces the left or right eye toward conspecifics. Our findings indicated that only the lateralization of the proportion of photoreceptor cells containing a single chromocenter is correlated with visual lateralization and not the focal length of the eye, density of photoreceptor cells, or minimum separatable angle. Additionally, this study revealed that light entering the eye reaches the argentea, the outer rim of the eye, and that the inner surface of the argentea reflects light towards the lens. The simulation results indicated that photoreceptor cells containing a single chromocenter in their nuclei efficiently collected reflected light on the outer segment. These results suggest that photoreceptor cells in the dominant eye collect more light from the outer segment, thereby enhancing weak light signals or contrast sensitivity.

Sepioteuthis cf sepioidea: a new cryptic species for American Loliginidae.

Ana Beatriz Bauer[2], Bianca Lima Paiva[1], Isabela Pereira Moura[1], Leilane Araujo Correa[1], Alan Erik Souza Rodrigues[1], Jonathan Stuart Ready[1], Carlos Eduardo Ferreira[3], Renato Junqueira Dantas[3], João Bráullio Luna Sales[1], Tatiana S. Leite[2]

1 Federal University of Para
2 Federal University of Santa Catarina
3 Fluminense's Federal University

Abstract

Loliginidae comprises several commercially important squid species present in neritic and coastal regions worldwide. Due to the high environmental heterogeneity of coastal and shelf habitats under distinct hydrological and oceanographic regimes, which directly influence the dispersal capacity of both adults and the paralarva stage, loliginids have played a significant role as organisms capable of indicating past biogeographic events. Recently, several genera inhabiting the Western Atlantic have shown the presence of cryptic species, indicating that this region is a source of hidden diversity for the family. *Sepioteuthis sepiodea* is the only species of the genus reported in this region, with a wide distribution between the north and south of the Western Atlantic, thus encompassing different biological and oceanographic regimes. The aim of this study was to investigate the possible presence of cryptic lineages within this species using molecular markers. Individuals were collected in Bahia and Rio de Janeiro, and the cox1 and Rhodopsin genes were sequenced. Additional sequences of the Sepioteuthis genus from GenBank were implemented in the database of the present study, and phylogenetic reconstructions of Maximum Likelihood and Bayesian Inferences were made. Theresults obtained show that *S. sepioidea* is composed of three distinct evolutionary lineages. One is restricted to the North Atlantic and the other two are found on the Brazilian coast, where all the clades formed have high support values. The results of this study highlight the need for integrative inferences in order to correctly identify the diversity of Loliginid species found in the Western Atlantic.

The importance of using molecular methods to identify octopuses marketed in Latin America: the vulgaris case.

Bianca Lima Paiva[1], Leilane Araujo Correa[1], Isabela Pereira Moura[1], Alan Erik Souza Rodrigues[1], Patricia Charvet[2], Vicente Faria[2], Manuel Haimovici[3], Jonathan Stuart Ready[1], João Bráullio Luna Sales[1]

1 Federal University of Para, Belem, Brazil
2 Federal University of Ceara, Fortaleza, Brazil
3 Fundation University of Rio Grande, RS, Brazil

Abstract

Cephalopods represent a growth area of commercial seafood products. Most of the growth in this market is the result of increased fisheries specially octopus products. During the commercialization of these organisms the removal of morphological characters used for identification impedes accurate identification of the final products as well several attempts are being made to diversify new products made with the species among them, canned, pre-cooked, roasted and others. This study therefore aimed to determine the efficiency of sequences of the 16S rRNA gene for identifying commercially important *Octopus* species sold in south and central America. A total of 76 samples were obtained from local markets and supermarket chains, from Northern, Northwestern and Southern Coast of Brazil, French Guyana and Mexico, sequenced, and identified using blast searches of GenBank at species level with similarity <98%. Five species of octopus were identified in the full dataset. All identified sequences on Blastn were recovered in highly supported clades. All samples from processed products of "octopus salad", identified as *Octopus vulgaris*, including the FAO fishing region, are genetically identical to *Octopus maya*, a species endemic to the Campeche Bank region, in Mexico, which is fished prohibited for up to six months a year. Animals sold under the generic name "octopus northeastern" were genetically similar to *O. americanus*, and one sample are genetically identical to *O. vulgaris*. All individuals collected at fairs in the northeast were genetically similar to *O. insularis*, however, the support value obtained was not statistically significant. Therefore, our results pointed to labelling errors in commercially available species of octopus and demonstrated the efficacy of the 16S for species identification and evaluation of labelling errors and commercial fraud.

Genomic signatures of light organ evolution in bobtail squid

Gustavo Sanchez[1], Fernando Ángel Fernández-Álvarez[2], Ainhoa Bernal-Bajo[3], Elizabeth Heath-Heckman[4], Raphael Lami[5], Margaret McFall-Ngai[6], Michele Nishiguchi[7], Spencer Nyholm[8], Oleg Simakov[9], Louise Allcock[10], Daniel Rokhsar[1]

1 Molecular Genetics Unit, Okinawa Institute of Science and Technology, Onna, Okinawa, Japan.
2 Spanish Institute of Oceanography, Centro Oceanográfico de Gijón (IEO, CSIC), Gijón, Spain
3 Spanish Institute of Oceanography, Centro Oceanográfico de Baleares (IEO, CSIC), Palma, Spain
4 Department of Integrative Biology, Michigan State University, East Lansing, MI USA
5 Sorbonne Université, UPVD, CNRS, UMR8176, Laboratoire de Biodiversité et Biotechnologies Microbiennes (LBBM), Observatoire Océanologique, 66650 Banyuls-sur-Mer, France
6 Department of Biosphere Sciences and Engineering, Carnegie Institution for Science, Pasadena, CA 91125, USA
7 Department of Molecular and Cell Biology, University of California, Merced, California, USA
8 Department of Molecular and Cell Biology, University of Connecticut, Storrs, Connecticut, USA
9 Department for Neurosciences and Developmental Biology, University of Vienna, Vienna, Austria
10 School of Natural Sciences and Ryan Institute, University of Galway, University Road, Galway H91 TK33, Ireland

Abstract

The light organ (LO) of bobtail squid is a coleoid morphological innovation colonized by luminescent bacteria that enables nocturnal hunting. The genomic underpinnings behind its origin remain unclear. Four transcription factors: SIX, EYA, PAX6, and DAC; are expressed in the LO and appear to have been co-opted from the generally conserved eye development program across animals. Following comparative genomics and regulatory data from the pioneering bobtail squid symbiosis model *Euprymna scolopes*, we identified key structural differences among these loci and their deep evolutionary origins. However, the extent of these changes in bobtail squid remains unknown due to limited taxonomic sampling and uncertain genomic completeness. To better understand the genomic basis of LO evolution, the Aquatic Symbiosis Genomics project sequenced chromosome-level genomes of seven decapodiforms, including species with and without LO. Our broad comparative analyses revealed a largely conserved genomic architecture and strong collinearity within clades, enabling the development of a lineage-specific ortholog dataset (CephBUSCO) to assess genome and proteome completeness in cephalopods. These validations allowed us to identify crucial changes associated with the LO emergence. First, bobtail squids with LO exhibit a unique fusion between two ancestral decapodiform chromosomes, indicating a derived karyotype. Second, we find bobtail squid-specific genomic constraints around the PAX6 and EYA loci, whereas DAC and SIX loci show similar rearrangement rates across LO and non-LO species. These findings help identify crucial changes during the co-option and emergence of LO-associated genomic configurations. We provide a model for the evolution of such constrained genomic configuration during decapodiform diversification.

Intrinsic and extrinsic motivations behind the exploratory behavior of *Octopus vulgaris* under environmental enrichment and deprivation

Catarina Caldeira-Santos[1], Mélanie Court[1], Julien Bacqué-Cazenave[2], Anne Sophie Darmaillacq[2], Rui Rosa[1, 3]

1 MARE - Centro de Ciências do Mar e do Ambiente
2 Normandie University
1, 3 MARE - Centro de Ciências do Mar e do Ambiente/Faculty of Sciences, University of Lisbon

Abstract

More exploratory animals tend to display enhanced survival rates, faster growth, better learning performance, and more frequent social interactions. However, this does not always translate into improved welfare measures, as exploration can correlate positively or negatively with induced stress. This study investigates the impact of emotional states on the exploratory behavior of prey (extrinsic factor) and novel objects (intrinsic factors) by Octopus vulgaris. Eight specimens were exposed to control and impoverished conditions (devoid of shelters, sand, shells and rocks) over five days in a cross-over experimental design. Daily 10-minute recording sessions were conducted to assess activity levels, latency to interact with objects, interaction duration, and interaction frequency. Octopuses exhibited significantly higher activity levels before and after stimulus introduction, as well as increased interaction frequency in deprived environments compared to control ones. Thus, octopuses in impoverished environments engage more readily with available stimuli regardless of novelty, suggesting increased boredom or stress. In contrast, those in control environments displayed lower but more selective interactions, potentially indicating positive welfare. These findings emphasize the crucial role of environmental supplementation in improving the welfare of captive octopuses, contributing to the ethical and scientifically informed management of *O. vulgaris* in controlled settings.

Three fingers and eight arms: curious pygmy squid living in the open ocean of Okinawa

Sato Noriyosi[1], Minemizu Ryo[2], Lu Chung-Cheng[3]

1 Tokai University
2 Ryo Minemizu Photo Office
3 National Chung-Hsing University

Abstract

The family Idiosepiidae includes the smallest cephalopods in the world, with nine known species distributed across the coastal regions of the Indo-west Pacific region, including South Africa, Thailand, Indonesia, China, Japan, and Australia. Members of this group possess a dorsal adhesive organ on the mantle, which allows them to attach to substrates such as seagrass for hiding or resting to avoid predation. Here, we report a newly discovered pygmy squid that displays several morphological traits never before observed within this family. A total of seven specimens – four males and three females – were collected from the open ocean off Okinawa. Uniquely, these individuals lacked both the dorsal adhesive organ and tentacles. Their bodies were generally reddish in color when alive, and their fins were located near the posterior end of the mantle. Additionally, their hectocotylus morphology was highly irregular, with the right one notably trifurcated. In addition to these morphological distinctions, mitochondrial DNA markers clearly indicate that this species is genetically distinct from members of the genus *Idiosepius*. Phylogenetic analyses place it closer to the genera Xipholeptos and Kodama, both recently erected as new genera within Idiosepiidae. This squid possesses a straight and narrow funnel-mantle locking apparatus, a feature shared with both Xipholeptos and Kodama. Based on both morphological and molecular evidence, we proposed that this new pygmy squid should not be placed within the genus *Idiosepius*.

Ecology of minor squid species from the waters around Hokkaido, since 2010's

Sawamura Masayuki[1]

1 HRO Kushiro Fisheries Institute

Abstract

To reveal the roles of minor squid species in the marine ecosystem, I compiled the ecology of minor squid species from the waters around Hokkaido since 2010's. As for the food habit, most pelagic squids fed on planktonic crustacean, squids (frequently cannibalism), and pelagic Pisces, and interspecific variations of food habit were smaller than intraspecific variations on body size classes. On the other hand, benthic species such as Rossia pacifica fed on the benthic invertebrates. As for the sexual maturation, migratory warm water species such as *Ommastrephes bartramii* was seldom matured in the feeding ground around Hokkaido, and it was suggested that the sexual maturation depends on the season. On the other hand, boreal species such as *Onychoteuthis borealijaponica* was matured in the plural seasons, and it was suggested that the sexual maturation depends on the body size. On *O. borealijaponica* and *Berryteuthis magister magister*, deflected sex ratio (about 1:2) was observed.

Morphological variation in *Spirula spirula* shell shape

Judy Stadler[1], Elizabeth Shea[1]

1 Delaware Museum of Nature and Science

Abstract

Spirula spirula is a small mesopelagic cephalopod with an internal, chambered, calcareous shell. Individual shells vary in the diameter of the shell whorl as well as the tightness of coiling. Past morphological analyses have demonstrated variation in overall shell shape, but whether this variation is due to population differences, species differences, or sexual dimorphism remains unknown. Multiple species of *Spriula* have been described, but recent molecular data suggests there is only one species with a worldwide distribution. We used undamaged dry shells from the Mollusk collection at the Delaware Museum of Nature and Science to test for differences in shell shape in specimens collected from the North Atlantic and the South Pacific Oceans. We used both landmark-based geometric morphology, and traditional morphometrics on a polar coordinate system to analyze specimens with a similar number of total segments and a fully intact first segment to confidently set repeatable landmarks. Preliminary results based on specimens collected from the east coast of Florida show that most useable shells had between 28 – 33 chambers, and that the distance between the whorls at the free end of the shell is variable. Analysis of larger specimens from around the world indicates that the whorl diameter stops increasing in shells with > 40 chambers, suggesting the onset of maturity or end of life.

A systematic review of Histioteuthidae (Verrill, 1881) Species Complexes in Aotearoa New Zealand

Benjamin Shirey[1], Kathrin Bolstad[1], Heather Braid[1]

[1]AUT Lab for Cephalopod Ecology & Systematics, School of Science, Auckland University of Technology, Auckland, New Zealand

Abstract

Histioteuthids are globally distributed and important prey for many apex marine predators. However, accurate characterisation of trophic interactions relies on stable taxonomy, and recent genetic results have revealed several *histioteuthid* species complexes, including three species from Aotearoa New Zealand. Therefore, the purpose of the present study is to use integrative taxonomy (morphology and genetics) to resolve the taxonomic status of three local species: *Histioteuthis aff. bonnellii, H. aff. eltaninae*, and *H. aff. atlantica*. Two sympatric species in the *H. atlantica* species complex have been identified in NZ waters: H. atlantica sensu stricto and H. *cookiana* (which was previously synonymised with H. atlantica). The *H. bonnellii* species complex appears to contain three potentially distinct species that are currently being evaluated to determine if they represent novel taxa, or species that have been previously synonymised. Clarifying the identity of local species will be essential in the future management of commercially and culturally important species that rely on these squids in New Zealand waters.

Genetic structure of *Sepia officinalis* in the Eastern Adriatic Sea based on mitochondrial COI gene

Mirela Petric[1], Zeljka Trumbic[1], Larisa Bogdanov[1], Zdravko Ikica[2], Svjetlana Krstulovic Sifner[1]

1 University of Split, Department of Marine Studies, Split, Croatia
2 University of Montenegro, Institute of Marine Biology, Kotor, Montenegro

Abstract

Sepia officinalis is one of the most important coastal fishery resources in the Adriatic Sea, contributing significantly to local economies, however facing challenges related to overfishing and habitat degradation. Considering that genetic characterization of exploited populations is critical for sustainable management, this study aimed to asses genetic diversity in S. officinalis populations across the Eastern Adriatic Sea by examining variation in mitochondrial COI gene. A total of 103 specimens from 5 sampling locations (Montenegro (N=27), Hvar (30), Split (N=9), Zadar (N=7) and Novigrad Sea (N=30)) were collected during winter of 2022. Among 38 haplotypes, we identified 19 polymorphic sites, of which 17 parsimony-informative and 2 singletons. Indication of substantial genetic variation with minimal nucleotide differences among individuals was revealed by high haplotype diversity (Hd = 0.960) and low nucleotide diversity (π = 0.0128). AMOVA analysis indicated significant low to high genetic differentiation among populations, with the overall pairwise Φst of 0.414. We have observed the area of genetic connectivity in Eastern Central Adriatic, encompassing Split, Zadar and Hvar. In contrast, local demes seem to be established in Novigrad Sea and along Montenegrian coast, suggesting limited gene flow. Our study reveals complex population structuring in S. officinalis across the Eastern Adriatic, challenging earlier assumptions of panmixia in the Western Adriatic. Future studies integrating nuclear markers, comparative Mediterranean sampling, and hydrogeographic modelling will clarify stock boundaries and inform sustainable joint management fisheries policies.

Modelling the Octopus vulgaris fishery in the Gulf of Cadiz with intra-annual multi-fleet generalized depletion models

Ignacio Sobrino[1], Arturo Jimenez[2], Ruben Roa-Ureta[3]

1 Spanish Centro Oceanográfico de Cádiz, Instituto Español de Oceanografía—Spanish National Research Council (IEO-CSIC), 11006 Cádiz, Spain; ignacio.sobrino@ieo.csic.es
2 Spanish Institute of Oceanography. Spanish National Research Council (IEO-CSIC), 11006 Cádiz, Spain ignacio.sobrino@ieo.csic.es
3 Independent consultant, Portugalete 48920 Bizkaia, Spain

Abstract

The octopus fishery in the Gulf of Cadiz (SW Spain) is composed of three distinct fleets: trawlers, pots and hooks. We built a database of weekly total catch, total fishing effort in boat-days, and mean weight of octopus in the catch, of ten fishing seasons (2015-2024). We then adapted the assessment framework developed for the Asturias (NW Spain) one-fleet small scale octopus fishery to our three-fleets fishery in Cadiz. This framework is based on intra-annual generalized depletion models that produce estimates of octopus total abundance, total biomass, recruitment, spawners, and natural and fishing mortality. Biomass estimates were then used to fit generalized surplus production models and spawning stock and recruitment models, thus allowing estimation of biological reference points useful for management. Following previous findings of pluviosity affecting future landings, our surplus production models included time-varying parameters that depended on pluviosity.

Isotopic niche of *Octopus maya* and *Octopus americanus* east of the Campeche Bank

Juliet Benítez-Solano[1], José Otilio Avendaño-Alvarez[3], Carmen Amelia Villegas-Sánchez[2], José Manuel Castro-Pérez[2], Rigoberto Rosas-Luis[1, 2]

1 Instituto Tecnológico Nacional de México / Instituto Tecnológico de Chetumal
2 SICIHTI- Instituto Tecnológico Nacional de México / Instituto Tecnológico de Chetumal
3 Unidad Multidisciplinaria de Docencia e Investigación, Facultad de Ciencias, Universidad Nacional Autónoma de México. Sisal, Yucatán. México

Abstract

The distribution of *Octopus maya* and *Octopus americanus* has been described separately, however, geographic overlap has been recorded for the east of the Campeche Bank. In this study, the trophic niche and degree of overlap between these species were analyzed using stable isotopes. To do this, individuals of *O. maya* and *O. americanus* were collected. Differences in isotopic values between species and sexes were evaluated and isotopic niche and niche overlap were calculated. The results indicated that females of *O. americanus* have smaller sizes compared to males of this species and both sexes of *O. maya*, which could be related to a non-reproductive period for *O. americanus*. Regarding the isotopic signatures, more positive carbon values were recorded in the females of *O. maya*, this suggests a displacement between less productive and more productive areas, while *O. americanus* showed more negative values that could indicate that it remains in less productive areas. Both species were positioned at a medium trophic level. Females of *O. maya* showed the largest isotopic niche between the sexes of both species, reflecting the mobility of this group of organisms and the possible variation in food sources. The largest niche overlap was recorded between *O. maya* males and *O. americanus* females, suggesting that these groups largely share food sources. These results show the existence of isotopic overlap between two species for which a separate distribution had been defined due to the effect of depth.

The Persistence of Memory: Behavioral Analysis and Arm Usage of a Nine-Armed *Octopus vulgaris*.

Sam Ellington Soule[1], Miguel Cabanellas-Reboredo[2], Ángel F. González[1], Hidde Juijn[2], Jorge Hernández-Urcera[1]

1 ECOBIOMAR Research Group, Institute of Marine Research (IIM-CSIC); Vigo, Spain.
2 Centro Oceanográfico de Illes Balears (COB-IEO), CSIC. Balearic Islands, Spain.

Abstract

Anatomical abnormalities in octopuses, whose behavior is facilitated by flexible, neuron-rich arms, offer insights into life histories and the neurological implications of understudied conditions such as bifurcation. Although documentation is scarce, we present in situ videos of nine-armed *O. vulgaris* with a functional bifurcated R1 arm. Analysis using RDAs and GLMs investigated the impact of the bifurcated arm on behavior and examined changes during growth. We revealed a differential usage between the bifurcated arms, alongside an initial specialization of the bifurcated arms for actions below the body, which decreased over time for only one of the arms as it grew. Furthermore, bifurcated and regrown arms were utilized more in safe behaviors than in risky ones, with more severely injured arms showing a higher frequency of use in safe behaviors. These findings contribute to the growing knowledge of arm usage in octopuses, suggesting that arm bifurcation may lead to branchial neural differentiation and potentially indicate post-traumatic associations in *O. vulgaris*

Characterizing Ontogenetic Shifts in Cephalopod Venom Gene Expression

Belkes Stambouli[1, 2], Mariam Gelashvilli[1], Alonso Delgado[1, 2], Mande Holford[1, 2, 3]

1 Harvard University, Cambridge, MA, USA
2 Harvard Museum of Comparative Zoology, Cambridge, MA, USA
3 American Museum of Natural History, NYC, NY, USA

Abstract

The distribution of *Octopus maya* and *Octopus americanus* has been described separately, however, geographic overlap has been recorded for the east of the Campeche Bank. In this study, the trophic niche and degree of overlap between these species were analyzed using stable isotopes. To do this, individuals of *O. maya* and *O. americanus* were collected. Differences in isotopic values between species and sexes were evaluated and isotopic niche and niche overlap were calculated. The results indicated that females of *O. americanus* have smaller sizes compared to males of this species and both sexes of *O. maya*, which could be related to a non-reproductive period for *O. americanus*. Regarding the isotopic signatures, more positive carbon values were recorded in the females of *O. maya*, this suggests a displacement between less productive and more productive areas, while *O. americanus* showed more negative values that could indicate that it remains in less productive areas. Both species were positioned at a medium trophic level. Females of *O. maya* showed the largest isotopic niche between the sexes of both species, reflecting the mobility of this group of organisms and the possible variation in food sources. The largest niche overlap was recorded between *O. maya* males and *O. americanus* females, suggesting that these groups largely share food sources. These results show the existence of isotopic overlap between two species for which a separate distribution had been defined due to the effect of depth.

Integrative analysis of digestive physiology, enzymatic function and microbiota in *Octopus maya*

Daisy Pineda-Suazo[1], Wendy Itzel Escobedo-Hinojosa[2], Francisco Guillén-Chable[1], Clara E. Galindo-Sánchez[3], Claudia Caamal-Monsreal[1], Carlos Rosas[1]

1 Multidisciplinary Teaching and Research Unit, Faculty of Sciences UNAM, Puerto de Abrigo s/n Sisal, Mpio, Hunucma, Yucatan, C. P. 97356, Mexico.
2 Chemistry Unit at Sisal, Faculty of Chemistry, National Autonomous University of Mexico, Puerto de Abrigo s/n, 97356 Sisal, Yucatán, Mexico.
3 Department of Marine Biotechnology, Functional Genomics Laboratory, CICESE, Ensenada, Baja California, Mexico.

Abstract

Understanding the digestive physiology of *Octopus maya* is essential for optimizing its culture in sustainable aquaculture systems. This work integrates biochemical, genomic, transcriptomic, microbiological, and *in vitro* approaches to characterize digestive function and identify enzymatic and microbial targets relevant to diet formulation. Enzymatic assays from digestive gland and gastric juice revealed active proteolytic processes and key enzymes involved in protein digestion. Genome- and metatranscriptome-based analyses identified cathepsin families (B, L, F, S, and Z) with conserved motifs and predicted subcellular localizations, suggesting intra- and extracellular roles in proteolysis. Based on this, a five-stage static in vitro digestion model was developed to simulate the digestive tract's physiological progression. The model showed high correlation with in vivo trials for protein, lipid, and cholesterol solubilization, validating its reliability to assess diet digestibility in *O. maya*. Additionally, the cultivable microbiota from the digestive tract was isolated, revealing proteolytic bacterial strains with potential as functional additives. Ongoing transcriptomic analyses aim to identify bacterial enzymes involved in macromolecule hydrolysis. This integrative research enhances understanding of host–microbiome–environment interactions and supports the development of functional diets using fisheries by-products adapted to the species' enzymatic profile. Together, these approaches contribute to the advancement of digestive biology in cephalopods and its application to scalable, community-based aquaculture systems.

Interindividual relationships of a solitary bobtail squid

Sugimoto Chikatoshi[1], Mao Yamada[2], Yuzuru Ikeda[2], Tsubokawa Tatsuya[1]

1 Keio University, Kanagawa, Japan
2 University of the Ryukyus, Okinawa, Japan

Abstract

Bobtail squids are generally solitary species and do not show schooling behavior. In spite of this characteristic, they show sympatric distribution with conspecifics while swimming at night in the field and proximity to neighboring individuals while burying and sitting on the bottom sand in the captive condition. In this study, we investigated the interindividual relationships of a solitary bobtail squid (Eumandya parva) to evaluate the sociality of the “unsocial” species. The bobtail squids were collected from both east and west sides of the mainland of Okinawa to prepare familiar and unfamiliar group. Around 10 immature squids from each side were separately reared in the laboratory. Although the interindividual distance between two squids while burying and sitting on the bottom sand changed with time, some pairs frequently showed the higher proximity than other pairs. In the experiment where a squid from the familiar or unfamiliar group was presented to the squids in the rearing tank through an isolated tank, the distance from each squid to the presented squid from the unfamiliar group became shorter than to the squid from the familiar group. Since the interindividual distance reflects a part of interindividual relationships, the bobtail squid may have a positive demand to interact with conspecifics. We will discuss the possible characteristics of the social interactions of the solitary species besides for the reproductive purposes. We will also discuss possibility of a marking method for the bobtail squid.

Sequential stereotyped body-arm postures underlie egg encapsulation in loliginid squid

Aline Taba[1], Dimas Sakamoto[1], Matheus Vieira[1], Alvaro Migotto[1], José Marian[1]

1 University of São Paulo

Abstract

Fertilization remains an obscure process in squids, which have spermatophore deposition sites separated from the female reproductive tract, and egg capsules with intricate jelly envelopes. Since fertilization probably occurs simultaneously with egg encapsulation, understanding the formation of the egg string should lead to a better understanding of fertilization in squids. To shed light on this topic, we have recorded the spawning behavior of Doryteuthis pleii in captivity. Using high-definition recording (4K UHD), adequate contrast and lighting, and a 70 x 50 x 40 cm aquarium, we recorded 6 females laying 184 egg capsules, totaling 255 minutes of behavior footage. The formation of each egg capsule takes 82s ± 33 s. Six sequential stereotyped body-arm postures underlie the formation of each capsule, i.e.: (I) dorsal arms arching (duration: 11 ± 3 s); (II) head retraction (22 ± 6 s); (III) flattening of the funnel and insertion of its distal tip between the base of the ventral arms (23 ± 7 s); (IV) ventral arms arching (30 ± 4 s); (V) arms expansion due to the presence of a complete capsule (18 ± 6 s); (VI) dropping tentacles followed by egg capsule deposition on the substrate. These data suggest that the egg capsule components (i.e., eggs, oviducal and nidamental secretions) are transferred to the space between the arms, where a considerable part of egg encapsulation occurs. We hypothesize that the distinct arm postures function as transient molds, which would help explain the formation of such a complex egg capsule.

Juvenile giant Pacific octopus, Enteroctopus dofleini, after settlement on the coast of southern Hokkaido

Takahara Hideo[1], Sano Minoru[1]

1 Mariculture Fisheries Research Institute, Hokkaido Research Organization

Abstract

After hatching and spending 2-3 months as a planktonic paralarva, *Enteroctopus dofleini* undergoes settling to a benthic juvenile stage. However, the early life history of settled octopus juveniles is unknown, except for a few records from studies in experimental laboratories or public aquaria. In the Autumn of 2021, a large-scale red tide affected the Pacific southeastern coast of Hokkaido, resulting in a dramatic decline in catches of octopus in this area. Recovery of the stock is urgently sought. To identify signs of recovery of the stock of *E. dofleini* and to investiate early growth, attempts were made to capture early settled octopus juveniles. The survey was conducted from July 2024 to March 2025. Traps were deployed on the seafloor at depths of 20-50 m and collected and replaced every two months. A total of 38 juvenile octopuses were found in the traps and nearby octopus pots. These juveniles ranged in body weight from 1.9 to 241.5 g. The water temperature range was 3.1-18.6°C. Using the collected octopuses, laboratory experiments are under way to understand the effect of water temperature on the growth of juvenile *E. dofleini*.

Comparison of ejaculate traits in three *Idiosepius* species with varied sperm competition mechanisms

Tanabe Ryohei[1, 2], Sugimoto Chikatoshi[3], Chung Wen-Sung[4], Iwata Yoko[5], Nakayama Arata[5], Hosono Shota[5], Sato Noriyosi[6]

1 Institute of Oceanic Research and Development, Tokai University, Shizuoka, Japan
2 School of Science and Technology, Tokai University, Kanagawa, Japan
3 Molecular Genetics Unit, Okinawa Institute of Science and Technology Graduate University, Okinawa, Japan
4 School of the Environment, The University of Queensland, St Lucia, QLD, Australia
5 Atmosphere and Ocean Research Institute, The University of Tokyo, Chiba, Japan
6 Department of Fisheries, School of Marine Science and Technology, Tokai University, Shizuoka, Japan

Abstract

Squid employ diverse and complex sperm storage strategies, such as differences in the number and morphology of sperm storage organs. Some unique cases show that females can selectively influence the fertilization success by discarding transferred sperm from the unwanted males. In this study, a systematic comparison among three species of the pygmy squid reveals insights into their sperm competition mechanisms and ejaculate traits: (1) *Idiosepius paradoxus* -males transfer spermatangia to the female's head surface; females often discard a portion before insemination. (2) *Idiosepius hallami* - males transfer spermatangia onto either the head surface or into a pocket-like structure located at the base of tentacles (spermatangia pocket); females remove those on the head shortly after copulation. (3) *Idiosepius kijimuna* –spermatangia are exclusively transferred into the female's spermatangia pocket; unlike *I. hallami*, females discard spermatangia by picking them up from the pocket with their buccal mass and ingesting them. Apart from behavioral variations, distinct differences are noted in the ejaculate traits, including relative testis size and the number of stored spermatophores (*I. paradoxus* > *I. hallami* > *I. kijimuna*). Differentials in sperm storage patterns may suggest that ecological demands are encoded for reproductive success in the pygmy squid, mediating with divergent sperm competition mechanisms to shape the evolution of ejaculate traits. This study confirms the pronounced interspecific differences in sperm storage patterns and how females respond to sperm removal amongst these three pygmy squid species dwelling in different habitats.

The Impact of Microbial Free Living Thermal Adaptation on Squid Host Recruitment

M. Thieme , D. Vakil, M. Nishiguchi

Abstract

The importance of beneficial bacterial throughout the animal kingdom is a well acknowledged phenomenon that contributes to the overall fitness of the host. This includes cephalopods, and, more specifically, the renowned and experimentally malleable model association between dumpling squids (Cephalopoda: Sepiolidae) and their bioluminescent partner Vibrio. With recent anthropogenic warming inducing stress on individuals and their microbial communities, examining how beneficial bacteria respond to these changes can provide insight into how the holobiont (host + symbiont) responds to a changing climate. The amenability of the sepiolid squid-Vibrio association for laboratory and field experimentation renders it a viable system to measure adaptation to changing temperatures. Four strains of V. fischeri were isolated from the light organs of various species of Euprymna (*E. scolopes*- Hawaii, ES114; *E. tasmanica*- Australia, ETBB1-C; *E. albatrossae*- Philippines, EAS005; and *E. morsei*- Japan, EM17). These symbionts were evolved in culture under three temperature conditions: high (32˚C), mid (25˚C), and low (18˚C) for 2000 generations. Key phenotypic changes were observed in bacterial traits critical to the formation of the symbiosis, namely luminescence. Single and competition infection experiments were completed to compare the colonization proficiency of the experimentally evolved V. fischeri to colonize the host squid against their ancestor; thus, providing a window into the impact of thermal stress on host-symbiotic evolution. Such colonization dynamics can provide insight into the ecological shifts of symbioses in the wake of increasing temperatures.

Shell coiling in the nautilus reflects conserved developmental gene expression patterns

Tochino Nanami[1], Hirota Kazuki[2, 3], Setiamarga Davin[2, 3]

1 Advance School for Ecosystem Engineering, National Institute of Technology (KOSEN), Wakayama College
2 Graduate School of Science, The University of Tokyo
3 National Institute of Technology (KOSEN), Wakayama College

Abstract

Mollusks exhibit a wide diversity of shell morphologies, with coiled shells most commonly found in the class Gastropoda. Genetic studies have shown that development-related transcription factors such as dpp are involved in controlling shell coiling in gastropods. Coiled shells also occur in cephalopods, although most of these, such as ammonites, are extinct. Most extant cephalopods have internalized, demineralized, or completely degenerated shells. The only living species with a true external shell is the nautilus, *Nautilus pompilius*, an early-branching member of the cephalopod lineage. The nautilus has a mineralized, planispiral, equiangular shell and is the only extant cephalopod with an external shell of this form. In this study, we investigated the genetic basis of spiral shell formation in the nautilus and compared it with that of gastropods. We analyzed gene expression using transcriptome data from dorsal and ventral mantle tissues, focusing on 85 Shell Matrix Protein (SMP) genes and several development-related genes involved in morphogenesis. Differential expression was found in 59 SMP genes, including Chitinase, PIF-like, and Tyrosinase-like proteins. In addition, 32 development-related genes, including dpp, RAR, wnt16, and Smad6/7, showed distinct expression patterns. Notably, dpp was highly expressed on the ventral side, where the shell coils more strongly. This resembles the expression pattern in gastropods, where dpp is highly expressed on the right side, corresponding to the direction of coiling. These results suggest that similar genetic mechanisms may control the formation of coiled shells in extinct cephalopods such as ammonites, and in mollusks in general.

Why bobtail squid mate long time - sperm removal or sperm transfer?

Tatsuya Toda [1], Inomata Masashi[1], Kawamura Tomohiko[1], Iwata Yoko[1]

1 The University of Tokyo

Abstract

Females of most cephalopod species mate with multiple males, leading to sperm competition which influences the fertilization success of each male. Cephalopod mating is the delivery of spermatophores. The spermatophores make a spermatophoric reaction to eject and attach spermatangia to the female. How females store sperm varies among species: some species store sperm released from spermatangia in seminal receptacle, and others store spermatangia on specific body parts. Sperm storage modes affect the sperm storage amount and the ease of sperm removal by other males, influencing the strength of sperm competition. Females of bobtail squid have a specialized organ for storing transferred spermatangia (bursa copulatrix). Previous studies reported prolonged copulation and suggested the possibility of sperm removal. However, it remains unclear whether sperm removal actually occurs during the prolonged copulation. Under the prediction that, if sperm removal occurs, it should be done before spermatophore transfer, we observed the mating behavior of *Sepiola birostrata* focusing on the timing of spermatophore transfer. The species conducted prolonged copulation, but no sperm removal behavior was observed. Spermatophore transfer occurred in the early stages of mating, suggesting that prolonged copulation is not for sperm removal but for subsequent processes to the spermatophore transfer. Next, we observed the spermatophoric reaction and found that the mating duration was longer than duration for sperm mass relocation and reaction completion. These findings suggest that the prolonged copulation would be not for sperm removal but to ensure the sperm implantation in the bursa copulatrix by holding females during the spermatophoric reaction.

Differences in habitat use by sex and male reproductive tactics in the spear squid *Heterololigo bleekeri*

Shun Tokioka[1, 2], Yasutoki Shibata[1], Yoji Narimatsu[1], Yonosuke Furukawa[3], Yoko Iwata[2]

1 Japan Fisheries Research and Education Agency
2 Atmosphere and Ocean Research Institute, The University of Tokyo
3 Ibaraki Prefectural Fishery Research Institute

Abstract

Males of the spear squid *Heterololigo bleekeri* exhibit size dimorphism associated with alternative reproductive tactics: large consort males pair with females, while small males (similar in size to females) employ sneaking tactic. Although their distribution expands from offshore waters to shallow spawning grounds (<100 m) during spring spawning season, little is known about how the distribution patterns relate to sex and male tactics. To reveal the habitat use of *H. bleekeri* during the spawning season, we collected catch data from a commercial trawler operating off northeastern Japan from February to April 2020–2023. For each tow, squid were classified into two size groups, large (consort males) and small (females and sneaker males), and the catches of each group were recorded with environmental data (bottom temperature, depth, month, year). GAMs were used to examine the effects of each variable on catch probability and density. Results showed distinct spatial patterns between the two groups. Small squid (females and sneaker males) were frequently caught deeper areas up to 260 m, with a peak density around 160 m. In contrast, large squid (consort males) were frequently found in shallower areas, with a peak density around 80 m. Small squid were frequently found at 9°C, with peak density at 8°C, whereas large squid did not show a clear relationship with the bottom temperature. These findings suggest that the habitat use of *H. bleekeri* varies by sex and male tactics. The temperature-independent but depth-dependent distribution of large males would be associated with territoriality in spawning substrates.

Mothers know best: Blue-lined octopuses (*Hapalochlaena fasciata*) discard weak and foreign eggs

Torin O'Connell[1], Fabio Cortesi[1], Wen-Sung Chung[1]

1 The University of Queensland

Abstract

Female blue-lined octopuses show prolonged maternal care of their eggs (6+ weeks). During maternal care, females protect their egg clutches without feeding and undergo rapid physiological declines, dying of exhaustion once their larvae have hatched. Among 27 brooding females observed, we noted several instances where the deceased eggs were removed from their clutches. To understand the role of chemosensory function during brooding, we recorded the females' behavioural responses when introducing four different types of eggs into their clutches: dummy eggs, their own healthy and deceased eggs, and foreign eggs from another female. The females gathered their own healthy eggs and rejected the dummy eggs instantly, demonstrating their ability to recognise the egg viability using their arms. In contrast, they took minutes examining the deceased eggs and hours examining the foreign eggs before discarding them. This behaviour may suggest that females can detect nuances of chemical cues between their own brood and others, facilitating brood recognition. The ability to assess embryonic health highlights the role of octopus chemotactile sensation in brood success. It also aligns with our neuroanatomical findings that female blue-lined octopuses maintain the integrity of the brain lobes responsible for chemosensory and arm control while most brain regions shrink during brooding.

Species records and identification of cephalopod paralarvae (Incirrata and Myopsida) in the surrounding waters of Taiwan

Wang-Ling Tsai[1], Kuo-Wei Yen[2], Chuan-Wen Ho[1]

1 Department of life sciences, National Chung Hsing University, Taiwan
2 Fisheries Research Institute, MOA, Taiwan

Abstract

The waters surrounding Taiwan host high cephalopod biodiversity, yet records of their early life stages remain scarce. This study investigated the composition and identification of cephalopod paralarvae through seasonal zooplankton sampling conducted from summer 2020 to spring 2022. A total of 1,157 individuals were collected, including 148 belonging to Myopsida and Incirrata. DNA barcoding identified over 3 species of Loliginidae, 17 Octopodidae, 2 Argonautidae, and 1 Tremoctopodidae. Among these, 12 species matched previously recorded adults in Taiwan; the remainder may represent undocumented or cryptic taxa. *Argonauta argo* (16.8%) and *Amphioctopus marginatus* (16.2%) were the most abundant species. Twenty-one species were distinguishable by traits such as sucker morphology, chromatophore patterns, and arm formula. Notably, basal sucker size and dense ventral chromatophores were proposed as diagnostic features of Amphioctopus and Callistoctopus, respectively. These findings provide new morphological data and highlight the need for continued research to resolve unidentified taxa and better understand cephalopod life history in the region.

Revealing the functional hierarchy of the cephalopod visual system

Konstantinos Tsaridis, Tomoyuki Mano, Yutaka Kojima, Thi Thu Van Dinh, Makoto Hiroi, Giovanni Masucci, Teresa Iglesias, Sam Reiter

Okinawa Institute of Science and Technology (OIST) Graduate University, Okinawa, Japan

Abstract

Cephalopods are marine invertebrates which display a range of sophisticated visually dependent behaviors, including hunting, communication, and dynamic camouflage. The cephalopod optic lobe (OL), a large brain region which receives input from the retina superficially, has independently evolved from, and appears to be organized radically different to, the well-characterized visual system of vertebrates. Therefore, through comparative neurobiological investigation we may be able to separate universal principles underlying visual information processing from group-specific features. Classical anatomical work on the OL has recently been supplemented by genetic, transcriptomic, and functional studies. To date, however, we lack a comprehensive description of the functional organization of the OL at cellular resolution. Here, we developed a novel method to head-fix awake juvenile squids (S. lessoniana) and perform two-photon calcium imaging and electrophysiological recordings using high density silicon probes (Neuropixels). Using calcium imaging, we recorded the activity of thousands of neurons across the superficial layers of the OL. Using Neuropixels, we recorded populations of neurons spanning the entirety of the OL volume. To characterize the visual tuning properties of OL neurons, we delivered a variety of visual stimuli spanning the three relevant properties of light:intensity, wavelength, and polarization. We used light-sheet microscopy to map the recorded neurons and their physiological properties to a functional OL atlas. We found that the superficial OL contains neurons organized retinotopically and belonging to a variety of functional subtypes. These include ON neurons, OFF neurons and direction selective neurons, responding in diverse ways to light intensity and polarization stimuli. Downstream in the deeper OL, visual responses become more complex in terms of their responses to light intensity vs polarization, and the interaction between spontaneous activity and visual modulation. LFP activity and single unit recordings revealed that retinotopic organization is preserved throughout the OL, and that receptive fields grow larger with OL depth. Together, these results suggest a functional hierarchy matching the tree-like anatomical organization of the OL. Lastly, we found that OL neurons do not respond to the chromatic stimuli presented at equiluminescent contrast, providing electrophysiological evidence for cephalopod color-blindness. This matched the behavioral sensitivity of these animals, assessed through tracking their neurally controlled skin patterns. Together, our study presents a significant methodological and neurobiological advance in understanding the cephalopod visual system.

Proposed Update IUCN Status of Teuthofauna from Thai Waters

Surangkana Tuanapaya[1], Jaruwat Nabhitabhata[2], Kittichai Tongtherm[3]

1 Reference Collection, Phuket Marine Biological Center, Phuket, Thailand
2 Centre for Marine and Coastal Studies (CEMACS), Universiti Sains Malaysia, Penang, Malaysia
3 Princess Maha Chakri Sirindhorn Natural History Museum, Prince of Songkla University, Hatyai, Thailand

Abstract

According to categories of the IUCN Red List of Threatened Species, five from nine, Endangered (EN), Vulnerable (VU), Near Threatened (NT), Least Concern (LC), and Data Deficient (DD), were assigned to one hundred and nineteen cephalopod species recorded from Thai waters. The number of species in the mentioned status comprised 1, 3, 8, 81, and 26 species, respectively. The status of 76 species were similar to the current assignment in the List, while the status of 43 species had been upgraded in this proposal based on current information and field observation. The status of *Nautilus pompilius* was proposed herein as EN with suspects as an isolated population. Nine sepiids were upgraded including one NT. One sepiolid were upgraded. All three idiosepiids were upgraded as VU due to habitat deterioration. Ten myopsids and eight oegopsids were upgraded. Twelve incirrate octopods were upgraded including seven species as NT. The status of sepiids, sepiolid, teuthiids and octopods were upgraded following the fishery information.

Resistance of East Asian common octopus *Octopus sinensis* in captivity to low water temperature

Ueda Nao[1], Takahara Hideo[1], Kawasaki Takuma[1]

1 Hokkaido Research Organization Mariculture Fisheries Research Institute

Abstract

The northern distribution limit around Japan of the East Asian common octopus (Octopus sinensis) was previously considered to be Aomori side of the Tsugaru Strait. However, in recent years, catches have surged on Hokkaido side of the Tsugaru Strait. In the autumn of 2024, a large number of octopuses have appeared around Muroran, where their distribution had not been previously confirmed. The lethal temperature for the O. sinensis is considered to be below 7℃, and overwintering and reproduction of octopus unknown around Muroran, where winter temperatures drop below 7℃. Therefore, this study aimed to understand the overwintering conditions of *O. sinensis* around Muroran by experiments to low temperatures. The rearing experiment used six octopus caught in the octopus pots around Muroran in December 2024. The octopuses ranged in body weight from 231 to 783 g. The octopus were fed twice a week. Filtered seawater was used for rearing, and the water temperature ranged from 5.3 to 9.0℃. During the rearing, there were 65 days when the water temperature dropped below 7℃. All octopuses died within 96 days. Among them, five octopuses decreased body weight. As a result of this study, it was suggested that O. sinensis has difficulty overwintering around Muroran. However, if winter water temperature increases in the future, they may be able to overwinter and reproduce.

CHROMAS: A Computational Pipeline to Track Chromatophores and Analyze their Dynamics

Johann Ukrow[1, 2], Mathieu Renard[1], Mahyar Moghimi[1], Gilles Laurent[1]

1 Max Planck Institute for Brain Research
2 Hasso Plattner Institute

Abstract

Cephalopod chromatophores are small dermal neuromuscular organs, each consisting of a pigment-containing cell and 10-20 surrounding radial muscles. Their expansions and contractions, controlled and coordinated by the brain, modify the animal's appearance during camouflaging and signaling. Building on tools developed by the Laurent Lab, we present a free and open-source software package to track and analyze chromatophore dynamics from high-resolution videos of behaving cephalopods. This suite of functions, which we call CHROMAS, segments and classifies individual chromatophores, compensates for animal movements and skin deformations, thus enabling precise and parallel measurements of chromatophore dynamics. Furthermore, CHROMAS features semi-automated tracking of chromatophore identity over development as well as novel tools for the analysis of anisotropic chromatophore deformations. A high-resolution tool for analyzing chromatophore deformations during behavior reveals details of their motor control and thus, their likely innervation. By analyzing thousands of chromatophores simultaneously and including statistical and clustering tools, this software allows for the revelation of the complex and distributed nature of the chromatophore motor units. We apply CHROMAS to the skins of the bobtail squid *Euprymna berryi* and the European cuttlefish *Sepia officinalis*, illustrating its performance with species with widely different chromatophore densities and patterning behaviors.

Assessing the Dynamics of European short-fin squid species: Trends and Environmental Forcing

Julio Valeiras[1], Esther Abad[1], Gema Hernandez-Milian[1], Graham J. Pierce[2]

1 Centro Oceanografico de Vigo (IEO-CSIC)
2 Instituto Investigaciones Marinas (IIM-CSIC)

Abstract

Cephalopods are characterized by rapid growth, short life spans, and marked sensitivity to environmental changes, often resulting in high variability in annual population abundance. Understanding the relative roles of environmental variability and fishing pressure is essential for informing effective management and ensuring sustainable exploitation. We assembled long-term data series on landings and survey catches of ommastrephids across the ICES area. Where possible, landings were standardized by fishing effort. Due to taxonomic uncertainties in many datasets, analyses were conducted at the family level. We applied Dynamic Factor Analysis (DFA) as the primary method to identify common trends in short-fin squid abundance across taxa and regions. DFA was complemented with exploratory analyses to assess variation by country, gear type, and data source (commercial vs. research surveys). Seasonal and regional patterns in survey data were also examined. To investigate potential drivers of the observed trends, we tested associations with large-scale and regional environmental variables, including the North Atlantic Oscillation (NAO), sea surface temperature (SST), and chlorophyll-a concentration, as well as with lagged fishery catches. Our findings provide insights into the environmental and anthropogenic factors influencing ommastrephid dynamics, supporting the development of adaptive, ecosystem-based management strategies. POSTER

Exploring the diversity of the buccal mass in the early life of cephalopods

Gabrielle A. R. Regmont[1], Erica A. G. Vidal[1]

1 Cephalopod early-life stages Laboratory. Center of Marine Studies, University of Parana - UFPR. Pontal do Paraná, PR, 83.255-976, Brazil.

Abstract

The buccal mass of cephalopods comprises chitinous beaks (upper and lower jaws) and a radula, which play crucial roles in prey capture and processing. The morphology of these structures varies across life stages and species, reflecting ecological adaptations. However, beak development during early life phases remains poorly understood. This study investigates the developmental patterns of the buccal mass of 21 species across major cephalopod groups — including some first-time records. Using photography and descriptive analysis of primordial beak structures (rostrum, crest, hood and lateral walls), followed by Multifactorial Analysis (MFA), we correlate morphological traits and beak measurements of paralarvae and juveniles to identify developmental trends. Key findings include significant patterns in the upper and lower jaws of cuttlefish and sepiolid juveniles, as well as in the upper beak of Octopodidae paralarvae. Specimens > 5 mm ML of Oegopsida, Myopsida, Idiosepiida, and Octopodidae exhibited dentition and a slit in their lower beaks. Pronounced pigmentation correlated with more developed primordial beak structures, while the radula exhibited species-specific patterns. MFA of upper beaks effectively differentiated cuttlefish, sepiolids, squids, and octopuses based on morphometric and developmental features, whereas lower beak analysis showed less distinct groupings. A correlation was observed between hatching mode and the development of the arm crown-beak complex: hatchlings with rudimentary arm crowns had underdeveloped beaks, while those with well-developed arm crowns exhibited beaks with fully formed primordial structures. Although limited by sample size, this study provides novel insights into the diversification of feeding structures in the early life phases of cephalopods.

First description of the paralarvae of the idiosepiid squid, *Xipholeptos notoides* (Cephalopoda: Idiosepiidae)

Gabrielle A. R. Regmont[1], Marcela Moni[2], Amanda Reid[3], Erica A. G. Vidal[4]

1 Graduate Program in Coastal and Oceanic Systems - UFPR. Pontal do Paraná, PR, 8355-976, Brazil.
2 Cephalopod early-life stages Laboratory. Center of Marine Studies, University of Parana - UFPR. Pontal do Paraná, PR, 83.255-976, Brazil.
3 School of Earth, Atmospheric and Life Sciences, Faculty of Science, Medicine and Health, University of Wollongong, Wollongong, NSW, 2522, Australia
4 Cephalopod early-life stages Laboratory. Center of Marine Studies, University of Parana - UFPR. Pontal do Paraná, PR, 83.255-976, Brazil.

Abstract

This study describes the early life phase of the Australian endemic idiosepiid squid, *Xipholeptos notoides* (Berry, 1921). Despite Australia's rich marine biodiversity, the early life phases and stages of cephalopods remain poorly understood. *Xipholeptos n*otoides is widely distributed across temperate southern Australia and is commonly found in seagrass habitats within bays and inlets. Like other Idiosepiid squids, this species possesses a specialized adhesive organ on the posterior dorsal mantle surface, which facilitates substrate attachment. We document the morphology and chromatophore patterns of paralarvae ranging from 1.0 mm (hatchlings) to 4.0 mm mantle length (ML). Hatchlings exhibit a bell-shaped mantle, laterally attached ear-shaped fins, short arms with biserial suckers, and an arm formula: II>I>III >IV. Tentacles are absent at hatchling, but begin developing as rudimentary buds at approximately 2.6 mm ML. Initially, the tentacles remain enclosed by a membrane before emerging. Paralarvae display dense body pigmentation with many large chromatophores. In the region of the adhesion organ the chromatophores are arranged in parallel. Beak analysis of smaller specimens reveals dentition and a slit in both the upper and lower jaws—features retained throughout the life cycle, suggesting paedomorphosis. These distinct morphological characteristics enable reliable identification of *Xipholeptos notoides* even at the hatchling stage, significantly advancing our understanding of this pygmy squid species.

Habitat utilization and environmental gradients experienced by the Atlantic Brief Squid *Lolliguncula brevis* (Blainville, 1823) near the southern limit of its distribution

Rafael Schroeder[1], Richard Schwarz[1], Erica A G Vidal[2]

1 Laboratório de Estudos Marinhos Aplicados. Escola Politécnica. Universidade do Vale do Itajaí (UNIVALI). Rua Uruguai 458, Centro, 88302-901, Itajaí, Brazil.
2 Cephalopod early-life stages Laboratory. Center of Marine Studies, University of Parana - UFPR. Pontal do Paraná, PR, 83.255-976, Brazil.

Abstract

The Atlantic brief squid *Lolliguncula brevis* is the only cephalopod capable of tolerating salinities below 20 for extended periods. Distributed in the Western Atlantic, it is abundant in estuaries from northern to southern Brazil, yet its ecology and ontogenetic movements remain poorly understood. This study examined its ontogenetic distribution and environmental gradients in the Paranaguá Bay Estuarine Complex (from 2014 to 2016) using quarterly samples collected from 12 sites. A total of 909 individuals were analyzed for size (mantle length, ML) and maturity stage (immature/mature). Juveniles (7–64 mm ML) were predominantly found in the outer estuarine areas (average salinity: 24.5), while adults (12–80 mm ML) were more common in the inner regions (average salinity: 23.2). No squid were caught in areas with average salinities below 13.8. Statistical analyses (DB-RDA, k-means, PERMANOVA) identified three distinct groups: Group 1 (summer): Adults associated with high salinity, turbidity, phosphorus and solar radiation; Group 2 (autumn-winter): Adults linked to high dissolved oxygen, specific sediments (clay, sands) and meteorological factors (wind, pressure); and Group 3 (spring): Juveniles in coastal areas, correlated with high salinity, nitrogen and coarse sands. A Flexible Discriminant Analysis (FDA) confirmed high classification accuracy (96%) in classifying these groups, reflecting seasonal adaptations. The species has a short life cycle (< 4 months), utilizing coastal waters for reproduction and estuaries as nurseries. Its distribution is influenced by salinity, oxygen levels, turbidity and sediment characteristics, indicating distinct habitat preferences at different developmental stages and life cycle phases.

Gut microbiome dynamics of juvenile octopus within its trophic network

Diego G. Vilarnau[1], Fernando Á. Fernández-Álvarez[2], Oscar Escolar[1], Massimo C. Pernice[1], Dolors Vaqué[1], Silvia G. Acinas[1], Roger Villanueva[1], Felipe Coutinho[1]

[1] Institut de Ciències del Mar (ICM-CSIC), Barcelona, Spain.
[2] Centro Oceanográfico de Gijón, Instituto Español de Oceanografía (IEO-CSIC), Gijón, Spain

Abstract

Microbiomes, defined as the communities of microorganisms present in a given environment, are a key aspect of animal biology. Microbial communities associated with several organs present mutualistic interactions essential in maintaining the metabolic and immune homeostasis of the host. The alteration of these communities, known as dysbiosis, can promote opportunistic and pathogenic colonization that can lead to negative effects on the host's health and well-being. In this study, we analysed the gut microbiome of the common octopus (*Octopus vulgaris*) during juvenile and adult phases, together with several species of fauna living alongside the juveniles, including known prey. The octopus microbiome was very variable, and trophic microbiome transmission in juveniles could not be assessed as the communities of prey and non-prey species shared many clades between them and compared with the octopus. Several bacterial taxons dominated compositions when they were present, which might represent opportunistic, and potentially pathogenic, colonizations. This observation was more prevalent in juveniles, probably because of the strong habitat and diet shifts after settlement that alter the bacterial communities that individuals are exposed to. This could be an overlooked factor in this transitional period, influencing the high mortality rates observed under culture conditions.

Exploring sperm cryopreservation and in vitro fertilization for the emerging cephalopod model organism *Euprymna berryi*

Miranda Vogt[1], Jack Koch[2], Emeline Manganella[1], Caroline Albertin[1]

1 Marine Biological Laboratory, Woods Hole, MA
2 Aquatic Germplasm and Genetic Resources Center, Baton Rouge, LA, USA

Abstract

The hummingbird bobtail squid, *Euprymna berryi*, has begun to emerge as a model organism important in fields such as neurobiology, RNA-editing, and animal-bacteria interactions. Mutant lines can be produced and bred year round in culture systems, but maintaining cephalopod lines is labor intensive and requires significant space. Currently there is no protocol for sperm cryopreservation or in vitro fertilization (IVF) for this benthic cephalopod species. Sperm cryopreservation and IVF are important tools for maintaining genetic lines and conducting high-throughput genetic manipulation of embryos and have been invaluable for cementing the utility of model organisms such as zebrafish and Xenopus. Building off sperm cryopreservation techniques such as those for *Illex coindetii* (a pelagic squid), oysters, and model organisms such as Xenopus, we have begun exploring the sperm cryopreservation process for *E. berryi*. We tested different sperm viability extenders, cryoprotectants, cryoprotectant concentrations, equilibration times, and freezing protocols. We show how sperm viability can be assessed for membrane integrity, motility, and the ability to fertilize eggs. As we have limited cultured *E. berryi* individuals, we utilized *Doryteuthis pealeii*, a readily available local squid, to guide and refine sperm experimentation. By modifying the IVF protocol for *D. pealeii* (Crawford 2002), we were able to produce a preliminary IVF protocol for *E. berryi*, though more work is needed to ensure efficiency. Once IVF and sperm cryopreservation procedures are optimized, genetically manipulated lines of *E. berryi* can be stored indefinitely and shared within the growing community.

Consquidering Populations: Evaluating Population Connectivity in Aotearoa New Zealand's Arrow Squids, *Nototodarus sloanii* and *N. gouldi* (family Ommastrephidae)

Connor J. Wallace[1], Heather Braid[1], Kathrin Bolstad[1]

1 AUT Lab for Cephalopod Ecology & Systematics (ALCES), School of Science, Auckland University of Technology

Abstract

Two arrow squid species (family Ommastrephidae, *Nototodarus sloanii* and *N.gouldi*) comprise the largest squid fishery in Aotearoa, New Zealand. This fishery is of substantial economic and sustenance value for people, with an average total annual catch of ~30500 tonnes valued at 140 million dollars (NZD). Aotearoa's arrow squids are also ecologically significant, being consumed by many species including the endangered pakake, New Zealand sea lion (*Phocarctos hookeri*). Although these squids are generally found in distinct geographic areas, the Ministry for Primary Industries (MPI) manages both species under a shared Total Allowable Commercial Catch (TACC) limit. This fishery spans nearly the entirety of Aotearoa's exclusive economic zone (EEZ). This study will be the first to investigate population structure in Aotearoa's arrow squids using simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs). By sequencing specimens from both species throughout their range, haplotype maps, observed and expected heterozygosity, genetic clustering and population differentiation will be determined. These measures will help detect the presence or absence of sub and meta-population dynamics, which will inform sustainable fisheries management. Comparisons will also be made between the utility of SSRs and SNPs as population genetic markers for arrow squids, another first in cephalopod research.

Geographic Distribution, Temperature, and Trophic Level as Multidimensional Drivers of Body Morphological Divergence in Cephalopods

Chao Wang[1], Xinjun Chen[1, 2, 3, 4, 5], Zhou Fang[1, 2, 3, 4, 5]

1 College of Marine living Resource Sciences and Management, Shanghai Ocean University
1, 2, 3, 4, 5 College of Marine living Resource Sciences and Management, Shanghai Ocean University/Key Laboratory of Sustainable Exploitation of Oceanic Fisheries Resources, Ministry of Education/National Engineering Research Center for Oceanic Fisheries/Key Laboratory of Oceanic Fisheries Exploration, Ministry of Agriculture and Rural Affairs/Scientific Observing and Experimental Station of Oceanic Fishery Resources, Ministry of Agriculture and Rural Affairs

Abstract

Cephalopods represent an evolutionarily significant molluscan lineage with marked species-specific adaptations shaped by environmental pressures. Previous cephalopod morpho-ecological studies focus primarily on single traits (e.g., mantle/fin length) versus distribution, with limited species coverage, failing to fully capture body geometry-ecology interactions. This study aims to quantify phylogenetic correlations between ecological and morphological traits in cephalopods, testing whether body shape diversification was driven by ecology. Using nuclear/mitochondrial markers, we built a molecular phylogeny and analyzed 680 species (size) and 483 species (shape), assessing links to distribution, temperature, and trophic level. Results showed that cephalopod body size and shape exhibited moderate phylogenetic signal, whereas ecological traits displayed weaker phylogenetic patterns. Species with broader distributions, wider temperature tolerances, and higher trophic levels had larger mantle lengths. Similarly, wider-ranging and higher-trophic species evolved longer, broader fins. Larger cephalopods (e.g., *Architeuthis dux, Mesonychoteuthis hamiltoni*) exhibited streamlined body shapes with higher hydrodynamic efficiency, whereas smaller cephalopods (e.g., *Sepiola rossiaeformis*) showed non-streamlined morphology. The findings revealed that multiple drivers shaped cephalopod morphology, demonstrating that body size and fin geometry constituted evolutionary adaptations for occupying distinct marine niches. Notably, streamlined large-bodied predators dominated open waters and higher trophic levels, while compact, non-streamlined forms thrived in specialized benthic and coastal environments. These insights advance our understanding of cephalopod morphological divergence and provide empirical support for biogeographic principles and niche theory.

Synapsys polarization projector reveals cuttlefish polarization vision alone enables prey capture.

Camilla Sharkey[1], Lisa Blackwell[2], Robert Scites[2], Larry Pezzaniti[2], Martin F. Wehling[3], Trevor J. Wardill[1]

1 University of Minnesota, EEB Dept, Saint Paul, Minnesota, 55117 USA
2 Polaris Sensor Technologies Inc., Huntsville, AL 35801, US
3 Nature-inspired Team, Sensor and Imaging Sciences Branch, Air Force Research Laboratory, Eglin Air Force Base, FL 32542, US

Abstract

To decipher how invertebrate polarization vision computations drive behaviour new technology is needed. We have developed, Synapsys, a wide-field ultraviolet-visible DMD projection technology that is capable of independently controlling colour, intensity, as well as degree and angle of polarization at high refresh rates (>1 kHz). This Synapsys projector now allows us to provide naturalistic and specifically modified stimuli capable of replicating polarization cues in nature within the spectral range of invertebrate photoreception. Dynamic polarization and intensity stimuli can be spectrally tuned in three wavelength bands, to suit a typical UV-, blue- and green- sensitive visual system. Three projector units delivering video onto polarization-preserving screens provides a 210° wide x 70° high field of view to the animal. The intensity and polarization state of each image pixel is controlled by the grey value of input image frames, resulting in full user control of DoLP and intensity at angles of 0, 45, 90 or 135°, in addition to intensity of three colour bands. We demonstrate the utility of this system for testing polarization vision in adult cuttlefish, *Sepia officinalis*. Preliminary data demonstrates that cuttlefish can use polarization vision alone for prey capture and full analysis should be ready for the October 2025 CIAC meeting.

Diverse musculature layers in 3 octopus species support precise motor control yet lack smooth types.

Trevor Wardill[1], Sarah West[1]

1 University of Minnesota, EEB Dept, Saint Paul, Minnesota, 55117 USA

Abstract

Octopus intrinsic arm musculature is often referred to as entirely obliquely striated muscle. However, only the transverse muscle layer has been systematically investigated for the presence of striated muscle fibers. Because molluscan muscle control can vary greatly depending on the muscle type (i.e. smooth vs striated), a systematic examination of each intrinsic muscle layer of the arm is necessary to understand its neuro-muscular control. Here, we use 2-photon microscopy to determine the presence or absence of striations in each of the eight layers and trabeculae of intrinsic arm muscle in *Octopus bimaculoides* (California two-spot octopus) and *Abdopus aculeatus* ("prickly" octopus). We also use scanning electron microscopy (SEM) to examine the arm of *Octopus bocki* (Bock's pygmy octopus). We confirm for the first time that each intrinsic arm muscle layer from multiple octopus species is obliquely striated. Furthermore, we find that the two layers of longitudinal muscle-- divided by the median oblique layer--exhibit different morphology. This occurs in all three species examined, with significantly lower muscle fiber density in the internal longitudinal layer of *O. bimaculoides* and *A. aculeatus*, suggesting additional functional muscle layers that would give octopuses even greater motor control precision of their arms. We are currently working on understanding the functional role of each muscle layer by developing approaches in behavioral sequencing (biomechanics & timing), pharmacology and electrophysiology methods.

Climate-related habitat variations of Humboldt squid in the eastern equatorial waters

Jian Wen[1], Wei Yu[1, 2, 3, 4], Xinjun Chen[1, 2, 3, 4]

1 College of Marine Living Resource Sciences and Management, Shanghai Ocean University, Shanghai 201306, China
1, 2, 3, 4 College of Marine Living Resource Sciences and Management, Shanghai Ocean University, Shanghai 201306, China/National Engineering Research Center for Oceanic Fisheries, Shanghai Ocean University, Shanghai 201306, China/Key Laboratory of Sustainable Exploitation of Oceanic Fisheries Resources, Ministry of Education, Shanghai Ocean University, Shanghai 201306, China/Key Laboratory of Oceanic Fisheries Exploration, Ministry of Agriculture and Rural Affairs, Shanghai 201306, China

Abstract

An integrated habitat suitability index (HSI) model was developed in this study for Dosidicus gigas in the eastern equatorial waters of the Pacific Ocean to explore climate-related spatial and temporal variability in the habitat distribution pattern based on three crucial environmental variables: sea surface temperature (SST), sea surface salinity (SSS) and chlorophyll-a concentration (Chl-a). Results revealed that the HSI model could accurately predict potential habitats for D. gigas. The habitat suitability varied significantly by month, with highest suitability in April and lowest in March. Besides, from December to May, the longitudinal gravity center of the fishing grounds (LONG) and the HSI overall shifted eastward and the latitudinal gravity center shifted northward then southward. In comparison to the warm ENSO phases in 2019, the cold ENSO phases in 2018 produced increased suitable habitat from December to May, leading to a significantly higher CPUE. Prospective high-quality habitats in 2018 primarily occurred in the western regions, with the exception of December, which resulted in a more westward distribution of LONG from January to May. High-quality habitats moved northward from December to February and southward from March to May 2018, compared to minor latitudinal movement in 2019. It was inferred that annual variations in squid abundance and distribution were largely affected by the SST-related habitat pattern of D. gigas in the eastern equatorial waters. Our findings suggested that D. gigas habitats clearly varied by month and year and were greatly influenced by climate-induced environmental changes.

Relationship between environmental food availability and food choice in *Octopus sinensis* d'Orbigny, 1841

Yamada Mayuko[1], Ono Hiroki[1], Sato Noriyosi[2], Hirohashi Noritaka[3]

1 Marine Biological Station, Shimane University
2 School of Marine Science and Technology, Tokai University
3 Faculty of Life and Environmental Science, Shimane University

Abstract

Octopuses are generalist predators that feed primarily on small crustaceans and bivalves. Although there are many studies on their diet, little is known about their basic food choice. A previous study reported that there were some differences in diet between populations, however, the cause of these differences remains unclear. It is generally accepted that animal feeding behaviors have evolved to maximize their own adaptations for growth. Therefore, the only factor that can influence basic food choice would be the availability of food resources in the environment. However, differences were observed in actual feeding menus among octopus individuals, suggesting that individual prey choice may affect their diet menu in addition to the basic food resource availability. In this study, we elucidated the relationship between food resources and feeding habits using East Asian Common octopus, *Octopus sinensis*. They live in shallow waters and their feeding behaviors can be easily observed. We set up the study site in the shallow-water area along the coast of Oki Island. In this area, we examined items found in the shell mounds near the octopus den openings and their environmental abundance throughout the year. The results showed that octopuses fed on many shellfish that were not collected in the resource abundance survey, and that the species composition did not necessarily match the shellfish fauna in the natural environment.

Migration Process of *Watasenia scintillans* (Firefly Squid) in the Japan Sea Estimated Using Statolith Analysis and Lagrangian Tracer Experiments

Yamaguchi Tadanori[1], Ootani Tetsuya[2], Takikawa Tetsutaro[1], Hirose Naoki[3]

1 Faculty of Fisheries, Nagasaki University
2 Tajima Fisheries Technology Institute, Hyogo Prefectural Technology Center for Agriculture, Forestry and Fisheries
3 Research Institute for Applied Mechanics, Kyushu University

Abstract

The firefly squid (*Watasenia scintillans*) is distributed across the western North Pacific, primarily inhabiting the Japan Sea, the Sea of Okhotsk, and the Pacific coast of Japan. During the day, adults reside at depths of 200 m or more near the seafloor, migrating upward to depths of 50–100 m at night. Their lifespan is approximately 12–13 months for females and 11–12 months for males. In the Japan Sea, spawning primarily occurs from April to June, when females aggregate around the 200 m isobath (shelf break) at the southern edges of the Tsushima Basin, Oki Trough, and Toyama Trough. To investigate the migration process of W. scintillans, we conducted statolith Sr/Ca ratio analysis to estimate empirical water temperatures and performed Lagrangian tracer experiments to simulate migration trajectories. Our findings suggest that firefly squid undergo daily vertical migrations in deep waters, maintaining positions within suitable temperature ranges. Additionally, they undertake an annual migration between nearshore spawning grounds and offshore feeding grounds in the southwestern Japan Sea, driven by ocean currents. Furthermore, the squid are likely to migrate toward nearshore waters in winter and mature, as decreasing excessively warm water temperatures and the persistence of suitable temperatures near the seafloor create favorable conditions for maturation. This may explain the observed pattern of squid reaching maturity in Japanese nearshore waters after winter.

The Japanese blue-lined octopus *Hapalochlaena cf. fasciata* release tetrodotoxin (TTX) from the body surface against predators: an experimental study for verification

Yamate Yuta[1], Takatani Tomohiro[2], Takegaki Takeshi[2]

1 Institute of Integrated Science and Technology
2 Graduate School of Integrated Science and Technology

Abstract

How to avoid predation is one of the most important issues for the survival of organisms, and the possession of toxins is an effective strategy of defense known in many taxa. The Japanese blue-lined octopus *Hapalochlaena cf. fasciata* possesses high levels of tetrodotoxin (TTX), a potent and lethal neurotoxin in its muscles and skin, leading to the hypothesis that TTX serves a defensive function. However, this hypothesis is based on the distribution and levels of TTX within the octopus, and it remains unclear whether TTX is actively secreted in response to the presence of predators. In this study, to examine the possibility of TTX being used for predator defense, experiments were conducted to test whether this species actually secretes TTX out of its body against predators. TTX concentrations were found to decrease in the arms (muscles and skin) of *H. cf. fasciata* following a three-day exposure to a potential predator (the moray eel). Moreover, TTX was detected on the body surface only in the presence of predators. These findings suggest that *H. cf. fasciata* secretes TTX from muscle and skin as a defense measure. Furthermore, TTX secretion could be triggered without direct physical contact with a predator, indicating that this species can detect predators through visual or olfactory cues and actively releases TTX in response.

Impact of water temperature experienced in early life of *Dosidicus gigas* on its adult growth

Dan Yating[1], Liu Bilin[1]

1 College of Marine Living Resources and Management, Shanghai Ocean University, Shanghai 201306, China

Abstract

The climatic variations experienced by cephalopods in their early life have a significant impact on the growth of individuals, with temperature being one of the most important factors. This paper investigated the effects of water temperature under different climatic variations on the growth of *Dosidicus gigas* during early life stages. Samples collected off Peru from 2008 to 2010 were analyzed for mantle length, age structure, mantle lengthbody weight relationship, age at the first sexual maturity, and growth rates of paralarvae and juvenile stages. The results showed that individuals experiencing El Ni˜no events during paralarvae and juvenile stages had smaller adult mantle length, shorter lifespan, and earlier sexual maturity, while those experiencing La Ni˜na events during paralarvae and juvenile stages had larger adult mantle length, longer lifespan, and later sexual maturity. In addition, we found that differences in sample collection locations had a more significant effect on adult age.

The mechanism of cephalopod cannibalism: accidental or intentional?

Kato Yoshiki[1], Hirohashi Noritaka[2]

1 Japan Fisheries Research and Education Agency Marine Fisheries Research and Development Center
2 Shimane University

Abstract

Cannibalism is one of the universal phenomena in many invertebrates that have acquired as a strategy for survival and reproduction. In cephalopods, cannibalism is also observed in wild and its occurrence remains largely obscure. For example, it was reported that approximately 60% of the stomach contents of the South African squid were derived from cannibalism, which may have a significant impact on population dynamics and fishery resources. Cannibalism also occurs frequently in captive environments such as aquariums. Therefore, understanding of the mechanism of the cephalopod cannibalism is an important research subject in the areas of fishery resource and aquaculture managements. Japan Fisheries Research and Education Agency has conducted fishing ground surveys of the squid resources, including Japanese flying squid, neon flying squid, Humboldt squid, New Zealand arrow squid, and Argentine shortfin squid, over the past 30 years. These surveys aimed to develop the effective commercial use of cephalopod resources and efficient fishing methods. The biological data obtained from these surveys were stored in a database by which the feeding behaviors of each species were analyzed. Statistical analysis using a generalized linear model revealed that cannibalism was particularly prevalent in mature females of the Humboldt squid and Neon flying squid. A leading hypothesis predicts that cannibalistic behaviors are triggered accidentally in severe environmental conditions with scarce food resources. In addition to the "unavoidable survival strategy" observed during emergent starvation, our results suggest that the Ommastrephidae may have developed a behavioral trait of "sexual cannibalism" as a reproductive strategy.

First assessment of mercury bioaccumulation in jumbo squid (*Dosidicus gigas*) off Peru

Zhenfang Zhao[1], Guanyu Hu[1], Xinjun Chen[1], Bilin Liu[1]

1 College of Marine Living Resource Sciences and Management, Shanghai Ocean University

Abstract

Jumbo squid, *Dosidicus gigas*, play a crucial role in marine ecosystems and are an important commercial species in the East Pacific. This study investigated the bioaccumulation characteristics of total mercury (THg) in jumbo squid (*Dosidicus gigas*) from the eastern Pacific Ocean, analyzing the influence of factors such as mantle length, sex, and feeding ecology. We analyzed the muscle tissue of 108 squid samples (small, medium, and large-sized groups) collected from June to December 2020. THg concentrations increased with mantle length, but all values remained within Chinese national edible standards. Females in medium and large-sized groups had significantly higher THg concentrations than males. Stable isotope (*δ13C* and *δ15N*) and fatty acid analyses revealed the feeding habits and trophic levels of different groups and their correlation with THg levels. Principal component analysis (PCA) identified body weight, *δ13C*, Eicosapentaenoic Acid, and THg as significant contributors to the first principal component, while Arachidonic Acid contributed to the second. Correlations between THg, *δ13C*, *δ15N*, and the Docosahexaenoic Acid/Eicosatetraenoic Acid ratio varied among groups. These findings highlight the importance of pollutant bioaccumulation research in cephalopods, providing insights into the biogeochemical cycling of pollutants in marine ecosystems. This study not only provides data on mercury levels in the muscles of giant squid, which can help evaluate the health risks of human consumption, but also suggest that future research should focus on the integration of pollutant research with biogeochemical techniques.

Maternal egg caring enhances embryo and newborn juvenile quality in *Amphioctopus fangsiao*

Jian Zheng

Abstract

Octopods with moderate egg size and fecundity evolved in two opposite reproductive strategies to respond to the environmental conditions: the k-strategy with large eggs and low fecundity, and the r-strategy with small eggs and high fecundity. Incirrate octopods with the former strategy have even evolved in extreme patterns: females care for their eggs until death without feeding and leaving their clutch. Egg-laying and egg-caring behavior have major consequences on both females and their offspring, but the effects of this major life transition on embryonic physiological and molecular mechanisms have not been well studied, and whether there is specificity in maternal egg-caring behavior is also unknown. Using *Amphioctopus fangsiao* as a model, this study observed the behavioral changes of the females in post-reproductive, detected the effects of egg caring on offspring quality. The octopus mother tended their eggs by guarding their den and gradually weakened until death. About 24% of females could meet with their post-hatching children. The egg care of octopuses was nonspecific, females might leave the shelter and abandon the egg clusters under stressful circumstances. In addition, egg care has a significant effect on the survival rate and development of the hatchlings. Through morphological and physiological analyses, microbiome, metabolome, and transcriptome sequencing, we found that octopus embryos without maternal caring may suffer from microbial contamination and high external pressure, resulting in oxidative stress and a disorder of many important metabolic pathways. These defense responses might consume much energy, causing the repression of the growth development and reduction of the survival rate of embryos. Our study extends the understanding of octopus egg caring, demonstrating additional empirical evidence for the role of benefit-cost tradeoff in determining the evolution of parental care behavior, and providing new insights towards knowing the differential reproductive strategy across different animal taxa in cephalopods.

Morphomics in cephalopod research: a direct comparison of two high-resolution 3D imaging techniques

Alexander Ziegler[1]

1 Bonner Institut für Organismische Biologie, Rheinische Friedrich-Wilhelms-Universität

Abstract

The application of three-dimensional (3D) imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) in biology - an approach termed morphomics - permits the study of whole cephalopod specimens in silico. In recent years, MRI and CT datasets have increasingly been used to describe new species, analyze organ systems non-invasively, and conduct digital volumetric analyses both in fossil and extant cephalopods. In the present study, key differences between two datasets of an exemplary cephalopod specimen (Octopoda: Cirrata: *Insigniteuthis bruuni*) gathered using preclinical 3D high-resolution MRI and CT are presented. The properties of each technique with regard to soft and hard part visualization are shown, with particular focus being placed on differences in achievable isotropic voxel resolution (and thus differentiable structural elements) as well as the application of contrast agents. Furthermore, approaches to and differences in visualization of selected organ systems are outlined and compared to traditional, invasive techniques such as dissection, histology or scanning electron microscopy. Finally, the value of the acquired datasets is put into perspective with regard to recent advances in taxonomy and systematics as well as in digital morphology and dataset transparency.

First report of vibriosis in oval squid

Lucia Zifcakova[1], Mehmet Arif Zoral[1], Zdenek Lajbner[1], Shinichi Nakamura[2], Masami Ueno[3], Nobuto Hayashimoto[3], Mayumi Kawamitsu[1], Giovanni D. Masucci[1], Nana Arakaki[1], Chen Chi Wang[1], Jonathan Miller[1]

1 Okinawa Institute of Science and Technology Graduate University
2 Laboratory of Veterinary Pathology, Faculty of Veterinary Medicine, Okayama University of Science (OUS)
3 ICLAS Monitoring Center, CIEA

Abstract

Sepioteuthis lessoniana species complex of oval squids harbors emerging model organisms for neuroscience, behavioral sciences, and also for commercial aquaculture. There is limited literature available on the pathogens and diseases that may affect squid in intensive aquaculture, and therefore further research is required. Our study identified 62 Vibrionaceae species using culture/DNA and RNA identification methods. *Vibrio harveyi* and *V. chagasii* were two most abundant pathogens in unhealthy oval squids. Vibrionaceae bacterial species infected oval squid simultaneously, while increasing morbidity and mortality in oval squid stocks at our experimental facility. The vibriosis symptoms included lethargy, loss of appetite, cachexia, skin erosions, vision loss, and “white arms”. Our histopathology findings showed that the epidermis of the abnormal specimen's arms bearded eosinophilic exudate and increased mucus secretion, which is evidence of the host’s innate immune response against vibriosis. Further examination of the fin epidermis in unhealthy specimens showed it was partially detached and ulcerated. The necrotic liver and kidney manifestations observed in infected animals indicated hepatic and renal damage. These findings will serve as a basis for future research aimed at understanding the negative effects of vibrio-related diseases on the overall health of cephalopods.

Morphologic and genetic variability in the family Gonatidae (Cephalopoda: Teuthida)

Anna Zolotova[1], Oleg Katugin[2], Yuri Kartavtsev[1]

1 A.V. Zhirmunsky National Scientific Center of Marine Biology Far Eastern Branch, Russian Academy of Sciences (NSCMB FEB RAS), Vladivostok, Primorskij kraj, Russia
2 Pacific Branch of Russian Federal Institute of Fisheries and Oceanography (TINRO), Vladivostok, Primorskij kraj, Russia

Abstract

Morphometric and molecular analyses of 12 species of the family Gonatidae from the Sea of Japan, Sea of Okhotsk, Bering Sea, Northwestern Pacific Ocean and Northwestern Atlantic Ocean suggested intrafamilial patterns and existence of cryptic species. Indices of fin length and width, funnel cartilage width, and arms widths appeared statistically significant characters that discriminate between species, based on multivariate analysis. Sequences from three mitochondrial (cytochrome c oxidase subunit I, 16S rRNA, and 12S rRNA) and two nuclear (18S and 28S) genes supported subdivision of the family into two distinct clades characterized by differences in the number of radular rows (five versus seven). Molecular evidence also suggested that two nominal widely accepted genera Gonatus and Gonatopsis form a single multi-species assemblage on the Gonatidae family tree, and may, therefore, constitute one polymorphic genus. Both genetic and morphologic differences were found between large-sized and small-sized Boreoteuthis borealis. Genetic analysis revealed species differences among squid morphologically identified as Gonatus berryi. Two genetically different groups of Gonatus pyros have been revealed, and further studies are needed to decide whether the observed differences are taxonomic.

Movements without joints, Octopus as inspiration to robotics

Letizia Zullo[1, 2], Orel Benita[3, 4], Beatrice Pistolato[1, 5], Janina Röckner[1, 5], Nir Nesher[3], Tal Shomrat[3], Benny Hochner[4]

1 Istituto Italiano di Tecnologia, Genova, Italy
2 Irccs Ospedale Policlinico San Martino, Genova, Italy
3 Ruppin Academic Center, Michmoret, Israel
4 Hebrew University, Jerusalem, Israel
5 Universita Degli Studi di Genova, Genova, Italy

Abstract

Octopuses are one of the major representatives of the Cephalopod class grouping the most complex marine invertebrates. Cephalopods diverged from vertebrates more than 500 mya, however they occupied the same environmental niche and had to compete with them for survival. This selective pressure is considered one of the driving forces for the development of a complex nervous system (NS) made up of a centralized brain and a distributed NS of the arm counting 2/3 of the total amount of neurons in the body.

Octopuses are animals capable of large variety of behaviors. Complex motions are produced through the integration of central motor commands and sensory feedback coming from the arms. However, octopus arms can also autonomously produce very fast stereotypical and reflex motions whose execution does not require a brain-centered feedback control mechanism. A question remains on how octopuses are able to control their arms with theoretically infinite degrees of freedom even in the absence of a point-to-point representation of body parts in the higher sensory-motor areas. I will showcase examples of solutions that octopuses developed to cope with the problem of body redundancy in motion, from lower to higher level of motor control. We will see how the octopus's brain and body co-evolved to allow for simple but unique solutions to an invertebrate. I will show how these solutions are of a great inspiration for soft-robotics and may reduce the computation load needed to control hyper-redundant soft structures.