# Assessing life-cycle and longevity of cold water octopods (Cephalopoda: Octopoda) using growth marks on hard body structures Richard Schwarz, Hendrik Jan T. Hoving, Uwe Piatkowski, GEOMAR Thorsten Reusch

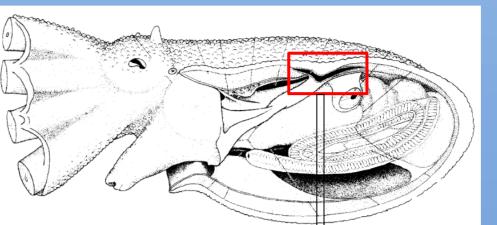
GEOMAR, Helmholtz Centre for Ocean Research Kiel, Düsternbrooker Weg 20, 24105 Kiel, Germany

#### Introduction

Life cycle investigations on cephalopods have focused mainly on commercially important squids and octopods. Age and growth studies for these semelparous marine molluscs are based on the interpretation of growth marks in hard body parts such as statoliths, gladius, beaks and stylets. For several species, the periodicity of increment deposition has been validated to be daily (Arkhipkin, 2004; Hermosilla et al., 2010; Rodríguez-Domínguez et al., 2013). Most studies to date have focussed on shallow and warm water species. These studies revealed fast growth and short life spans, the latter rarely exceeding 2 years. However, many cephalopods inhabit deep-sea and polar regions and for these species few attempts to determine longevity and growth rates have been made. Recent studies on the reproductive attributes of deep-sea octopods, show exceptionally long brooding times (Robison et al., 2014) and more than one reproductive cycle (iteroparity) (Hoving et al., 2015), both suggesting that these animals may live much longer than their shallow and warm water relatives . In order to assess how vulnerable or flexible deep water cephalopods are, and how they can cope with environmental change, it is crucial to understand their life cycle length and pace.

#### **Stylets**

The optimization of the technique for visualization of growth marks in stylets is still in progress but will follow the method developed by Barratt (2010).



# Aim

#### The objectives of this ongoing study are:

a) to test the hypothesis that octopods inhabiting cold waters (pelagic deep sea and benthic Antarctic) live longer and grow slower than warmer water ones; b) to determine which hard body structures are suitable for interpretation and quantification of growth marks in cold water octopods; c)to compare life length and growth in octopods with different modes of life (benthic, pelagic, benthopelagic) and phylogenetic groups (cirrate, incirrate, vampyromorph)

#### Material

Benthic Antarctic octopods: Pareledone aequipapillae, Megaleledone setebos, Muusoctopus rigbyae.

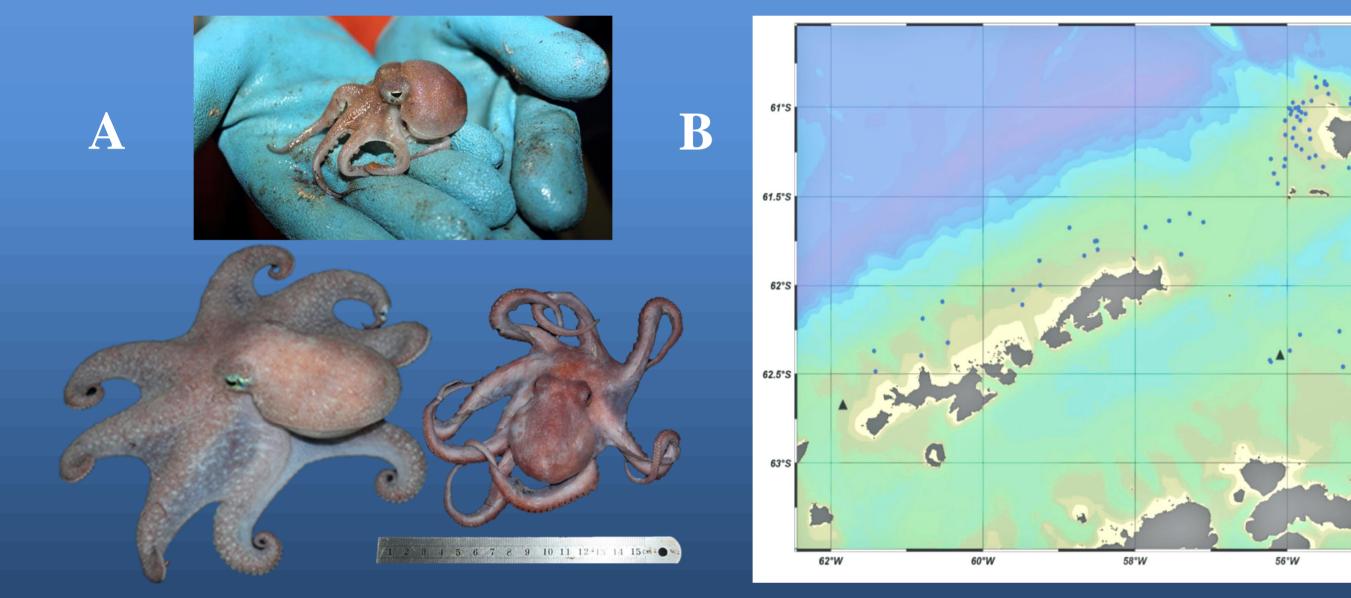
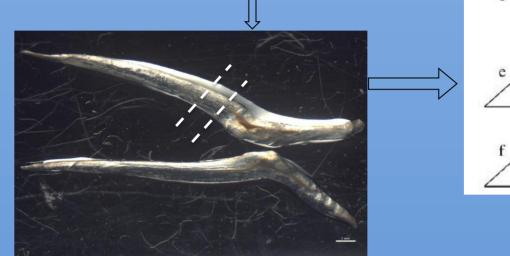
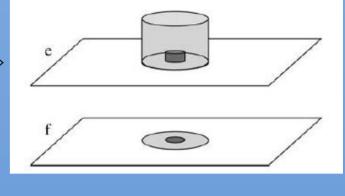


Figure 1 - A, The Antarctic octopods used in this study: Top - P. aequipapillae; Bottom: left- M. setebos; right - M. rigbyae; Photos:

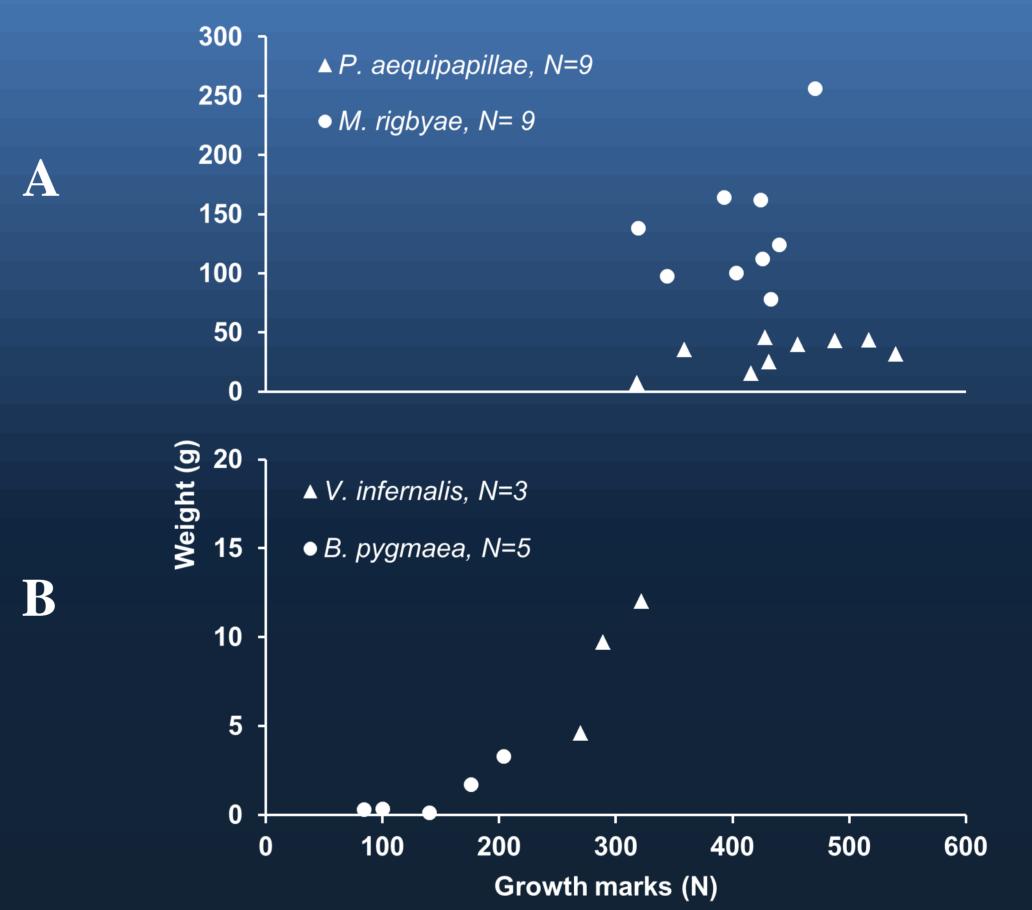




**Figure 4** – A schematic representation on how the stylets are extracted and prepared for visualization of growth marks, adapted from Bizikov (2004) and Barratt (2010).

# Results

The first results demonstrate that it is not possible to observe and quantify growth marks on the LWS in all species and specimens analyzed. Of the 34 beaks selected for methodology development, only 26 were readable, and it was impossible to visualize increments in the LWS of *M. setebos*. Only in *M. rigbyae*, RSS preparations produced observable growth marks. Depending on the degree of beak pigmentation we used reflected or transmitted light under stereomicroscope, from 50x magnification and above. In the Antarctic species (M. rigbyae and P. aequipapillae), animals ranging from immature to fully mature stages showed between 318 and 540 narrow and regular growth marks (between 10 and 30 µm). The examined pelagic octopod samples were composed only of juvenile immature animals. In these small specimens quantification of growth marks in beaks revealed 84 to 322 increments. Stylets were absent in deep-sea pelagic species and in Antarctic animals, only M. setebos presented this structure suitable for application of the methodology proposed by Barratt (2010), but no preparation produced satisfactory fields of view for visualization of growth marks. Scatterplots of increment number and weight are presented in figure 5.



Christoph Noever. **B**, The map shows the localization of the sampling stations, extracted from Lucassen 2012.

Pelagic deep-sea octopods: Bolitaenidae and Vampyroteuthis infernalis.



Figure 2 – The pelagic deep-sea octopods used in this study. At left, an unidentified member of the incirrate octopus family Bolitaenidae; on the right the vampire squid V. infernalis. Image source: MBARI.

#### Beaks

The interpretation and quantification of growth marks in the surfaces of the beak lateral walls (LWS) and rostrum sagittal sections (RSS) followed the methods described by Hernández-López et al. (2001) and Perales-Raya et al. (2010).

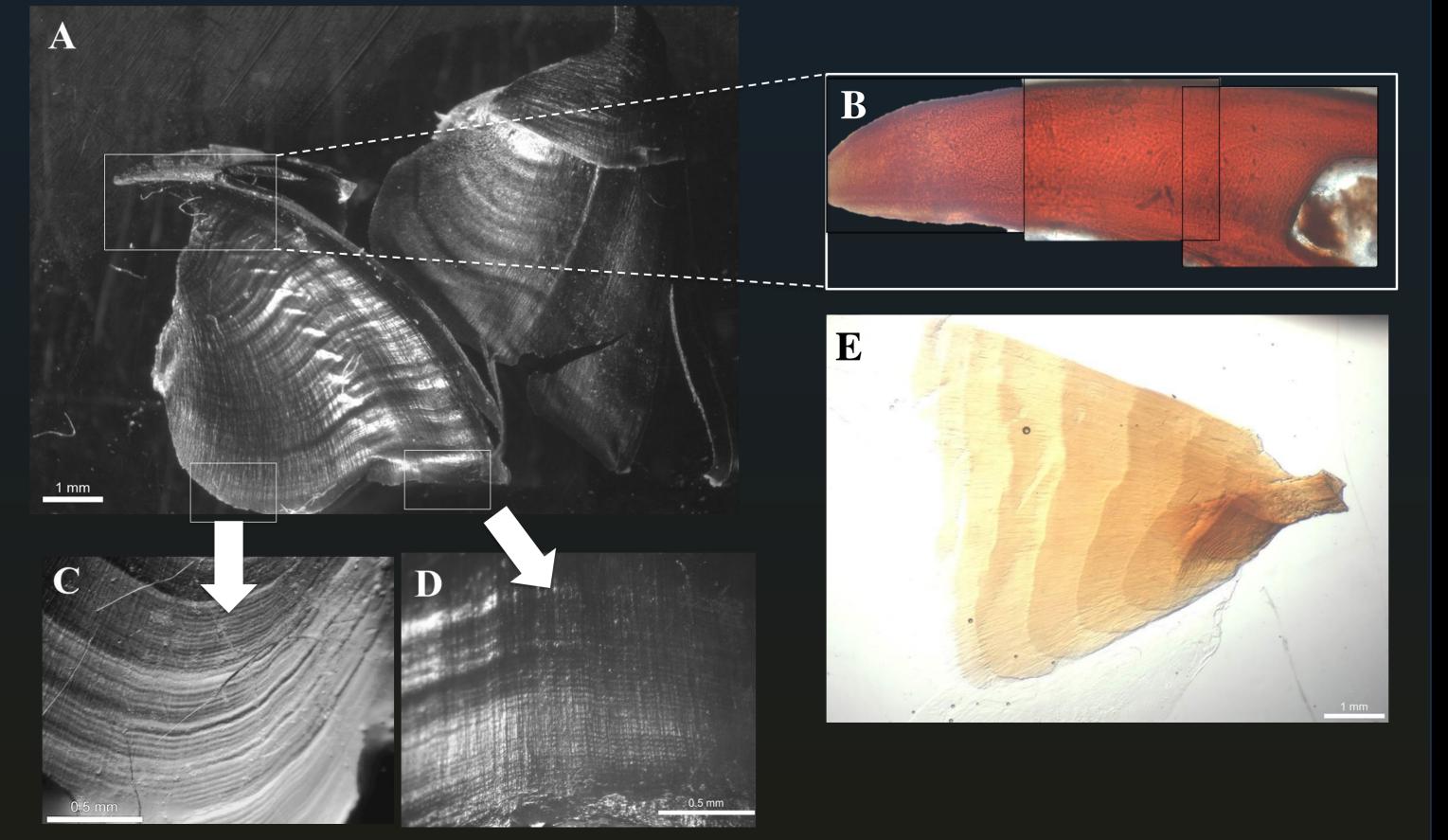


Figure 5 – Weight vs number of beak growth marks of the 26 animals examined in this study. A - Antarctic octopod species; Immature and mature animals of both sexes were plotted together. **B** - Increment observations in juvenile specimens of pelagic deep-sea octopods.

# Discussion

Although the deposition periodicity of the beak growth marks in the examined octopods are not validated, it has been demonstrated for several octopod species that such marks are formed on daily basis. Assuming daily deposition of beak increments for the analyzed species in this study would imply that they live longer than 1 year. The long brooding periods necessary for incubation of the eggs in cold water, could further extend the longevity of mature females.

These are the first attempts of age estimations using growth marks observation in beaks for the species analyzed here. Using the presented methodology, we aim to determine age at size for 300 more individuals. We also plan to perform age estimates in cirrate octopods, which will allow comparisons between lifespan in cold water octopods from different habitats.

# References

Figure 3 - A schematic representation of how growth marks are observed in octopods beaks. A - The sectioned upper beak of P. *aequipapillae*, under 10x magnification using reflected light stereo-microscope; B – Composite image of the rostrum sagittal section (RSS) under 100x magnification using transmitted light microscopy; C – Beak lateral wall of an unidentified Bolitaenidae, using transmitted light; D – Beak lateral wall of M. rigbyae using reflected light (50x); E – Section of an upper beak from M. setebos using transmitted light microscopy (10x).

Arkhipkin, A.I., 2004. Diversity in growth and longevity in short-lived animals: squid of the suborder Oegopsina. Mar. Freshw. Res. 55: 341–355. Barratt, I.M., Allcock, A.L. 2010. Ageing octopods from stylets: development of a technique for permanent preparations. ICES J Mar Sci 67: 1452-1457.

Hermosilla, C.A., Rocha, F., Fiorito, G., Gonzalez, A.F., Guerra, A., 2010. Age validation in common octopus *Octopus vulgaris* using stylet increment analysis. ICES J. Mar. Sci. 67: 1458–1463.

Hernández-López, J.L., Castro-Hernández, J.J., Hernández-García, V., 2001. Age deter- mined from the daily deposition of concentric rings on common octopus (Octopus vulgaris) beaks. Fish. Bull. 99(4): 679-684.

Hoving, H.T., Laptikhovsky, V.V., Robison, B.H. 2015. Vampire squid reproductive strategy is unique among coleoid cephalopods. Current Biology, 25(8): 322-323.

Lucassen, M. 2012. The expedition of the research vessel "Polarstern" to the Antarctic in 2012 (ANT-XXVIII/4), Berichte zur Polar- und Meeresforschung = Reports on polar and marine research, Bremerhaven, Alfred Wegener Institute for Polar and Marine Research, 652, 89 p.

Raya, C. P., Bartolomé, A., Teresa García-Santamaría, M., Pascual-Alayón, P., Almansa, E. 2010. Age estimation obtained from analysis of octopus (Octopus vulgaris Cuvier, 1797) beaks: Improvements and comparisons. Fisheries Research 106:171–176.

Robison, B.H., Seibel, B.A, Drazen, J. 2014. Deep-Sea Octopus (Graneledone boreopacifica) conducts the longest-known egg-brooding period of any animal. PloSOne, 9(7).

Rodríguez-Domínguez, A., Rosas, C., Méndez-Loeza, I., Markaida, U. 2013. Validation of growth increments in stylets, beaks and lenses as ageing tools in Octopus maya. Journal of Experimental Marine Biology and Ecology, 449: 194–199.

# Acknowledgements

