The Batsheva de Rothschild Workshop

Live foraminifera as a new model system for monitoring and reconstructing marine environments



### MEETING HANDBOOK & ABSTRACTS

### 10-16 September 2016, Eilat, Israel

#### SCIENTIFIC ORGANIZERS

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#### SPONSORS AND DONATIONS



Cover image: Porosononion sp.A from Hottinger et al. (1993) - graphical rendering by I. Voltski

#### **MEETING PROGRAM**

#### Friday September 9<sup>th</sup>

9:00 Tour at the old city of Jerusalem (leaving from Prima Royale Hotel, Jerusalem)

#### Saturday September 10<sup>th</sup>

Leaving Jerusalem at 9:00, visit at the Dead Sea 18:00 Arrival to Eilat 21:00: Icebreaker party (Harmony Yacht, North Marina, Eilat)

#### Sunday September 11<sup>th</sup>

8:00 Registration
8:30 Welcome Sigal Abramovich (Hilton auditorium)
8:35 Yossi Chen, CEO Eilat Municipal Tourism Corporation
8:40 Hanan Ginat, Chair of the Geological Society of Israel: The geological history of the Dead Sea Rift Valley

Morning: Session 1 – Foraminiferal biology and ecophysiology 09:00-11:00 Keynote lectures (4) 11:00-11:30 Coffee break & Art exhibition 11:30-12:45 Oral presentations (6) 12:45-14:00 Lunch (provided by Hilton)

### Afternoon: Session 2 – Geochemical proxies of foraminifera and Paleoceanography

14:00-16:00 Keynote lectures (4) 16:00-16:30 Coffee break & Art exhibition 16:30-17:45 Oral presentations (5) 17:45-19:00 Poster session & Art exhibition 19:00-21:00 Dinner

#### 21:00-22:00 Poster session + wine and snacks

Monday September 12<sup>th</sup> (Hilton auditorium) Morning: Session 3 – Ecology and morphology of foraminifera 8:30-10:30 Keynote lectures (4) 10:30 -11:00 Coffee break & Art exhibition 11:00-12:45 Oral presentations (7) 12:45-14:00 Lunch (provided by Hilton) Afternoon: Session 4 – DNA barcoding, metabarcoding and phylogenomics of foraminifera 14:00-16:00 Keynote lectures (2) and oral presentations (4) 16:00-17:00 Coffee break & Art exhibition 17:00-17:15 Eco Ocean presentation 17:15-19:00 FOBIMO (FOraminiferal BIo-MOnitoring) discussion by Frans Jorissen 19:00-21:00 Dinner 21:00-22:00 Poster session + wine and snacks

**Tuesday** September 13<sup>th</sup> IUI (transportation from Hilton Hotel and B center) Thematic workshops and Field collections

## Workshop 1 – Planktonic foraminifera collection and laboratory observation

#### Organisers: Jelle Bijma and Howie Spero

9:00-9:45 Introduction on planktonic foraminifera from the Gulf of Eilat by Jonathan Erez and Jelle Bijma

10:00-12:00 Cruise (1<sup>st</sup> cycle) collection of sediments by grab sampler.

12:00-14:00 Cruise  $(2^{nd} \text{ cycle})$  collection of sediments by grab sampler and snorkeling at the nature reserve  $(2^{nd})$ 

9:45-13:00 Observations on live plankton and planktonic foraminifera under the microscope (IUI teaching laboratory).
10:30-11:00 Coffee break
11:00-13:00 Tour at the underwater observatory (1<sup>st</sup>)
11:00-13:00 Snorkeling tour in the nature reserve (1<sup>st</sup>)
13:00-14:00 Lunch (at IUI)

#### **Workshop 2** – Recent benthic foraminifera from the Gulf of Eilat-Aqaba – Tribute to Zeev Reiss and Lukas Hottinger *Organiser:* Ahuva Almogi-Labin

14:00-14:30 Introduction by Ahuva Almogi-Labin
14:30-16:30 Laboratory Observation on diversity of benthic
foraminifera from shallow habitats (IUI teaching laboratory)
14:30-16:30 Tour at the underwater observatory (2<sup>nd</sup>)
16:30-17:00 Coffee Break
16:30-18:30 Snorkeling tour in the nature reserve (3<sup>rd</sup>)
17:00-18:00 Laboratory Observation on diversity of benthic
foraminifera from shallow habitats (IUI teaching laboratory).

18:30 Transportation to hotels

#### Wednesday September 14<sup>th</sup>

IUI (transportation from Hilton Hotel and B center). Thematic workshops and Field collections.

**Workshop 3 – DNA barcoding of benthic foraminifera** *Organisers:* **Jan Pawlowski** and **Maria Holzmann** 9:00-9:30 Introduction by Maria Holzmann

Workshop 4 – Foraminiferal symbiosis and ecophysiology Organisers: John Lee and Jonathan Erez 9:30-10:00 Introduction by John Lee and Jonathan Erez 9:30-14:00 Foraminiferal symbiosis and ecophysiology (IUI teaching laboratory).

10:00-10:30 Coffee Break

9:30-16:00 Barcoding of benthic foraminifera (DNA extraction and PCR amplification).

12:00-14:00 Lunch (at IUI).

14:00-15:00 Forams in their realm: Diving for Forams demonstration and discussions Beverly Goodman-Tchernov and Shai Oron.

#### Workshop 5 – Monothalamous foraminifera

#### Organisers: Susan Goldstein and Ivan Voltski

14:00-14:30 Introduction by Sue Goldstein

14:30-17:00 Monothalamid foraminifera - microscope observations

(IUI teaching laboratory)

15:00-16:00 Tour at the underwater observatory (3<sup>rd</sup>)

17:00-17:30 Organizing and packing collected material

17:30-18:30 Beers and snacks at the IUI beach

18:30 Transportation to hotels

#### Thursday September 15<sup>th</sup>

Transportation to Tel Aviv

8:30 Departure time-bus transportation (meeting point in front of the Hilton Hotel)
9:00-12:00 Archeological tour to the Timna Park (http://www.parktimna.co.il/en/)
12:00-12:30 packed lunch
13:00-14:30 Visit Kibbutz Neot Semadar and the Winery (http://neot-semadar.com/?lang=en)
15:30-17:30 Geological overview on the Makhtesh Ramon
20:00 Expected arrival to Tel-Aviv

Friday September 16<sup>th</sup> Tel Aviv-Jaffa and the beach Rock 9:00-12:00 Tour at the Jaffa Port and the flea Market- Shuk HaPishpeshim (http://en.shuktlv.co.il/category/jaffa-flea-market) 12:00-14:00 Sampling at the Beach Rock environment Free afternoon

#### SESSION SCHEDULE

#### Session 1 – Foraminiferal biology and ecophysiology Conveners: Emmanuelle Geslin and Joan M. Bernhard

Sunday, September 11<sup>th</sup> 09:00-11:00. Keynote lectures-30 minutes 11:00-11:30. Coffee break & Art exhibition 11.30-13.00. Oral presentations 12 minutes + 3 minutes discussion 18:15-19:00 poster session 21:00-22:00 poster session

#### **Keynote lectures:**

9:00-9:30 Bernhard J. M. Long-term culturing to assess impact of multiple stressors on a benthic foraminiferal community.
9:30-10:00 Geslin E., Lekieffre C., Nardelli P., Maire O., Barras C., Schweizer M., Thibault de Chanvalon, Langlet D., A. Meibom, Metzger E. Physiological functions of *Ammonia tepida* in different oxygenated conditions: evidence for dormancy under anoxia.
10:00-10:30. Goldstein S. T. Applications of the Propagule method in foraminiferal ecology.

10:30-11:00 **Wollenburg J.** High-pressure culturing – a major step towards a better understanding on deep-sea benthic foraminifers and the geochemical signals recorded in their calcareous shells.

#### **Oral presentations:**

11:30-11:45 **Wukovits J.**, Enge A. J., Bukenberger P., Watzka M., Wanek W., Heinz P. Laboratory feeding experiments with intertidal smaller benthic foraminifera

11:45-12:00 **Oron S**., Abramovich S., Almogi-Labin A., Erez J. Effect of pH and DIC on calcification and photosynthesis in *Operculina ammonoides*.

12:00-12:15 **Charrieau L. M.**, Schoon P. L., Chierici M., Groeneveld J., Kimoto K., Kritzberg E., Ljung K., Sasaki O., Toyofuku T., Filipsson H. L. Zombie foraminifera reveal impacts of ocean acidification in the Baltic Sea (DISCO project) 12:15-12:30 **Glock N.**, Dagan T., Eisenhauer A., Høgslund S., Liebetrau V., Revsbech, N. P., Roy A. S., Schönfeld J., Weissenbach J., Wein T., and Wöhle C. Temporal variability and different impacts on denitrification and respiration rates of *Globobulimina turgida* from Gullmar Fjord, Sweden 12:30-12:45 **LeKieffre C.**, Spero H., Russell A., Fehrenbacher J., Geslin E., Meibom A. Photosynthesis, carbon and nitrogen exchange between a symbiotic dinoflagellate and planktonic foraminifera host cell.

#### **Posters:**

**Bruisten B.**, Hallmann C. The Lipid Inventory of Rhizaria **Bukenberger P.**, Wukovits J., Enge A. J., Wanek W., Watzka M., Heinz P. The correlation between size and food uptake in *Ammonia tepida* a laboratory feeding experiment

**Enge A. J.**, Wukovits J., Wanek W., Watzka M., Witte U., Hunter W., Heinz P. Benthic foraminifera in the eastern Arabian Sea: Flourishing and feeding under oxygen depletion

**Frontalini F**, Greco M., Di Bella L., Lejzerowicz F., Caruso A., Cosentino C., Maccotta A., Giovanna S., Pawlowski J., Coccioni R. Assessing the effect of mercury pollution on cultured benthic foraminifera using DNA metabarcoding and morphological approaches.

**Frontalini F.**, Curzi, D., Cesarini, E., Canonico B., Giordano F.M., De Matteis R., Bernhard J. M., Pieretti N., Gu, B., Eskelsen J.R., Jubb A.M., Zhao L., Pierce E.M., Gobbi P., Papa S., Coccioni R. Effects of mercury pollution on *Ammonia parkinsoniana*: intracellular lipid accumulation and lysosomal compartment amplification.

**Heinz P.**, Nagy M., Quell G., Enge A. Test morphology of *Bolivina* aff. *B. dilatata* under oxygen depletion at the Indian margin (Arabian Sea)

**Kulaksiz M.**, Oertel A., Heinz P. Protein extraction and analysis of heat shock proteins (Hsp70) in living foraminifera

**Langlet D.**, Nardelli M. P., Metzger E., Geslin E. Effect of starvation and nourishment on respiration rates of intertidal benthic foraminifera.

**Nomaki H.**, Bernhard J. M., Ishida A., Tsuchiya M., Uematsu K., Tame A., Kitahashi T., Takahata N., Sano Y., Toyofuku T. Cellular ultrastructure and intracellular isotope localization in *Ammonia* sp. of oxygen-depleted environments: results of nitrate and sulfate labeling experiments.

**Stuhr M.**, Reymond C., Kucera M., Blank-Landeshammer B., Kollipara L., Sickmann A., Westphal H. Understanding the molecular basis for stress response in foraminifera and symbionts by proteome analysis.

**Wukovits J**., Enge A. J., Bukenberger P., Wanek W., Watzka M., Heinz P. Feeding strategies of the intertidal foraminifera *Ammonia tepida*.

# Session 2 – Geochemical proxies of foraminifera and Paleoceanography

Conveners: Jonathan Erez, Jelle Bijma and Howard J. Spero Sunday, September  $11^{th}$ 

14.00-16.00. Keynote lectures 30 minutes 16:30-17:45 Oral presentations 12 minutes + 3 minutes discussion 17:45-19:00 poster session 21:00-22:00 poster session

#### **Keynote lectures:**

14:00-14:30 **Erez J.** Biomineralization in perforate foraminifera and its implication for understanding paleoceanographic proxies 14:30-15:00 **Bijma J**. Biomineralization as the basis to understand proxie incorporation.

15:00-15:30 **Spero H. J.** Unraveling Planktonic Foraminifera Shell Biomineralization and Trace Element Variability Using Laser Ablation-ICPMS and NanoSIMS

15:30-16:00 **Rosenthal Y.** From Erlenmeyer to the sediment: a critical appraisal of proxy calibration methods.

#### **Oral presentations:**

16:30-16:45 **Barras C.**, Mouret A., Nardelli M. P., Petersen J., Metzger E., Filipsson H. L., Jorissen F. Experimental calibration of Mn incorporation in foraminiferal calcite.

16:45-17:00 Guamán F., Austin W., Austin H., Hicks N.

Quantification of the effects of ocean acidification on benthic foraminifera

17:00-17:15 **Toyofuku T.** Useful microenvironmental visualization to reveal foraminiferal calcification process.

visualization to reveal foraminieral calculation process.

17:15:17:30 Weinkauf M. F. G., Waniek J. J., Martini R.

Correlation between shell calcification intensity and geochemical composition in planktonic Foraminifera.

17:30-17:45 **Remmelzwaal S.**, Sadekov A., Schmidt D., Parkinson I. Assessing foraminiferal calcite as a recorder of Cr isotopes and concentrations.

#### **Posters:**

**Branson O**., Bonnin E., Perea D., Spero H.J., Zhu Z., Winters M., Hönisch B., Russell A. D., Fehrenbacher J. S., Gagnon A. C. Atom-scale insights into POM chemistry and function

**Bhatia R**., Wade B., Hilding-Kronforst S., Spratt J., Leng M., Thornalley D. Detecting foraminiferal photosymbionts in the fossil record: a combined micropalaeontological and geochemical approach

**Davis C. V.**, Fehrenbacher J. H., Russell A. D., Spero H. J., and Hill T. M. Mg/Ca: Temperature and Crust Calcification in Cultured *Neogloboquadrina pachyderma* and *Neogloboquadrina incompta*  **Hauzer H.**, Evans D., Muller W., Rosenthal Y., Erez, J., Na in foraminifera as a new proxy for ocean chemistry.

Russell A. D., Fehrenbacher, J., Davis C. V. Insights from laboratory culture experiments into the cause of high Ba/Ca in nonspinose planktic foraminifer *Neogloboquadrina dutertrei*. Zirks E., Krom M. D., Goodman-Tchernov B. N. Evolution of the Holocene oxygen minimum zone in the SE Levantine basin understanding the effect of the Nile and natural climatic change Titelboim, D., Sadekov A., Almogi-Labin, A., Herut, B., Kucera, M., Schmidt, C., Hyams-Kaphzan, O. and Abramovich S., Effect of future warming on benthic foraminiferal calcification: Mg/Ca evidences from field study on extremely heat tolerant species.

#### Session 3 – Ecology and morphology of foraminifera Conveners: Frans Jorissen and Ahuva Almogi-Labin

Monday, September 12<sup>th</sup> 8.30-10.30. Keynote lectures 30 minutes 11:00-12:45 Oral presentations 12 minutes + 3 minutes discussion

#### **Keynote lectures:**

8:30-9:00 **Kitazato H.**, Mishima K., Sinnger-Harii F., Growth strategy of permanently attached foraminifera: Case study of *Homotrema rubrum* (Lamarck).

9:00-9:30 **Tyszka J.**, Bickmeyer U., Raitzsch M., Bijma J., Topa P., Kaczmarek K., Mewes A. Chamber formation in globothalamean Foraminifera: From theoretical models to cytoskeleton staining experiments.

9:30-10:00 **Hohenegger J.** Methods for comparing living and dead assemblages of foraminifera.

10:00-10:30 **Goodman-Tchernov B. N.** Changes in foraminiferal assemblage and condition recognized in 2300 yr old tsunami deposit.

#### **Oral presentations:**

11:00-11:15 **Eder W.**, Woeger J., Kinoshita S., Hohenegger J., Briguglio A., The "natural laboratory" of *Heterostegina depressa*: evaluation of natural and laboratory growth by means of population dynamics

11:15-11:30 **Bouchet V.** Where are we standing? Where should we go? Review, limits and perspectives on current biotic indices based on benthic foraminifera

11:30-11:45 **Goleń J.**, Tyszka J., Komosinski M., Mensfelt A., Topa P. Constructing models of movement and sensing behavior in foraminifera.

11:45-12:00 **Guy-Haim T.**, Hyams-Kaphzan O., Yeruham E., Almogi-Labin A., Carlton J. T. "Jonah and the whale" -

Ichthyoendochory as a novel foraminifera invasion vector

12:00-12:15 **Tadir R.,** Hyams-Kaphzan O., Almogi-Labin A., Benjamini C. The long-term impact of sewage sludge input on live vs. dead foraminiferal assemblages of the Mediterranean coast, Israel.

12:15:12:30 **Chernihovsky N.**, Almogi-Labin A., Torfstein A. High-resolution temporal dynamics of planktonic foraminifera assemblages from sediment traps in the modern Gulf of Aqaba waters

12:30-12:45 **Seuront L**., Bouchet V. M. P. Motion behavior of intertidal benthic foraminifera: new approaches and ecological perspectives.

12:45-13:00 **Hyams-Kaphzan, O.,** Perelis Grossowicz, L., Almogi-Labin, A.

Benthic foraminifera of rocky reefs in the northern Israeli Mediterranean shelf in light of massive Lessepsian invasions

#### **Posters:**

**Ash-Mor A**., Almogi- Labin A., Ben-Avraham Z., Bookman R. Post-glacial resettlement of larger symbiont-bearing benthic foraminifera in the northern Gulf of Eilat/Aqaba.

**Enge A. J., Wukovits J.,** Gerg M., Heinz P. Interactions between intertidal foraminifera and turbellaria (Platyhelminthes) studied in the field and laboratory.

**Golikova E.**, Varfolomeeva M., Mikhailov D., Korsun S. Far north: modern salt marsh foraminifera from the subarctic White Sea.

**Kniazeva O.**, Korsun S. The Long Polar Night: Do Benthic Foraminifera Feed?

**Mikhailov, D.**, Foraminiferal propagules pelagic transport: sediment traps experiment.

**Wöger J.**, Shunichi K., Eder W., Briguglio A., Hohenegger J. Describing the growth of *Operculina:* determination of chamber building rates, maximum size and the use of growth functions in species determination.

**Zazu M.**, Micu D., Frontalini F. Assemblages of living benthic Foraminifera in a marine protected area from the Romanian Black Sea.

Session 4 – DNA barcoding, metabarcoding and phylogenomics of foraminifera Conveners: Jan Pawlowski and Maria Holzmann

Monday, September 12<sup>th</sup> 14.00-16:00. Keynote lectures (30 minutes) and oral presentations (12 minutes + 3 minutes discussion)

#### **Keynote lectures:**

14:00-14:30 **Holzmann M**., Molecular systematics of foraminifera: advances in DNA barcoding and phylogeny.

14:30-15:00 **Pawlowski J.**, Cordier T., Esling P., Lejzerowicz F., Visco J., Cedhagen T., Pochon X., Foraminiferal DNA metabarcoding applied to benthic monitoring: promises and challenges

#### **Oral presentations**

15:00-15:15 **Morard R.**, Quillévéré F., Darling K. F., Ujiià Y., Seears H. A., de Garidel-Thoron T., de Vargas C., Kucera M. Nomenclature for the nameless: A proposal for an Integrative molecular taxonomy of cryptic diversity exemplified by planktonic foraminifera.

15:15-15:30 **Sierra R.**, Holzmann M., Pawlowski J. Phylogenomics of Foraminifera.

15:30-15:45 **Schmidt C**., Morard R., Romero O., Kucera M. Identification of functional symbiont diversity in benthic foraminifera by a combination of algae culturing and genetic fingerprinting.

15:45-16:00 **Schweizer M.**, Darling K, Bird C, Austin B. Hidden twins from the antipodes: morphologically different but genetically identical cibicidids (benthic foraminifers) from Svalbard and Patagonia.

#### Posters

**Merkado, G.**, Oron, O., Holzmann, M., Pawlowski J., Ahuva Almogi-Labin A., Hyams-Kaphzan, O., Abdu U., Woeger, J., Briguglio, A., Abramovich, S. An unexpected case of genetic homogeneity within the morphologically diverse symbiont bearing larger benthic foraminifera *Operculina ammonoides*.

**Voltski, I., Abramovich, S.** Monothalamous foraminifera in marine habitats of Israel.

Weiner A. K. M., Tsuchiya M., and Kitazato H. Genetic diversity and mating behavior in foraminifera.

#### **Events**

17:00-17:15 Eco Ocean presentation.17:15-19:00 FOBIMO (FOraminiferal Bio-Monitoring).Discussion by Frans Jorissen.

#### ABSTRACTS

### Post-glacial resettlement of larger symbiont-bearing benthic foraminifera in the northern Gulf of Eilat/Aqaba

Ash-Mor A.<sup>1</sup>, Almogi- Labin A.<sup>2</sup>, Ben-Avraham Z.<sup>1,3</sup>, Bookman R.<sup>1</sup> <sup>1</sup> The Dr. Moses Strauss Department of Marine Geosciences, Leon H.Charney School of marine sciences. University of Haifa, Mt. Carmel, Haifa 31905, Israel. <sup>2</sup>Geological Survey of Israel, 30 Malkhe Israel, Jerusalem 95501, Israel. <sup>3</sup>The Department of Geosciences, Tel-Aviv University, Tel-Aviv 69978, Israel <u>ash anat@yahoo.com</u>

During the last glacial period, the increasing isolation of the Red Sea caused the northern Gulf of Eilat/Agaba (GEA) to become an extreme and hostile environment affecting the survival of many calcareous organisms. The sea water was much saltier (>50 psu) than present and colder by 40C, prev occurred only at the beginning of the Holocene. The timing of the reappearance of larger symbiont bearing benthic foraminifera (LBF), sensitive to lower temperatures and increasing salinity, is so far unknown. We use here new records from deep cores to trace the reappearance of this ecologically important group in the GEA. The LBF were examined in two piston cores taken from the western slope, at 316 m and 390 m covering the last ~13 ka and ~39 ka, respectively. The deep sea pelagic sedimentary record was interlayered by coarse sediment units, derived from the outer shelf. These units seem to be the outcome of Sediment Mass Transport (SMT) events associated with seismic activity occurring frequently in this area due to the complex fault system dissecting the gulf head. During the last glacial period no LBF occurred in the SMT units. Only facultative symbiont bearing Elphidiidae, known to have higher tolerance for hypersaline conditions, survived this extreme period and continued inhabiting the GEA. In the other Holocene core, the LBF Operculina ammonoides, Amphistegina papillosa and A. bicirculata are common components in all the SMT units. These LBF shells yield a maximum age of 11,759 BP. We suggest that this age is the first evidence for the time of re-settlement of symbiont bearing LBF in the northern GEA coinciding with the reappearance of the stenohaline planktonic foraminifera.

### Experimental calibration of Mn incorporation in foraminiferal calcite

Barras C<sup>1</sup>, Moure A<sup>1</sup>, Nardelli M.P.<sup>1</sup>, Petersen J<sup>1</sup>, Metzger E<sup>1</sup>, Filipsson H.L<sup>2</sup>, Jorissen F<sup>1</sup>
<sup>1</sup>LPG-BIAF UMR CNRS 6112, University of Angers, UFR Sciences, 2 bd Lavoisier 49045, Angers Cedex 01, France.
<sup>2</sup>Department of Geology, Lund University, Sölvegatan 12, 223 62, Lund, Sweden.
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The oceans have been subjected to low oxygen events of variable duration and intensity throughout geological time (e.g. sapropels, Oxygen Minimum Zones). In the context of recent climate change and increased anthropogenic activities in coastal areas, which both have an impact on dissolved oxygen availability, there is an increasing interest to better understand the mechanisms and evolution of such events in the past, to improve future predictions. The scientific community is currently exploring the potential of different redox sensitive trace metals as proxies for these events. Manganese is an element of high interest: in oxic conditions, manganese is present as insoluble oxy-hydroxydes whereas in hypoxic or anoxic conditions, the oxides are reduced to dissolved  $Mn^{2+}$ , available for incorporation in foraminiferal calcite. In this study, we investigated manganese incorporation in foraminiferal calcite by incubating two foraminiferal benthic species (Ammonia tepida and Bulimina marginata) in different dissolved manganese concentrations, under hypoxic conditions. The results of laser ablation ICP-MS measurements show a linear correlation between the Mn/Ca in calcite and seawater. These data clearly validate the use of Mn/Ca as a proxy of dissolved manganese in seawater and eventually of oxygen conditions. Moreover, our results highlight a clear species-specific effect on Mn incorporation: higher partitioning coefficients are measured in *B. marginata* (D=0.62) compared to A. tepida (D=0.08). We suggest that this is potentially due to differences in the biomineralisation processes of the two species since they calcified in similar conditions.

### Long-term culturing to assess impact of multiple stressors on a benthic foraminiferal community

Bernhard J. M. Woods Hole Oceanographic Institution, Department of Geology & Geophysics, Woods Hole, MA 02543, USA jbernhard@whoi.edu

Since the onset of industrialization, rising atmospheric carbon dioxide  $(CO_2)$  concentrations have increased oceanic  $CO_2$ , leading to decreases in seawater pH, resulting in a phenomenon called ocean acidification (OA). Simultaneously, rising global temperatures, also linked to higher atmospheric CO<sub>2</sub> concentrations, result in a more stratified surface ocean, reducing exchange between surface and deeper waters, leading to expansion of oxygen-limited zones (deoxygenation or hypoxia). Many studies have investigated the impact of one or two of these environmental stressors, but few experimental studies have focused on the simultaneous effects of these three stressors. I will describe a 10.5month experiment in which  $pCO_2$ ,  $O_2$ , and temperature were manipulated to evaluate the sole or combined influence of these stressors on the survival of a benthic foraminiferal propagule community. The community was collected from a ~75-m deep continental-shelf site south of Woods Hole, MA (USA). One agglutinated morphospecies, Bathysiphon cf. B. minuta, grew in each treatment. Occurrence of individual calcareous species was more restricted. Multidimensional scaling analysis indicates that  $pCO_2$  was not the most influential factor and that a compounded effect occurred in the triple-stressor treatment. Additional results on assemblage composition and growth will be presented. Supported by US NSF OCE-1219948.

#### Detecting foraminiferal photosymbionts in the fossil record: a combined micropalaeontological and geochemical approach

Bhatia R.<sup>1</sup>, Wade B.<sup>1</sup>, Hilding-Kronforst S.<sup>2</sup>, Spratt J.<sup>3</sup>, Leng M.<sup>4</sup>, Thornalley D.<sup>5</sup>

 <sup>1</sup>Dept. of Earth Sciences, University College London, UK
 <sup>2</sup>Dept. of Geology and Geophysics, Texas A&M University, USA
 <sup>3</sup>Core Research Laboratories, Natural History Museum, London, UK
 <sup>4</sup>NERC Isotope Geosciences Laboratory, British Geological Survey, Nottingham, UK
 <sup>5</sup>Dept of Geography, University College London, UK rehemat.bhatia.13@ucl.ac.uk

Modern symbiotic planktonic foraminifera exhibit cyclic Mg/Ca banding in their shell walls. Here we present electron microprobe data of well-preserved Eocene and Miocene planktonic foraminifera to investigate this feature in extinct taxa. In conjunction with  $\delta^{13}$ C and  $\delta^{18}$ O data, we further disentangle foraminiferal palaeoecology. Surface dwelling species Globigerinoides subquadratus and Acarinina praetopilensis and deep dwelling species Dentoglobigerina tripartita and Parasubbotina hagni were analysed. Geochemical results indicate distinct ecological preferences between species. All surface dwelling species exhibit Mg bands, whilst in thermocline dwelling species Mg bands were absent. The spatial distributions of the  $\delta^{13}$ C and  $\delta^{18}$ O data on a carbon-oxygen cross-plot also correlate with the occurrences of Mg banding observed in the microprobe maps. Surface dwellers were  $\delta^{13}$ C enriched, whilst thermocline dwellers were  $\delta^{18}$ O enriched. Having established these relationships, the evolution of photosymbiosis in middle and late Eocene hantkeninids was studied. Preliminary microprobe and  $\delta^{13}$ C data suggest an asymbiotic ecology in the middle Eocene and a photosymbiotic ecology in the late Eocene. The evolution of a photosymbiotic partnership in hantkeninids correlates with a depth habitat migration from deep to surface dwelling. Our data indicates that Mg banding is preserved in fossil planktonic foraminifera, and by combining micropalaeontological and geochemical data, more can be understood about extinct planktonic foraminiferal palaeoecologies.

### Biomineralization as the basis to understand proxie incorporation

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A mechanistic understanding of element transport and incorporation into calcifying organisms is the basis for translating empirical proxie relationships into robust tools for paleoreconstructions. Not only will it allow us to better understand the functional link between a target parameter and its geochemical signal but it will also unveil potential interactions with other biotic or physicochemical processes. There are currently two models proposed for the biomineralization in Foraminifera that are fundamentally different but maybe not mutually exclusive. One model, proposed by the group of Jonathan Erez is based on vacuolarisation of seawater while the other model (Nehrke, de Nooijer, Langer, Bijma) is based on active pumping of  $Ca^{2+}$  ions during chamber formation. I will introduce the trans-membrane transport model and discuss it in the context of additional, mostly experimental, data that has been generated over the last 30 years. As both proponents of the calcification models are present at the workshop, I expect that it will generate an interesting discussion on the state of the art of biomineralisation.

#### Where are we standing? Where should we go? Review, limits and perspectives on current biotic indices based on benthic foraminifera

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Over the last decades, concerns arose about the health of marine systems. It led to numerous legislations worldwide. These legislations enhanced the need to monitor and assess the ecological quality status (EcoQs) of marine areas. It requires that reference conditions and potential present-day deviations are defined. Benthic macrofauna invertebrates are the organisms mostly used to characterise EcoQs. Over the last few years, foraminiferists developped different indices to assess the EcoQ in order to promote the use of benthic foraminifera in biomonitoring studies. Different approaches have been considered, i.e. sensitivity-species approach and diversity indices. This state-of-the-art propose to assess the advantages and limits of existing methods so far. As the development of biotic indices based on benthic foraminifera is still in its infancy, it sounds relevant at this stage to settle and think of future works.

#### Atom-scale insights into POM chemistry and function

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The Primary Organic Membrane (POM) is integral to foraminiferal calcification. This sheet of complex carbohydrates and proteins is key in initiating and directing calcite growth in foraminifera, and sets the overall architecture of the entire test. Numerous studies have examined the POM using structural and chemical imaging, and proteomic techniques to reveal aspects of its broad-scale structure and chemistry. However, the transitory nature, atomic- to nanometer-scale, and geometric complexity of the POM has kept the in-vivo chemical interactions at this crucial interface outside our observational limits. We have captured a multi-scale chemical map of the POM in Orbulina universa using Atom Probe Tomography (APT), a time-of-flight mass spectrometry technique with Ångstrom-level spatial resolution, and time-of-flight secondary ionization mass spectrometry (ToF-SIMS), a 2D chemical imaging technique with sub-micron resolution. We quantitatively link these observations, revealing that the organic template in O. universa is uniquely enriched in both Na and Mg, and contributes to intra-skeletal chemical heterogeneity. Based on these measurements, we can estimate the influence of the POM on the Mg/Ca and Na/Ca palaeoproxies. Our APT analyses reveal the cation composition of the organic surface, offering evidence to suggest that cations other than  $Ca^{2+}$ , previously considered passive spectator ions in biomineralization, may be important in defining the energetics of carbonate nucleation on organic templates.

#### The Lipid Inventory of Rhizaria

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The microfossils of foraminifera, radiolaria and other rhizaria have been studied for well over a century and are important stratigraphic markers. Hardly anything is known about the corresponding molecular fossils. Biomarkers are the chemical remains of microorganisms that can be assigned to a particular biosynthetic origin and they can be preserved in sedimentary rocks for billions of years. The lipid inventory and molecular fossils of many eukaryotic algae are well studied and provide important insights into past ecosystem. Despite their abundance and ecological importance however, very little is known about the lipids and resulting molecular signatures of rhizaria. The lipid inventory of a single foraminiferal species, Allogromia laticollaris, was analysed in 2013 by Grabenstatter and colleagues and yielded surprising results: The 24-n-propylidenecholesterol produced by the for a minifera were previously considered to be only produced by marine pelagophyte algae and the geologic hydrocarbon product 24-n-propylcholestane is a widely applied biomarker of pelagophytes. Using organic geochemical techniques, we aim to elucidate the lipid inventory of additional foraminifera and other rhizaria including radiolaria. It is likely that other characteristic lipids with stable hydrocarbon skeletons are produced by some species that may potentially be used to provide evidence of the source organisms even in the absence of body-fossil preservation. This could even allow to trace rhizaria in deep time and elucidate their proposed Neoproterozoic emergence.

#### The correlation between size and food uptake in *Ammonia tepida* – a laboratory feeding experiment

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Ammonia tepida is one of the two dominating foraminiferal species of the Wadden Sea/Friedrichskoog and subject to many laboratory experiments because of its high abundance and availability. It is also a representative for shallow water environments, which are characterized by high productivity and abundant food sources. Productivity and high food availability are environmental factors that can control foraminiferal abundance and reproductive cycles. This could be related with the presence of sufficient suitable organic material to cover reproductive costs or to ensure high energy demands for juvenile growth. Freeze dried <sup>13</sup>C and <sup>15</sup>N labelled green algae (Dunaliella tertiolecta) was offered to investigate the relation of food uptake with growth stages of A. tepida. Sieves with 125 µm, 250 µm and 355 µm mesh size were used to separate different foraminiferal size classes. After two and four days of food incubation, triplicate samples of each size class were taken, decalcified and isotopically analyzed for differences in food derived carbon and nitrogen content within the foraminiferal cytoplasm. Ammonia tepida showed an increased phytodetrital carbon and nitrogen uptake in the smallest fraction. This could be explained by a higher energy consumption caused by growth processes and the formation of larger tests.

#### Zombie foraminifera reveal impacts of ocean acidification in the Baltic Sea (DISCO project)

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Ocean acidification is a consequence of current global climate change. The concomitant decrease in pH and carbonate ion concentration in sea water may have severe impacts on marine calcifying organisms, like foraminifera. This process may be amplified in coastal regions where erosion, land run-off, and eutrophication also contribute to lower the pH. Awareness of the effects of ocean acidification is increasing; however, it is still not clear how coastal marine ecosystems will react if the pH continues to decrease in addition to other environmental stressors such as temperature and salinity changes and also deoxygenation. The aim of our study is to investigate how benthic ecosystems will respond to pH changes as well as to other environmental stressors in the Baltic Sea region, and we use benthic foraminifera as one of the indicators. First results demonstrate large variations in living foraminiferal fauna with respect to species composition from five studied sites. The western Baltic (Skagerrak-Kattegat) stations have a higher species richness (50 species) compared to the two southern Baltic Sea stations (4 species). This can be linked to the large difference in salinity, ranging from 35 to 14 between the stations. More remarkable, the preservation of the foraminiferal shells is very different between the five stations. The foraminifera at the western stations have more or less pristine tests, while the tests of the southern stations for aminifera are partially to completely dissolved with only the organic inner linings still visible. Despite the lack of calcium carbonate tests, these "zombie" foraminifera are still alive, as determined from the CellTrackerTM Green labelling. These results can be linked with a recently observed negative trend in the Baltic Sea pH values, most likely in combination with other stressors such as deoxygenation, changes in salinity and temperature.

#### High-resolution temporal dynamics of planktonic foraminifera assemblages from sediment traps in the modern Gulf of Aqaba waters

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The Gulf of Aqaba (GOA) is recognized as a "natural laboratory" for the study of open ocean processes at high temporal resolution. Previous studies of planktonic foraminifera (PF) in the GOA focused on standing-stocks and species assemblages obtained from plankton tows and cores, yet information of the open water population dynamics are nonetheless still very poorly constrained. Here, we report the first systematic time-series of open-water PF assemblages in the northern GOA, collected using sediment traps. The traps, deployed vertically at five different depths (600m waterdepth), provide monthly resolution from January 2014 until present. These efforts aim to characterize the interplay between PF fluxes and species assemblages in the context of nutrient availability, water column stratification and terrigenous fluxes. Our results demonstrate strong seasonality of PF fluxes, whereby low fluxes are observed during the spring-summer months (min: 31±35 Ind. m-2 d-1), gradually rising to higher fluxes during the autumnwinter (max: 1570±790 Ind. m-2 d-1). This pattern follows the development of the mixed layer depth, but does not correspond to previous studies of the PF standing stock in the GOA from 1974-1976, possibly reflecting a change in the PF population over recent decades; indeed, a notable example is the total absence of G. sacculifer from our samples, despite the fact that it used to be the most dominant species 40 years ago. These discrepancies may also stem from our poor knowledge of the temporal and spatial PF distribution patterns in the GOA, emphasizing the importance of the current study. The results indicate that the most significant PF size fraction in terms of mass and fluxes is in the range of 63-125µm (87%, compared to 13% and 0.28%, for the 125-500µm and of 500-1000µm size-fractions, respectively), which has not previously been studied in this area.

#### Mg/Ca:Temperature and Crust Calcification in Cultured Neogloboquadrina pachyderma and Neogloboquadrina incompta

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The planktic foraminifers, Neogloboquadrina pachyderma and *Neogloboquadrina incompta* (formerly *N. pachyderma* var. dex.) were cultured at 6°C, 9°C, and 12°C and analyzed for Mg/Ca by Laser Ablation ICP-MS. Both species possess a texturally and geochemically distinct calcite "crust," added at the end of their life cycle. We report a linear Mg/Ca:temperature relationship through uncrusted shell material for N. pachyderma, and find that the von Langen (2005) calibration is also applicable to the uncrusted portions of the shell in *N. incompta*. Our observations of crust formation or lack thereof in laboratory cultures show that only 15% of N. incompta and no N. pachyderma added clearly crusted calcite. Culture observations of crusting in N. incompta support the hypothesis that crusting in the Neogloboquadrinids is added to the shell during the final 1-3 days of the life cycle. The low incidence of laboratory crust formation, despite successful gametogenesis, suggests that crusting is not required to complete the life cycle and could be related to an environmental cue. Other researchers hypothesize that the low apparent Mg/Ca and stable isotope offsets of Neogloboquadrinid crusts is caused by adding gametogenic calcite in cooler waters than those in which ontogenetic calcite is added. However, our observations demonstrate clearly that crust calcite has lower Mg/Ca than ontogenetic calcite even under constant temperature conditions. This suggests that the low Mg/Ca of planktic foraminifera crusts reported from the fossil record requires a separate temperature calibration from ontogenetic calcite.

# The "natural laboratory" of *Heterostegina depressa*: evaluation of natural and laboratory growth by means of population dynamics

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Heterostegina depressa has been the flagship species of laboratory investigations of larger benthic foraminifera (LBF) since the 70's. Even though earlier experiments gave much insight into chamber building processes, longevity and natural chamber building rates are understudied. In recent years, the appliance of laboratory growth rates on natural individuals has been strongly scrutinized. Therefore, the "natural laboratory" approach has been applied on Heterostegina depressa populations from Sesoko Jima, NW Okinawa, Japan. Using this, an averaged chamber building rate and averaged longevity of H. depressa can be calculated based on monthly samplings at fixed sampling stations. These results are later compared to specimens from laboratory cultures where ambient environmental conditions were simulated. Samples were collected by SCUBA in 16 monthly intervals around 20 and 50 meters water depths. Chosen live specimens of each sample were transferred to the culture aquarium and chamber building rate was observed using microphotography, while the remaining live population was dried and investigated by microCT. Frequency distributions of chamber number of the monthly sampled and dried individuals have been decomposed into normally distributed components. Means and standard deviations of the components of each month were extracted and could be used to calculate the maximum chambers number for every sampling interval of the components. Based on maximum chamber number, the natural chamber building rate could be estimated by the Bertalanffy function. The analyzed frequency distributions revealed two generations with similar chamber building rates, one starting in May and the other in September. Therefore, these two temporal separated generations per year explains the dominance of bimodal

distributions in monthly samples. Further, in comparison to the natural laboratory cultured individuals show stunted chamber building rates. This indicates, that growth patterns solely observed in laboratory investigations, are handicapped in comparison to natural observations, lacking a "natural" control group.

### Benthic foraminifera in the eastern Arabian Sea –Flourishing and feeding under oxygen depletion

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Benthic foraminifera at the Indian Margin (eastern Arabian Sea) live in high densities under anoxic to suboxic conditions at depths between 150 to 1200m. The concentration of dissolved oxygen, the flux of organic matter (OM) and biological interactions affect the abundance and species distribution of foraminifera within this Oxygen Minimum Zone (OMZ). A community showing low diversity but high abundance in the OMZ core region (<0.01 ml O<sub>2</sub>/l) shifts towards higher species numbers but lower individual accounts at greater water depths associated with lower OM fluxes, higher oxygen availability and stronger biological competition for food and space. The change in foraminiferal distribution along an OMZ depth gradient is also reflected in the feeding response of the different foraminiferal assemblages. A series of in situ labeling experiments, performed to study the short-term processing (<11 days) of organic carbon and nitrogen, and the nutritional demands of foraminifera, showed different food uptake patterns at three different oxygen concentrations. The response to the added food source varied between species, with depth, and over time. Faunal distribution and food uptake reflected the different energy demands and the variable influence of competition between foraminifera and other larger organisms at the different oxygen levels within the OMZ.

#### Interactions between intertidal foraminifera and turbellaria (Platyhelminthes) studied in the field and laboratory

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The frequent existence of turbellarian egg cocoons of on benthic for a minifera was observed in sediment collected in the intertidal of the German Wadden Sea. To learn more about the interac-tions between foraminifera and turbellarians in this extreme habitat, we studied these coccons in relation to presence and features of attachment on foraminifera from sediment samples. We were also able to study living turbellaria in the laboratory, their egg development as well as the hatching of juvenile worms. Observation of the actual cocoon deposition by turbellarians reveal that cocoons on foraminiferal tests derive from at least two turbellarian species and were fixed on tests of various foraminiferal species. The relative abundance of cocoons was highest for tests of living Haynesina germanica. Just few cocoons were present on Ammonia tepida, the most abundant species in these sediments. This might be theresult of cyst formation during feeding of Ammonia tepida, offering less available test surface for cocoon attachment. In general, foraminiferal tests seem to offer turbellarians a suitable hard substrate for cocoon attachment and living foraminifera might possibly act as dispersal vectors. SEM pictures reveal that the attachment of the cocoons leaves either corrosive marks in the outer layer of the foraminiferal test or depressional marks that are caused by incomplete formation of a new calcite layer during test growth and chamber addition. No signs of test penetration have been found. However, addition of new chambers can result in deformation of the foraminiferal test, caused by cocoon attachment at specific test positions.

#### Biomineralization in perforate foraminifera and its implication for understanding and development of paleoceanographic proxies

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Trace elements, stable isotopes and other ecologic and morphologic information obtained from the rich sedimentary archives of planktonic and benthic foraminifera shells are the main tools for paleoceanographic-paleoclimatic reconstructions. From the early days of using  $\delta^{18}$ O and  $\delta^{13}$ C it was clear that "vital effects" are playing a role in these proxies. With the introduction of trace elements (Cd, Mg, Sr, Ba, B, U, Li and others) it became clear that the process of biomineralization has a strong influence on the partition coefficients of these proxies. With the advancement of ICP techniques many stable isotopes of these trace elements were also measured and some of them (e.g.  $\delta^{11}$ B) are now standard tools in the field. In parallel the use of video, confocal, and electronic microscopy (TEM, SEM), direct observations on the biomineralization process in foraminifera became possible. We now have deeper understanding on this fundamental biogeochemical process and can use it to increase our confidence in existing proxies and to develop new ones. The incorporation of the membrane impermeant dyes Calcein and FITC-dextran into the shells of perforate foraminifera and the direct observations of seawater vaculoles at the calcification site prove that seawater is the main source of ions at the calcification site. pH elevation and a carbon concentrating mechanism facilitate the precipitation of calcite in the privileged space where calcification occurs. Using Li and its isotopes and  $\delta^{11}$ B in the benthic species Amphistegina lobifera we demonstrate how the mechanism of biomineralization is strongly bound into novel proxy development.

#### Assessing the effect of mercury pollution on cultured benthic foraminifera using DNA metabarcoding and morphological approaches

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Heavy metals such as mercury (Hg) occur in ecosystems from both natural sources and human activities, with large variations in concentrations and pose a significant health hazard through bioaccumulation and biomagnification. The aim of this experimental study is to assess the impact of different concentrations of Hg through time on the diversity of cultured benthic foraminifera. Tanks with concentrations of Hg up to 100 ppm were considered. Mesocosms containing 1-cm-thick sediment from each tank were subsampled at pre-established time intervals for geochemical (Hg content), morphological and molecular analyses. Morphological analyses were based on both Rose Bengal and the CellTracker Green (CTG) techniques. Molecular analyses comprised high-throughput sequencing (HTS) of foraminiferal DNA. A total of 6 datasets were generated: two from Rose Bengal morphological analysis (fraction > 63  $\mu$ m and fraction >125  $\mu$ m), one from CTG morphological analysis (fraction>125 µm), three from HTS run (one for each considered filter: Filter1, Filter10, Filter100). Species richness, Shannon and Fisher  $\alpha$  alpha indices were all negatively correlated with the Hg concentrations both in water and sediment regardless the considered datasets. Since the strongest correlations were observed among diversity indices and normalized fourth-root transformed HTS data (Filter1), the multivariate analyses (nMDS and Cluster Analysis) were based on this dataset only. The nMDS highlights the negative effect of Hg

pollution on the benthic foraminiferal assemblages at the highest concentrations, namely 10 ppm after three weeks and 100 ppm after two weeks of treatment. This experiment demonstrates the huge potential of metabarcoding for unraveling the impact of pollution on benthic foraminiferal assemblages.
#### Effects of mercury pollution on *Ammonia parkinsoniana*: intracellular lipid accumulation and lysosomal compartment amplification

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The responses of Ammonia parkinsoniana (Foraminifera) exposed to different concentrations of mercury (Hg) in the artificial sea water were evaluated at the cytological level. Foraminifera-bearing sediments were placed in mesocosms that were housed in aquaria each with seawater of a different Hg concentration. On the basis of confocal microscopy and the use of fluorescent probe incubations (Nile Red and Acridine Orange), the intracellular lipid accumulation and acidic compartment regulation were evaluated. Higher numbers of lipid droplets and the proliferation of lysosomes, both in terms of number and dimension, were associated with increased Hg concentrations. Lipid droplets and lysosomal proliferation might have acted as detoxification mechanisms induced by metal contamination. The application of the environmental scanning electron and scanning electron microscopies coupled with energy dispersive X-ray spectrometry revealed the presence of Hg in the organic lining at the basal part of pores and in the foramen/septa as well as in cytoplasm, mainly in the younger chambers.

# Physiological functions of *Ammonia tepida* in different oxygenated conditions: evidence for dormancy under anoxia

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Ammonia tepida is a common morphospecies in intertidal mudflats of the Atlantic coast. In Europe this morphospecies includes at least three different cryptic species: the phylotypes T1, T2 and T6. In the mudflats sampled along the French coast for these experiments the main phylotype is T6. Its vertical distribution in the sediment consistently shows high densities in the uppermost 2-4 mm where both oxygen and food are available. However numerous living specimens are also found deeper in anoxia. Several controlled laboratory experiments were carried out to 1) investigate the physiological functions of this morphotype in oxic and anoxic conditions, with and without nitrates, and 2) evaluate the respective contributions to C and N biogeochemical cycles. Our results showed that the survival rate in A. tepida (T6) is affected neither by oxygen nor nitrate concentrations, whereas its growth rate is strongly reduced by anoxia. Locomotion is also affected by anoxia, with both an increase of inactive periods and a decrease of the mean speed  $(2.19 \pm 0.66 \text{ mm.h-1 vs } 1.32 \pm 0.45 \text{ mm.h-1 in oxic})$ and anoxic conditions respectively). The nutritional function was quantified using a combination of TEM and NanoSIMS analyses on specimens, which have been previously incubated with 13C enriched diatoms. In oxic condition, diatoms were rapidly ingested. After one week, 13C was detected in high quantities in the whole cytoplasm and particularly concentrated in the lipid droplets, thus suggesting that carbon, was used for active cell metabolism, and

the excess of carbon can be stored into the cell. Conversely, after 1 day (and up to 28 days) under anoxia, the cytoplasm did not show any 13C enrichment, revealing a sharp decrease of the nutritional function under anoxia. A few entire diatoms were nevertheless observed in the cytoplasm after 1 day although they were not digested. The analyses of intracellular nitrate of *Ammonia tepida* specimens collected on French intertidal mudflats did not show almost any accumulation of nitrate in the cells. Moreover, the analyses by N2O microsensors did not allow for the detection of any denitrification activity, thereby suggesting the inability of *A. tepida* to respire nitrates under anoxia. Collectively, these observations revealed that *Ammonia tepida* (T6) is affected by anoxia and probably slows down its metabolism by entering dormancy. The contribution of this species to biogeochemical cycles under anoxia would be therefore potentially negligible.

#### Temporal variability and different impacts on denitrification and respiration rates of *Globobulimina turgida* from Gullmar Fjord, Sweden

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Benthic foraminifera from oxygen depleted environments are characterized by unique adaptations to the extreme environmental conditions. Various species are able to use nitrate as an electron acceptor via denitrification. Typically, the species from oxygen depleted habitats are characterized by a high porosity and increase their pore density with decreasing availability of electron acceptors (oxygen or nitrate) to optimize the intracellular uptake. These morphological adaptations bear a great potential to be used as a quantitative paleo-redox-proxy. Furthermore, recent modeling studies showed that foraminiferal denitrification is important for the nutrient budget in oxygen minimum zones. Nevertheless, studies about energy metabolism of foraminifera from oxygen depleted environments are very scarce. We determined denitrification and respiration rates of Globobulimina turgida from the Alsbäck Deep in Gullmar Fjord, Sweden, by measuring microprofiles of O<sub>2</sub> and N<sub>2</sub>O under acetylene inhibition,. Different incubation experiments have been conducted under varying conditions in respect to nitrate concentrations and pretreatment. For the denitrification experiments specimens were incubated under anoxic conditions. No N2O production was observed when the specimens were exposed to oxygen for an extended period of time before the incubation. When the specimens were kept under slowly induced anaerobic conditions until the incubation started N<sub>2</sub>O production was observed immediately. These results suggest that high oxygen concentrations inactivate denitrification relevant enzymes in G. turgida which are reactivated if

oxygenation falls below a certain threshold. If oxygen is depleted too fast *G. turgida* might become entrapped in anoxia, not able to synthesize the denitrification proteome due to the lack of usable electron acceptors. Individual denitrification rates showed a high temporal variability with distinct fluctuations during overnight incubations. Finally, specimens treated with antibiotics showed elevated oxygen respiration compared to untreated specimens which might indicate a stress response.

## Applications of the Propagule Method in Foraminiferal Ecology

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Many species of benthic foraminifera reproduce sexually by releasing numerous flagellated gametes directly into the surrounding seawater where syngamy occurs producing tiny zygotes. These in turn function as propagules, and they disperse over a range of spatial scales, both within and beyond the distribution of natal populations. Propagules, tiny juveniles, settle to the seafloor and form a "bank" in marine sediments. This propagule bank, broadly analogous to a seed bank, may include juveniles derived from both local and distant populations. Previous studies have shown that propagules of some species may remain dormant for months or even years and subsequently grow with exposure to optimal conditions. Propagule banks, which can be concentrated and separated from adult foraminifera and other larger members of the benthos by sieving, provide a novel experimental tool for addressing a variety of questions in foraminiferal ecology. Because propagule banks include juveniles of multiple species with different ecological requirements. taxonomically different assemblages can be grown from the same propagule bank by utilizing distinctly different but controlled environmental conditions. This experimental tool, the Propagule Method, affords us the ability to examine the effects of one parameter, for example temperature, while holding others (e.g., salinity, pH, alkalinity, oxygen, illumination, sediment source) constant. To date, the Propagule Method is being employed by several research groups, and has proven effective in examining species responses to different temperature and salinity regimes, exposure to specific pollutants or contaminants, food resources, and multi-stress factors. It also provides a tool for examining biogeographic patterns of dispersal, identifying opportunistic species, and assessing the potential for range expansion in a changing world.

# Constructing models of movement and sensing behaviour in foraminifera

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Foraminifera are used as excellent proxies applied for palaeoclimate and palaeoceanographic interpretations. Their observed fossil distribution depends on physiological, behavioural and ecological traits, biased by taphonomic processes. In order to interpret fossil records, we need to construct realistic models untangling its complexity. The eVolutus model is an attempt to reveal and test ecological and evolutionary hypothesis. Robust input data on physiology and behaviour of foraminifera are essential to perform reliable analysis. Among others, movement strategy and sensing ability are very important traits that have to be taken into account. They can enable for a ninifera to actively search for food and to find the best location in environment/sediment according to their ecological requirements. In trial version of eVolutus model we assumed that individuals have some sensing ability limited to specific area around the cell (sensing range). Studies of motion behaviour already showed that for a minifera respond to gravity, and probably react to different chemical signals or even in some cases to the light. Chemotaxis is a more complex phenomenon than geo- and phototaxis because organisms can possibly react to wide variety of different signals. Selected chemicals may indicate food sources or facilitate communication between individuals. It seems that chemotaxis can improve foraminiferal fitness, but its complexity makes it difficult to study. In consequence, we lack evidences on chemotactic sensing in foraminifera. However, there are data showing that these phenomena are ubiquitous among protists. Foraminifera share a lot of physiological and behavioural adaptations with other organisms, such as amoebae. We would like to address critical questions on for a miniferal behaviour, as well as to propose new experiments testing simple behavioural models. The research received a partial support from the Polish National Science Centre (DEC-2013/09/B/ST10/01734).

# Far north: modern salt marsh foraminifera from the subarctic White Sea

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Coastal salt marshes are highly-productive vegetated areas at the upper intertidal that develop in wave-sheltered estuaries, deltas, and semi-enclosed bays. While in mid latitudes salt marshes are numerous and prosperous, towards the polar circle their number and extension is strongly reduced, and they are scattered along low energy coasts in suitable areas. A few papers dealing with salt marsh foraminiferal fauna of subarctic Canada, USA and Norway have been published to date, and the foraminiferal assemblages of the Russian subarctic salt marshes have never been surveyed yet. Here we report on species compositions of the foraminiferans and vascular plants from the two salt marshes from the western White Sea coast. We identified 9 foraminiferal species: Jadammina macrescens, Balticammina pseudomacrescens, Trochammina inflata, Miliammina fusca, Ovammina opaca, Elphidium williamsoni, Elphidium albiumbilicatum, Ammotium salsum, Ammobaculites exiguus, which grouped into two distinct assemblages ('high marsh' and 'low marsh') each confined to certain vascular vegetation belt. Foraminiferans J.macrescens, B.pseudomacrescens, T.inflata resided at high marsh and were associated with reed foxtail Alopecurus arundinaceus, black-grass Juncus gerardii and sea plantain Plantago maritima, whereas M.fusca and E.williamsoni dwelled at low marsh in association with annual glasswort Salicornia pojarkovae and seashore alkali grass Puccinellia maritima. Foraminiferal fauna of the salt marshes was highly abundant (up to 3000 total living specimens per 10cm<sup>3</sup>), and numerically dominated by E.williamsoni (max 2048 ind./10cm<sup>3</sup>) and *M.fusca* (max 1148 ind./10cm<sup>3</sup>) at low marsh. The relative abundance of high marsh species was higher in Matrenin marsh, whereas low marsh species prevailed at Sukhaya Salma. The foraminiferal species diversity of the subarctic salt marshes studied is lower than in temperate regions and other subarctic marshes. The funding was provided by RFBR grant 14-04-93083.

#### Changes in foraminifer assemblage and condition recognized in 2300 yr old tsunami deposit

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The Gulf of Agaba-Eilat, located in the northern Red Sea, is considered low-risk with regard to tsunami activity because there are no modern records of damaging tsunami events and only one tsunami (1068 AD) referred to in historical records. However, given that the area was formed by and is located along the seismically active Dead Sea Fault, its population known to fluctuate in size and literacy in part due to its harsh hyper-arid climate, and the dearth of field studies addressing the presence or absence of tsunamigenic deposits, this assessment may be poorly informed. Here we show evidence from two offshore cores for a major paleo tsunami that occurred ~2300 years ago with a sedimentological footprint that far exceeds the scarce markers of the historically mentioned 1068 AD event. The interpretation is based on the presence of a laterally continuous and synchronous, anomalous deposit that includes allochtonous inclusions and unique structural characteristics. Based on sedimentological parameters, these inclusions could not be accounted for by other transport events, or other known background sedimentological processes. Foraminifers within the anomalous deposit are distinctive with regard to test size, abundance, and corrasion; though variations from core location to core location were also recognized. Those variations are discussed as associated to the specific environmental and bathymetric conditions per core location and are similar to reports from modern tsunami deposits.

# Quantification of the effects of ocean acidification on benthic foraminifera

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Anthropogenic activities since industrial times have caused a steady increase in the concentration of atmospheric carbon dioxide  $(CO_2)$ . The subsequent uptake of a large proportion of this  $CO_2$  by the ocean has changed seawater chemistry through the process of ocean acidification (OA). As a direct result of OA, there has been a decline in seawater pH, carbonate ion concentration  $[CO_3^{2-}]$ . and saturation state with respect to carbonate minerals ( $\Omega$ ). Although the effects of OA on marine calcifying organisms and ecosystems have been relatively well studied recently, there is still a lack of understanding with regards to benthic foraminifera. These ocean chemistry changes may, for example, affect for a growth rates and directly impact the functional structures of their tests. This study aims to quantify the effects of OA on intertidal benthic foraminifera through manipulative mesocosm experiments. Naturally co-occurring species of foraminifera (Haynesina germanica and Elphidium williamsoni) were collected from the Eden Estuary, St. Andrews, UK. Treatment at a constant temperature were subjected to seawater bubbled with equivalent atmospheric concentrations of CO<sub>2</sub> (400, 750, 1000 and 2000 ppm) replicating future scenarios of a high CO<sub>2</sub> atmosphere. Preliminary results show a significant effect on foraminiferal size and weight, especially at elevated CO<sub>2</sub> concentrations. Additionally, SEM images of specimens of Elphidium williamsoni cultured at high CO<sub>2</sub> concentrations show significant morphological modifications on test surface and also around the apertural area where functional feeding structures have been compromised by OA.

### "Jonah and the whale" - Ichthyoendochory as a novel foraminifera invasion vector

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Since the opening of the Suez Canal in 1869, hundreds of Lessepsian migrants, Indo-Pacific species originating from the Red Sea, have been extensively recorded in the Mediterranean Sea, including 68 species of benthic foraminifera. Most benthic foraminifera are holobenthic, i.e., have no planktonic stage and are permanent bottom-dwellers in sediment, on rocks, or as epiphytes on seaweeds and seagrass. Despite lacking a planktonic life stage, holobenthic marine organisms can disperse long distances through rafting and anthropogenic vectors such as aquaculture and shipping. Yet, none of these vectors are able to explain how the Lessepsian foraminifera migrants have rapidly invaded the Mediterranean Sea over the past century, successfully establishing sustainable populations. We present evidence for a new invasion vector explaining this long-distance dispersal. Living specimens of 24 benthic foraminiferal species were found in the fecal pellets of two herbivorous fish species – Siganus rivulatus and S. luridus, both invaders from the Red Sea. Gut analyses of siganids collected between 1955-1984 from the Gulf-of-Suez and the Eastern Mediterranean discover 16 benthic foraminifera species, known as Lessepsian migrants. A review of the first observation dates of S. luridus and of the most abundant non-indigenous foraminifera, Amphistegina lobifera, reveals congruent propagation patterns with a time lag of 4-11 years between discovery of the two species, respectively. We suggest that the incidental predation of benthic marine organisms by alien fish, followed by incomplete digestion and defecation of viable individuals, comprise the main introduction vector of these organisms into novel environments.

### Reconstructing past ocean-chemistry using foraminiferal Na/Ca: evidence from culture experiments with the benthic foraminifer *Operculina ammonoides*

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Trace elements to calcium ratios in foraminifera are widely used as paleo-environmental proxies. However, Ca has a short residence time in the ocean (~1My), resulting in secular variations in its concentration during the Phanerozoic. Sodium (Na) has a residence time of ~100My, hence variations in oceanic Ca may be deduced from foraminiferal Na/Ca if Na has a constant partition coefficient. Assuming a similar behavior of other elements, the paleoconcentrations of major and minor elements can be estimated relative to Ca in foraminiferal shells. To evaluate this working hypothesis, we cultured the benthic foraminifer Operculina ammonoides, an extant relative of the Eocene Nummulites, under varying Ca concentrations and different temperatures. Growth rates were monitored using alkalinity depletion and weight increase measurements. Prior to the experiment, the foraminifera were marked with calcein (fluorescent dye), to visibly indicate newly precipitated chambers as those that were not fluorescent. The newly grown calcite (spiked with 135Ba) was analyzed using LA-ICP-MS. Na has a constant partition coefficient in the experimental range and the changes due to temperature are small. Mg/Ca ratios vary with changes in Ca concentrations and temperature. However, the overall Mg-temperature sensitivity was lower than that of the Ca change. Lithium and strontium also have a constant partition coefficient with Ca but independent of changes in temperature. We suggest that Na uptake into shells of O. ammonoides is strongly dependent on seawater Na/Ca and therefore it can be used as a valid proxy to reconstruct seawater Ca in the geological past. High resolution Ca record will allow paleochemistry reconstructions and with it better understanding of the link between ocean-chemistry

and climate. Mg/Ca ratios will allow reliable paleotemperature reconstructions. Other elements (e.g. Li, Sr) also show clear partition coefficients with Ca and may be used to estimate their concentrations with time (and possibly other parameters).

## Test morphology of *Bolivina* aff. *B. dilatata* under oxygen depletion at the Indian margin (Arabian Sea)

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A strong oxygen minimum zone (= OMZ) at the Indian margin of the Arabian Sea exposes sediments and organisms to severe and persistent oxygen depletion. Benthic foraminifera represent an important faunal component in these sediments and sustain dense populations, like *Bolivina aff. B. dilatata*, which shows high dominance in the core region of the Indian margin OMZ. *Bolivina aff. B. dilatata* specimen at two water depths were studied to compare abundance and test morphologies between different oxygen levels (<0,0078 mL O2 L-1 at 540m; 0,053 mL O2 L-1 at 800m). No distinct differences between both stations were observed on deformation rate and generation mode. Test size varied between 540m and 800m, indicating larger growth at lower oxygen conditions. Significant changes were found comparing abundance of individuals, pore quantity on the tests and porequantity-to-surface-ratio.

# Methods for comparing living and dead assemblages of foraminifera

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Comparisons between living associations (LA) and dead assemblages (DA) are hampered by the fact that DA represent time-averaged associations, while LA reflect the status at the sampling time, influenced by population dynamics, seasonality in climate, food availability, as well as pollution, storms etc. Thus, a direct comparison of LA and DA has to consider these aspects when making ecological inferences about their relations. Besides species frequencies, statistical parameters like standing crop (densities) and diversity (species number and heterogeneity or dominance) describe assemblages. To compare LA and DA at a sample site using the above factors simultaneously the 'Incorporation Value' combines densities of LA and DA with correlation between these assemblages defined by IncorpVal = Receiving · Accordance · 200 While 'Receiving' characterizes the proportion of living associations on total assemblages, 'Accordance' marks the concordance in species composition between living association and dead assemblage. Correlation coefficients can be used, where the value 1 determines complete similarity or identity. The same magnitudes of 'Incorporation Values' can either be caused by high 'Receiving' combined with low 'Accordance' and vice versa. These relations are expressed in hyperbolic functions. For determining the influence in diversities from LA to DA, three parameters describing a vector based on species number and heterogeneity are used. Measures for species number (SN) and dominance (DM) must be normalized by  $x^* = x / x^*$ max x. Starting from the (normalized) origin of both diversity measures in LA, the vector's length marking the 'Intensity of Differences' in diversities and the angle determining the vector's 'Orientation' can be calculated. Angles between 0° and 90° indicate that LA are smaller in SN and DM than DA. Angles between 90° and 180° show stations where SN is higher in LA than in DA, but reverse for DM. When both SN and DM are higher in LA compared to DA, then the vector's orientation is located between 180° and 270°.

### Benthic foraminifera of rocky reefs in the northern Israeli Mediterranean shelf in light of massive Lessepsian invasions

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Rocky reefs, which are common in the northern Israeli shelf, constitute a highly diverse marine ecosystem rich in macroalgae and calcareous organisms, among them benthic foraminifera. This ecosystem has been suffering, during the last few decades, from continuous disturbance due to anthropogenic intervention. Benthic foraminifera, known to be sensitive ecological indicators were sampled by scuba diving from the rocky reefs of Akhziv (AK) and Carmel Head (CH). Different macroalgae species hosting living epiphytic foraminifera were sampled twice yearly at AK and four times a year at CH in three depth intervals between 2-18 m, during 2013-15. The numerical abundance ranges between 12-4855 specimens/10cc with no significant difference in standing stocks between AK and CH. However, the CH stations SP1 (18 m) and RD1 (7 m) differ significantly in their foraminiferal assemblage composition, probably due to water depth and macroalgae type. In total 68 epiphytic foraminiferal species were identified at both regions, including 8 symbiont-bearing species. Species richness per site varied between 3 and 42 with higher values at RD1 on the macroalgea Halopteris scoparia. Six benthic species dominate the living assemblage: Amphistegina lobifera, Textularia agglutinans, Pararotalia calcariformata, Hauerina diversa, Sigmamiliolinella australis and Tretomphalus bulloides. All these species except T. bulloides are known to be non-indigenous species, mostly of Lessepsian origin, and often compose up to 100% of the numerical abundance. The most common invasive species in 2013-14, A. lobifera, was replaced during 2015 in some sites by other species, such as, the invasive P. calcariformata and H. diversa or the native T. bulloides. The increasing numbers of non-indigenous species rapidly alter the native biota on rocky habitats causing difficulties in identifying habitat-biota relationship. This study indicates that Israeli rocky reefs represent a dynamic ecosystem prone to rapid changes, which need to be studied regularly.

### Growth strategy of permanently attached foraminifera: Case study of *Homotrema rubrum* (Lamarck)

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Most benthic foraminifera live in /on sediments or various substrates. Foraminifera keep species specific test shapes as they make different arrangements of chambers, surface ornamentations or compositions. In contrast, some benthic foraminifera attach permanently on hard substrate. These groups add new chambers on substrate surface or on own chambers. Morphology of these species is strongly controlled by each genetic characters and also related to undulation of substrates. Attached foraminifera should chase spaces with other creatures, such as coralline algae or bryozoan or even other species of attached foraminifera. If foraminifera fail to cover other creatures, foraminiferal shells are covered by other creatures and become dead. These attached foraminifera should possess species specific strategy for chamber formation and chamber additional speeds and patterns. Homotrema *rubrum* (Lamarck) is a red colored larger benthic foraminifera that is dwelled in coral reef environments in tropical-subtropical areas. The species have permanently attached on rubbles or rocks. The species show cryptic life that are distributed under stones on reef crest. It becomes to distribute on surface of stones at edge of reef crest and the offshore. This distributional pattern may strongly relate to tempo and mode of chamber formation. This means that growth speed of Homotrema spp. should be much higher than other organisms, such as coralline algae and bryozoan. We were continually measured growth speeds and patterns in the field during 1988-1990. We found that the species grew  $0.15-0.3 \text{ mm}^2$  / day. The growth speed of this group is one of rapidly growing species among benthic foraminifera. This growth strategy may succeed to live at coral reef environments where a lot of rapidly growing species dwell.

#### The Long Polar Night: Do Benthic Foraminifera Feed?

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The previously prevailing view of high-latitude marine communities as of being dormant during the polar night has started to change recently. The latest studies have shown that both, planktonic and benthic organisms show no expected metabolism deceleration; on the contrary, small omnivorous planktonic copepods stay active throughout dark season and continue diel vertical migrations. Benthic fauna as well sometimes demonstrates even higher abundance an d diversity at winter compared to other seasons. Benthic foraminiferans being one of the major groups of meiobenthos are of particular interest in this case. Although some foraminifera species are known to feed on bacteria, the majority of these protists keep to an algal diet, preying on diatoms and dinoflagellates. Given the seasonal transitions to long periods of darkness performed in high latitudes and leading to scarceness of primary production, one would expect foraminiferans to face absence of prey algae and following starvation, the latter resulting in either death or dormancy of the most part of population. In January 2015 and 2016, during the Marine Night cruise with RV Helmer Hanssen, we collected live specimens in the Kongfjorden area, Svalbard. All major species (Nonionellina labradorica, Islandiella helenae, Cassidulina reniforme, Elphidium excavatum, *Elphidium bartletti*) had brightly species-specifically colored cytoplasm implying they did not starve but had access to algal food. Transmission electron microscopy revealed that all specimens had well-developed mitochondria, Golgi apparatus and endoplasmic reticulum, therefore being metabolically active throughout the winter season. These results strongly indicate that high Arctic benthic foraminifera are not dormant during the polar night, and they access algal diet. Supported by Research Council of Norway grant 226417/E10 and RFBR grant 14-04-93083.

## Protein extraction and analysis of heat shock proteins (Hsp70) in living foraminifera

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Unicellular and higher organisms have developed several mechanism to deal with different environmental stress. such as anoxia, ultraviolet radiation, heavy metals or other chemicals. A family of highly conserved proteins called heat shock proteins (= stress proteins) play crucial role in these stressful situations. They are chaperone proteins and are evolutionary strongly conserved and ubiquitous. Stress proteins are associated with denatured or partially unfolded proteins in order to protect them from further denaturation and aid their refolding. They are located in the cytosol, mitochondria, endoplasmic reticulum, and nucleus. The Hsp70 is the most temperature sensitive group of the heat shock protein family, which comprise two main forms: a) constitutive: present all the time (heat shock cognates e.g. Hsc70) b) inducible: expressed in response to external stimuli (heat shock proteins e.g. Hsp70). Foraminifera are part of the marine benthos and exposed to all of above-mentioned stress factors in see water. The Hsp70 level in living foraminifera can be used as an indicator of natural and anthropogenic stress. To analyse the physiological response of different foraminifera (Ammonia tepida, Haynesina germanica, Heterostegina depressa) to varying stressful conditions, Hsp70 proteins have to be isolated. To optimize protein isolation and protein quantification in small and large benthic foraminifera, several isolation protocols were developed and tested, varying in buffers, solutions and incubation intervals. A comparison of these protocols will give an overview about changes and challenges of protein isolation in foraminifera.

### Effect of starvation and nourishment on respiration rates of intertidal benthic foraminifera

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Foraminiferal oxygen respiration rates are often used to quantify the role that living foraminifera play in the benthic organic-matter mineralization. It is now known that for a contribute up to 7% to benthic aerobic respiration. It has been previously shown that the foraminiferal respiration is dependent of the individual biovolume. However metabolic activity and then respiration rates could also strongly be affected by presence or absence of food. The quantification of the variability of respiration rates at different feeding states for a species is therefore crucial to avoid over- or underestimation of its role in organic carbon mineralization in a given ecosystem. In the present study we investigate, through experimental incubations, the effect of starvation and feeding on the respiration rates of the three most abundant species of intertidal mudflat ecosystems of French Atlantic coasts: Ammonia tepida, *Elphidium excavatum* and *Haynesina germanica*. We measured respiration rates of 5 to 7 specimens (in triplicates) incubated at different trophic conditions: 1) freshly sampled from natural sediment (control), 2) fed (2 days) with microphytobenthos collected at the sampling site, 3) starved (during 1 week). Respiration rates were measured using oxygen microsensors and all data were normalized to biovolume. Our results show a ~50% decrease in respiration rates after 7 days of starvation (compared to the control condition) in the three tested species. In A. tepida we observed no effect of feeding (compared to the control) suggesting that the food inputs provided are similar to those in the field. On the contrary, both E. excavatum and H. germanica showed a decrease in their respiration rates after 2 days of feeding, suggesting that these species do not consume the given microphytobenthos or that they were affected by the experimental conditions.

## Photosynthesis, carbon and nitrogen exchange between a symbiotic dinoflagellate and planktonic foraminifera host cell

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The planktonic foraminifera Orbulina universa lives in the photic zone of marine water columns and hosts photosynthetic microalgae (dinoflagellate) symbionts in its cytoplasm. During the day these dinoflagellates reside outside the host cell and during the night they migrate inside the foraminiferal cell. The hypothesis is that the dinoflagellates perform photosynthesis outside the host cell during day, forming photoassimilates and nitrogen-rich molecules, such as amino acids. By night, within the foraminifera, they transfer part of these photoassimilates and amino acids to the host cell. However the detailed mechanisms, and the time scales by which this transfer operates remain unknown. To answer this question, individuals of *O. universa* were incubated with <sup>13</sup>C-bicarbonate and <sup>15</sup>N-rich ammonium or nitrate during the natural day-night cycle. Specimens were sampled at intervals during the experiment, and immediately chemically fixed to preserve their cellular ultrastructure, allowing TEM imaging and subsequent NanoSIMS analysis. The NanoSIMS (Nanoscale Secondary-Ion Mass Spectrometry) is an analytical technique that allows to visualize the incorporation and transfer of isotopically labeled compounds in organisms with sub-cellular resolution. During the light phase, the symbionts fixed a large amount of <sup>13</sup>C as starch around the chloroplast pyrenoid and accumulated starch granules within the cell. After only 2 hours of incubation, carbon compounds were transferred to the host, where the <sup>13</sup>C-signal could be observed in lipid droplets. At the end of the night, the symbiotic microalgae were nearly devoid of starch granules, whereas the number of  ${}^{13}C$ labeled lipid droplets in the foraminifera had increased. The almost

complete disappearance of symbiont starch granules is the likely result of translocation of photoassimilated carbon from the symbionts to the host cell and dinoflagellate respiration. The symbiotic dinoflagellates were also integrating both nitrate and ammonium, the latter at a higher rate. The <sup>15</sup>N signal from ammonium could be observed in the foraminiferal cytoplasm, but its pathway of integration (through symbiosis or foraminifera itself) is not yet constrained.

### An unexpected case of genetic homogeneity within the morphologically diverse symbiont bearing larger benthic foraminifera *Operculina ammonoides*

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*Operculina ammonoides* (Gronovius, 1781) displays a remarkable phenotypic plasticity in respect to its planispiral shells from an inflated involute to strongly flattened relatively large evolute coils, with various intermediate forms. These morphologies often correspond to increase in water depths within the photic zone. *Operculina ammonoides* is a representative of the family Nummulitidae, which are among the most important fossil markers of Cenozoic carbonate platforms.

Analyses of SSU rDNA of living specimens from the Red Sea (Gulf of Eilat), North West Pacific Ocean (Japan), and the Mediterranean reveal a surprising genetic homogeneity between geographic locations, and no distinction between involute and evolute forms. CT images exclude the possibility of sexual dimorphism, as indicated by the presence of megalospheric proloculus (indicative to asexual forms) in both involute and evolute specimens. Genetic analyses of the diatom symbionts demonstrate that they belong to two clades within the cosmopolitan genus Thalassionema with no correlation to habitats water depths. The results suggest that the genetic homogeneity of the host foraminifera is accompanied by a strong symbiotic specificity. Our study suggests that the genetic homogeneity could be related to the ability of *O. ammonoides* to phenotypically adjust to different environmental conditions. Such phenotypic plasticity possibly allows the existing genotype to colonize new environments without imposing reproductive barriers that will promote cryptic speciation. This understanding has a direct implication for our interpretations of the paleoecology and taxonomy of fossil nummulitids. Moreover, Operculina ammonoides is an example for the genetic homogeneity of a widespread species.

## Foraminiferal propagules pelagic transport: sediment traps experiment.

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The dispersal ability of benthic foraminifera was highlighted by numerous studies. One of the possible mechanisms includes dispersion of "propagules" - small resting juvenile stages of the life cycle. They theoretically may be transported by water currents, and then deposited in sediment, where they stay inactive for years. They begin to grow, if environmental conditions become suitable for them. However, all evidences of this transport are indirect theoretical speculations or findings of allochtonous species in nontypical habitats. For trustworthy evidence, we combined two techniques: sediment traps for catching planktonic material, and, recently established (by E Alve and S Goldstein, 2014), "propagule method", for proving of existence of propagules in that material. We deployed pelagic sediment traps to accumulate plankton particles for 3 weeks, in the Chupa bay (White sea). Then we sieved accumulated samples - finer fraction with propagules passed through the sieve, and were cultivated for 6 weeks. After that we sieved samples again - grown propagules remained on sieve and were revealed as adult individuals of benthic foraminifera. So our experiments have proved the pelagic dispersal of foraminiferal propagules. Despite the finding of foraminiferal propagules in the pelagic zone, their abundance is surprisingly low, less than the number of adult foraminifera in the same planktonic samples. We propose some hypothesis explaining this fact, and hope, they will be useful for future studies, using sediment traps and "propagule method" simultaneously.

### Nomenclature for the Nameless: a Proposal for an Integrative Molecular Taxonomy of Cryptic Diversity Exemplified by Planktonic Foraminifera

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Investigations of biodiversity, biogeography and ecological processes rely on the identification of "species" as biologically significant, natural units of evolution. In this context, morphotaxonomy only provides an adequate level of resolution if reproductive isolation matches morphological divergence. In many groups of organisms, morphologically defined species often disguise considerable genetic diversity, which may be indicative of the existence of cryptic species. These units can be identified by unique DNA sequence motifs and allow studies of evolutionary and ecological processes at different levels of divergence. However, the nomenclature of genetically circumscribed units within morphological species is not regulated and lacks stability. This represents a major obstacle to efforts to synthesize and communicate data on genetic diversity for multiple stakeholders.

We have been confronted with such an obstacle in our work on planktonic foraminifera, where the stakeholder community is particularly diverse, involving geochemists, paleoceanographers, paleontologists and biologists, and the lack of stable nomenclature beyond the level of formal morphospecies prevents effective transfer of knowledge. To circumvent this problem, we have designed a stable, reproducible and flexible nomenclature system for genetically circumscribed units, analogous to the principles of a formal nomenclature system. Our system is based on the definition of unique DNA sequence motifs collocated within an individual, their typification (in analogy with holotypes), utilization of their hierarchical phylogenetic structure to define levels of divergence below that of the morphospecies, and a set of nomenclature rules assuring stability. The resulting molecular operational taxonomic units (MOTUs) remain outside the domain of current nomenclature codes, but are linked to formal morphospecies as regulated by the codes. We show how this system can be applied to classify genetically defined units using the SSU rDNA marker in planktonic foraminifera.

### Effect of pH and DIC on calcification and photosynthesis in *Operculina ammonoides*

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In an era of rapid global changes, models predict changes in carbonate system parameters, temperature, and oxygen saturation in the oceans. The extreme sensitivity of LBF to their environments makes them particularly good indicators for assessing the ecological and biological impacts of present and future climate and environmental changes. Studies investigating the biology, ecology, morphology, and symbiotic relationship with algae in LBF are essential for monitoring the ecological state of tropical and temperate ecosystems. They are also useful for understanding the impact of future climate and oceanographic changes such as the effect of ocean acidification on marine calcifiers. In this study we used Operculina ammonoides from the Gulf of Agaba-Eilat (Red Sea) as the selected model species. O. ammonoides is a tropical hyaline diatom-bearing LBF which can be found in a relatively large range of depth habitats. It is closely related to the fossil genus Nummulites (within the same subfamily), which were widespread throughout the Paleogene tropics/subtropics to the extent that they were the principal component of some shallow water carbonates. O. ammonoides show vulnerability to low pH, and no increased photosynthesis or calcification under higher DIC or CO<sub>2</sub> conditions. Initial low values of alkalinity or DIC did not affect calcification when pH was close to ambient levels in small, fast growing individuals, but large individuals revealed vulnerability to low DIC levels, a phenomenon we attribute to the large individuals' need for a large internal carbon pool. In general, O. ammonoides can cope more effectively with a low DIC supply compared to low pH,  $\Omega$ Ca or CO<sub>3</sub>. Our results call into question recent theories regarding LBF vulnerability to ocean acidification (OA) based on the microstructure of the shell, type of symbionts, and different activity of calcification enzymes based on Mg/Ca ratio.

## Foraminiferal DNA metabarcoding applied to benthic monitoring: promises and challenges

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Benthic foraminifera are powerful indicators of present and past environmental changes. Modern foraminifera have been widely used to demonstrate various types of anthropogenic impacts, either through morphogenetic changes or variations in taxonomic composition of foraminiferal communities. These studies are based on morphotaxonomic analysis of hard-shelled foraminiferal tests and demand a good taxonomic expertise, which impedes its application in routine biomonitoring. Recently, we have been exploring the possibility to use DNA metabarcoding to assess the impact of industrial activities on benthic foraminiferal communities. DNA metabarcoding consists in inferring the composition of species communities from the DNA present in environmental samples. In general, the taxon-specific DNA barcoding region is amplified with specific primers and sequenced using a high-throughput sequencing platform. Thousands of short rDNA sequences are then analysed in order to assess the richness of species and evaluate their relative abundance. Our pilot studies focus on assessing the impact on benthic communities of the organic enrichment associated with fish farming activities in Scotland, Norway and New Zealand. The results of these studies show a significant difference between taxonomic composition of foraminiferal community in impacted and non-impacted stations. The distribution of some foraminiferal species seems correlated to environmental variables related to organic enrichment. The for a miniferal diversity can be linked to macro-invertebrates-based indices commonly used in bio-assessment of marine aquaculture. The routine use of metabarcoding is still subjected to several limitations, such as the gaps in reference database, high genetic variations of some morphospecies, weak links between quantitative estimations inferred from genetic and morphology-based studies, various biases related to molecular techniques and data analyses. Although these challenging issues still need to be solved, the foraminiferal metabarcoding appears as a promising tool for more sensitive and cost and time effective environmental monitoring in the future.

# Assessing foraminiferal calcite as a recorder of Cr isotopes and concentrations

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Over the past decades low-oxygen conditions in the ocean have been spreading rapidly and are thought to continue to expand with the rise of global temperatures. The de-oxygenation of the oceans has major implications for the survival of fragile marine ecosystems. To understand the mechanisms of present deoxygenation the ocean's redox response to climatic events in the past needs to be investigated. An emerging redox proxy, Cr isotopes and concentrations, can potentially be used to reconstruct oceanic palaeo-redox conditions. Here we assess whether Cr in foraminiferal calcite faithfully record oceanic conditions. We have taken two approaches; Cleaning of core-top samples (Barker et al., 2003) as a preliminary assessment for the presence of Cr in the tests and high resolution LA-MC-ICP-MS of individual tests to reveal the distribution of Cr in core-top, down-core and sediment trap samples. These data suggest there is a fairly uniform presence of Cr throughout the foraminiferal test. There is a difference of up to two orders of magnitude in concentrations between sediment trap samples and core samples, which suggests the Cr signal in foraminifera could be influenced by the remobilisation of redoxsensitive metals in pore-waters. Whether these tests record a primary Cr isotope oceanic signature requires more detailed analysis of Cr concentrations and isotopes in foraminifera cultured under controlled conditions.enting stenohaline and stenothermic species survival. The full recovery of the planktonic and benthic foraminifera groups.

# From Erlenmeyer to the sediment: a critical appraisal of proxy calibration methods

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Paleo-interpretations of geochemical proxy records in foraminifera rely on the understanding of the various processes controlling the incorporation of geochemical signals in the calcifying tests of foraminifera and their preservation in the sediment. In this paper I will use results obtained from culture experiments, sediment traps and down core records to examine the application of several minorelements in planktonic foraminifera. Particularly, I will examine our recent studies of the elemental ratios B/Ca, Li/Ca, Na/Ca and Nd/Ca as potential proxies in paleoceanography. In comparing the different calibration methods I hope to critically assess the utility and limitations of each approach.

#### Insights from laboratory culture experiments into the cause of high Ba/Ca in non-spinose planktic foraminifer *Neogloboquadrina dutertrei*

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Observations of elevated Ba/Ca ratios in several non-spinose planktic species from plankton tows, sediment traps and core tops have puzzled researchers, since the seawater [Ba] implied by these ratios using the distribution coefficient (DBa  $\sim 0.15$ ) determined for spinose species is often >5 times higher than that observed in the deep thermocline waters that they inhabit. We conducted experiments with living N. dutertrei to establish whether a higher DBa for non-spinose species could explain these elevated calcite ratios. We raised the forams in 87Sr-labeled filtered seawater at constant temperature (16°C) and ambient pH (8.1), and used laser ICP-MS to quantify metal/Ca ratios and to identify the portion of shell grown under experimental conditions. Our results demonstrated that N. dutertrei DBa (0.13) is indistinguishable from that of spinose planktic species. We noted a dramatic difference between the Ba/Ca of calcite grown in filtered seawater compared to that of ocean-grown calcite. Whereas Ba/Ca of calcite cultured in ambient seawater ([Ba] = 33.9 nmol/kg) was generally 10 micromol/mol; intrashell Ba/Ca was also guite variable, often exceeding 50 micromol/mol. A calcite Ba/Ca ratio of 10 micromol/mol implies calcification from a fluid with [Ba] ~ 680 nmol/kg, nearly five times higher than the highest open ocean seawater [Ba] (~150 nmol/kg). Separate temperature and pH experiments demonstrated that Ba/Ca in N. dutertrei is insensitive to changes in these parameters, consistent with observations for spinose planktic species (Hönisch et al. 2016). Our results suggest that N. dutertrei calcifies at least partially within a fluid highly enriched in Ba. This environment could be provided by attachment to marine snow, in which barite crystals are found although barite is undersaturated in most ocean waters. The likely association of N. dutertrei and other non-spinose planktic foraminifera with marine snow should be considered when interpreting geochemical records from these species.

### Identification of functional symbiont diversity in benthic foraminifera by a combination of algae culturing and genetic fingerprinting

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Symbiont-bearing benthic foraminifera play an important role in global carbonate production. Their function is linked to the efficiency of their algal symbiosis. Like many other photosymbiotic organisms, individual foraminifera host multiple symbiont types, such as multiple algal strains or even multiple species of algae. Understanding the specificity of the symbiosis is essential for determining the resilience of the holobiont to global change stress. In this study we carried out algae culturing and genetic fingerprinting in a species of benthic foraminifera with an unusually heat-tolerant diatom symbiosis. Pararotalia *calcariformata*, and compared these results to the temperature sensitive Amphistegina lobifera where the symbiont diversity is known from algae culturing. We hypothesize that thermal tolerance is linked to symbiont type or symbiont diversity. Genetics and algae culturing was carried out on 40 P. calcariformata originating from four locations along the Israeli Mediterranean coast. The culturing approach unveiled a larger number of diatom species, whilst the genetic fingerprinting identified in most cases a single species belonging to *Minutocellus polymorphus*. This species has never been observed as symbiont before. Deep cloning of four specimens revealed that more diatom species were in association with the host, indicating that the culturing results are reliable, but the proportionality of the frustules in the culture does not reflect the life association. Thus, P. calcariformata hosts a consortium of multiple diatom symbionts, but the symbiosis is dominated by M. polymorphus. Interestingly, one DNA symbiont extraction yielded sequences from a different diatom species indicating that the other, less prominent, symbionts do not represent a "background" signal but can be functionally relevant. This finding is consistent with the concept of symbiont shuffling whereby the host can enhance its adaptive potential by maintaining (or being able to acquire) a broader spectrum of symbionts.

### Hidden twins from the antipodes: morphologically different but genetically identical cibicidids (benthic foraminifers) from Svalbard and Patagonia

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Cibicidids are a group of benthic foraminifers living from the intertidal area to the abyssal plain, often attached to hard or soft substrates (animals, seaweeds, pebbles). Based on the morphology of their test, they are traditionally separated between *Cibicidoides* (biconvex) and *Cibicides* (planoconvex), but molecular analyses have shown that this distinction is not justified, because a complete range of these shapes can be found within the same species and depend on the ontogeny of the organism.

Cibicidids were sampled in three sites around Svalbard and one site off NE Iceland. They were usually cemented to hard substrates such as shells or rocks and were found at depths between 148m and 700m. After documenting their morphology with Scanning Electron Microscope (SEM) images, the specimens were crushed for DNA extraction and amplification of a partial 3' SSU (small subunit) rDNA fragment. The sequences were found to be very similar to published sequences of Cibicidoides variabilis from Patagonia and ITS (Internal Transcribed Spacer) region was therefore sequenced to ascertain the degree of similarity between both populations. Results showed complete ITS sequence identity, confirming that they were the same genetic type as in Patagonia. In opposition to their genetic identity, Arctic specimens have a regular coiling habit while Patagonian express chaotic coiling. This explains why the morphospecies C. variabilis, was not identified in Svalbard until it was genetically characterised. This example illustrates the limitation of morphologically-based taxonomy and highlights the need to use a three-stage approach to taxonomy

based on strict morphological criteria to link molecular identification and traditional taxonomy. This approach comprises genotyping, the production of a formal morphological description of the SEM images associated with the genetic type and then the allocation of the most appropriate taxonomic name by comparison with the formal type description.

# Motion behaviour of intertidal benthic foraminifera: new approaches and ecological perspectives

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Motion behaviour of benthic foraminifera have been so far poorly studied. Displacements have been described using simple parameters i.e. distances and mean speed. Detailed study of trajectories is informative on the ecology of a species. Indeed, motion is involved in the search for food, escape strategies in the presence of a predator and in reproduction strategies. In this study, we propose to described both the geometrical and stochastical parameters of their trajectories, species-specific variability and specimen-specific variability. This approach is critical to understand the ecology of foraminifera, as the adaptive value of individual variability in motion behaviour is increasingly acknowledged as a competitive advantage in temporally and spatially complex landscapes. In June 2015, three intertidal benthic foraminifera typical of temperate mudflats were sampled; Ammonia tepida, Cribroelphidium excavatum and Havnesina germanica. Their motion behaviour was investigated continuously with time-lapse photography in the laboratory. We assessed their displacements in control conditions and both their geotactic and phototactic responses. The detailed nature of motility, investigated in terms of both geometric and stochastic complexity of their motion behaviour, was consistently characterised by a strong interspecific, inter-individual and intra-individual variability. Specifically, A. tepida and H. germanica were characterised by an intensive search behaviour, while C. excavatum adopted an extensive search strategy. This approach offers new perspectives in our understanding of the ecology of benthic foraminifera.
#### **Phylogenomics of Foraminifera**

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The deep relationships of Rhizaria have been studied using phylogenomic tools, but little is known about the higher-level phylogeny of Foraminifera, based almost exclusively on SSU rDNA sequences. A few studies have used other phylogenetic markers such as actin. B-tubulin and RNA polymerase but single marker phylogenies yield poorly resolved trees. When using the non-conventional markers very few species can be analyzed due to the many constraints to amplify these genes. We sequenced the transcriptomes of 12 species in Globothalamea, Monothalamids (Reticulomyxa filosa, Psammophaga sp.) and Tubothalamea (Sorites sp.) to recover hundreds of orthologous genes for the phylogenomic reconstruction of the tree of Foraminifera. Also, we sequenced the transcriptome of Amphistegina sp., for which no molecular data is available, to confirm its phylogenetic relationship within Foraminifera. In this first multigene study on Foraminifera, we aim to resolve the position of many common foraminiferans such as Ammonia spp. and Elphidium spp. and provide a useful tool for elucidating the phylogenetic position of species that are difficult to place on the tree.

#### Unraveling Planktonic Foraminifera Shell Biomineralization and Trace Element Variability Using Laser Ablation-ICPMS and NanoSIMS

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Laboratory experiments with living planktic foraminifera have revealed a wealth of information on the biological and environmental parameters controlling shell geochemistry for Paleoceanographic applications. However, far fewer studies have explored the fundamental biology of these planktic organisms from the perspective of biomineralization and intrashell geochemistry. In the past decade, we have conducted a series of experiments on the spinose species, Orbulina universa to explore the mechanisms of biomineralization and chamber formation via elemental or isotope labeling (e.g. 25Mg, 43Ca, Ba) to document cation transport and incorporation into shell calcite with laser ablation ICPMS and NanoSIMS. The results have revealed new insight into the mechanisms and patterns of chamber formation and organic matrix production in O. universa. In this presentation, I will review our techniques and present laboratory data from our recent experiments.

### Understanding the molecular basis for stress response in foraminifera and symbionts by proteome analysis

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The proliferation of calcification by coral reef building organisms greatly relies on the mutual association with photosymbiotic algae, albeit making the holobiont more sensitive to light and temperature fluctuation. To evaluate the adaptive capacity of photosymbiontbearing reef organisms such as large benthic foraminifera (LBF), it is essential to understand the underlying stress response mechanisms at the cellular level. To date, probing proteins in host and symbiont cells and partitioning both components are major obstacles for ecological studies. Recent advances in protein analysis in foraminifera e.g. expression of RuBisCO and Hsp70 provide new physiological insights, but these low- to mediumthroughput analysis are elaborate and target only single proteins. To overcome these limitations, we applied bottom-up proteomics workflow and mass spectrometry-based label free quantification to LBF holobionts. Changes in relative abundances of proteins in response to different environmental stress conditions are thereby determined. Protein identification by database and homology searching allows for the partial assignment of proteins either to the host or the photosymbionts. In a temperature stress experiment on Amphistegina gibbosa, we quantified 6,000 peptides, which are associated to 1,600 proteins of phylogenetically related species ( $\geq 1$ unique peptide; 1% false discovery rate). Among the symbionts, photosynthesis-related proteins were significantly reduced, while higher abundance of stress response proteins such as Hsp70 indicates thermal stress. In the foraminiferal host, high water temperatures relatively increased proteins involved in metabolic pathways and in building the cytoskeleton. These changes were reflected in measured physiological parameters e.g. oxygen production, which demonstrates how thermal stress impacts the

protein expression of symbionts and related shifts in the hosts metabolism. The presented proteomics approach offers new opportunities to study component specific molecular responses in LBF and provides novel insights into their adaptive mechanisms towards environmental changes, especially ocean warming.

## The long-term impact of sewage sludge input on live vs. dead foraminiferal assemblages of the Mediterranean coast, Israel

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The largest known point source of nutrient introduction into the ultra-oligotrophic eastern Mediterranean is the sewage sludge outlet of the Shafdan Wastewater Treatment Plant at Palmachim, at 36 m water depth 15 km south of Tel Aviv. Change in benthic foraminiferal living assemblages (LA) in the vicinity of the outfall over ten years of sludge injection revealed a pattern of natural amelioration controlled by storm intensity and frequency. In this study, differences between LA and dead foraminiferal assemblages (DA) were tracked along the productivity fall-off at three stations at increasing distance from the outfall, as well as long-term environmental impacts using only the DA. The most common species in the DA were similar in all stations but differed in abundance. For a miniferal abundance was lower near the outlet than at the more distal stations, both regarding live and dead assemblages. In the LA, Ammonia tepida was dominant over at least the last ten years. Typical DA over the past 6,000 years had high dominance of either Ammonia parkinsoniana and A. tepida (undivided) or *Porosononion subgranosus*, species that dominated the DA together with Ammonia beccarii but hardly occurred in the LA. The changes between the LA and DA are attributed to in-situ bioturbation or lateral transportation from nearby unpolluted environments. Ammonia parkinsoniana and P. subgranosus are the most common species known from soft sandy sediments along the Israeli coast, between 10-40 m, while A. beccarii occurs on the rocky environment at 30-50 m water depth. Thus, three main mechanisms contributed to produce the present structure of the DA: (1) Sewage introduction at the Shafdan outlet; (2) Lateral transport from nearby submerged rocky environment or soft sediments by storms; (3) Sedimentary processes and bioturbation implying a "past memory" of the pre-polluted environment.

#### Inferred calcification limits of the thermo tolerant benthic foraminifera *Pararotalia calcariformata & Lachlanella* - Mg/Ca records from a field study.

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Being major calcium carbonate producers foraminifera play an important biogeochemical role in any marine ecosystem and are a major part of the global carbon cycle. An ideal opportunity to study the effect of expected rise in oceans temperatures on calcification ability is presented by *Pararotalia calcariformata*, a thermally tolerant species that inhabits a heat polluted area along the northern coast of Israel, where it is exposed to temperature up to 36<sup>o</sup>C. Specimens of both species were collected over a period of one year from two stations that were monitored using in-situ temperature data loggers: A heat-polluted station located about 300 meters from the discharge point of the warm water and from an undisturbed station, located about 18 km north of the heat plume representing natural environment. Single-chamber Mg/Ca element ratios of these specimens were obtained using laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS).

In both species and in both stations specimens calcified at the warmest temperatures measured and did not calcify at the lowest temperatures they were exposed to. This indicates that in temperature at least up to  $36^{\circ}$ C the ability to calcify is limited by colder rather than warmer temperatures. *P. calcariformata* has higher minimum calcification threshold ( $20^{\circ}$ C) than *Lachlanella* ( $14^{\circ}$ C) and while it is highly tolerant to elevated temperatures our results show it is sensitive to lower temperatures that inhabit their growth. This provides interesting insights about the effect of temperature on calcification of marine protozoa and their responses to future global warming.

## Useful micro environmental visualization to reveal foraminiferal calcification process

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Calcareous foraminifers have been considered as one of the major carbonate producer in ocean. Their tests are key tools as paleoceanographic indicators in various studies because their tests have been archived as numerous fossils in sediment for geologic time and various environmental information are brought by population, morphology and geochemical signatures. Meantime, the calcareous test itself is interested by many scientists. The knowledge about the environmental controls on carbonate precipitation has been described for couples of decade using by various approaches. Scientists wish to understand that minute foraminiferal control of material uptake into calcareous tests from ambient seawater are of great interest. Our previous studies showed the potential to understanding the biomineralization of foraminifers by the application of fluorescent indicators. Recently, we apply the method to show the spatial distributions of cytological calcium and pH of living cell and micro-environment around foraminiferal specimen. Observed results show that foraminifers control pH and concentration of calcium. Meantime, the surrounding water environments are also altered by foraminiferal activity. These observations results will help to consider how the geochemical compositions arranging on the foraminiferal test, sensitivity of pH proxy of boron and others.

### Chamber formation in globothalamean Foraminifera: From theoretical models to cytoskeleton staining experiments

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Globothalamea (Pawlowski et al., 2013) is the most widespread class of Foraminifera that includes species adapted to all marine environments, including both benthic and planktonic habitats. In consequence, globothalamean shells are used as crucial proxies for deciphering past global changes and monitoring recent environmental dynamics. There are three research approaches to study Foraminifera that are based on (1) overall shell morphology, (2) chemical composition of the shell, and (3) molecular composition of organic compounds (e.g. DNA, RNA, proteins). The problem is that these three approaches show huge gaps between each other. The only way to bridge the gaps is to focus on morphogenetic and biomineralization processes observed on living organisms. These processes depend on sophisticated relationships between genetic, epigenetic, structural, and environmental factors. We have started morphogenetic modelling that follows simple iterative algorithms simulating basic shell patterns in Globothalamea. More complex arrangements of chambers result from ontogenetic interactions between chamber shapes and position of their apertures. Therefore, the clue to understand growth of globothalamean shells is to disentangle their chamber and aperture formation processes. The most efficient methodology includes a high resolution fluorescent confocal microscopy combined with fixed and live staining protocols adapted to experiments on foraminifera. In our preliminary experiments, we labeled the major cytoskeletal proteins (filamentous actin, tubulin) during the chamber formation in Amphistegina lessonii d'Orbigny. The staining results show that chamber shape is predefined by a globular structure (globopodium), which acts as a dynamic organic scaffold supported by the F-actin meshwork. This meshwork seems to interact with microtubules and associated proteins, which are involved in the morphogenesis and biomineralization of skeletal structures. Extracellular calcium carbonate nucleation takes place within a delimited zone beneath the lamellipodial structures, which envelope the shell during its primary and secondary biomineralization. The research received a partial support from the Polish National Science Centre (DEC-2013/09/B/ST10/01734).

#### Monothalamous foraminifera in marine habitats of Israel

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In the monumental taxonomic catalogue, Hottinger & al. (1993) documented a large number of benthic foraminifera from the Gulf of Eilat. Similar studies were performed by other researchers in various regions of the Mediterranean. With rare exceptions, monothalamous foraminifera fell out of the scope of those faunal accounts; likewise, they were largely ignored in subsequent ecological studies which used this taxonomic framework. Despite being abundant in many marine habitats, monothalamids are not easy to reveal and identify; many species are undescribed and the only signature of their presence is contained in the environmental sequence data. In order to expand our knowledge of the taxonomy and biology of this enigmatic group of foraminifera, we are searching for monothalamids in the Gulf of Eilat and on the Mediterranean seaboard of Israel. The integrative taxonomic approach involves microscopy and DNA sequencing of individually isolated cells, deposition into type collection and a taxonomic checklist construction. In just a couple of small nearshore areas on the Mediterranean coastline, more than 20 species were revealed. The exploration is ongoing in several other locations, notably in reefal habitats and Halophila beds of Eilat. The effort to identify and catalogue monothalamous foraminifera demonstrates the scale of the unknown biodiversity, which is important for future ecological research and management of marine ecosystems.

#### Genetic diversity and mating behavior in foraminifera

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Many groups of foraminifera are characterized by the formation of elaborate shells, which provide detailed morphological features, useful for species classification. Since the majority of works focuses on their fossilized shells, a comprehensive morphotaxonomy has been established. Yet, genetic analyses revealed an even higher diversity on the molecular level, hidden within the traditional morphospecies. These cryptic species are marked by large genetic distances and differentiated distribution patterns, implying that cryptic species rather than morphospecies represent the level of species. As a consequence, today we are facing a conflict between the morphological species concept and the interpretation of genetic diversity. The biological meaning of both is still unclear and the relationship between the genetic divergence and the level of species or populations remains uncertain. In order to overcome this conflict, we try to combine aspects of morphological variability, genetic diversity and reproduction to achieve an integrative approach for species delimitation in foraminifera. To this end, we use benthic foraminifera from rocky shore environments around the coast of Japan, which can be maintained and bred in laboratory cultures. We screen morphospecies for hidden genetic diversity as well as slight morphological variability. The goal of our study is to increase the number of genes used for the delimitation of cryptic species and for molecular phylogenies in order to increase the reliability of such approaches. In addition, we carry out breeding experiments to observe the mating behavior between genetically divergent lineages to detect the degrees of genetic divergence that correspond to reproductive isolation or represent population genetic variability. Understanding the connection between morphological and genetic diversity and the mating behavior of foraminifera allows putting the observed diversity in an evolutionary context and is thus essential to understand aspects of diversification and adaptation in the group.

### Correlation between shell calcification intensity and geochemical composition in planktonic Foraminifera

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The geochemical composition of planktonic Foraminifera shells is widely used as environmental proxy to reconstruct ambient seawater parameters, but recently shell calcification intensity (i.e. the size-normalized weight of the shell) has also been applied to reconstruct oceanographic parameters. While carbonate saturation of the seawater is the main factor influencing calcification intensity, it is also mediated by other environmental factors. Likewise, shell geochemical composition was shown to be very variable even across specimens from the same species and locality, increasing uncertainties when only few individuals are available for analyses. Because both proxies are closely linked to the process of shell calcification over time, it is reasonable to assume a correlation between calcification intensity and geochemical composition that can help to better constrain those proxies, deriving more robust environmental reconstructions. Such an analysis requires high-resolution, long-term observations, as provided by sediment trap material. Here, we therefore use material from sediment trap Kiel 276 from the North Atlantic, spanning two years with a resolution of 1-2 months, to investigate calcification intensity and shell geochemical composition, and their relationship to each other. Preliminary results of the Globigerinoides ruber (pink)/ruber (white)/elongatus plexus reveal distinct differences in shell size and calcification intensity between the species, which can be correlated with environmental factors (positive with temperature, negative with nutrient availability) in the absence of large carbonate system changes. In the next step, the geochemical composition of the same shells will be analysed using single shell mass spectrometry. This will lead to a better understanding of the calcification processes influencing both parameters.

# Describing the growth of *Operculina*: determination of chamber building rates, maximum size and the use of growth functions in species determination

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Over a period of 16 months (April 2014-July 2015), living specimens of the genus Operculina were collected from 20 and 50 m depth around the Island of Sesoko (Okinawa, Japan) in a series of monthly sampling. A minimum of eight living specimens (4 smallest and 4 largest) per sampling were separated and cultured in a long term experiment designed to approximate conditions in the field as closely as possible. A set up allowing recognition of individual specimens enabled consistent documentation of chamber formation, which in combination with u-CT-scanning after the investigation period permitted the assignment of growth steps to specific time periods. These data were used to fit various mathematical models to describe growth (exponential-, logistic-, generalized logistic-, Gompertz-function) and chamber building rate (Michaelis-Menten-, Bertalanffy- function) of Operculina. The mathematically retrieved maximum lifespan and mean chamber building rate found in cultured Operculina were further compared to first results obtained by the simultaneously conducted "natural laboratory approach". Even though these comparisons hint at a somewhat stunted growth and truncated life spans of Operculina in culture, they represent a possibility to assess and improve the quality of further cultivation set ups, opening new prospects to a better understanding of their ecological requirements. Furthermore, the obtained growth functions can represent a useful tool in the diagnosis and differentiation of species.

#### High-pressure culturing – a major step towards a better understanding of deep-sea benthic foraminifers and the geochemical signals recorded in their calcareous shells

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Over the last years I have invented and successfully applied highpressure chambers (-250bar) and supportive seawater circuits to culture deep-sea sediments and their benthic foraminiferal fauna at in Situ pressure (Wollenburg et al., 2015). Our experiments lasted from 4 to 6 months and showed that in this time span from all foraminiferal species that lived at the respective coring site, specimens had experimentally reproduced, including the first experimental offspring of Cibicides wuellerstorfi. So far we have used the new technology to elucidate the influence of methane on the carbon isotope composition of seep foraminifera and the influence of pH on deep-sea benthic foraminifers boron isotope. Although these experiments were successful in so far that they showed the influence of the considered parameter, calculations were complicated by the fact that neither the reproduction time could be exactly determined nor was it possible to avoid geochemical processes in the sediments for the long experimental time span. Recently, we have invented and patented a new series of high-pressure (500bar) culture chamber designed for use under a microscope, allowing continuous observations on growth and reproduction, measurement of fluorescent probes and Raman spectroscopy. Culturing single specimens in small volume chambers eliminates the problems associated with biochemical processes in the sediment and allows a perfect control of the elemental and isotopic composition of the water, a prerequisite for calibration studies. The talk will show the opportunities and problems encountered in working with high-pressure culturing devices

### Feeding strategies of the intertidal foraminifera Ammonia tepida

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The intertidal is an extreme environment with strongly fluctuating conditions. High physiological requirements result in communities with low biodiversity consisting of few well adapted species. Adaptations include feeding strategies like food specification or high levels of food exploitation abilities. Ammonia tepida is a common species, which typically occurs in high abundances in tidal flats. To test if its feeding behaviour is driven by variations in patterns of organic flux, laboratory feeding experiments were performed. Individuals of A. tepida were isolated from sediments collected in autumn in the intertidal of the German Wadden Sea. The specimens were fed with dual isotope labelled ( $^{13}$ C and  $^{15}$ N) freeze dried microalgae. Food was offered in form of a single high pulse of organic matter as well as at a constant, steady input rate of organic matter. The latter approach was run in parallel at 20°C and 25°C. Neither the two different food modes nor the two temperatures triggered contrasting reactions within the first days. Nevertheless, a generous food uptake at constant feeding demonstrates a distinct food uptake behaviour. In contrast, the isotope signals level off towards the end of the single pulse experiment. This is most likely related to the degeneration of the algal material, limiting the food uptake. Cytoplasmatic content of phytodetrital nitrogen relative to carbon is high compared to results from other investigations on spring populations. This might be related to the abundant food availability or distinct nutritional demands in autumn populations. The all over high levels of food affinity in A. tepida are comparable with species successfully dominating other extreme marine benthic habitats characterised by high continuous flux of organic matter. The high food consumption in A. tepida could cover metabolic costs for adaptations to the harsh and rapidly fluctuating environmental conditions.

## Laboratory feeding experiments with intertidal smaller benthic foraminifera

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Observations regarding the feeding behavior of foraminifera support the definition of their role in marine food webs and nutrient cycles. Ammonia tepida and Haynesina germanica typically represent a major part of the benthic community and concurrently build up a high amount of the benthic biomass in intertidal flats. These environments are also characterised by abundant food sources due to high primary production and fluxes of organic matter. When inquiring about patterns of distribution and abundances in foraminifera, food source related mechanisms can be revealed by applying laboratory feeding experiments. Stable isotope labelling serves as a powerful tool for tracing food source derived compounds like carbon and nitrogen. Methods used in our studies include the cultivation of microalgae in isotope labelled medium, the isolation of live foraminiferal individuals from sediment samples, and their incubation in the laboratory setting. For the subsequent elemental analysis, appropriate amounts of foraminiferal biomass need to be identified depending on individual carbon or nitrogen content. Recently run culture experiments in our laboratory revealed a preference of chlorophytes over diatoms in A. tepdia, and identified temperature as a critical factor for nutrient uptake in *H. germanica*. Different time related endurance of the two species at low food input hint for a struggle of A. tepida when coping with low availability of organic matter. However, sustaining both species for extended cultivation periods is difficult and challenges the achievement of long term observations.

### Assemblages of living benthic Foraminifera in a marine protected area from the Romanian Black Sea

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The study was conducted in a marine protected area (MPA) near the port of Mangalia, Romania in the Western Black Sea. The MPA is a biodiversity hotspot for marine habitats and species with high conservation value, harboring the highest diversity of species along the Romanian coast, including species listed in the Red List at national and regional (Black Sea) level. A quantitative study of the foraminiferal assemblages was performed on several sediment types during two sampling campaigns in May and September 2015, using a Van Veen grab. Sampling stations were distributed along a perpendicular transect going from the shore down 115 m depth. Living benthic foraminifera were identified in 11 samples. Assemblage parameters (foraminiferal abundance, frequency, diversity, dominance and constancy) were calculated to describe the foraminiferal assemblages and quantify seasonal variations between spring and autumn. The benthic foraminiferal assemblages were found to be poorly diversified with an overall 24 identified taxa. The most abundant taxa are Ammonia tepida (Cushman, 1926), Ammonia ammoniformis (d'Orbigny, 1846) and Cribroelphidium poeyanum (d'Orbigny, 1826). Higher foraminiferal diversity is occurs at the shallower stations and in spring as compared to autumn. KEY WORDS: living benthic foraminifera. Romanian Black Sea. MPA.

#### Evolution of the Holocene oxygen minimum zone in the SE Levantine basin understanding the effect of the Nile and natural climatic change

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Several anoxic, organic-rich sediment layers, called sapropels, are found in the Eastern Mediterranean Sea (EMS). They are mostly examined in water depths below 1800 m, but shallower water depths are poorly studied by now. This study will focus on the most recent sapropel S1 from the early Holocene. Comparison of existing data of sediment cores from the Levant Basin from 500 to 1800 m water depth show different timing in the beginning and end of sapropel S1 depending on water depth and distance to the Nile outflow. Several (SL112, 9509, PS009PC) sediment cores of Israel show an interruption of sapropel S1 around 8.2 ka BP but in sediment core 9501 (close to Cyprus), the sapropel ends at this time. New cores will be taken of the Israeli coastal shelf to investigate sapropel S1 in transect from shallow to deep. On these cores, planktic foraminifera will be analyzed for surface salinity, productivity and for dating. Diversity and oxygen indices will be calculated on benthic foraminifera to determine the development of Oxygen Minimum Zones (OMZ) through space and time. The purpose of this study is to investigate how changes in Nile discharge influenced biogeochemical processes in the Levant Basin and to determine the development of Sapropel Intermediate Water (SIW) over time. This study will help to foresee future trends in expansion of OMZ in coastal marine systems specially created by increased nutrient supply from adjacent river systems.

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