Scientific Cruise Report

Sargasso Sea Environmental Assessment – European Eel Larval Studies (SEA-EELS)

01 April – 29 April 2015

Chief Scientist: Reinhold Hanel
Captain: Ralf Schmidt
Two years after the completion of a cruise with the Research Vessels POLARSTERN, SONNE, METEOR, MARIA S. MERIAN, POSEIDON, ALKOR, HEINCKE, or ELISABETH MANN BORGESE, the scientific exploitation of the samples and data obtained have to be documented in a Scientific Report by the chief scientist. This includes the progress with regard to the scientific objectives as outlined in the original cruise proposal and the publication of the results in scientific journals.

Citation:

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1. General Information

- Cruise No. MSM41
- GPF identification of the cruise proposal MerMet 14-46
- Chief Scientist Prof. Dr. Reinhold Hanel
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Project Title

Sargasso Sea Environmental Assessment – European Eel Larval Studies

Acronym

SEA-EELS

St. Georges, Bermuda – Dockyards, Bermuda

01 April – 29 April 2015

Publication overview

- 8 peer reviewed publications in scientific journals
- 1 book chapter
- 2 Master theses
- 2 Bachelor theses
- 1 Research report of a student’s project
Detailed list of publications resulting from cruise MSM41

(Participants of the cruise in bold letters)

a) Peer-reviewed publications


b) Book publications

c) Other publications

Master theses:

Lischka, Alexandra; November 2015; Cephalopods of the Sargasso Sea - Distribution patterns in relation to hydrographic conditions. 112 pp. Christian-Albrechts-University Kiel

Petersen, Imke; August 2016; Differences in the rafting assemblages associated with floating plastic debris and floating Sargassum in the Sargasso Sea. University of Bremen

Bachelor theses:

Hellenbrecht, Lea; January 2018; Larval size and distribution of Ranzania laevis and Masturus lanceolatus (Tetraodontiformes:Molidae) across thermal frontal zones in the Sargasso Sea. University of Hamburg

Hintzki, Lisa; 2018; Metagenomic analysis of microorganisms of the domain bacteria and archaea on microplastic out of the Sargasso Sea by next-generation-sequencing. Fachhochschule Lemgo/University Bielefeld.

Research report of a student's project

Potrafke, Chantal; 2017; DNA-Extraktionsmethoden im Vergleich: Marines Mikroplastik als biologische Nische für Biofilm-Bakterien in der Sargassosee. University Bielefeld.
2. Summary

This interdisciplinary research cruise assessed the hydrographic and ecological situation of the central Sargasso Sea pelagic community. Special focus was given to the early life history stages (leptocephali) of the two Atlantic eel species of the genus Anguilla (A. anguilla and A. rostrata).

Data on the distribution of A. anguilla leptocephali were used to analyze their drift. According to this modelling approach, the majority of leptocephali remain trapped in the Sargasso Sea, whereas only a small proportion of leptocephali are entrained into the Gulf Stream system. Hence, spawning success may be highly sensitive to oceanographic factors. Leptocephalus larvae of non-Anguillid species were also examined to comparatively study gut contents and hence food preferences. A more detailed analysis of the geographic size distribution of Ariosoma balearicum leptocephali of two revealed species-level differences in myomere-counts and DNA sequence identity, providing evidence for the existence of two cryptic species with different larval dispersal strategies in the Sargasso Sea region.

The consistent presence of early life history stages of slender sunfish Ranzania laevis in the samples throughout the study area confirmed spawning activity of this species in the Sargasso Sea.

Regarding cephalopods, a new and comprehensive data set on early life stages from the Sargasso Sea was collected. The subtropical convergence zone marked a significant faunal frontier for several species. The early life stages of Pyroteuthis margaritifera and representatives of the hooked-squids were the most abundant species and occurred throughout the whole study area. Some species like the glass squid Leachia lemur were only encountered in the northern water masses, whereas other squids of this family were distributed throughout the whole area. In contrary, early life stages of the flying squid, Hyaloteuthis pelagica tended to be more abundant in the southern stations.

During the cruise, assemblages associated to floating Sargassum as well as to floating marine debris were analysed. The taxonomic analysis revealed a substantial overlap in the spectra of species associated with seaweeds and floating debris. Yet, multivariate analyses showed a significant difference in the structural composition of assemblages from different types of substrata, suggesting that the occurrence of floating marine debris can change the rafting community of this region by providing an alternative type of substratum. Due to the strong numerical dominance of floating seaweeds in that region the overall effects on the rafting community are still restricted to single floating items. However, a continuous increase in floating debris density may induce significant shifts in the overall future composition and functioning of the unique rafting community of the Sargasso Sea.

The low lipid levels of most pelagic animals in the Sargasso Sea indicate that the low primary production in this oligotrophic system is not sufficient for the accumulation of extensive lipid reserves or that the regular, but limited food supply of tropical waters does not require specific adaptations to overcome seasonal periods of food scarcity. Among the different krill species, specialisations in feeding behaviour and dietary composition were indicated by trophic marker analyses with several species occupying the rather low trophic position of herbivores, whereas other species had a higher index of carnivory. Eel larvae had surprisingly low \( \delta^{15}N \) ratios, which may be explained by feeding on marine snow particles. \( \delta^{13}C \) signals indicate two different ultimate carbon sources of primary production, either from phytoplankton or from Sargassum spp. macroalgae drifting at the surface. Herbivores mainly grazing in Sargassum macroalgae and carnivores feeding on these grazers had significantly different \( \delta^{13}C \) ratios than zooplankton and other consumers relying on phytoplankton primary production as ultimate carbon source.

Microplastics were sampled for analyses of their associated microbial communities in comparison with free-living and particle-attached communities from the surrounding water. Microplastics were collected from all surface stations, while no visible microplastics were found in 125 m depth.

During the MSM41 cruise, novel information on the ecology of several groups of organisms in this unique oceanic region could be obtained. There is still some work in progress and it is expected that further insights and publications based on data from MSM41 will become available in the future.

Collaboration between scientists and cooperation with the crew of R/V Maria S. Merian were excellent.
3. Scientific Results

Importance of hydrographic conditions and trophodynamics in the pelagic community of the Sargasso Sea for the reproduction of the endangered European eel

(R. Hanel, M. Miller, L. Marohn, H. Westerberg, K. Wysujack, M. Freese, J.-D. Pohlmann)

One of the primary objectives of MSM41 in April 2015 was to collect eel larvae, so called leptocephali, of the two Atlantic freshwater eels *Anguilla anguilla* (European eel) and *Anguilla rostrata* (American eel), both spawning in the Sargasso Sea, together with leptocephali of oceanic and other marine Anguilliform species for a better understanding of their ecology and community structure. The MSM41 survey was not intended to be a stand-alone project. Instead, it represented a further step in our efforts to establish a continuous time-series of Sargasso Sea surveys to monitor abundance as well as horizontal and vertical distribution of eel larvae dependent on hydrographic conditions during the eel’s spawning period. However, the aim was also to reveal additional information on trophic interactions and the food web structure of the pelagic community of the Sargasso Sea.

During the MSM41 survey, an Isaacs-Kidd Midwater Trawl (IKMT) with a 6.3 m² mouth-opening and a mesh size of 500 µm was used for catching all sizes of eel larvae and other planktonic organisms. Some leptocephali were also collected by the smaller mouth-opening gear deployed during the survey, which were the MOCNESS depth sampling trawl (*N* = 30 leptocephali, 14 stations, 11 species, 9 families), the Manta surface net (*N* = 44, 5 stations, all *Ariosoma balearicum*), the Multinet vertical plankton sampler (*N* = 1, *Nemichthys scolopaceus*), and a large-mesh IKMT (mesh size in cod-end 5 mm) (*N* = 130, 10 stations, 22 species). Leptocephali were sorted out of the plankton samples immediately after catch and then identified on board using a dissecting microscope before being preserved in ethanol or frozen.

Leptocephali were widely distributed in the study area and were collected at every station. A total of 362 *Anguilla* leptocephali ranging from 6 – 46 mm total length (average: 16.2 ± 4.5 mm) were collected and 1721 marine eel leptocephali were collected (6 – 405 mm), with 10 juvenile eels of the Serrivomeridae (*N* = 7) and Derichthyidae (*N* = 3) also being collected. The distributions and abundances of anguillid and marine eel leptocephali (~ 44 species, 14 families) showed various different patterns in the transects of stations that sampled at a range of latitudes between 22.5°N and 31°N and from 70°W and 61°W.

Genetic species identification of the *Anguilla* larvae revealed the following results: 242 *A. anguilla*, 117 *A. rostrata*, 1 potential hybrid with *A. anguilla* and 2 potential hybrids with *A. rostrata* on the mother’s side.

**Distribution and drift of *A. anguilla* leptocephalus larvae**

The data on distribution and size of leptocephalus larvae *A. anguilla* obtained during this cruise were included in an approach to model larval drift and time of spawning (Westerberg et al. 2018), using current data from two global oceanographic assimilation models. The study revealed an overall growth rate of the leptocephali of 0.18 mm·day⁻¹, which is close to earlier estimates. Accordingly, the study indicated an extended spawning period from December to March. For the leptocephali caught during the MSM41-cruise, the modelling revealed that individuals caught in the eastern part of the area predominately came from the west, while leptocephali caught in the western area came mostly from an easterly direction. If the assumptions about growth rate and passive drift holds, this means that spawning takes place over a 1–2 × 10⁶ km² area. The fact that the velocity data from the two models correlate reasonably well with concurrent ADCP measurements, in combination with the fact that both models give similar general results, support the conclusion.
The drift was also calculated forwards for approximately 1 year, resulting in an unexpected outcome. According to this modelling approach, most leptocephali remained in the area south of the Subtropical Frontal Zone. One conclusion is that the majority of leptocephali remain trapped and possibly die in the retention area. A small proportion of leptocephali are entrained into the Gulf Stream system. An implication is that the spawning success may be highly sensitive to oceanographic and climatic factors that alter the dispersion of leptocephali out from the retention area. Comparisons revealed that the proportion of leptocephali entrained to the Gulf Stream is highly variable between years.

**Marine eel leptocephalus larvae**

Marine eel leptocephali were widely distributed across the study area, including to the north of the 22°C front. The larvae of the mesopelagic snipe eels (Nemichthyidae, $N = 615$, ≤ 4 species) were most abundant at the northern stations, and especially north of the frontal zone, but they appeared to be spawning at various latitudes. Other abundant species of larvae, such as those of the congrid eel *Ariosoma balearicum* ($N = 362$), were most abundant within the frontal regions of the more central parts of the transects, but were widely distributed mostly at a narrow size range of about $80 – 100$ mm. Small larvae of the other mesopelagic species of the sawtooth eels (Serrivomeridae, $N = 204$), the longneck eels (Derichthyidae, $N = 137$) and the gulper eels (Eurypharyngidae, $N = 76$) were collected, which indicates these species were spawning offshore. However, the larvae of the other species of eels whose adults live in coastal areas, such as *A. balearicum*, other Congridae ($N = 46$), Chlopiidae ($N = 94$), Muraenidae ($N = 109$), and Moringuidae ($N = 20$) were all large in size and had been transported offshore into the sampling area.

**Gut contents of leptocephali**

Leptocephali sampled during the MSM41 cruise were included in a study of gut contents of anguillid larvae and several families of larger marine eel larvae based on direct observations and photographs (Miller et al. 2019b).

The gut contents of leptocephali consisted of amorphous material that sometimes flowed out of the intestine. Appendicularian houses and fecal pellets and other visible objects were sometimes present. High-magnification microscope images showed the presence of many spherical objects, amorphous and other materials that were likely related to bacteria, protists, fungi, or other organisms. The presence of filter structures confirmed that large oval objects were appendicularian houses. The gut contents of *A. anguilla*, *E. pelecanoides*, and *Kaupichthys hyoproroides* leptocephali appeared to contain round appr. 40 mm heterotrophic thraustochytrid protists (class Labyrinthulomycetes) that likely colonized marine snow materials consumed by the larvae. These observations support the hypothesis that leptocephali, whose teeth structure and relative teeth sizes change with growth, primarily target overlapping size ranges of marine snow particles as a food source in the Sargasso Sea, and the particles contain many types of organisms and other materials including discarded appendicularian houses and probably hydrozoan tissues. Heterotrophic thraustochytrid protists that have been found in marine snow particles elsewhere were possibly distinguished in leptocephalus gut contents for the first time, suggesting that they can be consumed by leptocephali along with aggregates.
Distribution of two myomere-count types of Ariosa balearicum

In conjunction with data from other cruises (2011 - WH352, 2014 - WH 373, 2017 - WH404), the standard IKMT tows data obtained during MSM41 were also used to examine the geographic size distribution of two myomere-count types of Ariosa balearicum leptocephali (Miller et al. 2019a).

Myomere count subsamples of larvae during each survey indicated that the majority of larvae had counts in the HC range of ≥128 TM for the main size class. The TM counts of the HC leptocephali in the standard IKMT tows were not statistically different among years (p = 0.53; Kruskal-Wallis), with mean TM only ranging from 131.0 to 131.5 myomeres.

High-count (HC) larvae were consistently mostly between 80 and 100 mm in size (60–132 mm; 87.9 ± 6.8 mm) as observed previously, and frequently had narrow size ranges. The smallest larvae in each survey ranged from 60 to 72 mm (N = 9 total larvae <70 mm), and from 4 to 26 large larvae, 110–172 mm, were collected during each survey (N = 55 total).

The usually larger low-count (LC) larvae (78–176 mm; 111.4 ± 26.7 mm) were more abundant in western or central areas.

Mitochondrial 16 S rRNA sequences of HC and LC larvae showed species-level differences, providing evidence of the existence of two cryptic species with different larval dispersal strategies in the Sargasso Sea subtropical gyre region.

Further aspects of leptocephalus larvae - saccopharyngiform eel mysteries

Samples of the MSM41 cruise likely have also helped to solve a long-standing mystery about a certain type of leptocephali (Poulsen et al. 2018). Based on mitogenomic sequence data from rare new specimens, it was shown that the long-speculated-about larval form referred to as „Leptocephalus holti“, which was thought to possibly be the larva of the rare orange-colored eels of Neocyema (5 known specimens; speculated to belong to the Cyematidae) are actually the larvae of the one-jaw eels of the family Monognathidae. The genetic analysis of the MiFish 12S rRNA DNA sequences of eight specimens of Leptocephalus holti leptocephali, including individuals from the MSM41 cruise, show for the first time that these are the larvae of the Monognathidae and are not cyematid larvae.

Larval distribution of ocean sunfishes Ranzania laevis and Masturus lanceolatus in the Sargasso Sea

The IKMT-samples obtained during the cruise were also used to examine abundance and size distribution of sunfish larvae. Sunfishes or Molidae are a rarely encountered family within the teleost order Tetraodontiformes and most details of their reproductive biology including times and places of spawning and their larval ecology are rather unclear. Spawning of two species of Molidae was suggested in the Sargasso Sea before, but comprehensive data on larval distribution from this area or elsewhere have never been published. Overall, 365 larvae of slender sunfish (Ranzania laevis) and 18 larvae of sharptail mola (Masturus lanceolatus) were caught. The 18 Masturus larvae were mostly larger and evenly distributed over the study area. Therefore, it was concluded that there was no active spawning of M. lanceolatus in the area during the time of the cruise. In contrast, Ranzania larvae were caught primarily inside and south of a thermal frontal zone with increasing abundances toward warmer surface layers in the southeast of the study area. Due to the consistent presence of young Ranzania, it can be assumed that spawning activity was ongoing throughout the month of April, 2015. Hence, our findings confirm the Sargasso Sea as a spawning area for R. laevis.
**Scientific outcome**

- 5 peer reviewed paper
- 1 bachelor thesis (Lea Hellenbrecht)

**Sample storage**

Samples of eel larvae and other fishes (preserved in ethanol or frozen) and the plankton samples (preserved in ethanol) will be held at the Thünen Institute of Fisheries Ecology while being analyzed further, but arrangements will be made in the future to transfer appropriate samples to museums for long-term archival.

**Plankton biodiversity and the pelagic food web of the Sargasso Sea**

(W. Hagen, H. Auel, F. Buchholz, C. Buchholz, M. Kaufmann)

**Background and Objectives**

One major objective of the MSM 41 SEA-EELS (Sargasso Sea Environmental Assessment – European Eel Larval Studies) research cruise onboard R/V MARIA S. MERIAN was the comprehensive assessment of meso- and macrozooplankton species composition, biomass and trophic interactions throughout the Sargasso Sea. The pelagic food web of the Sargasso Sea and its primary and secondary production provide the nutritional basis for the leptocephalus larvae of the European (A. anguilla) and American (A. rostrata) eels as well as many other anguilliform fish species and the meso- and bathypelagic communities in one of the deepest basins of the Atlantic Ocean.

The specific objectives of this work package included:

- To quantify the distribution of phytopigments in the Sargasso Sea as proxy for phytoplankton biomass and primary production;
- To quantify meso- and macrozooplankton abundance and biomass;
- To assess phyto- and zooplankton species composition in relation to the hydrographic regime;
- To trace trophic interactions and predator-prey relationships throughout the pelagic food web of the Sargasso Sea by means of trophic biomarkers including fatty acids and stable isotopes (C, N).
- In co-operation with other research teams on board: To elucidate and better understand the nutritional basis of the neuston community and animals associated with drifting Sargassum seaweed, i.e. whether they more rely on phytoplankton production as food-web basis or primary production by Sargassum itself.

The ultimate goal is to quantitatively describe the pelagic food web of the Sargasso Sea, on which the survival, growth, and recruitment success of eel larvae is based.

**Work at Sea and Results**

To assess the standing stock and the composition of the phytoplankton community, water samples of 10 L each were taken at 43 stations for later analysis of the phytopigments by HPLC (High Pressure Liquid Chromatography).

In addition, a more detailed method will be applied to analyse the contribution of smaller organisms, like nano- (2-20 µm) and picoplankton (<2 µm). Aliquots of 3.2 ml were preserved in cryovials with
0.2 ml of 20% paraformaldehyde for later analysis by flow cytometry to determine type and abundance of the main nano- and picoplankton groups (e.g. prymnesiophytes, Prochlorococcus, Synechococcus, picoeukaryotes).

Mesozooplankton (size fraction between 200 µm and 20 mm) was sampled by stratified vertical hauls with an opening/closing net system (Hydro-Bios MultiNet Midi, mesh size: 200 µm, mouth opening: 0.25 m²) at a total of 20 stations along five N-S transects at 70°W, 67°W, 61°W, and 58°W between 30°N and 24°N.

All MultiNet samples were screened immediately after the catch and larger organisms, including mainly decapods and mesopelagic fishes, but also euphausiids, mysids, amphipods, pteropods, heteropods, chaetognaths, salps, siphonophores, and calanoid copepods, were sorted from the catch and deep-frozen at -80°C for biochemical analysis of trophic biomarkers (fatty acids, stable isotopes). At stations along the transects at 70°W, 67°W, and 61°W, the remaining samples were preserved in a 4% formaldehyde-seawater solution for quantitative analysis of mesozooplankton abundance and species composition. By contrast, the remains of the MultiNet samples obtained at stations along the transects at 64°W and 58°W were deep-frozen as bulk samples at -80°C for precise determination of mesozooplankton biomass and biochemical analysis of trophic biomarkers.

In order to collect and assess the abundance and biomass of larger and usually rarer meso- and macrozooplankton (size fraction between 2 mm and 20 cm), stratified trawls with a Multiple Opening Closing Net with Environmental Sensing System (MOCNESS, mesh size: 330 µm and 2 mm, mouth opening: 1 m²) were conducted at 23 stations along the same five N-S transects.

At stations along the central N-S transect at 64°W, the MOCNESS was deployed deeper to a maximum sampling depth of 1.000 m with depth intervals of 0 to 125 m, 125 to 250 m, 250 to 500 m, and 500 to 1.000 m during the downward cast (with 330 µm mesh) and 1.000 to 750 m, 750 to 500 m, 500 to 250 m, and 250 to 0 m during the upward cast (with 2 mm mesh).

To establish the isotopic signature of the baseline of the pelagic food web, seston samples were collected from the mixed surface layer (5 to 25 m depth) and/or chlorophyll maximum (85 to 150 m).

The permanent stratification between warm (>22°C) surface waters and colder water below the thermocline limits nutrient replenishment to the euphotic zone. This nutrient deficit strongly limits the primary production of phytoplankton (e.g. diatoms, dinoflagellates), which results in a very low food supply for the zooplankton. Maximum densities of the algae were not as usually concentrated at the surface, but occurred in 120 to 140 m depth. There is still sufficient light at this depth due to the extremely clear water of the Sargasso Sea and more nutrients are available in this depth layer than near the surface. Very small algae with a size of ≤0.02 mm play a crucial role in this layer.

Cyanobacteria dominated the surface phytoplankton at the southernmost stations between 24° and 22.5°N. These microorganisms have the unusual ability to utilize molecular nitrogen from the atmosphere as fertilizer for growth instead of nitrate, a successful strategy to cope with the general nutrient deficit in the “blue desert” of the Sargasso Sea.

Owing to the extremely limited food supply, zooplankton stocks were also very low compared to other regions. In particular copepods, which generally prevail in plankton communities with 50 to 80% of biomass globally, were rarely found in the Sargasso Sea. At the surface there were only minute copepods <1 mm.

At most stations, zooplankton biomass was dominated by deep-sea shrimp (Decapoda), myctophids and other deep-sea fish.
Similar to the copepods, euphausiids were found in much lower densities in the Sargasso Sea than in other regions of the Atlantic, but with higher species numbers. At night, several krill species concentrated in higher densities near the thermocline, where food accumulates, to “graze” and hunt for prey. Hence, the omnivorous krill may be a competitor for food, but also a predator of the eel larvae. Altogether, 23 euphausiid species were identified which is 27% of the total species repertoire of only 86 species worldwide. This high diversity demonstrates the typically tropical biodiversity pattern in the area. Horizontal distribution and abundances reflected the influence of currents and different water masses within the study area.

Microscopic examination of the stomach content of some krill specimens (*Nematoscelis tenella, Nematoscelis microps, Thysanopoda aequalis, Thysanopoda tricuspidata*) revealed the presence of small pennate diatoms (cf. *Navicula* and *Pseudo-nitzschia*), some small dinoflagellates (Gymnodiniales and *Cladophysis*) and coccolithophores (*Prismnesiophyceae, Discosphaera tubifera, Rhabdosphaera, Syracosphaera cf. pulchra*, cf. *Helicosphaera carteri*).

**Results of Trophic Marker Analyses**

After the cruise, deep-frozen zooplankton samples were analysed for trophic biomarkers to elucidate food-web structure, dietary spectra, trophic positions, and pathways of energy flow in the pelagic ecosystem of the Sargasso Sea. In total, 309 samples of 66 zooplankton species were analysed for lipid content and/or fatty acid composition. The collection included all dominant pelagic taxa with highest taxonomic resolution in euphausiids (krill). In addition, 160 zooplankton samples were analysed for $\delta^{15}$N and $\delta^{13}$C stable isotope (SI) ratios to establish their trophic level and carbon source. The analysis of some additional samples, in particular regarding the stable isotope baseline of the food web, is still pending.

Preliminary results show that the lipid content of most pelagic organisms in the Sargasso Sea is rather low (10 to 15% dry mass) indicating that the low primary production in this oligotrophic system is not sufficient for the accumulation of extensive lipid reserves or that the regular, albeit limited food supply of tropical waters without seasonality does not require specific adaptations to overcome seasonal periods of food scarcity. Among the different krill species, specialisations in feeding behaviour and dietary composition were apparent with several species occupying the rather low trophic position of herbivores, whereas other species had a higher index of carnivory in line with mouthparts adapted to raptorial feeding. Accordingly, several krill species had rather low $\delta^{15}$N ratios indicative of herbivorous feeding, while others showed higher SI signals of omnivory. Eel larvae had surprisingly low $\delta^{15}$N ratios, which may be explained by feeding on marine snow particles.

Another interesting feature of the pelagic ecosystem of the Sargasso Sea is the fact that there are two different ultimate carbon sources of primary production, either from phytoplankton or from *Sargassum* spp. macroalgae drifting at the sea surface. These two primary producers differ in their $\delta^{13}$C signal, which can be traced throughout the food web. Herbivores mainly grazing in *Sargassum* macroalgae and carnivores feeding on these grazers had significantly different $\delta^{13}$C ratios than zooplankton and other consumers relying on phytoplankton primary production as ultimate carbon source.
Ecology of the *Sargassum* seaweed community

(K. Koch, L. Sielhorst)

The recent concern about declining *Sargassum* algae calls for research on the stress ecology of *Sargassum*. Consequently, this subproject addressed the question whether the hitherto contrasting reports on the overall biomass and spatial vs. temporal variability might be based on variation in the hydrography of the Sargasso Sea current system or rather due to environmental stress. By multifactorial stress assessments using a combination of temperature, radiation and nutrient levels, it was intended to explore, if there are physiological reasons for an apparent decline in *Sargassum* abundance. In the field *Sargassum* floating on the sea surface is exposed to high irradiances of PAR as well as UV radiation. While it is evident that the species has developed adequate protective measures, as e.g. high xanthophyll cycle pigment turn-over as well as phlorotannin concentration, it is not known how efficient these strategies will operate under varying temperature and nutrient regimes.

**Latitudinal comparison of physiological algal status**

For a latitudinal comparison of the physiological algal status, at least five replicate individuals of *Sargassum natans* were collected at 13 positions with a rake from the Fast Rescue Boat. The positions were mainly equivalent to the regular stations of the cruise grid; however, some positions were also located between these stations. On board of the R/V MARIA S. MERIAN, pulse amplitude in vivo variable chlorophyll *a* fluorescence of photosystem II was measured with a pulse amplitude-modulated fluorometer (Diving-PAM). Moreover, samples were shock-frozen for later analysis of photosynthetic pigments (e.g. xanthophyll cycle pigments, light-harvesting versus photoprotective pigments), antioxidants and phlorotannins.

At two positions (23°N 61°W with 25.8°C ambient seawater temperature and 28°N 61°W with 24.1°C ambient seawater temperature), larger amounts of *S. natans* were collected with the FRB. This algal material was challenged in short-term (3 h) ship-based culture experiments at one of three different temperature levels (ambient, +5°C and +10°C) in order to identify temperature threshold values for emerging physiological stress. The temperature stress was followed by a three hours recovery period under ambient temperature conditions. During the experiments, the maximum quantum yield of PS II (Fv/Fm), an estimate of the maximum portion of absorbed light quanta used in PSII reaction centers, was monitored continuously with the Diving-PAM. In addition, samples were taken for the determination of the physiological algal status (photosynthetic pigments, antioxidants, phlorotannins) at the beginning of the experiment, after three hours of temperature stress and after three hours of recovery.

Generally, three hours of temperature stress resulted in a decline of the Fv/Fm values in algal samples from 23°N 61°W and 28°N 61°W. Exposure to ambient as well as +5°C temperatures lead to decreases of approximately 20-40% of initial values, whereas +10°C temperatures resulted in more than twice as high declines. In the following three hours of recovery, the algae were able to recover partly (80-90% of initial values) from the temperature stress.
Abundance, distribution patterns and community structure of pelagic cephalopods
(U. Piatkowski, A. Lischka)

Background and Objectives

In the Sargasso Sea cephalopods and especially their early life stages form the bulk of the oceanic micronekton/macroplankton, together with euphausiids, decapod shrimps, and early life fishes. Although they are well-known top predators and essential members of pelagic food webs, our knowledge on the cephalopod fauna of the Sargasso Sea is still very poor. Therefore, it was our aim during cruise MSM 41 of R/V Maria S. Merian to study the species composition, distribution patterns and community structure of cephalopods, and to collect tissue samples of the majority of species for further studies on barcoding, food web analysis, and morphology.

Work at Sea and Results

The major gear to sample cephalopods was an IKMT with a mouth opening of ca. 6.2 m² that was equipped with a net of 500 µm sized meshes. At each standard station of the five transects the IKMT was sampling down to a depth of 300 m in a double oblique fashion. In total, we collected cephalopod specimens from 49 IKMT stations.

Immediately after the net was recovered, the sample was taken from the cod-end bucket and maintained in cooled seawater before and during processing in the ship’s laboratory. The processing included the sorting of all cephalopod specimens from each sample, their identification to the lowest possible taxon, measurement of dorsal mantle length and other body parts, and taking pictures of selected specimens. Afterwards, specimens were preserved in ca. 85% ethanol, or deep-frozen at -20°C. A few specimens were preserved in buffered 8% formaldehyd-seawater solution. In total, we obtained the impressive number of 2,406 specimens from the IKMT. They belonged to 17 families and at least 25 different species (see Table 1).

Additionally, we sampled 88 specimens that were caught by other nets (n = 60 specimens from the IKMT with 5000 µm mesh size; n = 18 specimens from the MOCNESS with mesh sizes of 330 or 2000 µm, respectively; n = 10 specimens from the Manta trawl).

Our results show that the abundance of cephalopods varied strongly between night and day catches; with night catches showing a higher abundance and species diversity. Furthermore, the subtropical convergence zone was found approximately between 27°N and 28°N during most transects which marked a significant faunal frontier for several species. The surface water temperature changed in those latitudes from approximately 22°C to 24°C from north to south. The early life stages of the fire squid, *Pyroteuthis margaritifera* and representatives of the hooked-squids (family Onychoteuthidae) were the most abundant species and occurred throughout the whole investigated area. Some species like the glass squid *Leachia lemur* (family Cranchiidae, see Fig. 1a) were only encountered in the northern water masses, whereas other squids of this family, like *Helicocranchia papillata* (Fig. 1b), were distributed throughout the whole research area (see Fig. 2). In contrary, the early life stages of the flying squid, *Hyaloteuthis pelagica* (family Ommastrephidae) tended to be more abundant in the southern stations. Abundance, diversity, and distribution pattern of the cephalopods confirmed the results of an earlier study, which investigated abundance, distribution and diversity of early life cephalopods sampled by Bongo (500 µm mesh size) and IKMT (300 µm mesh size) in the top 300 m along a north to south transect from 31° to 25°N at a longitude of 62°W in March 1993.
Table 1. Cephalopods caught during MSM 41 with the IKMT (500 µm mesh size). Cephalopod taxa are listed in alphabetical order.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Transect 1</th>
<th>Transect 2</th>
<th>Transect 3</th>
<th>Transect 4</th>
<th>Transect 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abraliopsis morisii</td>
<td>7</td>
<td>18</td>
<td>4</td>
<td>0</td>
<td>17</td>
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<tr>
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<td>14</td>
<td>11</td>
<td>3</td>
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<td>1</td>
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<td>3</td>
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<td>9</td>
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<td>10</td>
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<td>8</td>
<td>9</td>
<td>20</td>
<td>19</td>
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<tr>
<td>Cranchiidae indet.</td>
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<td>4</td>
<td>4</td>
<td>6</td>
<td>0</td>
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<tr>
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<td>20</td>
<td>11</td>
<td>18</td>
<td>46</td>
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<tr>
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<td>3</td>
<td>4</td>
<td>1</td>
<td>3</td>
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<tr>
<td>Helicocranchia papillata</td>
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<td>27</td>
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<tr>
<td>Helicocranchia sp.</td>
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<td>87</td>
</tr>
<tr>
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<td>7</td>
</tr>
<tr>
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<tr>
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<tr>
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<td>2</td>
<td>5</td>
<td>0</td>
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<td>1</td>
<td>2</td>
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<tr>
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<td>83</td>
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<tr>
<td>Onychia carribaena</td>
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<td>10</td>
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<td>4</td>
<td>11</td>
</tr>
<tr>
<td>Pyroteuthidae indet.</td>
<td>3</td>
<td>3</td>
<td>14</td>
<td>77</td>
<td>56</td>
</tr>
<tr>
<td>Pyroteuthis margaritifera</td>
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<td>5</td>
<td>5</td>
<td>14</td>
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<td>41</td>
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<tr>
<td>Thysanoteuthis rhombus</td>
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<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Chiroteuthidae indet.</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Vitreeledonella richardi</td>
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<td>1</td>
<td>0</td>
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<tr>
<td>Planctoteuthis sp.</td>
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<td>1</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>259</strong></td>
<td><strong>378</strong></td>
<td><strong>541</strong></td>
<td><strong>571</strong></td>
<td><strong>657</strong></td>
</tr>
</tbody>
</table>

It is important to note that the distribution patterns of eel leptocephali and euphausiids were similar to those of cephalopods, indicating that these micronektonic groups share the same habitat.

After final identifications and comparisons with a reference collection of early life cephalopods at GEOMAR, Kiel multivariate statistical analyses were applied to our dataset to examine differences in cephalopod species composition and abundance and to describe faunal assemblages in the investigated area and how they are related to hydrographic conditions. For this purpose, samples were classified by hierarchical agglomerative cluster analysis using the group-average linking method and ordinated non-metric, multi-dimensional scaling techniques (MDS plots).
Figure 1. Distribution of the cranchiid squids *Helicocranchia papillata* (top) and *Leachia lemur* (bottom) along the IKMT station transects during MSM 41 with the IKMT (500 µm mesh size).
Scientific outcomes

Work during the cruise was running perfectly, and we were able to collect a new and comprehensive data set on early life cephalopods from the Sargasso Sea. The results provided new and unique data on distribution and taxonomy of cephalopods from a subtropical ocean. The cephalopod fauna and distribution patterns in relation to the hydrographic conditions during the sampling were published in 2017 in the journal *Marine Biodiversity*:


Further species list outcomes and comparisons to an earlier cruise (WH373) of the MSM41 cruise were included in Alexandra Lischka’s master thesis:


The samples collected during this cruise furthermore contributed to the research on biodiversity of the blue oceans of subtropical oceanic regions as we were able to discover two new species of the family Onychoteuthidae, which doubled the number of known species for that area. The results were published in 2017 in the journal *Hydrobiologia*:


These samples were further included in a global revision of the family Onychoteuthidae and published 2018 in the journal *Molecular Phylogenetics and Evolution*:


We were also able to include data from this cruise into a chapter of the German book *Faszination Meeresforschung*, published in 2017:

Overall, we were able to use the cephalopod material collected during MSM41 for 1.) publication in three peer-reviewed journals of the scientific community, 2.) the completion the Master thesis of Alexandra Lischka and 3.) publishing a book chapter for a broader audience, all within three years after the cruise.

Sample Storage and Data Policy

Formaldehyde- and ethanol-preserved samples as well as frozen samples obtained during MSM 41 will be stored at GEOMAR, Kiel. The samples will be processed and analysed by researchers at GEOMAR in co-operation with other scientists (e.g., BreMarE, Bremen). Geo-referenced data sets, such as cephalopod abundance and length distribution, will be archived and made publicly available through the PANGAEA World Data Centre, jointly operated by the University of Bremen and the Alfred Wegener Institute for Polar and Marine Research. As far as DNA sequences of cephalopod species, in particular COI gene sequences used for DNA barcoding, are required for species identification or confirmation of taxonomic classification Cephalopod sequences obtained during this cruise were submitted to GenBank Sargasso Sea Onychoteuthid Biodiversity’ (project code: SSONY).

Rafting assemblages on floating Sargassum spp. and marine debris

(L. Gutow, I. Petersen)

Huge amounts of flotsam accumulate in the subtropical convergences of all major oceanic basins. In the Sargasso Sea, this leads to high densities of floating seaweeds of the genus Sargassum as well as increasing amounts of floating anthropogenic debris. Floating Sargassum hosts a diverse and well investigated community of rafting organisms comprising about 100 species of marine invertebrates and fish. Similarly, floating marine debris provides substratum for the settlement of marine species. However, the knowledge about rafting communities on floating marine debris is still very limited and studies comparing the rafting communities on natural and anthropogenic floating objects are almost entirely lacking.

During the cruise MSM 41 of the research vessel Maria S. Merian, 42 clumps of floating Sargassum, comprising the species S. natans and S. fluitans, were collected at 14 stations. Additionally, 28 items of floating marine debris were collected at 12 stations. The associated assemblages of rafting species were analyzed taxonomically as well as functionally with regard to the feeding type of the organisms. The species were determined to species level during the cruise if possible. Species that could not be determined onboard were preserved for later determination. The compositions of the rafting assemblages on the two types of substrata were compared based on presence/absence data. Finally, a visual survey was conducted to quantify floating seaweeds and floating marine debris on transects along the entire cruise.

The taxonomic analysis revealed a substantial overlap in the spectra of species associated with seaweeds and floating debris. However, multivariate analyses clearly showed a significant difference in the structural composition of assemblages from different types of substrata. Assemblages on Sargassum were comparatively homogeneous whereas a high heterogeneity among assemblages from debris items reflect the heterogeneity of floating marine debris. Species richness of the rafting assemblages was higher on single Sargassum clumps than on debris items. However, species area curves suggest that the diverse compilation of floating marine debris provides settlement substrata for an overall wider spectrum of species than does floating Sargassum. Species were encountered on floating marine debris, which are not typical representatives of the well established community on
floating seaweeds of the Sargasso Sea. The differences in the taxonomic composition of the rafting assemblages was associated with functional differences with regard to the feeding type of the organisms, again with a higher heterogeneity in feeding types on floating debris.

In the Sargasso Sea, densities of floating *Sargassum* were much higher than densities of floating marine debris. Both seaweeds and debris were heterogeneously distributed within the investigated area, however, with different distribution pattern for both types of flotsam.

The results of this study suggest that the occurrence of floating marine debris in the Sargasso Sea can change the rafting community of this region by providing an alternative type of substratum. Due to the strong numerical dominance of floating seaweeds in that region the overall effects on the rafting community are still restricted to single floating items. However, a continuous increase in floating debris density may induce significant shifts in the overall future composition and functioning of the unique rafting community of the Sargasso Sea.

The results have not been published in peer-reviewed journals yet, but a manuscript is presently in an early stage of preparation. Furthermore, the master thesis of Imke Petersen “Differences in the rafting assemblages associated with floating plastic debris and floating Sargassum in the Sargasso Sea” was successfully finished in August 2016 at the University of Bremen.

Preserved samples of specimens from taxonomic analysis of the rafting communities on floating Sargassum and anthropogenic debris are stored at the Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research at Bremerhaven, Germany. Data will be archived and made publicly available through the PANGAEA.

**Exploring the microplastic-associated microbial communities**

(S. Oberbeckmann, S. Mothes)

As part of the Atlantic Garbage Patch, the Sargasso Sea is an optimal sampling area for plastic research. Therefore, during the cruise MSM41, microplastics were sampled for analyses of their associated microbial communities in comparison with free-living and particle-attached communities from the surrounding water. Microplastics were collected from all surface stations, while no visible microplastics were found in 125m depth.

The main goal was to understand the unique bacterial communities that form on plastic waste in aquatic systems. First results show that differences between bacterial communities on plastic and in the water column exist. Certain genera were found on Sargasso Sea microplastics, which are frequently being detected on plastics across the world (e.g. *Erythrobacter*). Now, bacterial biofilms from Sargasso Sea microplastics will be analysed alongside plastics taken from the Mediterranean and the Baltic Seas to find reoccurring patterns in community membership and structure, potentially hinting at the ecological role of these communities. To accomplish this, total DNA was extracted from Sargasso plastic particles and the V3 and V4 variable regions of the 16S rRNA gene was successfully amplified. The amplified fragments were then sequenced on the Illumina MiSeq platform and the sequences are currently being processed using bioinformatical tools.

Publishing of the results in a peer-reviewed journal ist foreseen for the near future.

**Scientific outcome**

1 Bachelor thesis (Lisa Hintzki)

1 Research report of a student’s project (Chantal Potrafke)