ALKOR - Berichte

Teaching cruise (BMARSYS) on the Southern Baltic Sea

AL530

14.10.2019 – 18.10.2019, Kiel (Germany) – Kiel (Germany) **MARSYS-16**

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1 Cruise Summary

1.1 Summary in English

Our current Bsc-MARSYS cohort was at the time twice as large as planned (37 students on 18 slots) and was promptly joined by another double cohort in the 2019/20 term. Thus, we applied for additional "emergency" ship time in October 2019 utilizing "Verfügungstage".

This was a teaching cruise aimed at training the students in methods commonly used in biological oceanography, with a strong focus on plankton ecology and evolution. Methods ranged from CTD runs, BONGO and WP2 sampling to on-board incubation and metabolic measurements of phytoplankton samples, and to identification and distribution analysis of zooplankton samples. The course aimed specifically at giving the students a better understanding of the invisible world that ultimately makes and breaks the fate of aquatic ecosystems (Falkowski 1998; Raven & Falkowski 1999). Marine microbes have short generation times and live in large populations, making them ideal model organisms to detect how evolution works (Schaum & Collins 2014; Schaum *et al.* 2017; 2018; Schaum 2018). But these organisms do not live in a void, rather, biotic and abiotic parameters shape their evolutionary responses (Scheinin *et al.* 2015; Hattich *et al.* 2017; Schaum *et al.* 2017; Bach *et al.* 2018). Evolutionary biology often ignores ecological drivers and ecology does not usually consider rapid evolution. Our student projects, instead, aimed to disentangle the relative contributions of ecology (e.g. community turn-over), immediate responses (in situ metabolic responses within the same generation), plastic responses (a few generations) and evolution (dozens to hundreds of generations) to Baltic Sea picoplankton responses in a changing world. This includes changes to the mean of environmental parameters, as well as to the variability of these parameters. Specifically, with the Baltic Sea samples taken in different seasons, the students are now able to answer these specific questions:

- Are picoplankton (Worden *et al.* 2004) communities coming from more variable areas better able to rapidly adjust their phenotypes on time scales ranging from single to many generations?

- How does the contribution of phytoplankton primary production change through seasons and space? How will it change regarding different temperature ranges?

- How can we better describe the physiology and biogeography of tiny picoplankton

 $(-1 \mu m - 3 \mu m)$ hosts and their giant viruses?

1.2 Zusammenfassung

Unsere Bsc-MARSYS-Kohorte war in 2019/20 doppelt so groß wie geplant (37 Studenten auf 18 Plätzen) und wurde im Semester 2019/20 prompt durch eine weitere Doppelkohorte ergänzt. Daher beantragten wir im Oktober 2019 eine zusätzliche "Notfall"-Schiffszeit unter Verwendung von "Verfügbarkeitstage".

Es handelte sich um eine Lehrfahrt mit dem Ziel, die Studenten in den in der biologischen Ozeanographie üblichen Methoden auszubilden, wobei der Schwerpunkt auf der Ökologie und Evolution des Planktons lag. Die Methoden reichten von CTD-Läufen, BONGO- und WP2- Probenahmen über Inkubations- und Stoffwechselmessungen von Phytoplanktonproben an Bord bis hin zur Identifizierung und Verteilungsanalyse von Zooplanktonproben. Der Kurs zielte insbesondere darauf ab, den Studenten ein besseres Verständnis der unsichtbaren Welt zu vermitteln, die letztlich das Schicksal der aquatischen Ökosysteme bestimmt (Falkowski 1998; Raven & Falkowski 1999). Marine Mikroben haben kurze Generationszeiten und leben in großen Populationen, was sie zu idealen Modellorganismen macht, um zu lernen, wie Evolution funktioniert (Schaum & Collins 2014; Schaum et al. 2017; 2018; Schaum 2018). Aber diese Organismen leben nicht in einem Vakuum, vielmehr prägen biotische und abiotische Parameter ihre evolutionären Reaktionen (Scheinin et al. 2015; Hattich et al. 2017; Schaum et al. 2017; Bach et al. 2018). Die Evolutionsbiologie ignoriert oft die ökologischen Driver, und die Ökologie berücksichtigt in der Regel keine rasche Evolution. Unsere Studentenprojekte zielten also darauf ab, die relativen Beiträge der Ökologie (z.B. community sorting), der unmittelbaren Reaktionen (in situ metabolische Reaktionen innerhalb derselben Generation), der plastischen Reaktionen (einige Generationen) und der Evolution (Dutzende bis Hunderte von Generationen) zu den Reaktionen des Ostseepicoplanktons in einer sich verändernden Welt zu entwirren. Dazu gehören Änderungen des Mittelwertes der Umweltparameter sowie der Variabilität dieser Parameter. Mit den in verschiedenen Jahreszeiten entnommenen Ostseeproben sind die Studierenden nun in der Lage, diese spezifischen Fragen zu beantworten:

- Sind Pikoplankton (Worden et al. 2004) Gemeinschaften, die aus variableren Gebieten stammen, besser in der Lage, ihre Phänotypen auf Zeitskalen von einer bis zu vielen Generationen schnell anzupassen?

- Wie verändert sich der Beitrag der Phytoplankton-Primärproduktion durch die Jahreszeiten und den Raum? Wie wird er sich in Bezug auf verschiedene Temperaturbereiche verändern?

- Wie können wir die Physiologie und Biogeographie des winzigen Pikoplanktons besser beschreiben?

2 Participants

2.1 Principal Investigators

2.2 Scientific Party

2.3 Participating Institutions

IMF Hamburg University

2.4 Crew

3 Research Program

3.1 Description of the Work Area

The spatial focus lies on the Western Baltic Sea, and the Arkona Basin. The training includes collecting samples from all major compartments of the ecosystem, from coastal to open waters in a 3-dimensional distribution. To achieve a holistic understanding of the environment under investigation, students learn how to take, prepare and pre-analyze samples on board as well as post-cruise-processing of collected data or samples in the laboratory. Some samples are analysed on board using on-board incubations. Below, we provide a map detailing the cruise trajectory.

Figure 3.1: Cruise track of AL530. All sample stations are depicted by green, filled dots. We provide positions per gear in Table 6.1.

3.2 Aims of the Cruise

As mentioned above, this was a teaching cruise. The main aims and objectives are therefor related to teaching outcomes. The cruise aimed at training the students in methods commonly used in biological oceanography, with a strong focus on plankton ecology and evolution. Methods ranged from CTD runs, BONGO and WP2 sampling to on-board incubation and metabolic measurements of phytoplankton samples, and to identification and distribution analysis of zooplankton samples.

3.3 Agenda of the Cruise

The course aimed specifically at giving the students a better understanding of the invisible world that ultimately makes and breaks the fate of aquatic ecosystems (Falkowski 1998; Raven & Falkowski 1999). Marine microbes have short generation times and live in large populations, making them ideal model organisms to detect how evolution works (Schaum & Collins 2014; Schaum *et al.* 2017; 2018; Schaum 2018). But these organisms do not live in a void, rather, biotic and abiotic parameters shape their evolutionary responses (Scheinin *et al.* 2015; Hattich *et al.* 2017; Schaum *et al.* 2017; Bach *et al.* 2018). Evolutionary biology often ignores ecological drivers and ecology does not usually consider rapid evolution. Our student projects, instead, aimed to disentangle the relative contributions of ecology (e.g. community turn-over), immediate in different seasons, the students are now able to answer these specific questions:

- Are picoplankton (Worden *et al.* 2004) communities coming from more variable areas better able to rapidly adjust their phenotypes on time scales ranging from single to many generations?

- How does the contribution of phytoplankton primary production change through seasons and space? How will it change regarding different temperature ranges?

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Students gained, alongside gathering first answers and insights to these open questions in ecology and evolution, experiences in taking and size-fractioning phytoplankton and virus samples (using plankton nets and Niskin bottles in combination with step-wise filtration), cryopreservation for a variety of molecular techniques, as well as a series of metabolic measurements used to describe the phenotypes of these samples. Zooplankton and small larvae distributions and abundances were also characterized. The students rotated through these questions on a scheme that allowed for all of them to participate in all aspects of work on deck, driving gear, and dealing with samples.

4 Narrative of the Cruise

RV ALKOR departed from GEOMAR pier on the 14th of October 2019 at 07:54 am and headed to the first sampling station in the Kiel Bight (KB3, see below). Over the course of the cruise, we conducted several daily zooplankton hauls with Bongo and WP2. A CTD haul was also carried out at each station. Due to the time involved in preparing the phytoplankton samples (especially size fraction filtering and metabolic measurements – these amount to ca 6-7 hours per station), the Niskin bottle was dispatched for sampling at every other station only. In addition, hydroacoustic data obtained with four different echosounder frequencies (38, 70, 120 and 200 kHz) were continuously recorded. After having taken samples at four stations in the Kiel Bight, we steamed to the Arkona basin, where another four stations were sampled daily until the $17th$ of October 2019. Unfortunately, the weather worsened increasingly in the evening, and therefore all but one night had to be spent sheltering at or near a harbour (Sassnitz and Roenne). Luckily, we were still able to carry out all experiments as planned and steamed back to the Mecklenburg Area (starting with MB3, see below) for our final day of sampling. We spent the contingency day dealing with the final samples (especially metabolic measurements/on-board incubations) and cleaning the laboratory spaces. Below, we provide a summary table of which gear was dispatched on which day (including the greater biogeographical region that the gear was used in; see section 7 for a more detailed list of all stations).

4.1: Details on which samples were taken with which equipment throughout the cruise

Day Date Station Name/Region Gear

5 Preliminary Results

As this was a teaching cruise, the main objective was that the students learn to work under the specific conditions of a cruise, including methods that may not be applicable for work in a 'standard' laboratory and how to organize a laboratory that is in perpetual motion. Nevertheless, the students were able to work on genuine scientific questions, which I am providing a short summary of below:

1. Can we still see a signature of the summer heatwaves in the latest CTD profiles?

We had found a clear warming signal in the summer cruises, especially during the AL524 cruise. In October, the warming signal was indeed still prevalent compared to data taken in 2017 at the same time of year, and in many regions, this was accompanied by lower than usual oxygen levels.

Figure 5.1: CTD profiles taken along the transect from Kiel to Arkona. Upper left: Temperature in ^oC, with warmer temperature (up to 15°C) in red hues, and colder temperatures in blue and purple hues (coldest temperature around 10ºC). Upper right: Salinity, with higher salinities in red hues, and lower salinities in blue and purple hues. Thermo-haloclines were well established in all but the shallowest regions. Middle left: Chlorophyll a (approximate values, µg/L), high levels in red hues, lower levels in blue and purple hues. Middle right: Oxygen saturation (here in %), with high levels in red hues, lower levels in blue and purple hues. Bottom: Transect as recreated with DataView.

2. Does grazing pressure through zooplankton vary between the biogeographically distinct regions of the Kiel Bight, the Mecklenburg Bight, and the Arkona Basin?

In this pilot study, the students tracked growth rates of phytoplankton from each 'Niskin Bottle' station in three biological replicates for two treatments each. The first treatment contained the full phytoplankton community, and zooplankton <300µm, of which a count and identification was carried out under the dissecting microscopes to make sure the amount of grazers per sample was about equal between samples. The second treatment contained the full phytoplankton community <35 µm, and no zooplankton (this sample was also briefly checked for the presence of grazers). Every day, a 200µL aliquot of the sample was frozen away in sorbitol and measured on a flow cytometer upon return to Hamburg, allowing the students to calculate growth rates of phytoplankton with and without grazers from the three regions. They found that the effects of grazers (added to be present at 10% of the phytoplankton biomass as per

theory (Ives & Cardinale 2004; Patrick L Thompson 2015)) was negligible here, with a much stronger impact of sampling region. Samples from the Mecklenburg region grew the fastest, and samples from the Arkona Basin, the slowest. The most variable responses were found in samples from the more variable Kiel region. Where there was a trend for growth rate to be affected, samples with the grazer fraction generally grew faster, likely due to recycling of nutrients.

Figure 5.2: Growth rates in samples from three regions (Kiel, Mecklenburg, Arkona) for phytoplankton communities with (red) and without (green) grazers. There was a weak trend for higher growth rates in the presence of grazers, but this trend was dwarfed by the effect of region on growth rates (see above). Boxplots are displayed as is standard with girdle band indicating the median.

3. Are zooplankton from the Bornholm Area metabolically more vulnerable to changes in salinity than zooplankton from the Kiel Area?

Working on ice, the students first characterized zooplankton from WP2 and BONGO samples using the dissecting microscopes. They agreed that samples from the much gentler WP2 haul were in better condition than those from the BONGO haul. One group proceeded to asses diversity and distribution of zooplankton species in the BONGO and WP2 samples and the other continued with the metabolism experiment.

Using the WP2 samples, they then picked at least three copepodes of similar size from each region for each experimental salinity and transferred them to a salinity gradient on a Presens SDR® sensor dish (a type of entirely non-invasive oxygen optode, one copepode per electrode). Zooplankton were given 20 minutes to adjust to the new environment (as a back-up respiration data were already recorded at this point in time). Respiration (as descrease in oxygen) was then recorded for a further 10 minutes. The students then compared whether copepods from the three regions investigated here differed in their responses to salinity. They found that copepods from the more variable Kiel and Mecklenburg region respired significantly more when transferred to salinities deviating up to five PSU either way from their sampling station salinity (Figure 4A). Samples from the Arkona Basin on the hander entered metabolic depression in salinities deviating from those at the sampling station (Figure 4B). This effect was reversible, with samples from the Kiel and Mecklenburg stations quickly returning to baseline values – a process that took much longer (up to 2 hours) in samples from the Arkona Basin.

Figure 5.3 : Copepode respiration rates for samples from the Kiel/Mecklenburg area (pooled here as they were not statistically different from each other) on the left (A) and the Arkona Basin samples on the right (B). Green for salinity at sampling station, red for lower salinity, and blue for higher salinity. Boxplots are displayed as is standard with girdle band indicating the median.

4. What are the relative contributions of acute (within minutes), seasonal (comparison across seasons), and long-term (comparison across basins) changes in mean temperature on picophytoplankton community metabolism? Do picophytoplankton behave differently than communities made up from larger phytoplankton?

This ongoing research question combines data from the cruises of the last two years. Here, we are presenting data from AL530 only. To answer these questions, the students took surface water samples along the cruise track of AL530. On board, they measured photosynthesis and respiration of two different size fractions (0.2-2 μm and 0.2-37.5 μm) using a clark-type oxygen electrode immediately after sampling. The temperature gradient spanned 5ºC -30ºC. They also assessed photosynthesis and respiration rates over a gradient of salinity. Furthermore, aliquots of water samples passed through an 0.45 µm filter were set aside to isolate viruses. Picoplankton were later isolated by dilution from the $\langle 3\mu m\rangle$ fraction and confirmed by 18S sequencing. Preliminary analyses of the temperature curves (see Figure 5) show that the size fractions differ in their metabolic activity, but also point to differences between the examined regions along the salinity and temperature gradient of the Baltic Sea.

Figure 5.4: The temperature performance curves for all size fractions and all regions were unimodal, allowing us to extract the steepness of slope to the optimum (A), the intercept (B), and the temperature T_{opt} at which rates are the fastest. Again, there was no significant difference between Kiel and Mecklenburg samples, and they were therefore pooled. Samples from the Kiel region had on average gentler slopes, lower intercepts, and higher T_{opt} than samples from the Arkona area, pointing towards larger

metabolic thermal tolerance in the Kiel samples. All data had been normalised for biomass upon returning to Hamburg. All boxplots displayed as is standard.

Further, we have had ongoing lysis successes between 18S-confirmed Ostreococcus samples and ultra-filtrated seawater, pointing toward the presence of lytic host-virus pairs across the Baltic Sea Basins, especially in the Kiel Bight

6 Station List AL530

6.1 Overall Station List

7 Data and Sample Storage and Availability

a) The station list meta data (time, position, gear) has been transferred to the DOD.

b) CTD data will be quality checked and transferred into PANGAEA.

c) A cruise summary report (CSR) has been sent the BSH.

d) The cruise leader confirms the data transfer from a) and b) in his cruise report.

e) The cruise leader will supply detailed information about the analysis of samples and long term storage of the data and samples in his cruise report. Diplomatic mandatory data transfers to visited states will be conducted by the cruise leader.

8 Acknowledgements

We most sincerely thank the captain - Jan Lass - and the entire crew of RV ALKOR for their outstanding support throughout the cruise, Magarethe Nowicki and Richard Klinger for their tireless help in preparing the cruise and Svend Mees (GEOMAR) for his support in technical equipment for the cruise. We also thank Tim Heimann for being an extremely valuable student helper on deck. Finally, we also thank all bachelor students on AL530 for their enthusiasm and motivation throughout the cruise.

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