

THE BOOK OF POSTERS



Cephalopod Science from Biology to Welfare

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Cephalopod Science from Biology to Welfare

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Cladistic analyses of cuttlefishes (family Sepiidae): How informative are morphometrics?

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Background

Reconstructing the sequence of events that has led to extensive speciation in many regions of the world remains one of the biggest frontiers for exploration in marine evolutionary science. To tackle these key questions, we have examined the evolutionary history of a well known group of molluscs: the cuttlefishes, family Sepiidae. The family comprises three genera: *Sepia*, *Metasepia* and *Sepiella*. Khromov [1], however, also recognised the following subgenera – *Acanthosepion*, *Anomalosepia*, *Doratosepion*, *Hemisepius*, *Rhombosepion* and *Sepia* – based mostly on discrete morphological characters of the cuttlebone and of soft parts.

The overall aim of this project is to ¹construct a comprehensive multi-gene molecular phylogeny of the group that explains sepiid radiation. We will then ²map morphological characters onto it to further understand development and evolution within the group. ³We will also construct a morphological "super tree", based on both discrete and continuous data, and look for potential overlap with molecular phylogeny to discuss reliability of morphology in cladistics.

Here, we present results from our analyses of morphological continuous data, and thus provide the first step and a framework for a long overdue revised classification of this diverse group.



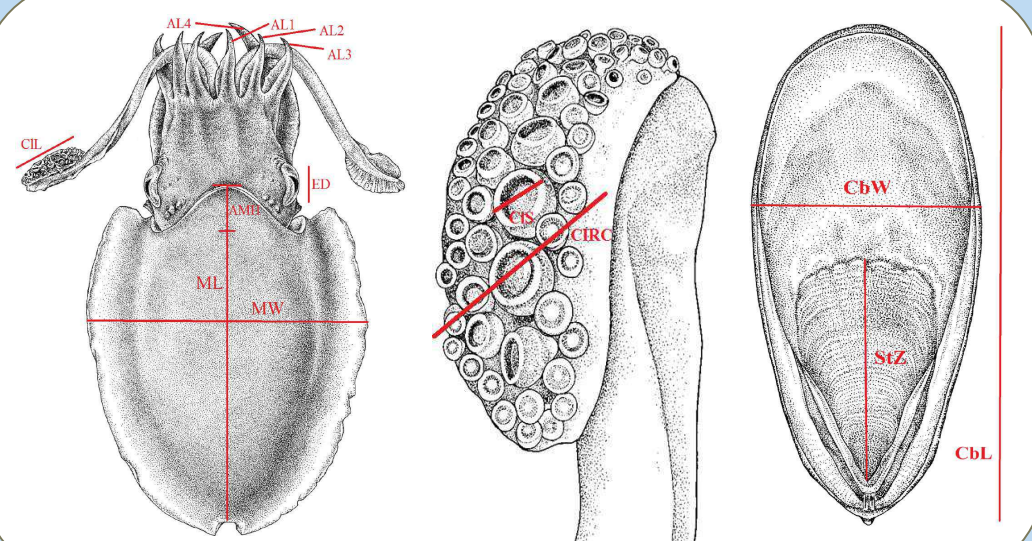
Questions

We incorporated morphometrics, statistical and phylogenetic analyses to test the following questions:

Q1: Are phylogenetic trees, based on continuous data, reliable in informing us of evolutionary relationships?

Q2: Do analyses of continuous data detect existing sepiid genera, and Khromov's subgenera?

Morphometrics



Measured parameters: Mantle Length (ML), Mantle Width (MW), Anterior Mantle to Head length (AMH), Funnel Length (FuL), Eye Diameter (ED), Arm Length 1-4 (AL 1-4), Arm Sucker diameter 1-4 (AS 1-4), Club Length (CIL), Club Sucker diameter (CIS), Cuttlebone Length (CbL), Cuttlebone Width (CbW), Striated Zone length (StZ), Club Row Count (CIRC)

Analyses

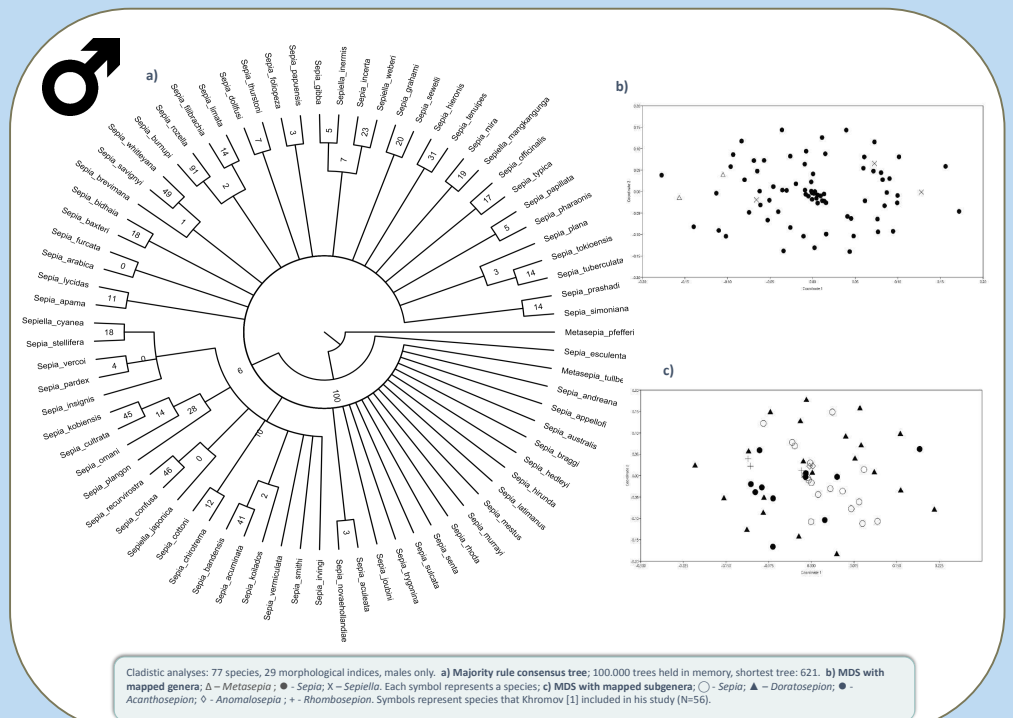
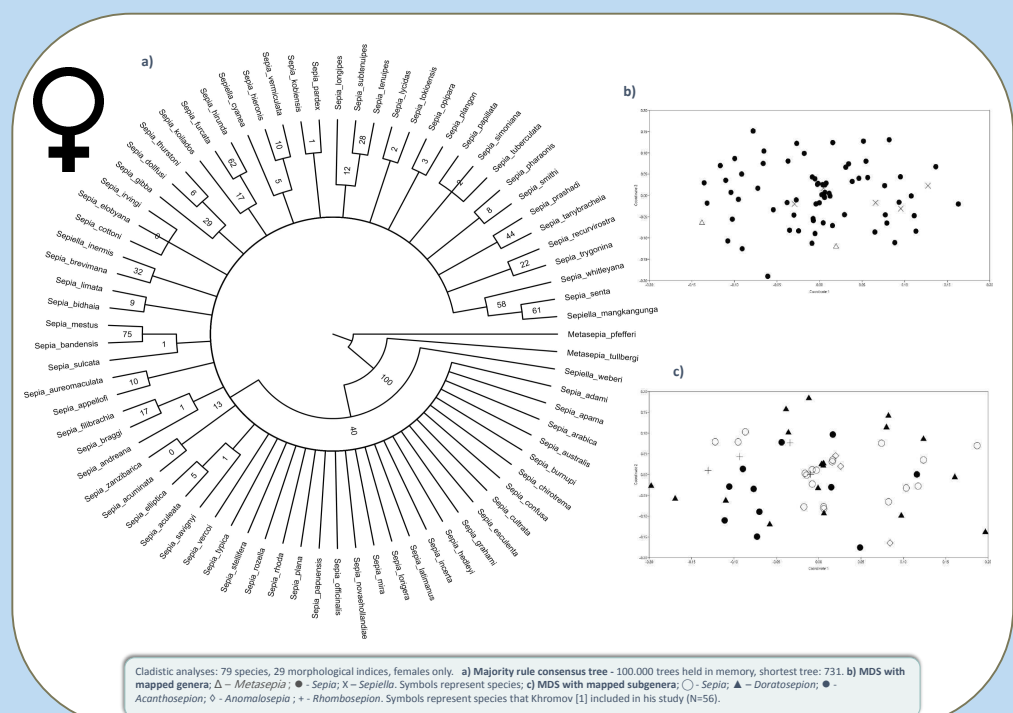
From raw data, we calculated indices and used K-Means clustering in XLSTAT [2] to find natural breaks in individual index ranges. We analysed this matrix in TNT [3] and produced a variety of phylogenetic trees (strict consensus & majority rule consensus of all indices/only those with good elbows).

Using PAST [4], we constructed sex-specific multidimensional scaling (MDS) plots and mapped existing sepiid genera and subgenera.

Results

1) Phylogenetic trees based on continuous data alone seem to contain some relevant information, but fail to resolve deeper phylogeny. For example, some sister-taxon combinations show high bootstrap support throughout different trees, while others lose resolution when analysed differently (e.g. when omitting indices with less well-defined elbows).

2) Our analyses failed to detect clustering of species that belong to same currently accepted genera, or Khromov's subgenera [1]. This might be the result of poor overlap between our and Khromov's dataset. The latter is mostly composed of discrete characters, with special focus on cuttlebone morphology. Here, we examined and quantified a wider array of both hard and soft parts.



References

- [1] Khromov, D. N. (1987). System and phylogeny of the cuttlefish family Sepiidae (Cephalopoda). Zoologicheskyy Zhurnal, 66(8), 1164-1176; [2] Addinsoft, S. (2013). 2013: XLSTAT Software; [3] Goloboff, P. A., & Catalano, S. A. (2016) TNT version 1.5, including a full implementation of phylogenetic morphometrics. Cladistics, 32(3), 221-238; [4] Hammer, Ø. (2016). PAST: PAleontological Statistics software package, v3. 06.

Introduction

The *Octopus cf. vulgaris* (Cuvier, 1797) is a cosmopolitan species with unknown distribution limits. Recently, studies including taxonomic and molecular analysis show that the Brazilian *O. vulgaris* is a cryptic species of this complex and therefore must be redescribed. In order to understand the biological and ecological aspects of a Brazilian *O. cf. vulgaris* populations in Southern Brazil, we evaluated the habitat and the spatial and temporal distribution at shallow waters around a continental island. This species is the main target of artisanal and industrial octopus fisheries at this region.

Materials and methods

The *O. cf. vulgaris* was studied around the coast of Florianópolis Island, southern Brazil (Fig.1). Data were collected at 7 sampling areas in five periods: Sep/Nov 2014, Jan 2015, Mar/Jul 2015, Jan 2016 and Jan 2017.

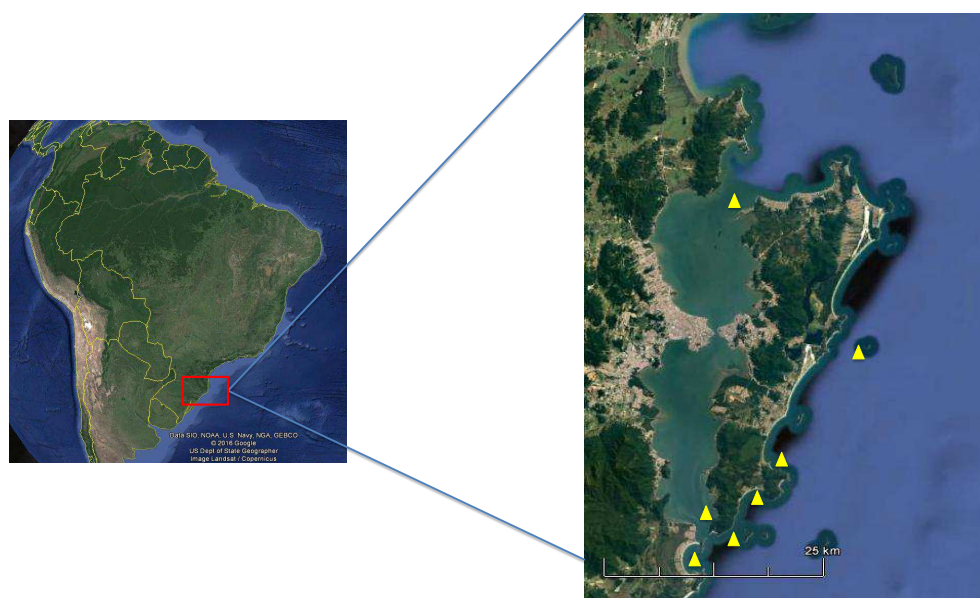


Figure 1. Area of the study around the coast of Florianópolis Island, Southern Brazil (27° 35' 48" S; 48° 32' 57" W). Legend: ▲ indicate the sampled areas.

Two techniques was used: visual census by free and scuba diving to register octopuses in the natural environment (Fig. 2A) and sampling using a longline pot fishery (Fig. 2B). The type of substrate and depth were recorded at 0-5, 5-10 and 10-15 m. The water temperature ranged from 19-24 °C and salinity around 35 ppt. Size, sex and stage of macroscopic maturity of the individuals were recorded.



Figure 2A. *Octopus cf. vulgaris* registered by free diving in Florianópolis Island.



Figure 2B. *Octopus cf. vulgaris* captured using longline pots in Florianópolis Island, southern Brazil

Results and discussion

Overall, 83 octopuses were recorded (P=19, M=27, G=37) and the sex ratio was 1M:1F. The larger specimens were found in winter with 135-175 mm ML ($160 \pm 21,8$); following by specimens with 92-188 mm ML ($135 \pm 25,4$) in autumn, 105-174 mm ML ($128 \pm 24,2$) in spring and 131-138 mm ML ($135 \pm 3,2$) in summer. From the 70 specimens assessed for the maturity stage, the highest % of mature males and females were found in winter (M = 77%, F = 23%) and in autumn (M = 79%, F = 21%), while the immature individuals were found mainly in autumn 64% and in spring 27%, specially females (Fig. 3).

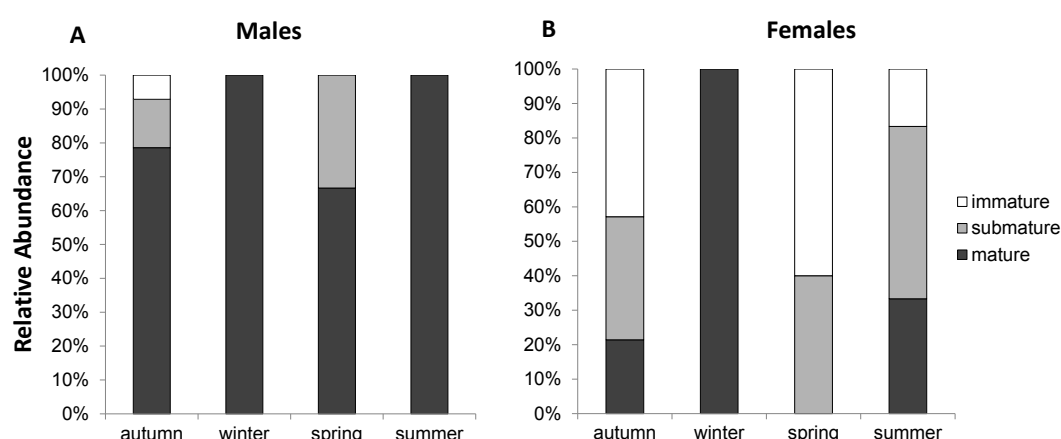


Figure 3. Abundance of males (A) and females (B) of *Octopus cf. vulgaris* in different maturity stages from Florianópolis Island.

Octopuses were found in sandy bottom, interface between rock and sand, between rocks and in soft bottom and were more abundant in sandy bottom for all size classes (Fig 4). The greater abundance were recorded at 5-10m (n=64) and less abundance at 0-5m (n=13) and 10-15m (n=6). When compared to *O. vulgaris sensu stricto*, the *Octopus cf. vulgaris* in Florianópolis coast occurs in slightly warmer waters, what could accelerate the development and growth.

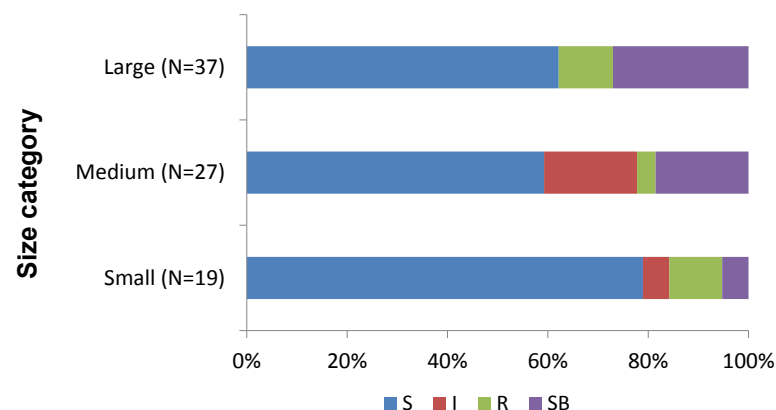


Figure 4. Occurrence of *Octopus cf. vulgaris* size categories across four bottom types. Legend: S=sandy bottom; I=Interface between rock and sand; R=between rocks and SB=soft bottom.

Conclusion

The peak of reproduction in this region occurs during the winter and not during the spring as observed for *O. vulgaris sensu stricto*, probably due to the milder temperatures in this season found in southern Brazil. The results points the spawning period correspond to end of winter and beginning of spring once the greater number of juveniles was found in spring and summer.

Financial support

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IS THERE ANY REASON WHY OCTOPUSES GO BORING OR NOT THE SHELL OF A BIVALVE?

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INTRODUCTION

Many species of benthic octopuses perform perforations on the shells and exoskeletons of their prey through the combined action of the radula (chitinous structure for scraping, typical of molluscs) and saliva. In the laboratory, it has been observed that the octopus accesses the bivalve muscle basically in three ways: pulling both shells, breaking those that have a thin shell and drilling with the radula those that can not be opened by the first two ways. In this sense, it has been found that *Enteroctopus dofleini* in captivity perforates only those shells that it can not open, because they have a greater closing force. The closure of the valves is performed by one, two (and in some species up to three) adductor muscles that are in the internal region of each valve leaving a well marked scar.

In this study, analysis was done to determine the possible relationship between octopus size and prey size, as well as to determine the factors that influence shell boring.

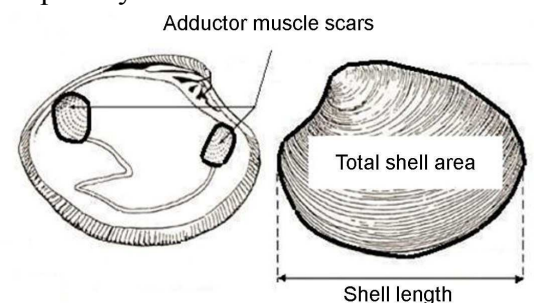
Predator / prey sizes. The length of the octopus mantle and the length of the shell (SL in cm) of the most common bivalves found in the accumulations outside their respective shelters were measured. The octopuses were grouped into three length intervals and the shells were in 5 size intervals, in the 1-12 cm LC range. With both measurements the frequency percentage was obtained by intervals of shell size preferred by small, medium and large octopuses.

Percentage of perforation in relation to the size of the shell. The percentage of perforation was calculated for each of the most important species in the diet, considering the perforated and non-perforated shells of each species.

Relationship between the percentage of perforation of the shells and the size of the octopus. From the frequency of shells with perforations consumed by the small, medium and large octopuses.

Relationship between the percentage of perforation of the shells and the relative area of the muscle in the most important bivalve-prey. As an indicator of the closing force, the adductor muscle relative area was obtained as the ratio between the area occupied by the abductor muscles and the total area of the shell. Photos of the right valve (13 to 20 individuals per species and of different sizes) were taken and the areas of the shell and the scars of the adductor muscles were measured using Image Scan Pro software.

Statistical Analysis. An ANOVA analysis was applied to find possible specific differences of the ARM among the bivalve species, followed by a posteriori Tukey test.

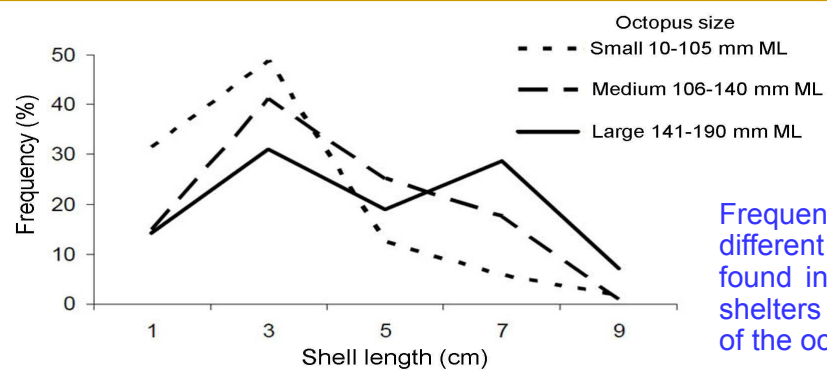


RESULTS

A total of 575 bivalve shells was collected from the accumulations of hard remains of the refuges, and were distributed in 29 taxa.

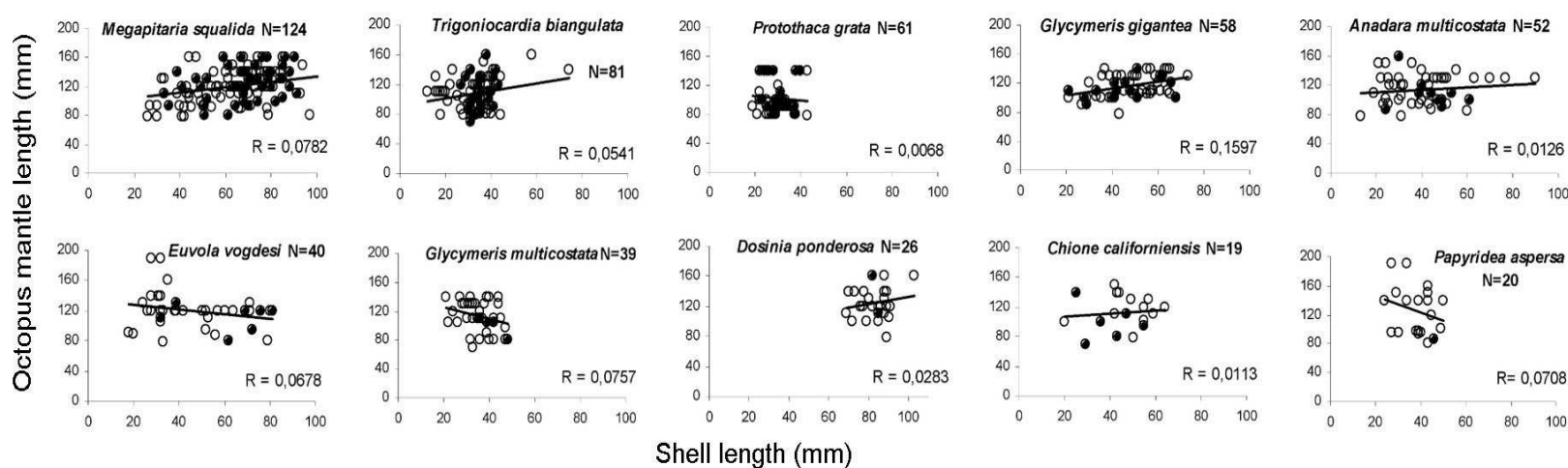
Predator / prey sizes. Small, medium and large octopuses consume bivalves of all sizes, however, small were concentrated in 3-5 cm SL bivalves; being the most frequent length interval in the diet of all.

No significant relationship was found between the sizes of predatory octopuses and that of the bivalve shells found in their shelters ($r = 0.06$, $P > 0.05$)



Frequency of occurrence of different sizes of bivalve shells found in *Octopus bimaculatus* shelters according to the size of the octopus.

Percentage of perforation in relation to the size of the shell. No significant relationship ($r = 0.06$ to 0.1 , $P > 0.05$) was found between the sizes of predatory octopuses and shells in any of the 10 species.



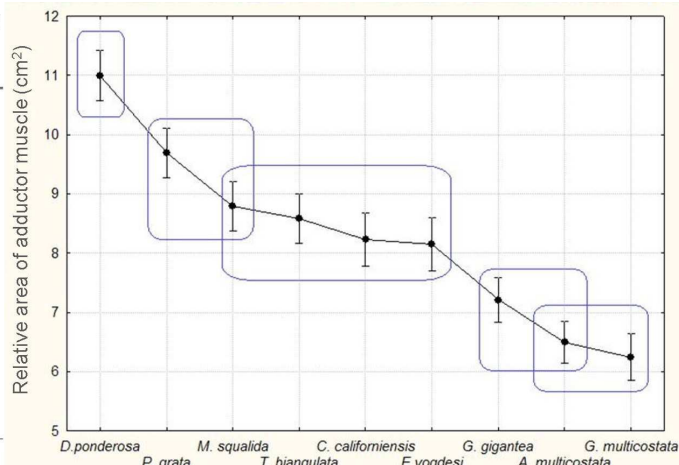
Shell / predator size ratio of the ten most consumed bivalves by *Octopus bimaculatus* in Bahía de Los Angeles, B.C., Mexico.

- perforated shells
- non-perforated shells

Relationship between the percentage of perforation of the shells and the size of the octopus. Octopuses of all sizes drill in a proportion of about 40% the shells of their bivalve prey.

By obtaining the relative adductor muscles (ARM) area for the 10 most consumed bivalves, practically the same order of species found was obtained for the percentage of perforation. The ARM of *P. aspersa* was not calculated due to the morphology of the species.

| Prey species | Boring percentage | Total N | Perforated shells N | Relative area of adductor muscle |
|---------------------------------|-------------------|---------|---------------------|----------------------------------|
| <i>Dosinia ponderosa</i> | 92.3 | 26 | 14 | 10.98 |
| <i>Protothaca grata</i> | 60.7 | 61 | 15 | 9.69 |
| <i>Megapitaria squalida</i> | 33.3 | 81 | 15 | 8.79 |
| <i>Trigonocardia biangulata</i> | 37.9 | 124 | 15 | 8.59 |
| <i>Chione californiensis</i> | 31.6 | 19 | 13 | 8.27 |
| <i>Euvola vogdesi</i> | 27.6 | 58 | 18 | 7.21 |
| <i>Glycymeris gigantea</i> | 17.5 | 40 | 13 | 6.51 |
| <i>Anadara multicostata</i> | 19.2 | 52 | 21 | 6.50 |
| <i>Glycymeris multicostata</i> | 12.8 | 39 | 17 | 6.24 |
| <i>Papyridea aspersa</i> | 5 | 20 | — | — |



Mean and standard deviation of relative muscle areas for the nine most consumed species of bivalves by *O. bimaculatus* in Bahía de Los Angeles, B.C., Mexico. The rectangles show the affinity groups according to the differences provided by the ANOVA.

CONCLUSIONS

The octopus prefers to force bivalves rather than perforate them. Although it means less physical effort, it can expose them more to predation and can mean a lot of time if the reward is poor (small size).

It was useful to evaluate the proportion of the mark of the adductor muscle with respect to the shell of its bivalve preys, since it is the muscle responsible for keeping the valves closed, so it can be a useful tool to know the preference that can have towards certain Bivalve species. However, there are still other factors that could favor consumption on certain bivalve species, such as thickness and roughness of the shell and the affordability of the prey (accessibility).



INTRODUCTION

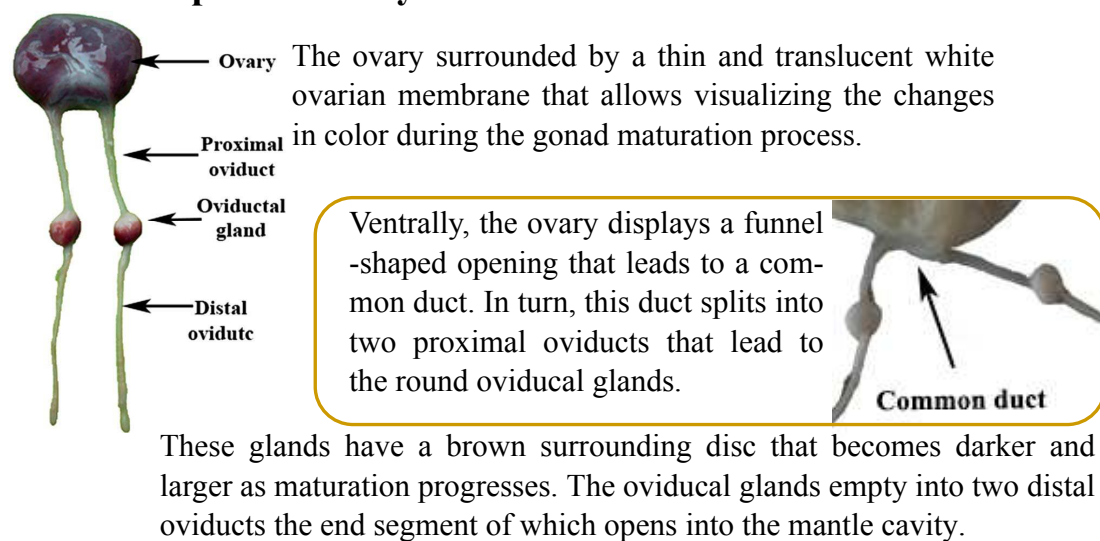
Octopus hubbsorum is a commercially important species from the Mexican Pacific, but its reproductive aspects have been scarcely studied. Several studies have investigated the macroscopic and histological features of the reproductive system of octopods, focusing primarily on the ovary and its maturation process, but detailed histological descriptions of the reproductive system in both sexes are not available.

In this study, we provide morphological and histological descriptions of male and female reproductive systems in the octopus *Octopus hubbsorum*.

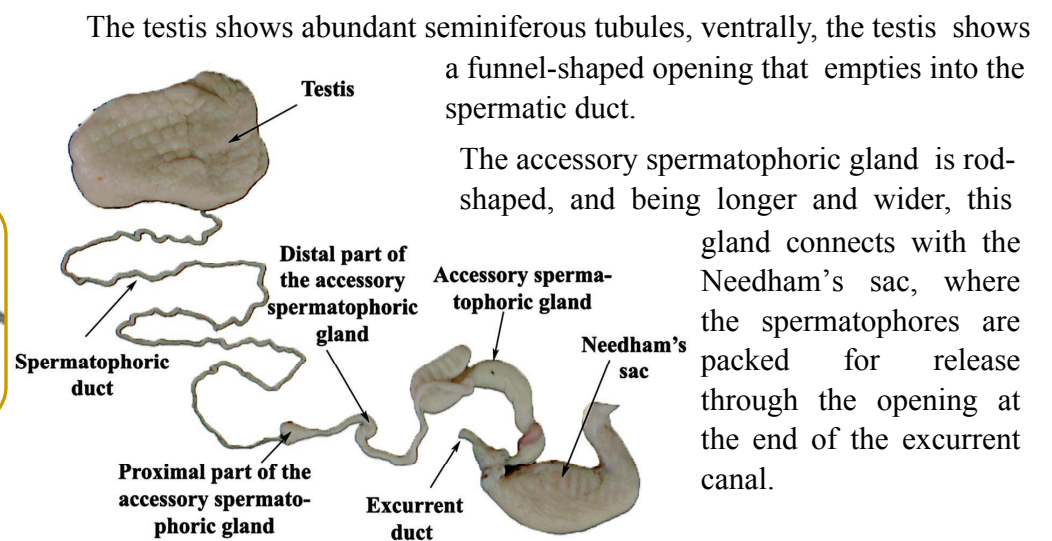
RESULTS

MORPHOLOGICAL DESCRIPTION

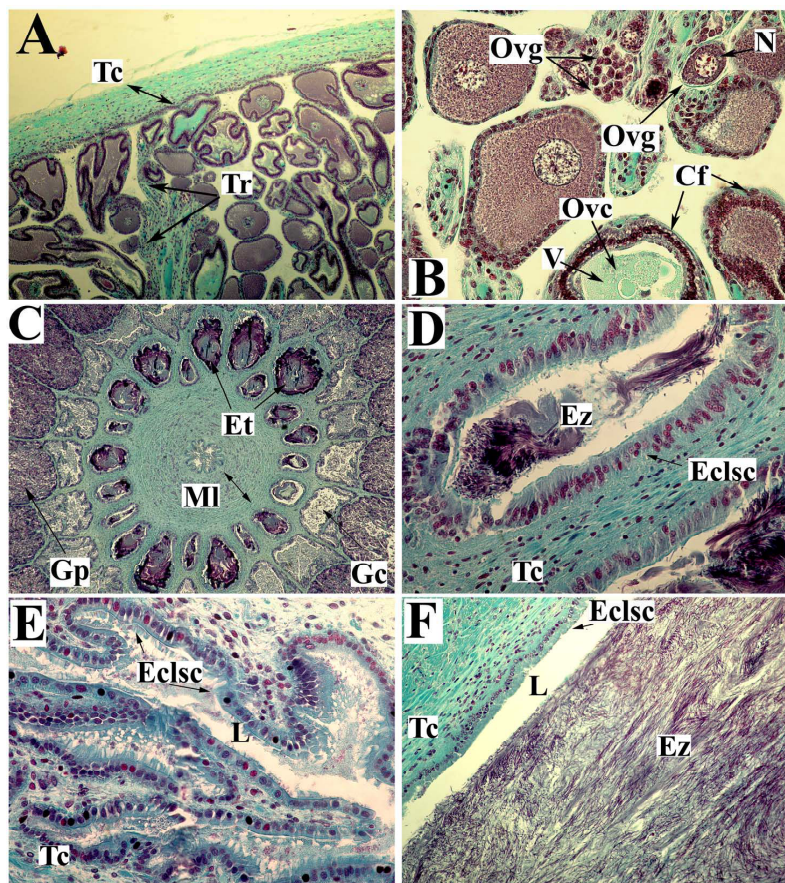
Female reproductive system



Male reproductive system

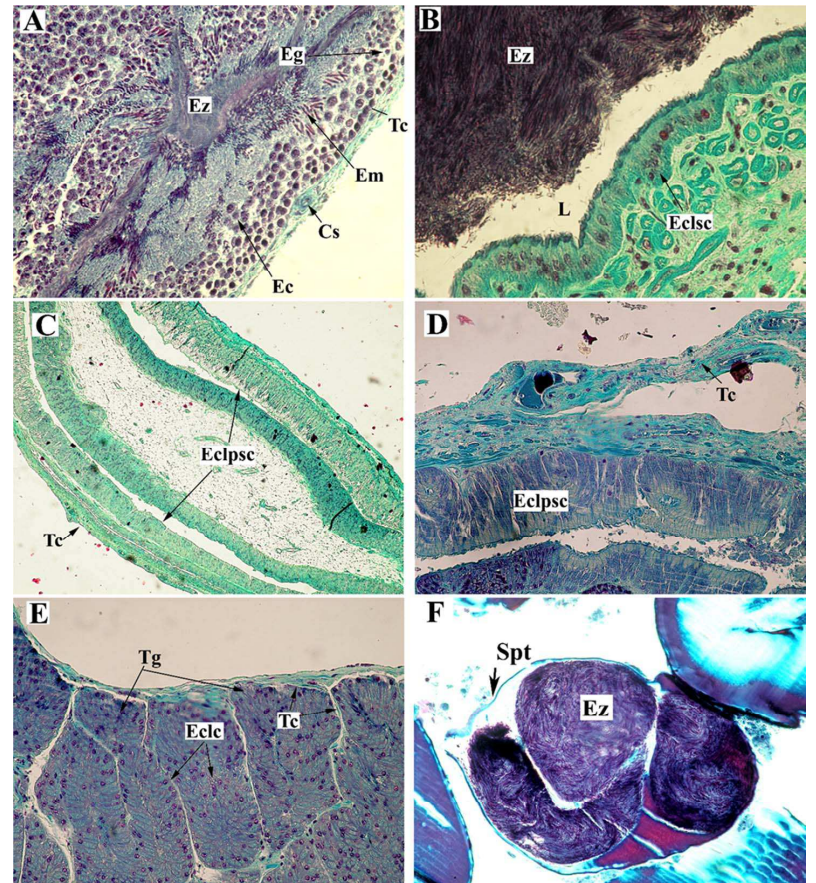


HISTOLOGICAL DESCRIPTION



Micrographs of the female reproductive system of *O. hubbsorum*. A, B) Developing ovary; C) Oviductal gland cross section; D) Spermatheca, Cross section; E) Longitudinal section of a proximal oviduct; F) Detail of the wall of the distal oviduct.

Abbreviations: Cf, Follicle cells; Eclsc, ciliated simple columnar epithelium; Et, spermatheca; Ez, spermatozoa; Gc, central gland; Gp, peripheral gland; L, lumen; MI, smooth muscle; N, Nucleus; Ovc, Oocyte; Ovg, oogonia; Tc, Connective tissue; Tr, Trabeculae; V, vitellum.



Micrographs of the male reproductive system of *O. hubbsorum*. A) Detail of seminiferous tubules; B) Detail of highly compacted spermatozoa of the spermatophoric duct; C, D) The proximal and distal regions of the accessory spermatophoric gland; E) The accessory spermatophoric gland; F) the Needham's sac.

Abbreviations: Cs, capillary; Ec, spermatocytes; Eclpsc, pseudostratified ciliated columnar epithelium; Eclc, ciliated columnar epithelium; Eclsc, simple, ciliated columnar epithelium; Eg, spermatogonia; Em, spermatids; Ez, spermatozoa; L, lumen; Spt, spermatophore; Tc, connective tissue; Tg, glandular tissue.

CONCLUSIONS

The anatomy of the reproductive system of *O. hubbsorum* fully corresponds to the classic model for the genus *Octopus*. The histological characteristics of the female reproductive systems shows spermathecae in the oviductal glands lined by simple ciliated columnar epithelium; as storage progresses, the spermathecae enlarge. Besides, it has two different glandular tissues which might supply secretions to coat or bind eggs. The male reproductive system of *O. hubbsorum* revealed differences in the epithelium vs. other octopus species, as it consists of a columnar epithelium whereas in *O. insularis* and *O. bimaculoides* it is made of cubic epithelium. The Needham's sac has a pseudostratified ciliated columnar epithelium that surrounds and nurtures spermatophores.



Predator recognition in cuttlefish embryo

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¹: Human and animal ethology laboratory (EthoS)– University of Caen (France)

²: Institute of Systems Neuroscience - National Tsing Hua University (Taiwan)

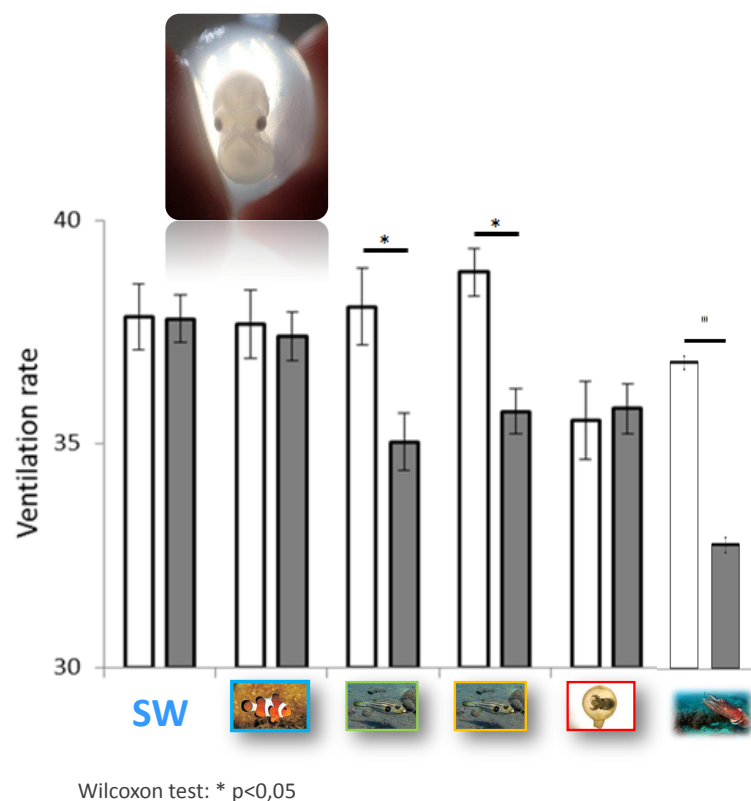
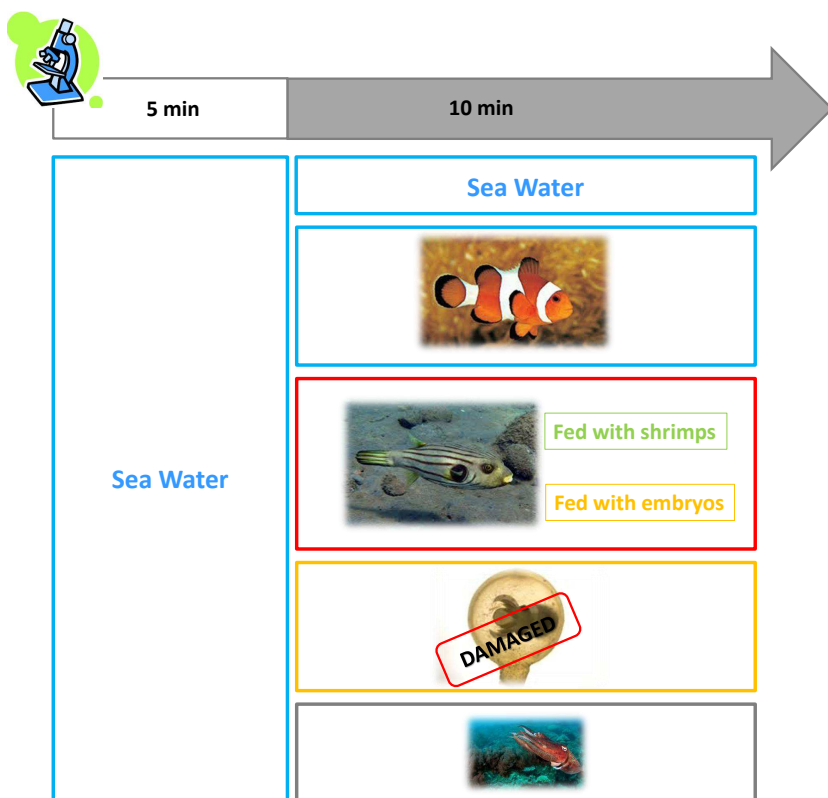
corresponding author: anne-sophie.darmaillacq@unicaen.fr

Introduction

Predator recognition plays a crucial role in the **survival** of individuals. In many vertebrate species, adults provide food to their young and ensure their protection. In the **absence of direct parental care** after birth or hatching, juveniles need to cope on their own to avoid predators by the mean of auditory, chemosensory or visual cues. Being able to **recognize a predator, innately** or by **learning**, increases the probability of survival, and the sooner, the better. **Fish and amphibian embryos**^{1,2,3,4} have been shown to respond to predatory chemical cues and to be able to learn a predatory threat when the predator odour is **paired** with conspecific **chemical alarm cues**. The aim of this study is to test whether cuttlefish **embryo** can recognize a threat through chemical **and** visual cues, and whether they can learn about a danger from chemical **and** visual cues in *Sepia pharaonis*.



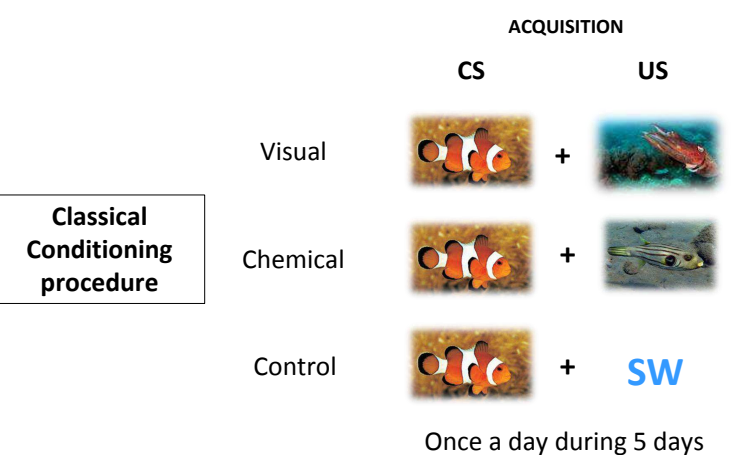
Innate predator recognition



Ventilation Rate

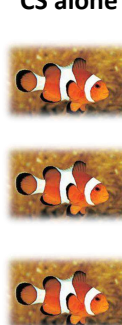
Innate **CHEMICAL** and **VISUAL** (data not shown) recognition of a potential **DANGER** (carnivorous fish AND ink)

Learned predator recognition

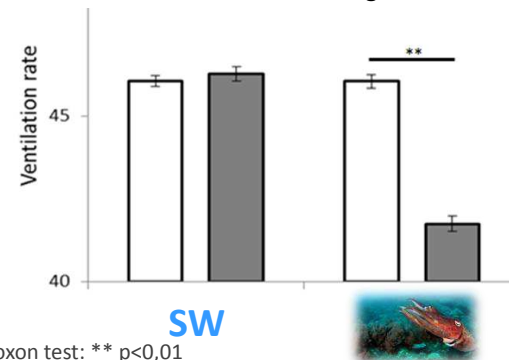


RETENTION

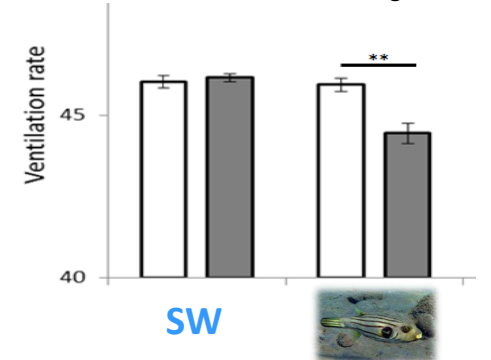
CS alone



Visual conditioning



Chemical conditioning



Learned VISUAL and CHEMICAL recognition of a potential **DANGER** by associating a threatening cue with a neutral fish

Discussion - Conclusion

Here, we show **for the first time** in a cephalopod that:

- Embryos can **innately** recognize a threat from both chemical and visual predatory cues.
- Embryos are also capable of using this information to **learn** about predation risk before they hatched.

This recognition could lead to a number of antipredator behavioural adaptations after hatching and prepare juveniles to their post-hatching ecological niche.

These results shed new light on the cognitive capabilities of embryos and the development of defensive behaviour in cuttlefish.

¹ Mathis et al. (2008). Proc R Soc B 275, 2603–2607; ³ Oulton et al. (2013). Plosone 8(10): e76061;

² Dixon et al. (2012). Anim Behav 84, 45–51;

⁴ Atherton & McCormick (2015). Anim Behav 103, 1–6;

Reproductive Success is Affected More by Maternal than Embryonic Stress in the Cuttlefish, *Sepia officinalis*

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Scientific interest in the influence of **stress** is growing due to increasing awareness of its effects on health, population structure and animal husbandry. **Maternal** and **embryonic stress**—stress that occurs during reproduction or embryonic development—appears especially influential. The effects can be positive, altering offspring phenotype to combat a perceived threat. More often however, reacting to stress can deplete resources and detract from offspring fitness^{1,2}.

Does maternal stress affect reproduction and offspring?

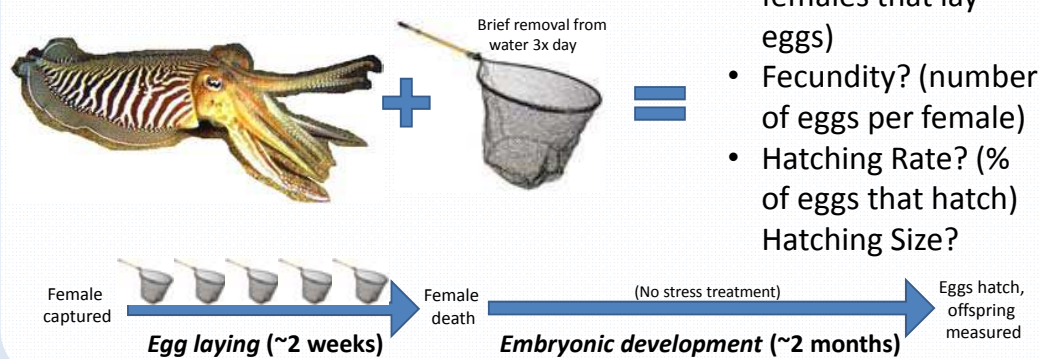
Does embryonic stress affect offspring?

Which type of stress affects offspring more?

The **cuttlefish *Sepia officinalis*** is a cephalopod mollusc inhabiting the Mediterranean and Atlantic coasts of Europe and North Africa. It is economically important for a well-established fishery and aquaculture industry and is a model species in biological research³. Cuttlefish reproduce only once at the end of life (although intermittent or continuous egg-laying may occur over several days or weeks⁴).

We tested the effects of stress applied to spawning female cuttlefish and to developing embryos. Maternal stress consisted of brief removal from the water 3x day ("Stressed Mother"), while Predator cues from seabass ("Predator") and bright LED light 6x a day ("Light") were used as embryonic stressors. We then compared the relative influence of these stressors on offspring.

Maternal Stress



Embryonic Stress

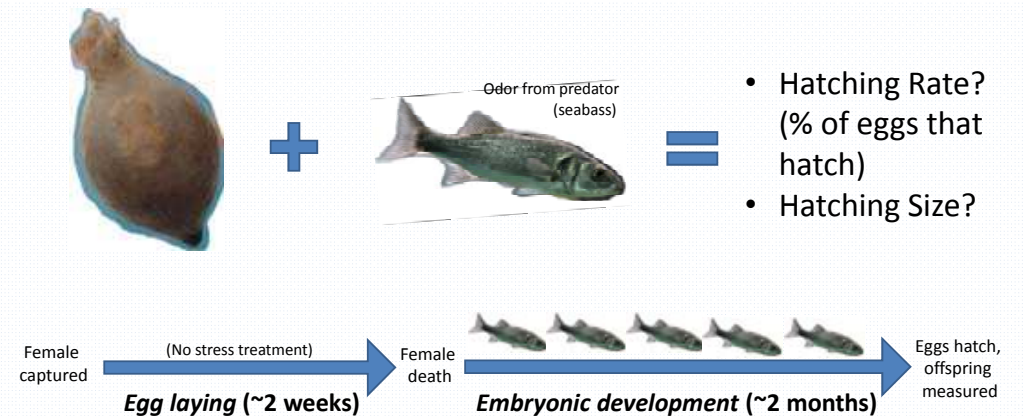


Table 1) Proportion of each treatment group that laid eggs (Fisher exact test, N = 39).

| | Control Mother | Stressed Mother | |
|----------------|----------------|-----------------|---------|
| Egg Layers (%) | 78.95 | 60.0 | p = 0.3 |

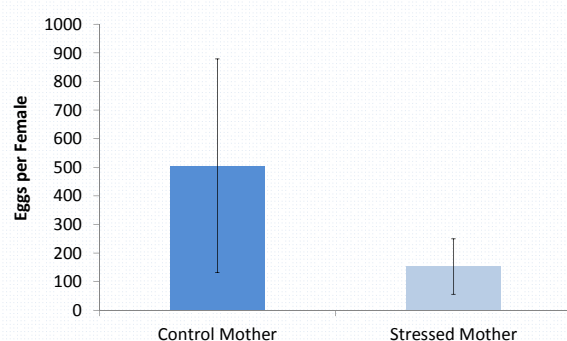


Table 2) Hatching Rate (chi-square test, N = 3600) and Hatching Size (mean dorsal mantle length (DML) ± s.d.; independent T-test, N = 169).

| | Control Mother | Stressed Mother | |
|-------------------|----------------|-----------------|------------|
| Hatching Rate (%) | 57.14 | 22.27 | p < 0.0001 |
| Mean DML (mm) | 8.56 ± 0.75 | 8.41 ± 0.92 | p = 0.26 |

Table 3) Hatching Rate (Cochran-Mantel-Haenszel chi-square test, N = 5628) and Hatching Size (mean dorsal mantle length (DML) ± s.d.; two-way ANOVA (with "stress treatment" and "mother" as factors), N = 231. Not shown on table: p < 0.001 for mother factor).

| | Control | Predator | Light | |
|-------------------|-------------|-------------|-------------|----------|
| Hatching Rate (%) | 57.14 | 59.33 | 63.38 | p > 0.99 |
| Mean DML (mm) | 8.56 ± 0.75 | 8.64 ± 0.73 | 8.71 ± 0.69 | p = 0.22 |

Maternal Stress was associated with fewer eggs per female and a lower Hatching Rate. Among **Embryonic Stress** groups, there was no significant difference in Hatching Rate.

No group showed a significant difference in Hatching Size.

Overall, **Maternal Stress** seemed to have a stronger effect on Hatching Rate than Embryonic stress.

These findings suggest ways to mitigate negative stress effects in cuttlefish aquaculture (*i.e.* reduce handling of spawning females).

It also suggests that maternal experience may have a larger impact on offspring than previously realized, a possibility worth exploring in other animals.

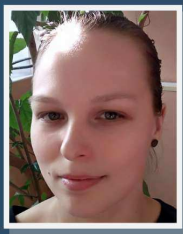
¹ Braastad, B. O. (1998). Effects of prenatal stress on behaviour of offspring of laboratory and farmed mammals. Applied Animal Behaviour Science, 61, 159–180.

² Schreck, C. B. (2010). Stress and fish reproduction: the roles of allostasis and hormones. General and comparative endocrinology, 165(3), 549–556.

³ Bloor, I. S., Attrill, M. J., & Jackson, E. L. (2013). A review of the factors influencing spawning, early life stage survival and recruitment variability in the common cuttlefish (*Sepia officinalis*). Adv. Mar. Biol., 65, 1–65.

⁴ Boletzky, S. V. (1987). Fecundity variation in relation to intermittent or chronic spawning in the cuttlefish, *Sepia officinalis* L. (Mollusca, Cephalopoda). Bulletin of marine science, 40(2), 382–388.

Size and weight of commander squid (*Beryteuthis magister* Berry, 1913) in 2005 and 2016.



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Introduction

The schoolmaster gonate squid (*Beryteuthis magister*, Berry, 1913) is the main commercial cephalopod species in Russian waters. Considerable volume of it is caught in the area of the Northern Kuril Islands. Dependence of body weight from individual size of species is an important characteristic which is used for stock assessment (Bizikov V., Golub A., 1996.). Hence, investigation of size-weight characteristics and variation of these characteristics in relation to sex and maturity stages are so essential. The main aim of present study is investigation of sex-based, maturity-based and interannual variability of length and weight in schoolmaster gonate squid inhabiting the main fishing grounds adjacent to the Northern Kuril Islands.

Results and discussion

Annual changes

Relationship between individual weight and mantle length was well fitted by power function (fig. 1), parameters of function did not differ significantly between sexes. At the same time curves of weight/length ratio in relation to maturity of squids could be divided into three segments.

For example, in 2005 during period of intensive growth (stages 1-3 in females and stages 1-4 in males) weight/length ratio increased very fast (fig. 4A). The relationship between weight/length and maturity stages in squids during maturation (stages 3-5 in females and stages 4-5 in males) showed slight retardation of growth. Both sexes showed equal types of weight/length ratio during periods of immaturity and maturation. But the most significant differences were observed among mature individuals (stage 6). The weight/length ratio of females decreased by 0.2 g/mm, while the weight/length ratio of males didn't show any changes regarding to previous maturity stage.

The same type of changes was observed in 2016 with the exception of the fact that the first period of intensive growth in females lasted to the 4th stage (fig. 4B). Also we observed changes of weight/length ratio in males during maturity. At post spawning stages weight/length ratio in adult males continued to increase and reached maximum in 1.5 g/mm, while in mature females it significantly decreased.

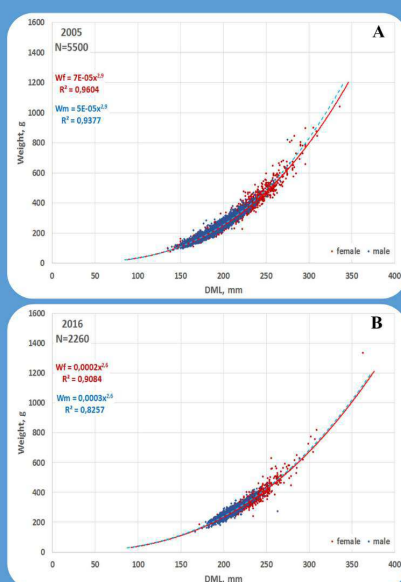


Fig. 1. Mean weight and mantle length relation in squids

Materials and methods

The data were collected by scientific observers during two commercial cruises in the Northern Kuril Island fishing ground in 2005 and 2016. Specimens for biological analysis were sampled randomly from catches. For each individual the mantle length was measured with a precision to the nearest 1 mm, and weight up to 1 g; sex, maturity stage (Nigmatullin ChM et. al., 1996), degree of stomach fullness and the condition factor were recorded. This study presents the results of data analysis obtained from 5500 and 2260 specimens during summer-autumn seasons 2005 and 2016 respectively. Based on biological analysis data, the following parameters were calculated: relationship between individual weight and mantle length; relationship between individual weight (or mantle length) and maturity stages; weight to mantle length ratio and changes of these characteristics during maturation.



Interannual variability

Squids of the same maturity stages had significant differences in mean length (fig. 2), weight and weight/length ratio in 2005 and 2016 (fig. 3, fig. 4) as well. For instance, mean mantle length of immature females and males was 50 mm less and 20 mm less in 2005 respectively, and mean weight of immature squids showed the same patterns in 2016. On the other hand there were differences in patterns of weight and length changes. Comparison of the data obtained in 2005 and 2016 revealed that in 2005 the growth rates in both sexes were higher and squids reached much larger size at maturity. The same situation was observed with weight increase rates. It should be noted that in spite of insignificant increase of mean length in adult males at post-spawning stages (5-6) in 2005 this parameter increased considerable in 2016.

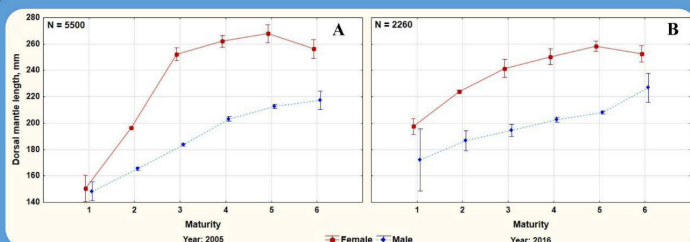


Fig. 2. Mean mantle length of squids during maturation in 2005 (A) and 2016 (B)

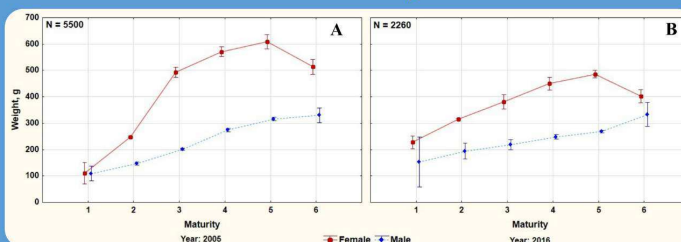


Fig. 3. Mean weight of squids during maturation in 2005 (A) and 2016 (B)

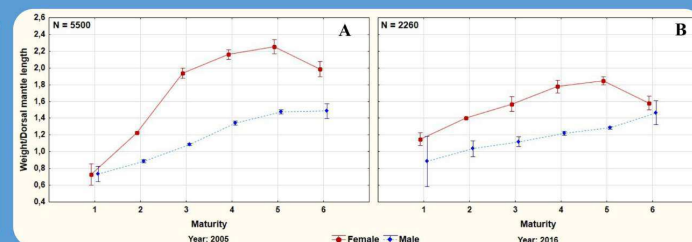


Fig. 4. Weight/length ratio of squids during maturation in 2005 (A) and 2016 (B)

Conclusions

The analysis of data obtained during both research cruises showed considerable reduction of weight in females at spawning stages. Such significant decrease of weight/length ratio could be the consequence of spawning. However, according to our observations, the mean mantle length in females also decreased. We believe that smaller average size (and weight) of spawning females in comparison with maturing and mature females in catches could indicate to heterogeneous composition of squid aggregations in the Northern Kuril Island fishing ground. Finally this decrease of weight/length ratio could be a result of combined effects of spawning and heterogeneity of aggregations.

Size-weight characteristics and growth parameters visibly differed in 2016 from data obtained in 2005. On the one hand mean mantle length and weight of immature squids were significantly higher in 2016, but on the other hand we observed smaller length and weight of mature individuals which were caused by lower growth rates and weight/length ratio. Such changes of size-weight characteristics in squids may be related to the warming changes in hydrological regime observed in this region in 2014-2016.

Literature cited

- Bello, G. 1991. Growth by weight versus growth by length in cephalopods. In P. Jereb, S. Ragonese & S. von Boletzky, eds. *Proceedings of the International Workshop held in the Istituto di Tecnologia, Della Pesca e del Pescato*. 1: 35-37.
- Bizikov V., Golub A. 1996. Weight-length ratio of squid *Beryteuthis magister*. In: Jelizarov AA (ed.) Commercial aspects of biology of commander squid *Beryteuthis magister* and of fishes of slope communities in the western part of the Bering Sea. Moscow, VNIRO Publ, pp 38-40 (in Russian)
- Merella, P., A. Quetglas, F. Alemany and A. Carbonell, 1997. Length-weight relationship of fishes and cephalopods from the Balearic Islands (western Mediterranean). *Naga ICLARM Q.* 20(3/4):66-68.
- Nigmatullin ChM, Laptikhovsky VV, Sabirov RM 1996. Reproductive biology of squid *Beryteuthis magister*. In: Jelizarov AA (ed.) Commercial aspects of biology of commander squid *Beryteuthis magister* and of fishes of slope communities in the western part of the Bering Sea. Moscow, VNIRO Publ, pp 101-124 (in Russian)



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Statolith shape variability in *Berryteuthis magister*

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1. Goals

Squid statoliths are the unique structure that can be used for ageing studies, investigation of ontogenetic habitat changes during ontogenesis or affiliation of a specimen to specific geographic grouping.

The main aim of this study is to investigate shape variability of schoolmaster gonate squid (*Berryteuthis magister*, Gonatidae) statoliths.

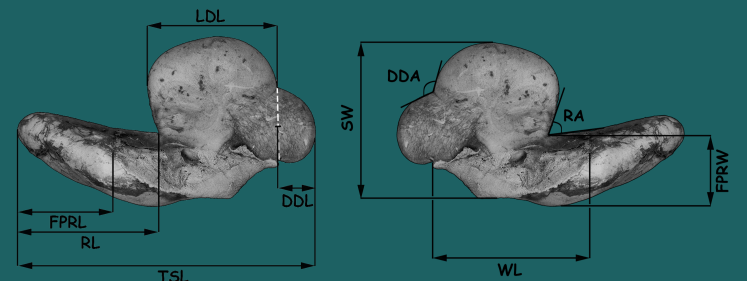
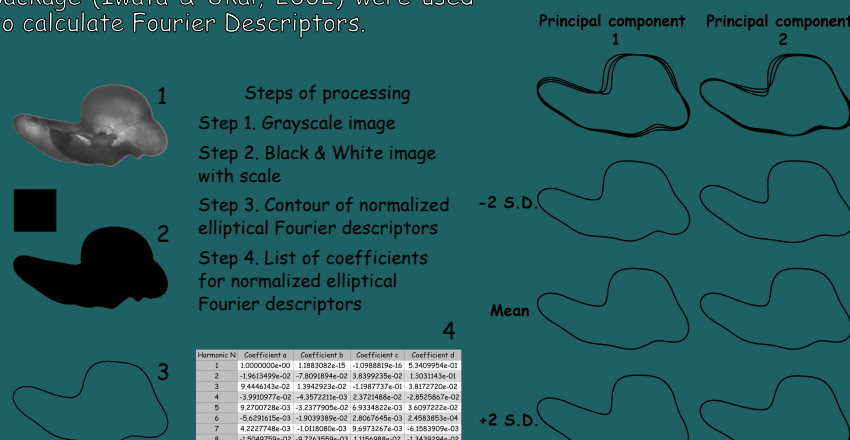
The first goal is to test different methods of statolith shape description in order to accept them for variability studies.

The second goal is to test gender, maturity-based and geographical variability of *Berryteuthis magister* statoliths.

2. Methods

Two different methods were applied to shape description — normalized Fourier descriptors and set of standard measurements.

Coefficients of Normalized Fourier Descriptors (below) were calculated following standard methods (Afanasyev et al, 2017). SHAPE software package (Iwata & Ukai, 2002) were used to calculate Fourier Descriptors.



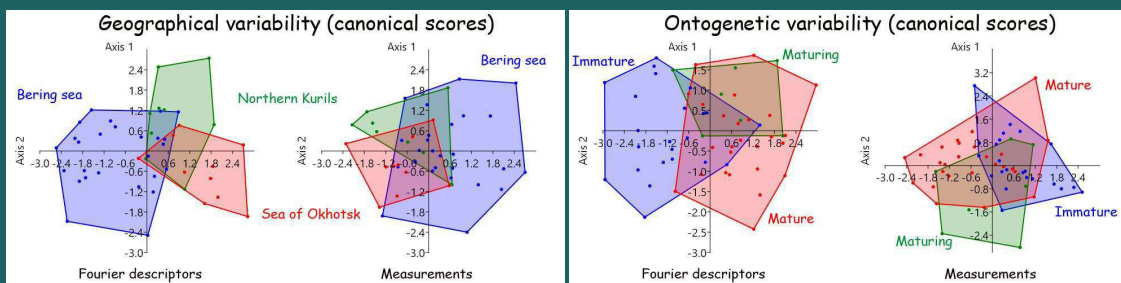
Set of basic measurements (above) included total statolith length (TSL), rostrum length (RL), free part of rostrum length (FPRL), wing length (WL), dorsal dome length (DDL), lateral dome length (LDL), statolith width (SW), basis of rostrum width (BRW), rostrum angle (RA) and dorsal dome angle (DDA).

3. Results

MANOVA of principal components extracted from the normalized Fourier descriptors showed significant gender, maturity-based and geographic differences. At the same time, MANOVA based on measurements showed differences only between sexes and regions.

| Multivariate Tests of Significance | | | | | | | | | | | |
|------------------------------------|-------|---|------|--------|---------|------|-----------------------|------|--------|---------|------|
| Effect | Test | Normalized elliptical Fourier descriptors | | | | | Standard measurements | | | | |
| | | Value | F | Effect | Error | p | Value | F | Effect | Error | p |
| Intercept | Wilks | 0.96 | 1.85 | 10 | 406 | 0.05 | 0.99 | 1.03 | 5 | 405 | 0.40 |
| gender | Wilks | 0.95 | 2.16 | 20 | 812 | 0.02 | 0.97 | 2.30 | 10 | 810 | 0.02 |
| maturity | Wilks | 0.95 | 1.73 | 20 | 812 | 0.02 | 0.97 | 1.25 | 10 | 810 | 0.25 |
| region | Wilks | 0.96 | 1.30 | 20 | 812 | 0.05 | 0.98 | 1.03 | 10 | 810 | 0.47 |
| gender*maturity | Wilks | 0.94 | 1.30 | 20 | 812 | 0.17 | 0.98 | 0.80 | 10 | 810 | 0.63 |
| gender*region | Wilks | 0.96 | 0.86 | 20 | 812 | 0.64 | 0.98 | 0.97 | 10 | 810 | 0.47 |
| maturity*region | Wilks | 0.89 | 1.15 | 40 | 1541.36 | 0.24 | 0.98 | 0.51 | 20 | 1344.18 | 0.97 |
| gender*maturity*region | Wilks | 0.90 | 1.06 | 40 | 1541.36 | 0.37 | 0.95 | 0.59 | 20 | 1344.18 | 0.47 |

classify individuals by region and maturity affiliation with high precision. Some results of this study are presented below. In addition, we obtained classification matrixes where Northern Kuril region were not considered as independent (a priori probability = 0). This allowed us to identify the origin of individuals caught there.



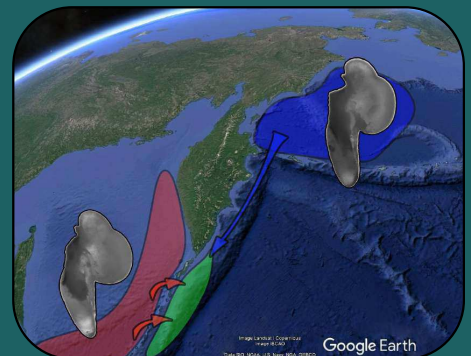
Geographical affiliation (Classification matrix)

| Rows: Observed classifications | | | | | | | | | |
|------------------------------------|----------------------|---|----|----|-----------------------|-----------------|----|-----------------|---------|
| Columns: Predicted classifications | | | | | | | | | |
| | Group | Normalized elliptical Fourier descriptors | | | Standard measurements | | | Percent correct | p-value |
| | | Percent correct | BS | NK | SO | Percent correct | BS | NK | SO |
| Females | Bering sea (BS) | 92.3 | 24 | 2 | 0 | 88.5 | 23 | 1 | 2 |
| | Northern Kurils (NK) | 55.6 | 3 | 5 | 1 | 50 | 4 | 4 | 0 |
| | Sea of Okhotsk (SO) | 70 | 1 | 2 | 7 | 70 | 3 | 0 | 7 |
| | Total | 80 | 28 | 9 | 8 | 77.3 | 30 | 5 | 9 |
| Males | Bering sea (BS) | 66.7 | 10 | 5 | 0 | 46.7 | 7 | 8 | 0 |
| | Northern Kurils (NK) | 91.9 | 3 | 34 | 0 | 86.5 | 5 | 32 | 0 |
| | Sea of Okhotsk (SO) | 33.3 | 0 | 4 | 2 | 16.7 | 0 | 5 | 1 |
| | Total | 79.3 | 13 | 43 | 2 | 69 | 12 | 45 | 1 |

4. Discussion

Our study confirmed that statolith shapes could be used for identification of population affiliation and showed their usability for maturity stage and gender identification. Standard measurements reflect intersexual variability better than normalized Fourier descriptors. Ontogenetic variability is represented by measurements and descriptors equally. Statolith shapes obtained using descriptors are much more suitable for geographical variability studies.

Moreover, our studies confirmed the presence of complicated structure of squid's aggregations in Western coastal area of the northern Kuril Islands. Majority of mature males had their origin from the Sea of Okhotsk which corresponds to distribution scheme proposed by Katugin (Katugin et al, 2013). At the same time the largest part of mature females migrated from the Bering sea, which confirmed hypothesis of Alexeyev (Alexeyev, 2007).



5. Future challenges

Our first goal for future studies is to collect statoliths of *Berryteuthis magister* from isolated parts of its range in order to study of the spatial aspect of squid population structure.

The second goal is to apply described methods to other cephalopod groups.

Acknowledgements

We are grateful to Dr. P. Afanasyev for inspiration of this study and helpful recommendations. We thank Dr. A. Buyanovski for positive and constructive discussion of our study. Also, we thank Dr. A. Golikov for help in taking SEM photos of statoliths.

Literature cited

Alexeyev D.O. 2007. The role of North Kurile Islands area in functional structure of populations of schoolmaster squid *Berryteuthis magister* (Berry, 1913). VNIRO proc. 147, p.246-265 (in Russian, English summary).

Afanasyev P., Orlov A., Rolskiy A. 2017. Comparative analysis of the otoliths form as a tool of species identification and study of the population organization of various fish species. Russian journal of zoology, vol 96 №2, p. 192-200.

Iwata, H. & Y. Ukai. 2002. SHAPE: a computer program package for quantitative evaluation of biological shapes based on elliptic Fourier descriptors. J. Hered., 93: 384-385.

Katugin O.N., Shevtsov G.A., Zuev M.A., Didenko V.D., Kulik V.V., Vanin N.S. 2013. *Berryteuthis magister*, Schoolmaster Gonate Squid. Advances in Squid Biology, Ecology and Fisheries. // Part II. Oegopsid Squids. New-York, Nova Science Publishers. P. 1-48.



COMPARATIVE STUDY OF AGE ESTIMATION IN WILD AND CULTURED *Octopus vulgaris* PARALARVAE. Effect of Temperature and Diet



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Abstract

We estimated the age in 100 wild paralarvae collected during spawning season in NW Spain, where temperature was about 14.5°C. We also analyzed 50 paralarvae (0 to 22 days old) reared at 14°C and 33 paralarvae reared at 21°C (0 to 30 days old) which is the optimal temperature under culture conditions. Age estimation using anterior region of paralarvae beaks was improved. Results at 21°C matched with true age supporting daily deposition, whereas less than one increment/day was recorded at 14°C. General Linear Model (GLM) analysis showed that temperature significantly ($p < 0.05$) influenced in the increment deposition in captivity, but not the diet ($p > 0.05$). In the natural environment, ages ranged between 0 and 8 days presuming daily deposition. Nevertheless, considering the possible influence of temperature, we used the mean value at capture to correct with GLM equation the age estimations of wild paralarvae.

Introduction

Wild and cultured populations of common octopus (*Octopus vulgaris* Cuvier, 1797 and multiple *O. vulgaris*-like species since Amor *et al.* 2016) have a high economic value worldwide. However the survival of the species during its planktonic early life is critical for the population success. Difficulties in collecting wild paralarvae and the complexity to estimate their age has prevented the accurate study of the ecology and feeding of planktonic stages in the natural environment and the comparative analysis of wild and cultured paralarvae of similar ages. This comparative approach could serve as a guide to establish the requirements of these early developmental stages and improve their low survival under captivity.

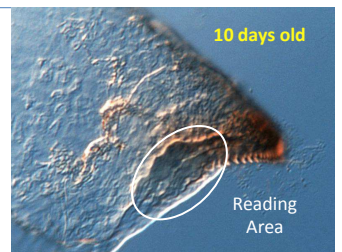
Aims. The present study aims to address the effect of temperature and diet under culture conditions. It was used to interpret the age estimated in wild paralarvae collected during its planktonic early stage.

Paralarvae

- 33 paralarvae cultured at 21°C and 50 paralarvae cultured at 14°C in 2015 (Nande and Otero, 2015) with two different crustacean preys (*Artemia* and *Maja brachydactyla* zoeae). Paralarva photo: 40X
- 100 wild paralarvae from NW Atlantic during spawning season of 2015 and 2016. Oceanographic vessel "José María Naváz" (IEO). Sampling: Multinet 200 µm, 2m diameter. Hauls at 10-20m depth, 15 min, 2 knots speed

Age estimations

- Counting the parallel thin increments (rings) observed in the anterior region of upper jaws, as described in Perales-Raya *et al.* (2014). Maximum width of reading area (WRA) was measured in all individuals
- Transmitted light with Nomarski Differential Interference Contrast. 400X magnification. Two readings, precision estimated by Coefficient of Variation (CV) (Campana, 2001)



Results and Discussion

CULTURE

Fig. 1. Increments in the beaks

Optimal thermal conditions (21°C) → 1 increment/day with any diet
Cold temperature (14°C) → <1 increment/day with any diet

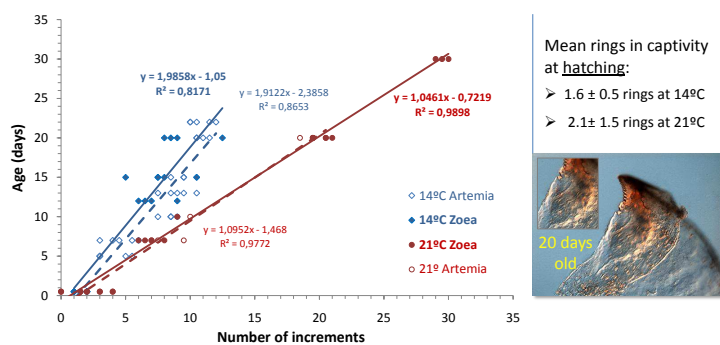
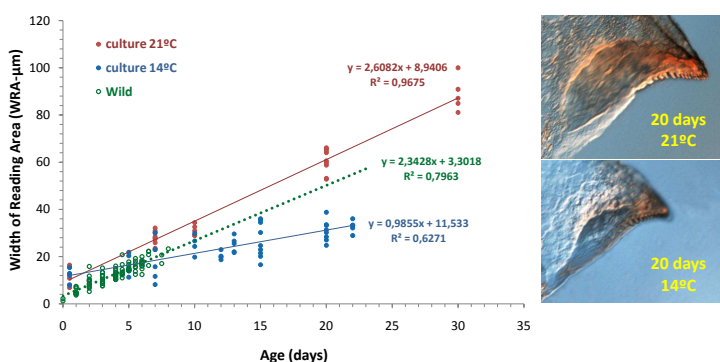
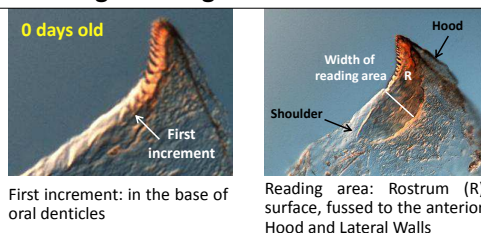


Fig. 2. Beak growth in captivity

Lowest WRA at 14°C (both diets). Wild specimens showed intermediate values and similar growth trend to individuals cultured at 21°C



Age reading IMPROVEMENTS



| Paralarvae group | Mean CV |
|----------------------|-------------|
| Cultured 21°C (N=33) | 2.97 ± 2.05 |
| Cultured 14°C (N=50) | 3.53 ± 4.32 |
| Wild (N=100) | 2.94 ± 5.96 |

Age Precision: Coefficient of Variation (CV)

Beaks of paralarvae reared at 14°C showed <1 increment/day, whereas those cultured at same conditions but optimal temperature (21°C, Hamasaki and Morioka, 2002) displayed daily increments, as validated by Perales-Raya *et al.* (2014).

GLM analysis from our cultured data showed temperature significantly influencing increment deposition ($p < 0.05$), whereas diet did not ($p > 0.05$). GLM coefficients resulted in this equation:

$$AGE = \frac{NI - 4.604 + T \cdot 0.176}{T \cdot 0.074 - 0.612}$$

NI: number of increments
T: temperature

Low temperature in captivity may result in physiological stress and disruption of increment deposition (e.g. Campana and Neilson 1985, Morales-Nin 2000), whereas wild specimens are possibly less affected by any thermal adverse conditions. Results for wild paralarvae are presented assuming daily deposition in the natural environment (Fig. 4A) and also considering the possible influence of temperature in the periodicity of increment deposition (Fig. 4B).

WILD

Fig. 3. Capture location of wild paralarvae and oceanographic data

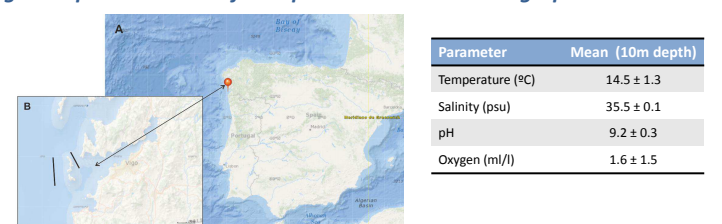
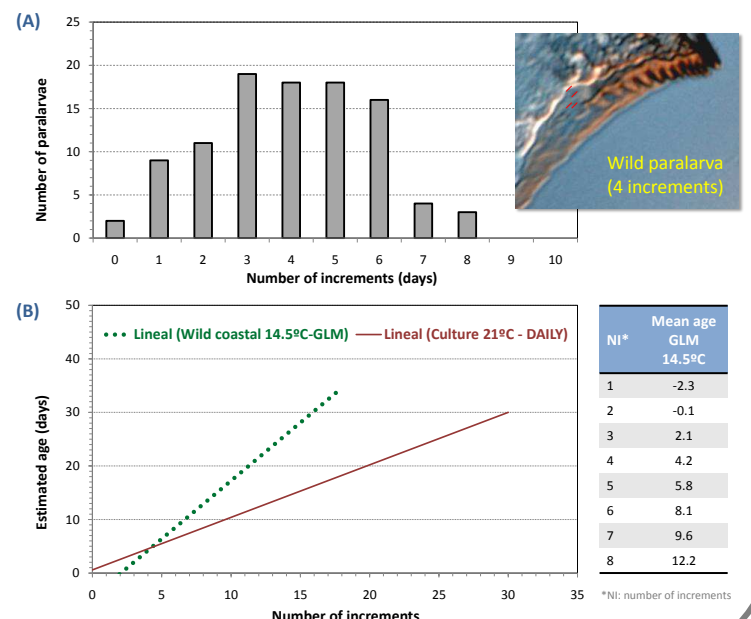


Fig. 4. Age of wild paralarvae

Numbers by age (A). Model (GLM) for age adjustment considering the effect of temperature in the increment deposition, and table with mean ages obtained (B)



Conclusion

Beak increments in octopus paralarvae reared at optimal thermal conditions (21°C) have a daily deposition, however cold temperature conditions (14°C) decrease the number of increments. Culture results were considered for age estimation in paralarvae from the natural environment.

References

- Amor M.D., Norman M.D., Roura A., Leite T.S., Gleadall I.G., Reid A., Perales-Raya C., Lu C.C., Silvey C.J., Vidal E.A.G., Hochberg F.G., Zheng X., Strugnell J.M. 2016. Morphological assessment of the *Octopus vulgaris* species complex evaluated in light of molecular-based phylogenetic inferences. *Zool Scr* 00:000-000
- Campana S.E., Neilson J.D. 1985. Microstructure of fish otoliths. *Can J Fish Aquat Sci* 42(5):1014-1032
- Campana S.E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *J. Fish Biol.* 59:197-242.
- Hamasaki K., Morioka T. 2002. Effects of temperature on egg incubation period, and paralarval survival and growth of common octopus, *Octopus vulgaris* reared in the laboratory. *Suisan Zoshoku*, 50, 407-413.
- Morales-Nin B. 2000. Review of the growth regulation processes of otolith daily increment formation. *Fish Res* 46(1):53-67
- Nande M. and Otero J.J. 2015. Relationship between the standard growth rate (SGR%) and synthesis of arms in common octopus paralarvae (*Octopus vulgaris*, Cuvier 1797) fed by different preys, spider crab zoeas vs. *artemia*. The digestive tract of cephalopods workshop (COST Action FA1301). Cascais, Lisbon (Portugal) November 24th.
- Perales-Raya C., Almansa E., Bartolomé A., Felipe B.C., Iglesias J., Sánchez F.J., Carrasco J.F. and C. Rodríguez. 2014. Age validation in *Octopus vulgaris* beaks across full ontogenetic range. Beaks as recorders of live-events in octopuses. *Journal Shellfish Research*, 33(2): 481-493.

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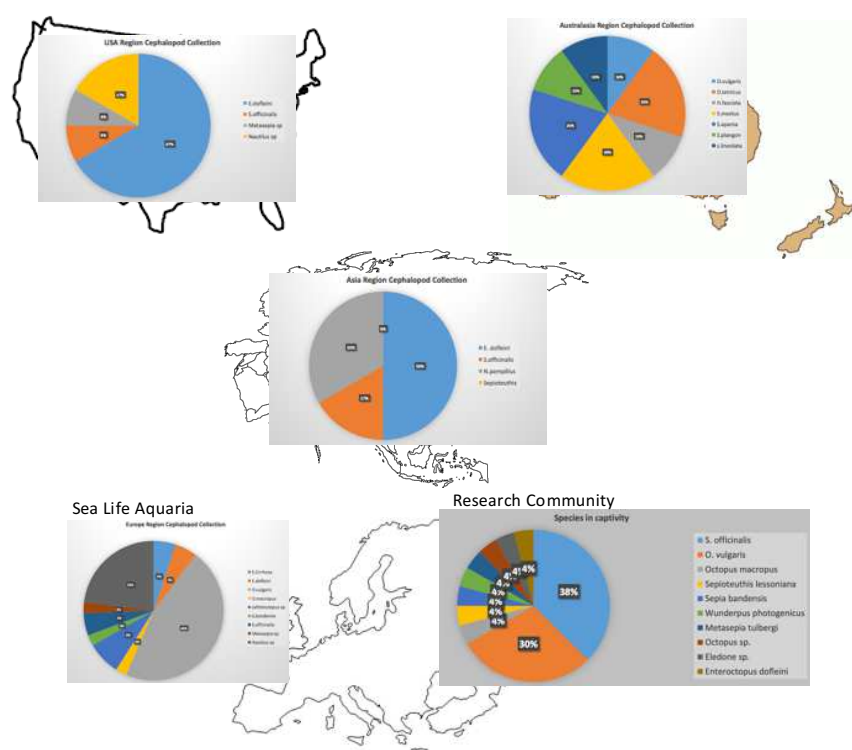


The Public Cephalopod: A review of public aquarium cephalopod collections

Kerry Perkins *, Marie Collins, Chris Brown, *Correspondence address, Sea Life Brighton, Marine Parade, Brighton, BN2 1TB1

A review of cephalopod welfare and husbandry was conducted globally in over 40 SEA LIFE aquariums in 2016. A questionnaire detailing tank sizes, species and husbandry information were collated and analysed for trends. We hope to demonstrate the value of the sharing of information globally and between different areas of expertise to gain a better understanding of cephalopod husbandry

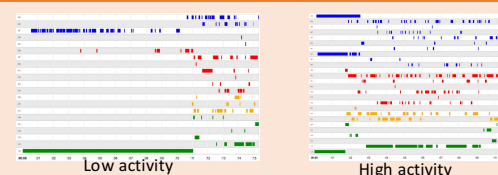
Geographical Species Breakdown



Welfare

Monitoring Activity

| Colour Key | Behavioural Category |
|------------|----------------------|
| A | Aquarist |
| P | Postural |
| C | Chromatic |
| L | Locomotor |



- Plotting the behaviour of Aquarist and octopus
- Can determine periods of high and low activity
- Is detailed down to postural, chromatic and textural components
- Can be plotted over long periods of time

Using public to monitor activity

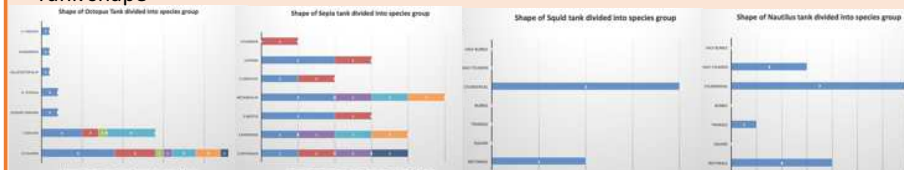


- Computer program built for portable device
- Public able to record octopus behaviour with assistance from staff member

Husbandry

Health Monitoring

Tank shape

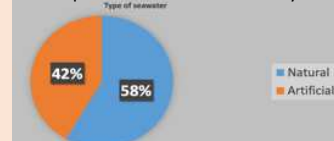


Rectangular tanks are found across all 4 groups, Sepia have the most variation in tank shape

Sea Life Aquarium

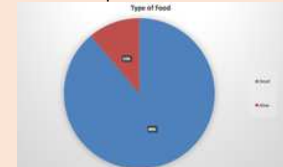


European Research Community

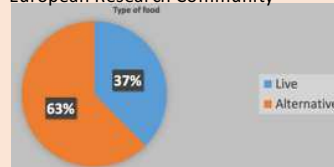


More sites use artificial seawater than the European Research community

Sea Life Aquarium

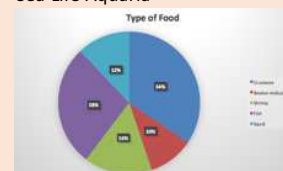


European Research Community

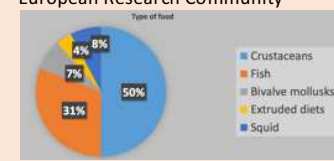


Dead food is the preferred food substance for aquaria, this is most likely due to the higher presence of Octopus and Nautilus in the sample which will accept dead food

Sea Life Aquarium



European Research Community



In both research and Aquaria Crustaceans are the major food group being fed

Histology

- Histology conducted over 10 years and compared for any commonalities
- Different cephalopod families seem to have different parasite loads

Survival rates

- Determine external factors affecting survival rates
- Looking at transport stress and longevity

Growth Rate

- Regular weighing regime across sites to determine growth parameters against feed type

Future Analysis

- Compare previous mortality records against cephalopod questionnaire

Parasite fauna of post spawning female *Octopus maya* (Cephalopoda: Octopodidae) in the Yucatan Peninsula, Mexico

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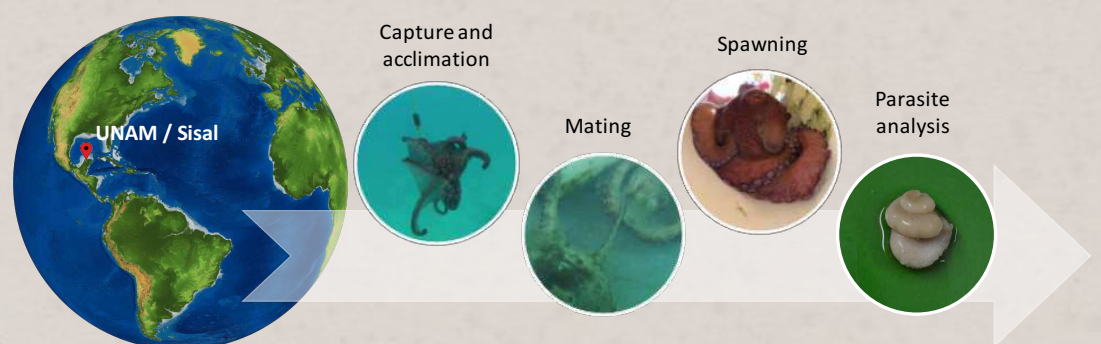


Introduction

There is an increasing interest on the use of cephalopods for aquaculture purposes. Despite the benefits, one of the disadvantages of aquaculture is the increase of the parasites normally found in wild populations (Castellanos-Martínez and Gestal, 2013), that may be responsible for disease outbreaks. Nonetheless, studies on the parasite fauna of cephalopods are limited (Hochberg, 1990; Castellanos-Martínez and Gestal, 2013). *Octopus maya* is an endemic species of the Yucatan Peninsula and is considered one of the main species for aquaculture. However, little information on the parasitology of this species is available. Thus, we aimed to evaluate the parasite fauna of post spawning *O. maya* females.

Material and Methods

Adult *O. maya* were captured in the coast of Sisal (YUC, Mexico) and acclimatized to the National Autonomous University of Mexico (UNAM) for 2 weeks. After mating, the females (n=22) were separated until spawning and evaluated for the presence of parasites immediately after spawning (0) and at subsequent moments (10, 20, 30 and 40 days after spawning (DAS)). Eyes, buccal mass, kidneys, digestive gland, gills, systemic and branchial hearts, and gastrointestinal were analysed.



Results

Coccidian parasites of the genus *Aggregata* were found in the intestine, caecum and gills with prevalence ranging from 75-100% in the different DAS (Table 1). Cestode larvae *Prochristianella* sp. were identified in the buccal mass, with prevalence of 100% in all DAS, with the exception of the females in 30 DAS, which presented prevalence of 50% (Table 2). No differences were found in the mean abundances ($p=0,6987$) and mean intensities of infection ($p=0,2571$) in the different DAS (Table 2). This parasite was also found in the intestine of *O. maya* females immediately after spawning and 10 DAS with prevalence of 25% (data not shown).

Table 1: Total prevalence and in the different organs by *Aggregata* sp. in *Octopus maya* females analysed immediately after spawning (0) and at subsequent days after spawning (10, 20, 30 and 40 DAS). P: prevalence (%); IO: infected octopus; EO: examined octopus.

| DAS | Intestine P | Caecum P | Gills P | Total IO/EO | P |
|-------|----------------|-------------|------------|----------------|-------|
| 0 | 25,0 | 75,0 | 0 | 4/4 | 100,0 |
| 10 | 0 | 75,0 | 25,0 | 3/4 | 75,0 |
| 20 | 20,0 | 80,0 | 20,0 | 4/5 | 80,0 |
| 30 | 50,0 | 50,0 | 0 | 3/4 | 75,0 |
| 40 | 0 | 100,0 | 40,0 | 5/5 | 100,0 |
| Total | 18,2 | 77,3 | 18,2 | 19/22 | 86,3 |

Table 2: Parasitological indexes of cestode larvae *Prochristianella* sp. in the buccal mass of *Octopus maya* females analysed immediately after spawning (0) and at subsequent days after spawning (10, 20, 30 and 40 DAS). IO: infected octopus; EO: examined octopus; P: prevalence(%); MA: mean abundance; MI: mean intensity of infection, followed by standard deviation and minimum and maximum values in parenthesis.

| DAS | IO/EO | P | MA | MI |
|-------|-------|------|---------------|------------------------|
| 0 | 4/4 | 100 | 121.00±84.63 | 121.00±84.63 (8-203) |
| 10 | 4/4 | 100 | 192.50±122.17 | 192.50±122.17 (58-354) |
| 20 | 5/5 | 100 | 176.40±134.76 | 176.40±134.76 (38-401) |
| 30 | 2/4 | 50 | 34.00±53.22 | 68.00±62.63 (24-112) |
| 40 | 5/5 | 100 | 167.20±111.02 | 167.20±111.02 (21-332) |
| Total | 20/22 | 90,9 | 141.27±112.21 | 155.40±107.73 (8-401) |

Discussion

Aggregata sp. (Protozoa: Apicomplexa) is the most common parasite found in cephalopods all over the world (Hochberg, 1990; Gestal et al., 2010). Cephalopods have an important role in the life cycle of Trypanorhyncha cestodes (Stunkard, 1977), acting as intermediate or paratenic hosts, and the elasmobranchs the definitive hosts (Hochberg, 1990). From our knowledge, this is the first record of *Aggregata* sp. in *O. maya* and it is the first time that cestode larvae are reported in the buccal mass of cephalopods. Both the coccidian *Aggregata* sp. and the cestode larvae *Prochristianella* sp. are transmitted by food chain. Our results demonstrated that even after 40 DAS maintained in the laboratory without feeding, *O. maya* females were parasitized. Since the acclimation until the spawning, the females were fed on frozen crabs or artificial diet, which suggests that these animals were already infected with both parasites before capture.

References

- Castellanos-Martínez, S., Gestal, C. (2013) Pathogens and immune response of cephalopods. J. Exp. Mar. Biol. Ecol. 447, 14–22.
- Gestal, C., Pascual, S., Hochberg, F. (2010) *Aggregata bathytherma* sp. nov. (Apicomplexa: Aggregatidae), a new coccidian parasite associated with a deep-sea hydrothermal vent octopus. Dis. Aquat. Org. 91, 237–242.
- Hochberg, F.G. (1990) Diseases of Mollusca: Cephalopoda. In: Kinne, O. Diseases of Marine Animals, Vol. III. Cephalopoda to Urochordata. Hamburg: Biologisches Anstalt Helgoland, pp. 47-227.
- Stunkard, H.W. (1977) Studies on Tetraphyllidean and Tetrahynchidean metacestodes from squids taken on the New England coast. Biol. Bull. 153, 387–412.



Bioaccumulation and biomagnification of metals in *Octopus hubbsorum* from the Santa Rosalia mining harbor, Gulf of California, Mexico

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Introduction

Several studies demonstrate that octopuses have the capacity to bioaccumulate high concentrations of metals in their tissues¹. The quantification of these elements has become subject of increasing concern around the world due to the high demand of these organisms for human consumption². In addition, as prey of numerous species, octopuses are transfer vectors of metals to higher trophic levels; while as predators of prey with high content of these elements, such as crustaceans and bivalves, octopuses present bioaccumulation and biomagnification of metals³.

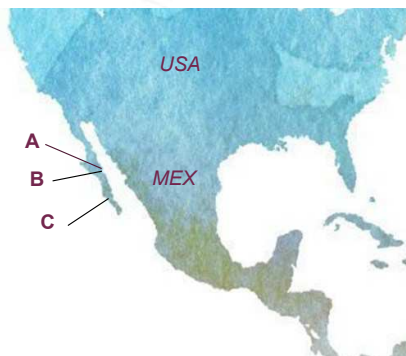
In Mexico, most of the octopus fishery on the Pacific coast is based on the capture of one species: *Octopus hubbsorum*⁴. In the Gulf of California, the harbor of Santa Rosalia is the main locality for the capture of this species⁴. However, due to its extensive mining history, the coastal sediments of this site are heavily contaminated by some metals (**Mn>Cu>Zn>Pb>Co>U>Cd**)⁵.

Despite its importance for the ecosystem and human health, to date there is no known study that evaluate the concentration of metals in octopus from Mexico or in the American continent.

The objective of this study was to determine the bioaccumulation and biomagnification of metals in *O. hubbsorum* from the mining harbor in Santa Rosalía and in La Paz Bay, as a control.

Materials and Methods

Levels of 21 metals were determined in mantle, digestive gland, branchial hearts and gills of 23 octopuses collected in June 2016, from three localities in Mexico:



A: hotspot zone in Santa Rosalia
B: 2km far from the hot spot in Santa Rosalia
C: unpolluted zone (La Paz Bay), as control

The determinations were performed at the Canadian Rivers Institute, UNB, Canada, by using thermal decomposition and Inductively Coupled Plasma Optical Emission Spectrometry.

To assess the biomagnification, the biomagnification factor was calculated by locality, considering the proportion of each metal in *O. hubbsorum* with respect to the total concentration in one of its main potential prey: the clam *Megapitaria squalida*.

The concentration of each metal was compared between sexes, tissues and localities using ANOVA or Kruskal-Wallis tests in the software STATISTICA ($\alpha=0.05$).

Results and Discussion

Metal levels

- The elements with higher concentrations in *O. hubbsorum* were **Mg>Cu>Fe>Zn**.
- Concentrations of metals obtained in the present study are within the ranges reported for other octopus species in the world^{6,7}. The exception are Co, Fe and Mn in the digestive gland and branchial hearts. (Co was almost 100 times higher than previous reports).
- This is the first report for **Al, La, Mg, Sr and Ti** in any species of octopuses.

Metal concentration vs. biological parameters

Size and weight

There are discrepancies about the effect of these parameters on the bioaccumulation of metals in octopuses⁸. In the present study this source of variation was removed examining organisms of similar sizes and weights (7-11 cm mantle length, 28-44cm total length, 228-710 g total weight)

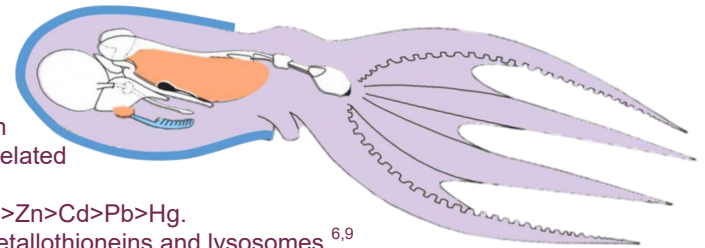
Sex

The lack of relation between metal concentration and sex agrees with previous works^{2,6}.

Differences between tissues

The higher concentrations of metals were found in the digestive gland and branchial hearts, organs related to detoxification physiology in cephalopods¹².

- Digestive gland**- higher concentrations of **Cu>Zn>Cd>Pb>Hg**.
Attributed to affinity to metallothioneins and lysosomes^{6,9}.
- Branchial hearts**- higher concentrations of **Ni>Co>Mn>Sr>Al>V>Cr**.
Attributed to the presence of adenochromes, Fe-rich pigments^{7,10}.
- Mantle**-lower concentrations of all metals, in accordance with other works in cephalopods^{2,7}.
- Gills**- mantle-like concentrations, because it is an organ of short-term storage¹¹.



Differences between localities

Santa Rosalia A vs. B

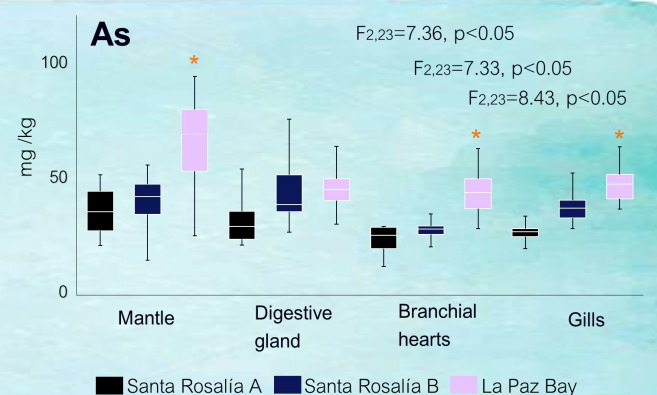
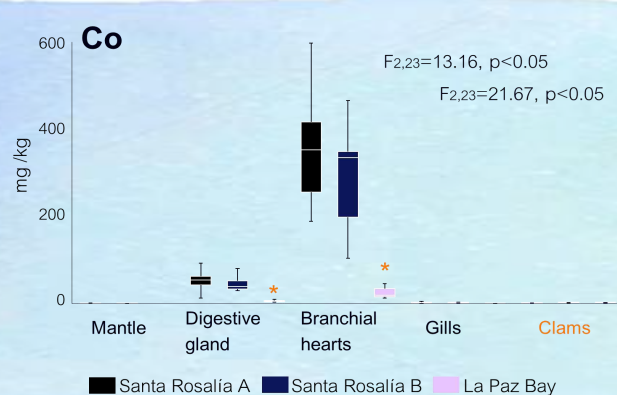
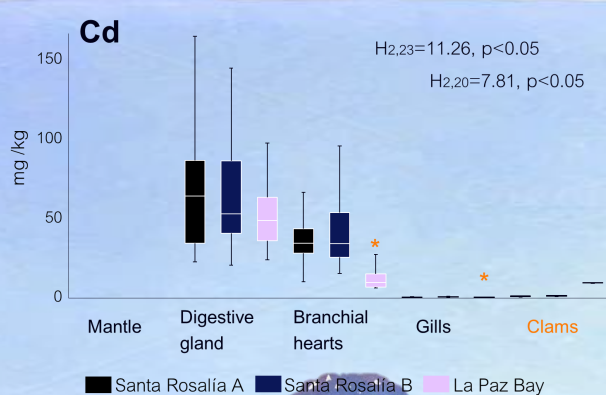
In both localities, octopus showed similar concentrations of metals, possibly due to the displacement of the octopuses between sites and/or the similar bioavailability of metals in each one⁵.

Santa Rosalia A and B vs. control locality

-Cd, Co, Cr, Mn, Ni, Pb and Zn were higher in octopuses from Santa Rosalía (in all tissues except mantle), reflecting the main metallic contaminants reported in Santa Rosalía⁵, except for Cu, Zn and U.

Explained because: Cu and Zn are essential elements, and U is also a contaminant present in La Paz Bay¹².

-Although is not considered an area with high pollution¹³, **Ag, As, Cr, Hg, Ni and Sr** were higher in octopuses from La Paz Bay, in all tissues. (**As y Hg** were the most important). Explained because: Arsenic is not detoxicated by the digestive gland and may increase if the organisms of this locality consume more crustaceans³. Hg can be accumulated from water in muscular and gills tissues^{2,9}.



Biomagnification (Cd, Co, Cu, Fe, Mn, Ni, Pb and Zn)

Biomagnification was evident in all localities, mainly in digestive gland and branchial hearts, with concentrations ranging from one to 300 times higher than in *M. squalida* whole tissues. However, the present study only represents a general indication, since molluscs constitute only 30% of the diet of *O. hubbsorum*²³, so it is suggested that future studies consider other prey such as crustaceans.

Concern for public health

Concentration of metals in *O. hubbsorum* muscle did not exceed maximum permitted levels allowed for human consumption in Mexican (NOM 1995 and NOM 2009) and international regulations (Codex Alimentarius, European Community y Food and Drug Administration).

References

- Rjeibi *et al.*, 2014. Interspecific and geographical variations of trace metal concentrations in cephalopods from Tunisian waters.
- Mshana and Sekadende, 2014. Assessment of Heavy Metal Pollution in *Octopus cyanea* in the Coastal Waters of Tanzania
- Bustamante *et al.*, 1998. Cephalopods as a vector for the transfer of cadmium to top marine predators in the north-east Atlantic Ocean.
- López-Uriarte *et al.*, 2005. Range extension for *Octopus hubbsorum* Berry, 1953 (Mollusca: Octopodidae) in the Mexican Pacific.
- Shumilin *et al.*, 2011. Assessment of Geochemical Mobility of Metals in Surface Sediments of the Santa Rosalia Mining Region, Gulf of California.
- Raimundo *et al.*, 2004. Geographical variation and partition of metals in tissues of *Octopus vulgaris* along the Portuguese coast
- Napoleao *et al.*, 2005. Elemental characterization of tissues of *Octopus vulgaris* along the Portuguese coast
- Raimundo *et al.*, 2010. Metallothioneins and trace elements in digestive gland, gills, kidney and gonads of *Octopus vulgaris*
- Marigómez *et al.*, 2002. Cellular and Subcellular Distribution of Metals in Molluscs
- Nakahara *et al.*, 1982. Uptake and excretion of Cobalt-60 taken up from seawater by *Octopus vulgaris*.
- Machreki-Ajmi *et al.*, 2008. The effect of in situ cadmium contamination on some biomarkers in *Cerastoderma glaucum*.
- Servicio Geológico Mexicano, 2014.
- Rodríguez *et al.*, 2006. Element concentrations in some species of seaweeds from La Paz Bay and La Paz Lagoon, Mexico.

Acknowledgements



The embryonic olfactory system in the common cuttlefish *Sepia officinalis*

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Background

Olfaction in cephalopods is poorly understood.

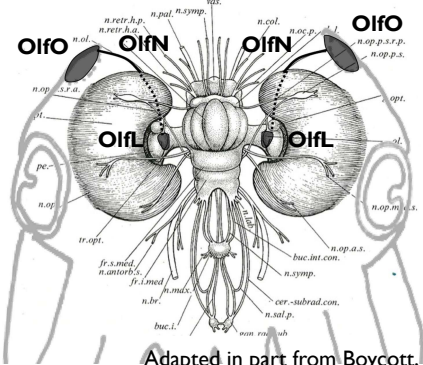
The olfactory organ was named more than 100 years before the first behavioral studies demonstrated its functionality. Kölliker (1844) described pit-like structures above the eyes in octopus and squid. Zernoff (1869) and Watkinson (1909) suggested the large ciliated cells in their ultrastructure were chemosensory. Young, Messenger, Boycott, and Woodhams included the olfactory lobe in their detailed mapping of the entire cephalopod central nervous system (CNS). They inferred, based purely on innervation patterns using Cajal silver stain, that this lobe was where olfactory information was processed. The first experiments to demonstrate the functionality of the olfactory epithelium were published in the 1980s (Boyle; Chase & Wells).

Today, there is still much to be understood about the olfactory system, including its neurotransmitters and structural organization. Using modern histochemical and molecular techniques, we describe neurotransmitters and neuropeptides involved in the olfactory system of the common cuttlefish, *Sepia officinalis*, to better understand the intricacies of this chemosensory system.

Objective

To explore and describe the complexities of the olfactory system in *Sepia*.

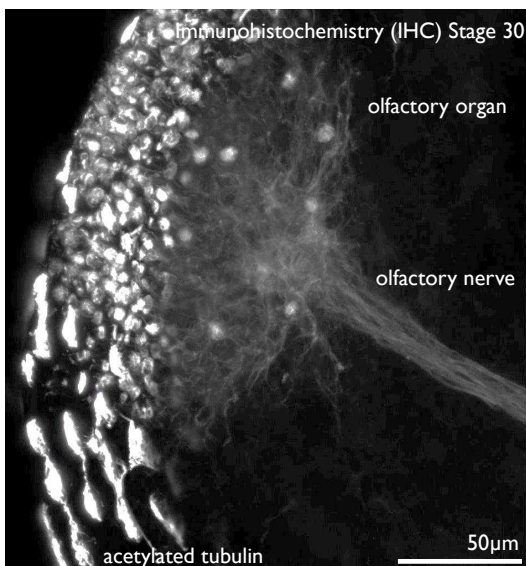
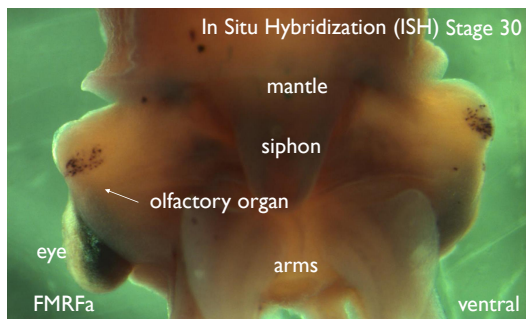
Sepia officinalis



Adapted in part from Boycott, 1961

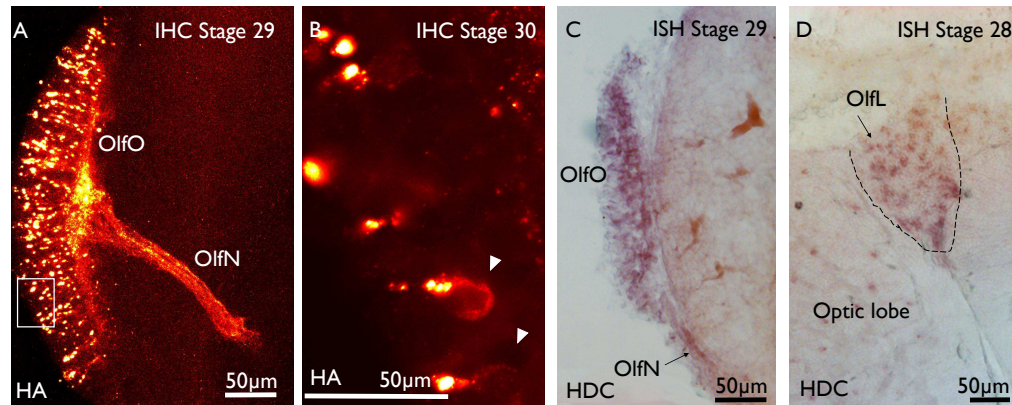
OlfO: olfactory organ (ventral side)

OlfN: olfactory nerve
OlfL: olfactory lobe (dorsal side)



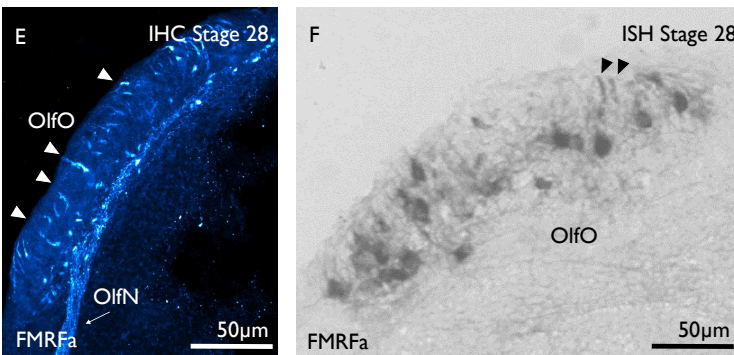
Figures A-O are transverse sections of brain across the anterior-posterior axis.

Histamine (HA) and Histidine decarboxylase (HDC)

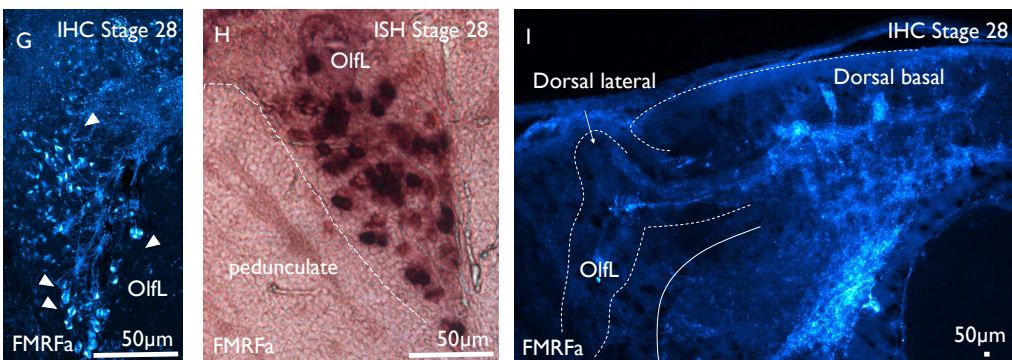


HA is a neurotransmitter in the olfactory organ and nerve (Fig. A) that at high magnification (boxed area) seems to be in the dendrites of dimly stained sensory cells, (arrowheads pointing to cell bodies, Fig. B). In addition, HDC mRNA is present in neurites, possible cell bodies, and the nerve (arrow) in the olfactory organ (Fig. C), as well as in neurons in the olfactory lobe (Fig. D).

FMRamide and related peptides (FMRFa)

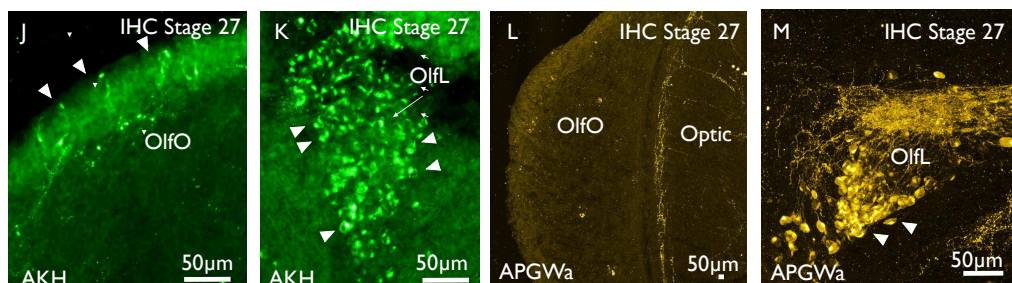


The olfactory organ contains FMRFa and related peptides in the dendrites and axons of dimly stained sensory neurons (arrowheads, Fig. E). The axons then bundle into the nerve (arrow). FMRFa cell bodies and potential dendrites (arrowheads, Fig. F) are visible using an antisense FMRFa mRNA probe.



FMRFa is also present in cell bodies (arrowheads, Fig. G) and fibers of the olfactory lobe. ISH confirms the presence of FMRFa neurons (Fig. H). FMRFa is also abundant in the rest of the CNS and demonstrates the interconnectivity of the olfactory lobe to the dorsal basal and dorsal lateral lobes (Fig. I).

Adipokinetic hormone (AKH) and APGWamide (APGWa)



Previous studies indicate that the olfactory system is involved in reproduction. We therefore decided to look at two reproductive peptides, AKH and APGWa. The olfactory organ contains some AKH neurites (arrowheads, Fig. J) and dimly stained cell bodies. While AKH is limited to a few cells in the olfactory organ, it appears in numerous cell bodies in the olfactory lobe (arrowheads, Fig. K). APGWa appears absent from the olfactory organ in comparison to the fibers in the optic lobe (Fig. L). APGWa is present in the olfactory lobe, both in neurons (arrowheads) and fibers that appear to project to the optic gland (Fig. M).

A special thank you to:

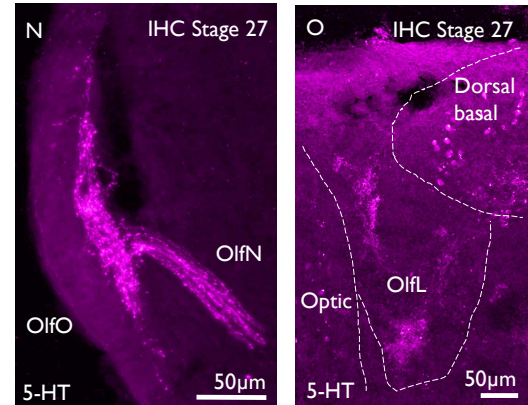
Halifax: N. Merovitch, J. Doyle, G. Beach, M. Secord, A. Wallace, & Drs. A. Gaudin, M. Stoyek, A. Quinn, P. Torkkeli, & S. Adamo

Paris: B. Imarazene, A. Rio-Cabello, & Drs. A. Andouche, Y. Bassaglia, & Prof. L. Bonnaud

Concarneau: Drs. S. Auzoux-Bordenave, & A. Badou



Serotonin (5-HT)



5-HT is localized in the neuropil below the olfactory organ. These fibers extend through the olfactory nerve (Fig. N) and connect to the neuropil in the olfactory lobe. 5-HT is widely distributed throughout the neuropil of the CNS (Fig. O).

Discussion

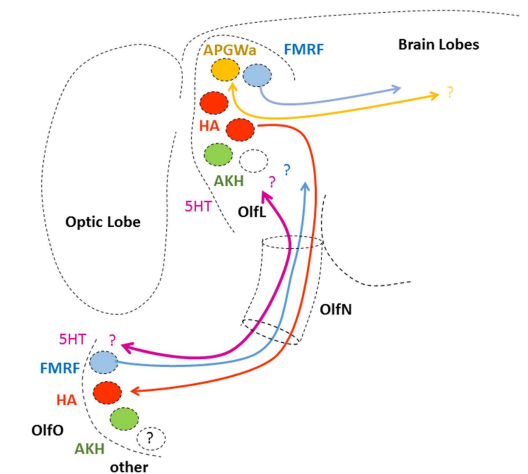
The olfactory system is complex and contains several neurotransmitters and neuropeptides.

We have identified HA as a potential efferent neurotransmitter as it most likely originates in the HA neurons of the olfactory lobe and terminates in neurites in the olfactory epithelium.

At least one class of sensory neurons in the olfactory organ appears to contain FMRFa related peptides. Due to the additional presence of FMRFa neurons in the olfactory lobe, we posit that FMRFa has an additional second-order processing function.

5-HT neurons do not appear in either the peripheral epithelium or lobe. Instead, 5-HT appears to be localized in neuropil under the organ and in the lobe, which are connected via the olfactory nerve. Functionality and directionality will be determined in future studies.

AKH neurites are present in the olfactory epithelium, further supporting previous studies that suggest involvement in pheromone detection. Because APGWa has no apparent projections to the olfactory organ, but is abundant in the olfactory lobe and CNS, it may be involved in the processing of olfactory stimuli.



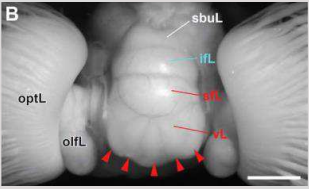
There are several other classes of neurotransmitters that have been associated with the olfactory systems in other molluscs which we would like to verify in cephalopods. These include glutamate, GABA, nitric oxide, and dopamine. These additional studies will assist in a description of the entire olfactory system and lead to a greater understanding of distance chemoreception in cephalopods.

Analysis of stem cell markers in the adult *Octopus vulgaris* brain

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INTRODUCTION

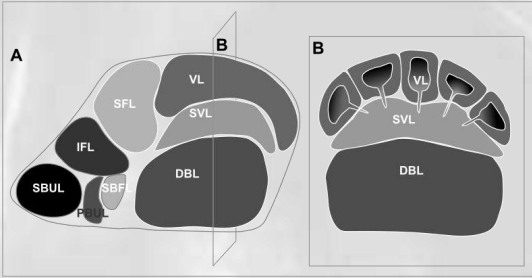


Top down view on the fixed *Octopus vulgaris* brain: supraesophageal mass localized between the two optic and olfactory lobes.
olfL, olfactory lobe; optL, optic lobe; ifL, inferior frontal lobe; sbuL, superior buccal lobe; sFL, superior frontal lobe; VL, vertical lobe
From Shigeno et al., 2015

The basic invertebrate molluscan nervous system plan has evolved into a highly centralized brain in the *Octopus vulgaris*, a solitary predator capable of problem solving and social learning. The diversity of neural cell types, as well as the key molecular components responsible for this intelligent divergence of the octopus from other invertebrates remain elusive. Furthermore, the molecular factors underlying the generation of this neuronal diversity are not well understood.

Here, we attempt to visualize postnatal neurogenesis in the adult *Octopus vulgaris* brain using immunohistochemical stainings for markers of proliferation. Using the recently published *Octopus bimaculoides* genome, we identified orthologues of Sox2 and SLC6A1; markers for stem cells and differentiated neurons (GABA transporter) respectively. We used *in situ* hybridization to map the expression of these orthologues on the supraesophageal mass of the *Octopus vulgaris*.

Via immunohistochemistry, we visualized the presence of phosphorylated histone H3 (PH3), a marker for mitotic chromosome condensation and found that the presence of this marker correlates with the expression of Sox2 in the region of the vertical lobe. Furthermore, a high proportion of *Octopus vulgaris* white body cells is positive for PH3 when freshly isolated and after 3 days in culture.



Schematic representation of the supraesophageal mass
A. Midline section showing the different lobes and B. coronal section showing the five gyri of the vertical lobe.
DBL, dorsal basal lobe; IFL, inferior frontal lobe; P/SBUL, posterior/superior buccal lobe; SBFL, subfrontal lobe; SFL, superior frontal lobe; SVL, subvertical lobe; VL, vertical lobe

IN SITU HYBRIDIZATION

M. musculus

D. melanogaster

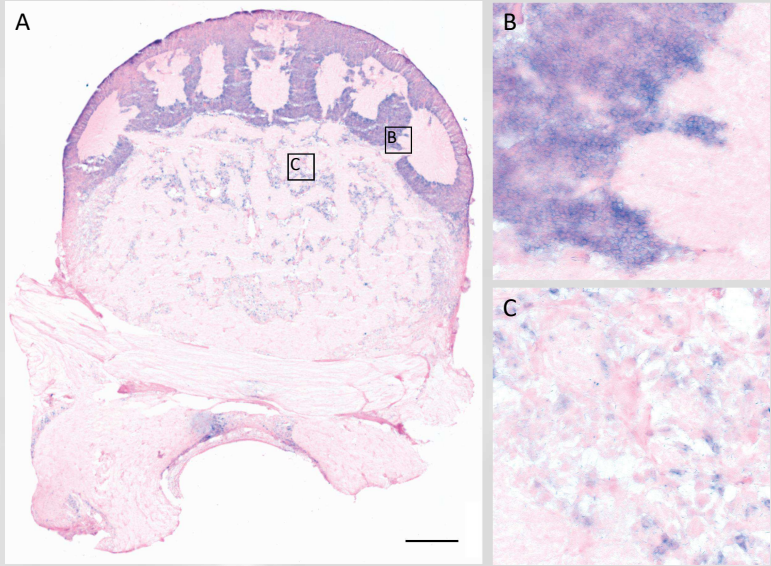
O. bimaculoides

O. vulgaris

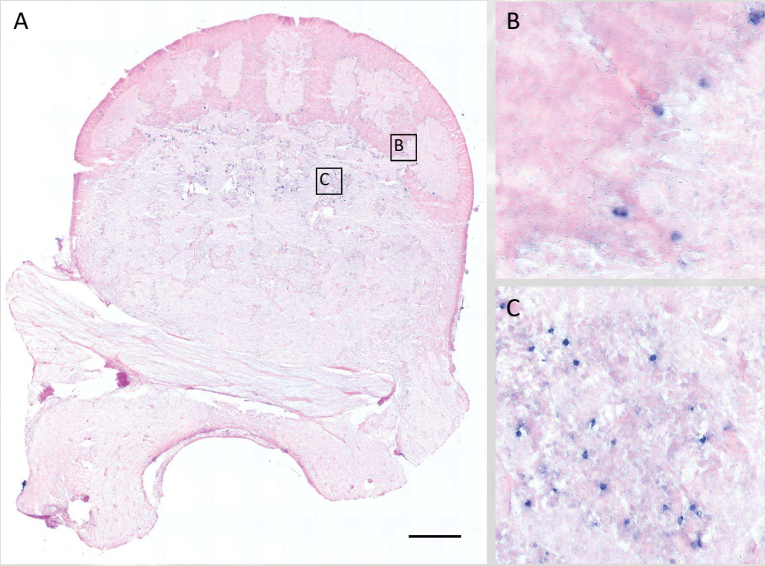


Expression of Sox2 shows presence of putative stem cells in vertical and subvertical lobes

Expression of Slc6A1 shows presence of postmitotic neurons in vertical and subvertical lobes



Expression of ovSox2 in the adult *Octopus vulgaris* supraesophageal mass.
A. We used *in situ* hybridization to visualize the expression of ovSox2 in the supraesophageal mass. ovSox2 mRNA is present in the amacrine cells of the vertical lobe (B) and in the cells of the subvertical lobe (C). Scale bar represents 500 μ m

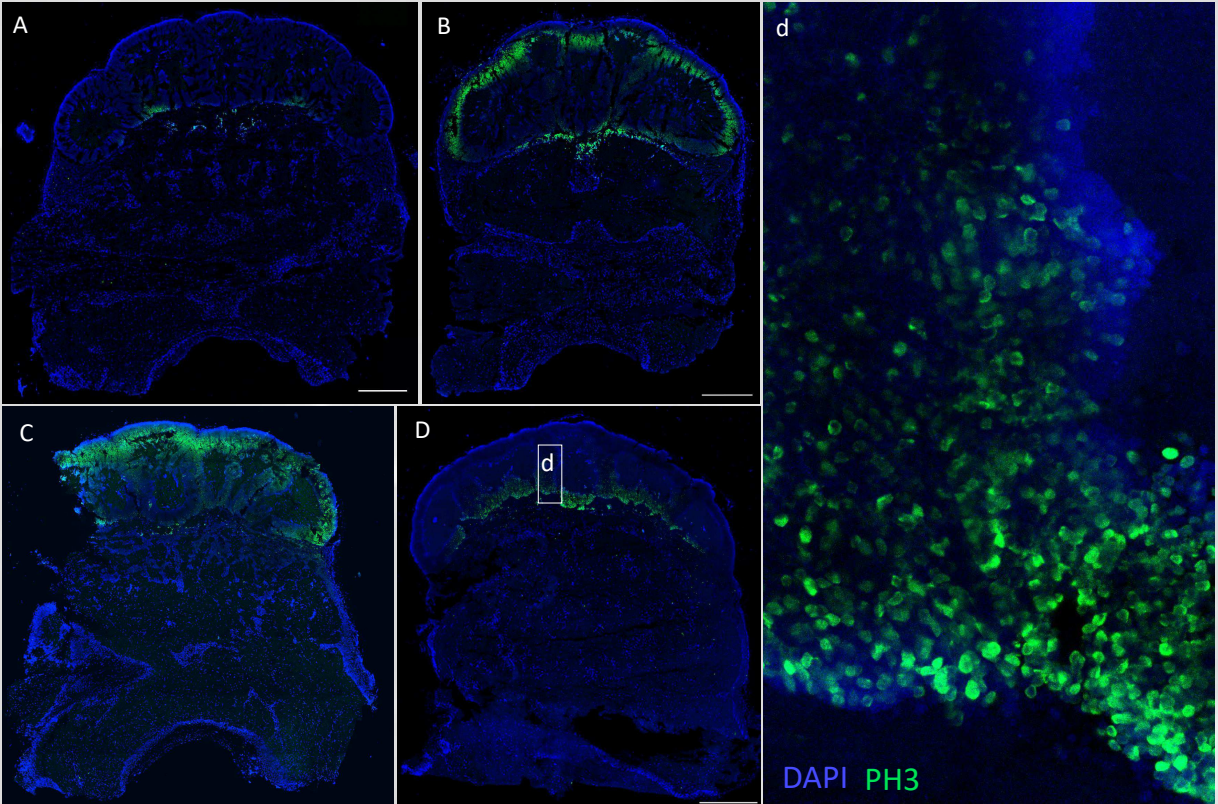


Expression of ovSlc6A1 in the adult *Octopus vulgaris* supraesophageal mass.
A. We used *in situ* hybridization to visualize the expression of ovSlc6A1 in the supraesophageal mass. ovSlc6A1 mRNA is present in a small subpopulation of the vertical lobe (B) and in a subset of subvertical lobe cells (C). Scale bar represents 500 μ m

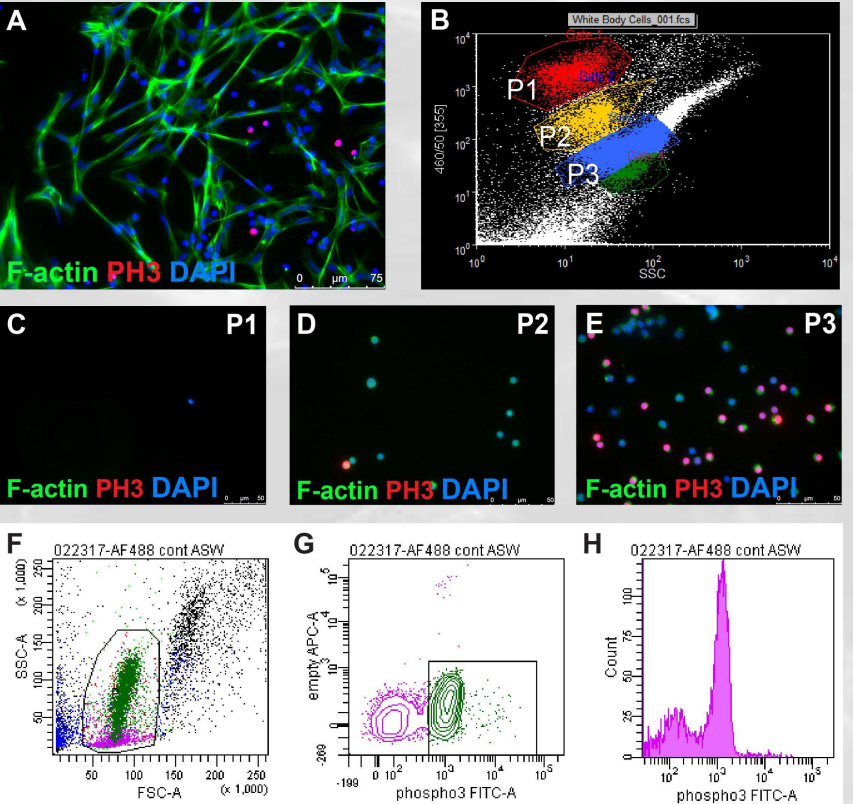
HISTONE H3 PHOSPHORYLATION

Presence of phospho-histone H3 (pSer28) in the vertical lobe of the supraesophageal mass is dynamic

Phospho-histone H3 (pSer28) positive cells are enriched in a fraction of white body cells



Presence of phospho-histone H3 (pSer28) in the adult *Octopus vulgaris* supraesophageal mass.
A-D. Stainings in 4 different animals show PH3 positive cells in the vertical lobe in different patterns. d. Magnification shows that the PH3 signal originates from the nuclei. Scale bar represents 500 μ m



Presence of phospho-histone H3 (pSer28) in adult *Octopus vulgaris* white body cells.
A. After 3 days in culture, both differentiated and undifferentiated cells can be distinguished shown by presence of F-actin and PH3 respectively. B. FACS sorting of freshly isolated white body cells incubated with Hoechst enables separation of different populations. C-E. Subsequent fixing and staining reveals population 3 (P3) is enriched in PH3 positive cells. F-H. FACS sorting of fixed and stained white body cells shows a high proportion of PH3 positive cells (peak in H: corresponding to green cells in F and G).

Larval and juvenile cephalopods from the North Atlantic.



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Introduction

Three transatlantic passages from Galway, Ireland to St. Johns, Newfoundland were conducted onboard *RV Celtic Explorer* in 2014, 2015 and 2016. Cephalopod samples were mainly collected using a pelagic trawl, with a focus on sampling across a warm core eddy on the western part of the transect (Fig 1). A multinet and a ringnet were also used to collect specimens, however out of the 306 cephalopods caught only 11 were taken in a ringnet and six in a multinet.

Table 1: List of specimens collected

| Species | 2014 | 2015 | 2016 |
|---|------|------|------|
| <i>Abraliopsis (Abraliopsis) morisii</i> * Verany, 1839 | | | 4 |
| <i>Ancistroteuthis lichtensteini</i> (Férussac, 1835) | 6 | 5 | 46 |
| <i>Bathothauma lyromma</i> Chun, 1906 | 1 | 1 | |
| <i>Brachioteuthis beanii</i> Verrill, 1881 | | 5 | 6 |
| <i>Brachioteuthis</i> sp. | | | 3 |
| <i>Chiroteuthis veranyi</i> (Férussac, 1834) | 1 | | 5 |
| <i>Chiroteuthis mega</i> (Joubin, 1932) | | | 3 |
| Taoniinae indet. | | | 2 |
| <i>Chtenopteryx</i> sp. | | | 2 |
| <i>Cranchia scabra</i> Leach, 1817 | | | 1 |
| <i>Eledone cirrhosa</i> (Lamarck, 1798) | | 1 | |
| <i>Gonatus steenstrupi</i> Kristensen, 1981 | | 5 | 13 |
| <i>Haliphron atlanticus</i> Steenstrup, 1861 | | | 2 |
| <i>Helicocranchia pfefferi</i> Massy, 1907 | | 1 | |
| <i>Heteroteuthis dagamensis</i> Robson, 1924 | | 2 | |
| <i>Histioteuthis reversa</i> (Verrill, 1880) | | 1 | 5 |
| <i>Histioteuthis bonnellii</i> (Férussac, 1835) | 3 | 16 | 9 |
| <i>Histioteuthis</i> sp. | | 1 | 1 |
| <i>Lampadioteuthis megaleia</i> Berry, 1916 | | 1 | 5 |
| <i>Leachia lemur</i> (Berry, 1920) | 1 | 2 | 3 |
| <i>Mastigoteuthis agassizii</i> Verrill, 1881 | 4 | | 1 |
| <i>Mastigoteuthis magna</i> Joubin, 1913 | | | 4 |
| <i>Octopoteuthis sicula</i> Rüppell, 1844 | 3 | 5 | 17 |
| <i>Onychoteuthis</i> sp. | | | 2 |
| <i>Pterygioteuthis gemmata</i> Chun, 1908 | | 1 | |
| <i>Pyroteuthis margaritifera</i> (Rüppell, 1844) | 1 | 10 | 71 |
| <i>Taonius pavo</i> (Lesueur, 1821) | | | 2 |
| <i>Teuthowenia megalops</i> (Prosch, 1849) | 1 | 2 | |
| <i>Todarodes cf. sagittatus</i> (Lamarck, 1798) | | 1 | 11 |

*= *Abraliopsis pfefferi* (see Bello, 2005)

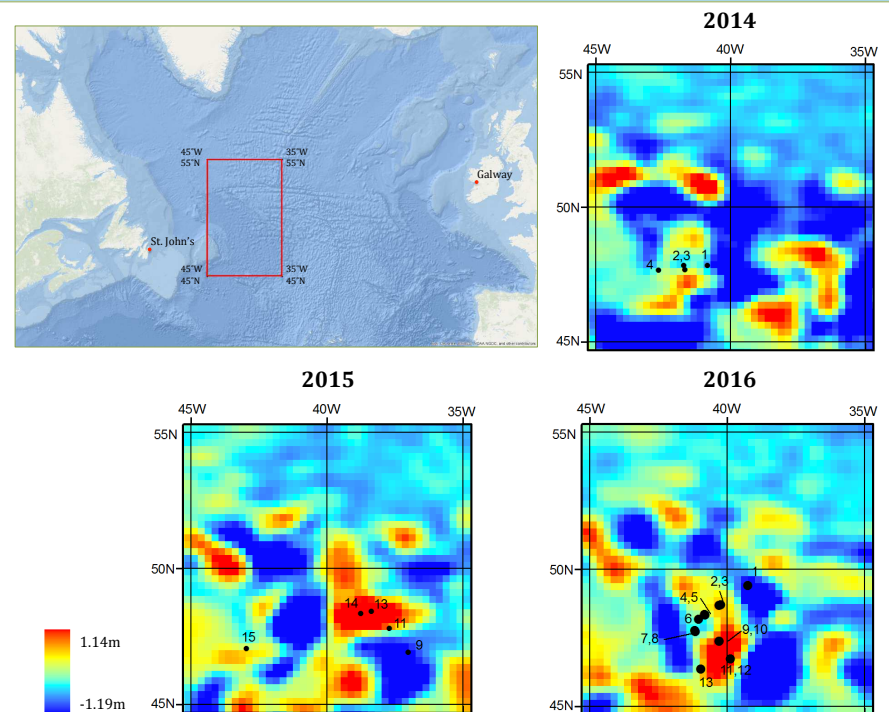


Fig 1: Position of trawl stations aboard the *RV Celtic Explorer* on warm core eddy: 2014, 2015, 2016. (Note that in 2016 two trawls were conducted at each station). Maps were created using sea level altimetry anomalies, downloaded via AVISO (Archiving, Validation and Interpretation of Satellites Oceanographic Data). Note that sea level altimetry has been used as a proxy for temperature.

Our Data

Once collected, the samples were fixed in ethanol. They were barcoded using the Folmer region of the COI gene (Folmer et al., 1994) and compared to sequences available on Genbank. Specimens from 2014 and 2015 were also morphologically identified by MV. Specimens from 2016 have yet to be identified by MV but were identified by comparing the sequences to the 2014 and 2015 sequences and to sequences available on Genbank, in the knowledge that not everything on Genbank has been correctly identified. Haplotype networks were built using TCS and were used to identify species networks. Species were then placed in a phylogenetic context using RAXML. The specimens with no matching sequences that were identified to species level were identified by MV as *Bathythauma lyromma*, *Helicocranchia pfefferi*, *Lampadioteuthis megaleia*, *Pterygioteuthis gemmata* and *Todarodes cf. sagittatus*. MV also looked at the 2015 *Histioteuthis* sp. specimen but could not identify it to species level as the specimen was too damaged. The *Onychoteuthis* sp., and *Brachioteuthis* sp. were identified to genus level by comparing the sequences from our specimens and all other available oegopsid sequences on Genbank. The specimens described as Taoniinae indet. were identified by MT and LA based on a closed mantle and will be identified further by MV.

Some species were found further north than we may have expected (Fig 2). Some specimens, e.g., the *Todarodes* (Fig 3) were interesting from a taxonomic point of view.



Fig 2: Top and bottom left, *Heteroteuthis dagamensis*: Top right and bottom centre, *Ancistroteuthis lichtensteini*; Bottom right, *Lampadioteuthis megaleia*

Some of the specimens collected are known from a more southerly distribution (Jereb and Roper, 2005 and 2010). We therefore assume that the following were brought north within the eddy: *Lampadioteuthis megaleia*, *Ancistroteuthis lichtensteini*, and *Heteroteuthis dagamensis* (genetically identical to sequences from specimens collected in the Gulf of Mexico by Heather Judkins, identified by MV as *Heteroteuthis dagamensis* (Judkins et al., 2016).

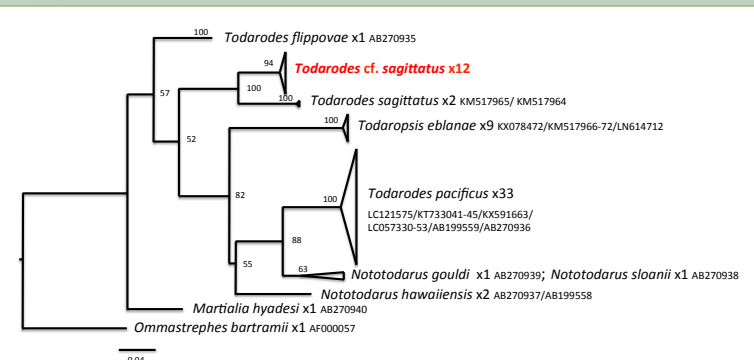


Fig 3: Neighbour-joining tree based on partial sequences of COI. Samples from recent transatlantic cruises shown in red. Other sequences from Genbank.

References & Acknowledgements

Bello, G. (2005). *Abraliopsis morisii* vs. *Abraliopsis pfefferi* (Cephalopoda: Enoplateuthidae): which is the right name? *Journal of Conchology* 38(5), 561-565.

Folmer, O., Black, M., Hoeh, W., Lutz, R. and Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3, 294-299.

Gebhardt, K. and Kneibelsberger, T. (2015). Identification of cephalopod species from the North and Baltic Seas using morphology, COI and 18S rDNA sequences. *Helgolander Marine Research*, 1-13.

Jereb, P. and Roper, C.F.E. (2005) *Cephalopods of the World: An annotated and illustrated catalogue of cephalopod species known to date. Volume 1: chambered nautilus and sepioids*. FAO, Rome.

Jereb, P. and Roper, C.F.E. (2010) *Cephalopods of the World: An annotated and illustrated catalogue of cephalopod species known to date. Volume 2: myopsid and oegopsid squids*. FAO, Rome.

Judkins, H., Vecchione, M. and Rosario, K. (2016). The presence of *Heteroteuthis dagamensis* in the Gulf of Mexico. *Bulletin of marine science* 92(1), 51-57.

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Todarodes cf. sagittatus

One specimen was collected in 2015 and 11 in 2016. The specimen from 2015 was morphologically identified by MV as *Todarodes sagittatus* however it is genetically distinct from the *T. sagittatus* collected in the North Sea by Gebhardt and Kneibelsberger (2015). A haplotype network was built using TCS and a neighbour-joining tree using RAXML which showed that the specimens from both 2015 and 2016 clustered separately from any of the sequences available on Genbank.