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Cruise report FRV Walther Herwig III Cruise 465

02.03. - 23.04.2023

Studies on the early life stages of the European eel in the central Sargasso Sea

Cruise leader: Prof. Dr. Reinhold Hanel

On its 465th cruise, the FRV Walther Herwig III went for the fourth time since 2011 to the central Sargasso Sea to carry out studies on the distribution and abundance of the early developmental stages of the European eel (*Anguilla anguilla*). The study area ranged from 31°-19°N and 67°-64°W with 45 stations being sampled along two north-south transects with an Isaacs-Kidd Midwater Trawl (IKMT).

The primary objectives of the survey were the investigation of distribution and abundance of leptocephalus larvae with focus on the European and American eel and to further characterize the hydrography of the spawning area of these species. The study also aimed at better understanding the influence of abiotic factors on potential eel spawning sites and on the distribution of eel larvae. Together with the results of the 342nd, the 373rd and the 404th voyage of the Walther Herwig III in 2011, 2014 and 2017, respectively, as well as the 41st voyage of RV Maria S. Merian in 2015, this cruise aimed at delivering insights into possible changes of the leptocephalus abundance and assemblage in the Sargasso Sea and to shed light on the influence of varying hydrographic conditions on the distribution of eel larvae. For this purpose, all caught leptocephalus larvae were sorted from the catch, identified, measured and preserved. In addition, gut and tissue samples were taken for gut content and virus analyses and depth-stratified catches with an IKMT-MultiNet were conducted to record diurnal changes in the depth distribution of early developmental stages.

The analysis of environmental DNA (eDNA) was tested to detect the presence of eels in water samples of different depths. Additional aspects of this survey included an assessment of occurrence, abundance and distribution of spiny lobster and sunfish larvae as well as of the gelatinous zooplankton.

Originally, it was planned to assess the accompanying fish fauna at selected stations with a pelagic trawl and to compare the catch composition with the catches of a large mesh-size IKMT (4 mm). However, due to technical problems with one of the winches, the operation of the pelagic trawl was not possible and the assessment of the mesopelagic fauna could only be made with the 4 mm-IKMT. This net was also deployed at two stations along the return transit from Bermuda to Bremerhaven. Hydroacoustic data targeting the mesopelagic fauna were recorded during the entire survey.

A special aspect of this survey was the participation of a documentary filmmaker (Hans Dortmans, Doxy-Films, Netherlands), who was shooting footage for a documentary on eels.

Distribution List:

BMEL Ref. 613/614 BLE, Ref. 523 Schiffsführung FFS Walther Herwig III TI, FI TI, SF TI, OF TI – Präsidialbüro (Michael Welling) TI-Reiseplanung Forschungsschiffe (Dr. Norbert Rohlf) Personalrat MRI, Institutsteil Fisch Deutscher Fischerei-Verband e.V. Bundesamt für Seeschifffahrt und Hydrographie Helmholtz-Zentrum für Ozeanforschung, GEOMAR Fahrtteilnehmer

The cruise

The Walther Herwig III left Bremerhaven for St. George's, Bermuda, on the evening of March 3 2023 with one day delay due to technical problems. Unfavorable weather conditions further delayed the journey resulting in a late arrival in Bermuda on the evening of March 18 with a delay of 4 days.

All 12 members of the scientific crew boarded the vessel in the morning of March 19. Bad weather in the research area and technical problems of the harbor's pilot boat further delayed departure by two days and the voyage to the study area started from St George's in the morning of March 23 with a total delay of seven days.

The scientific work started after reaching the first station (at 67°W 31°N) on March 23 and continued south along a latitudinal transect in steps of 1° or 0.5°, depending on catch efficiency (Fig. 1). After ending the first transect at 67°W 22°N, the second transect started at 64°W 19°N with one intermediate station between the two transects. The distance between the transects was 3° longitude. Last station was at 64°W 31°N. Originally, it was planned to sample three transects, but the third transect had to be cancelled due to the above-mentioned delay. Nonetheless, it was still possible to extend the two transects to stations far south to investigate the potential distribution of eel larvae in waters around the Puerto Rico trench within the EEZ of the British Virgin Islands.

At all stations, plankton sampling was conducted with a 500 μ m mesh size IKMT in double oblique tows from the surface to 300 m depth. Additionally, at all stations a hydrographic profile was generated through CTD casts, recording salinity, temperature, oxygen and Chlorophyll-a concentrations down to 500 m depth. At two stations, the IKMT was used in combination with a 500 μ m mesh-size MultiNet-Midi, enabling depth-stratified plankton sampling. Water samples from the CTD rosette for eDNA analyses were taken and filtered at three stations. At two stations a new eDNA sampling approach was tested by attaching small amounts of filter material to the IKMT. The material was stored for subsequent eDNA analysis at the Thünen Institute of Fisheries Ecology in Bremerhaven and partner institutions.

Leptocephalus larvae of all Elopomorph species were sorted out of the catches immediately after each haul. Freshwater eel larvae of the genus *Anguilla* were separated and measured for total body length. For each specimen, a tissue sample was taken for genetic species identification and guts were removed and stored in RNAlater for subsequent metagenomic gut content analyses. The remaining parts were preserved in ethanol. Leptocephali from other Elopomorph families were identified to the lowest possible taxonomic level, measured and stored frozen at -20°C. Potential *Anguilla* eggs were preserved in Chelex resin and genetically analyzed on board using a qPCR assay. In addition, fish larvae of all other taxonomic groups and palinurid phyllosoma larvae were sorted out of the catches and preserved in ethanol for subsequent taxonomic analyses. Selected gelatinous plankton organisms were sampled, measured and preserved and all mesopelagic fishes were sorted from catches and further analyzed on board or stored. The remaining plankton samples were fixed in ethanol and stored.



Figure 1. Map of the sampling stations where the IKMT was deployed to collect Anguilla leptocephali.

The scientific work was concluded on April 06 2023. On April 7, the Walther Herwig entered the port of St. George's and on the evening of April 8, a reception for invited guests from different Bermudian institutions and the German consulate were held. On April 9, two scientists from the Charles University in Prague boarded the ship for sampling along the transit back to Bremerhaven, while the initial scientific crew left the ship. The same day, the vessel departed for Bremerhaven where it arrived in the early morning hours of April 23.

During the 465th cruise of the Walther Herwig III the following station work was carried out:

Isaac Kidd Midwater Trawl (500 μm mesh) 47 tows				
Isaac Kidd Midwater Trawl (4 mm mesh)	3 tows			
IKMT-MultiNet (500 μm mesh)	3 tows			
CTD Probe	48 casts			
eDNA-samples	5 stations (3 water samples + 2 towed filter samples)			

Descriptions of research aspects and first results

Leptocephalus larvae and eggs (preliminary results)

R. Hanel, L. Marohn, M. Freese

A total of 1590 Elopomorph leptocephalus larvae out of 26 species and 15 families were collected during WH465. The most abundant species were *Nemichthys scolopaceus* (Slender snipe eel, N=235) and *Ariosoma balearicum* (Bandtooth conger, N=213), while the most species-rich families were Chlopsidae (false morays, 5 species) and Congridae (congers, 4 species). Regarding Freshwater eel or anguillid leptocephali, 245 specimens where caught, including 87 European eel larvae (*Anguilla anguilla*, size rage 8-43 mm) and 158 American eel larvae (*Anguilla rostrata*, size range 7-25 mm). Based on the frequency of *A. anguilla* and *A. rostrata* larvae in the catches, their abundance and distribution will be compared to earlier studies in order to assess potential changes over time, also considering the influence of hydrographic conditions during the surveys.



Figure 2. Anguilla anguilla leptocephalus larvae collected during the WH465 survey.



Figure 3. Marine eel leptocephalus larvae collected during the WH465 survey. A) Ilyophinae (a subfamily of the Synaphobranchidae or Cutthroat eels) with a pronounced rostral cartilage, B) *Gymnothorax miliaris* (Goldentail moray), C) *Xenomystax concroides* (Bristletooth conger) and D) lateral pigments of *Chlopsis bicolor* (Chlopsidae)

Fish eggs showing characteristics of anguillid eggs (i.e. large perivitelline space, oil droplet, diameter of about 1 mm) found in the plankton catches were photographed and genetically tested for a potential match or mismatch with either *Anguilla anguilla* or *Anguilla rostrata* based on a qPCR assay specifically developed for the detection of low concentrations of anguillid eel DNA. In total, about 130 fish eggs were selected and tested. None of the tested eggs were found to be of the genus *Anguilla*. The extracted DNA was stored for a subsequent DNA barcoding for species identification.



Figure 4. Fish eggs caught during WH465.

Epipelagic gelatinous zooplankton in the southern Sargasso Sea

F. Lüskow

On-board approach

After joint sorting of plankton samples (and separation of all leptocephali, eggs, phyllosomas, etc.), gelatinous zooplankton larger than 4–5 mm, *e.g.*, pyrosomes, medusae, heteropods, doliolids, and salps, were identified to species (or genus) level and measured in size (diameter or body length). If occurring in sufficient numbers (or being of adequate size), specimens were frozen in groups (or individually) at -20°C for stoichiometric analysis. Some specimens (salps, pyrosomes, and cubozoans) were fixed in 90% ethanol for subsequent genetic identification. All other specimens were returned to the bulk zooplankton sample and fixed in ethanol.

Preliminary results and outlook

In total, about 4500 gelatinous zooplankton specimens belonging to 26 taxonomic units were identified and measured. Most of them (N=4165) originated from standard 300 m double-oblique IKMT tows (Table 1). About 900 specimens were frozen for stoichiometry and *circa* 30 specimens were individually fixed in ethanol for genetic analysis. Among the gelatinous organisms, doliolids accounted for the vast majority (N=3284). Doliolids were encountered at all standard IKMT stations. The next most abundant taxon was the salp *Salpa fusiformis* that was collected on 36 out of 43 IKMT stations. Third in frequency of occurrence was *Salpa aspera* (N=180), while the hydromedusa *Bougainvillia* spp. (*B. niobe* and *B. platygster*) were encountered at more stations (31 out of 43), but usually in low numbers. Other taxonomic groups (two species of heteropods, nude ctenophores, and scyphozoans) were irregularly caught (Fig. 5). Neustonic species like *Physalia physalis* were noticed drifting at the ocean surface in the study area, but only the small hydrozoan *Porpita porpita* was caught. Noteworthy are three specimens of the cubozoan *Alatina alata*, which have not previously been collected in this open-ocean region. The diversity of taxa (especially that of salps) was higher at the southern stations compared to stations near Bermuda, despite lower overall abundance. Whether this trend holds for other species groups and is statistically significant, will need to be analysed at a later stage. Compared to the species richness of salps, relatively few hydromedusa species were collected, which may be indicative of the destruction of fragile species. Sturdy calycophoran siphonophores that were encountered in high abundances, were not enumerated or identified to species level (see results of the 2017 Walther Herwig III expedition). Samples frozen for stoichiometry will be prepared for analysis later in 2023 in Bremerhaven, while salp and cubozoan specimens fixed in ethanol will be sent to collaborators in Norway and Germany.



Figure 5. Some characteristic species of gelatinous zooplankton collected in the southern Sargasso Sea in spring 2023, beginning in the upper left corner, ending in the bottom right corner: heteropod *Cardiapoda* sp., salp *Traustedtia multitentaculata*, hydrozoan *Bougainvillia* sp., cubozoan *Alatina alata*.

Phylum	Class	Genus/Species	Specimens	Stations	Presence (% stations)	Size range (mm)	Specimens (Class)
Chordata	Thaliacea	Cyclosalpa polae	4	3	7.0	15-32	3977
		Doliolidae spp.	3284	43	100.0	3-25	
		<i>Helicosalpa</i> sp.	15	6	14.0	10-65	
		Pegea confoederata	4	4	9.3	6-7	
		Pyrosoma atlanticum	14	11	25.6	4-71	
		Salpa aspera	180	7	16.3	5-54	
		Salpa fusiformis	335	36	83.7	5-87	
		Soestia zonaria	18	4	9.3	9-32	
		Thalia cicar	48	5	11.6	5-15	
		Thalia orientalis	60	13	30.2	3-12	
		Traustedtia multitentaculata	15	4	9.3	8-48	
Cnidaria	Anthozoa	Anthozoan larva	3	3	7.0	4–5	3
	Cubozoa	Alatina alata	3	3	7.0	15-52	3
	Hydrozoa	Bougainvillia spp.	104	31	72.1	2-12	112
		Geryonia proboscidalis	2	2	4.7	22-23	
		Hydromedusa sp. 1	1	1	2.3	25	
		Hydromedusa sp. 2	1	1	2.3	11	
		Hydromedusa sp. 3	2	2	4.7	9-12	
		Liriope tetraphylla	1	1	2.3	10	
		Porpita porpita	1	1	2.3	5	
	Scyphozoa	Nausithoe sp.	3	3	7.0	12-17	11
		Pelagia cyanella	7	4	9.3	6-48	
		Periphylla periphylla	1	1	2.3	35	
Ctenophora	Nuda	Beroe sp.	2	2	4.7		2
Mollusca	Gastropoda	Cardiapoda spp.	55	22	51.2	9–93	57
		Clione limacina	2	2	4.7	5-9	

 Table 1. Diversity and number of collected gelatinous zooplankton species in standard 300 m double-oblique IKMT tows.

Evolutionary adaptations of selected mesopelagic fish

Z. Konvičková, V. Kaufman

The deep sea is an extreme environment that challenges its inhabitants in many respects. For illustration, deep sea organisms have to cope with the hydrostatic pressure, light scarcity or limited access to food. They therefore evolved numerous adaptations to thrive in ocean depths. While studying deep sea fishes, we are still revealing new adaptations for life in depth, often unparalleled among vertebrates. Many of these adaptations include the sensory systems such as vision and olfaction. In this project, we mainly focused on evolution of vision in mesopelagic fishes and its coevolution with bioluminescence.

Mesopelagic fishes possess visual adaptations on many levels – eye shape and morphology (such as tubular eyes), yellow filters in the lens or retina, or modified photoreceptors in the retina with different types of visual pigments within them. For example, a unique novel visual sensory system has been recently discovered in three deep sea lineages – this system is based solely on multiple rod cells resulting from numerous rhodopsin gene duplications. Additionally, in some other fish lineages the identity of the cone and rod cells (clearly distinguished among all vertebrates) has been challenged as some unknown, intermediate cell types have been proposed. One of the main objectives of the project was, therefore, focused on adaptations related to vision to understand how the visual system of mesopelagic fish works on the molecular and single-cell level.

The mesopelagic zone lies between 200 and 1000 metres of depth with 1000 metres being the maximum depth where the light from the surface can penetrate in sufficient intensity to support vision. However, surface light will often only penetrate much shallower depending on water clarity and angle of the sun rays entering the water. Below the limit depth for the surface light, bioluminescence remains the only source of light enabling vision. The ability to emit light is widespread among marine organisms. Most of the deep-sea bioluminescence occurs in wavelengths between 440 and 520 nm with its peak around 475 nm – these wavelengths correspond to the blue part of the visible spectrum and spread best in water. Visual system of deep-sea organisms, including fishes, is thus usually tuned to detect these wavelengths. However, dragon fishes (order Stomiiformes, family Stomiidae) are known to produce light of longer wavelengths, emitting even in the red part of visible spectrum. Furthermore, it was demonstrated that some species also possess visual pigments specifically tuned to detect their long-wavelength bioluminescence which provide these species with their private communication channel. Bioluminescent organs of dragon fishes are very diverse (positioned on the cheek, on the chin barbel, or along the body), and are known to differ between species. Dragon fishes also produce their own luciferase for bioluminescent reaction. The aim of our project is to explore the genetic basis of the luciferase enzyme to reveal if the morphological diversity of bioluminescent organs has its parallel at the molecular level. We will also study chemical properties of pigments present in bioluminescent organs that influence the colour of emitted light. Additionally, we will search for correlation between the colour of emitted light and the visual abilities of dragon fishes.

During WH 465 survey, over 28 individuals belonging to eight fish orders were sampled for the study of visual adaptations and bioluminescent organs. All individuals were processed in a standardized way – standard and total length was measured, species was determined morphologically, and fin clip was taken for subsequent molecular determination.

Eyes of all species were sampled for multiple purposes. Most eyes will be used for the retinal transcriptome analysis focusing on visual pigments (opsins) and genes of phototransduction cascade in the photoreceptors. We aim to reconstruct expression profiles of these genes, of which visual abilities of the species will be inferred. Comparative sampling will serve to compare fishes from various

phylogenetic groups (Fig. 6) and to understand evolution of these genes in the extreme habitat of the deep sea.



Figure 6. Phylogenetic overview of sampled orders and the design of the comparative approach for the molecular study. The main goal is to compare orders possessing different adaptations, such as multiple rod opsin genes, unclear rod vs. cone identity, or tubular eyes

Furthermore, several target species/families were selected for more detailed study of their visual abilities, namely focusing on the single-cell transcriptomics. This method will reveal the composition of the photoreceptor genes in the single rod (or cone) cells, and hence will aid understanding how vision works. This is particularly important for the lineages with the novel multiple rod-based visual system (families Diretmidae, Myctophidae, Stylephoridae), and those with challenged rod vs. cone identity (family Scopelarchidae), but also for those with extreme morphological adaptations such as tubular eye which have not been thoroughly studied yet on the molecular level (family Opisthoproctidae and the genus *Argyropelecus*).

Bioluminescent organs of dragon fishes (Fig. 7) were sampled for the analysis of transcriptome and for the liquid chromatography. We sampled bioluminescent barbels and cheek photophores of 11 individuals belonging to 5 species. Abovementioned sampling of eyes of these will enable us to search for correlation between the colour of emitted light and the visual abilities of these fish by comparing the eye transcriptome (and rhodopsin sensitivity) and bioluminescent spectra.

We managed to catch one individual of the silver spinyfin (*Diretmus argenteus* (Fig. 7)), species with extraordinarily rich repertoire of rod opsin genes. Both eyes of this specimen will be analysed by singlecell sequencing. We have also successfully covered some of the diversity of other target species: namely myctophids, genus *Argyropelecus*, scopelarchid species *Benthalbella infans* (closely related to the genus *Scopelarchus* in which cone-to-rod developmental progression has been recently discovered, and five species of dragon fishes (Stomiidae) were obtained. Additionally, muscle and liver tissue of selected species was sampled to obtain high quality genome. High quality genome will be used for detection of photoreceptor genes with very low expression level as well as genes that are not expressed at all. It will also enable us to reconstruct genome architecture surrounding the genes and their regulatory regions. Furthermore, we sampled black skin of several species for histological and transcriptomic study.

All obtained samples (preserved in various fixative solutions) will be processed and held at the Department of Zoology, Faculty of Science, Charles University, Prague. Most of the analyses will be performed at the same place, some samples might be sent to collaborators for specific analyses after further arrangements.



Figure 7. Fish species sampled on WH465; From left Diretmus argenteus, Chauliodus danae, Diaphus rafinesque

Participants

01 Prof. Dr. Reinhold Hanel	19.03. – 09.04. TI-FI (cruise leader)
02 Dr. Lasse Marohn	19.03. – 09.04. TI-FI
03 Dr. Marko Freese	19.03. – 09.04. TI-FI
04 Tina Blancke	19.03. – 09.04. TI-FI
05 Sarah-Jane Reyelt	19.03. – 09.04. TI-FI
06 Sree Lakshmi Santosh	19.03. – 09.04. TI-FI
07 Jennifer Bogun	19.03. – 09.04. TI-FI
08 Ina Becker	19.03. – 09.04. TI-FI
09 Lisanne Hoch	19.03. – 09.04. TI-FI
10 Dr. Florian Lüskow	19.03. – 09.04. University of British Columbia, Vancouver, Canada
11 Dr. Josefin Sundin	19.03. – 09.04. Swedish University of Agricultural Sciences, Drottningholm, Sweden
12 Hans Dortmans	19.03. – 09.04. Documentary filmmaker, Doxy-Films, Amsterdam, Netherlands
13 Zuzana Konvičková	09.04. – 24.04. Charles University Prague, Czech Republic
14 Vit Kaufman	09.04. – 24.04. Charles University Prague, Czech Republic

In the name of the scientific participants, I would like to thank Captain Arne Schwegmann and his crew for their support and cooperation throughout the trip.

Prof. Dr. Reinhold Hanel

Appendix

List of stations

Station	Date	Time (UTC)	Lat (°N)	Long (°W)	Gears
001	24.03.2023	00:40	31°00.05	66°59.77	CTD, IKMT-S
002	24.03.2023	09:15	30°00.21	66°59.91	CTD, IKMT-S
003	24.03.2023	18:00	28°59.91	66°59.92	CTD, IKMT-S
004	24.03.2023	23:19	28°30.23	67°00.38	CTD, IKMT-S
005	25.03.2023	05:47	28°00.05	66°59.45	CTD, IKMT-S
006	25.03.2023	14:28	27°30.28	67°00.14	CTD, IKMT-S
007	25.03.2023	20:16	27°00.13	66°59.44	CTD, IKMT-S
008	26.03.2023	02:15	26°30.11	67°00.64	CTD, IKMT-S
009	26.03.2023	08:42	26°00.17	66°59.82	CTD, IKMT-S
010	26.03.2023	14:33	25°30.12	66°59.76	CTD, IKMT-S
011	26.03.2023	20:32	25°00.26	67°00.11	CTD, IKMT-S
012	27.03.2023	02:45	24°30.12	66°59.50	3 x CTD, 2 x IKMT-S, 2 x IKMT-MN
013	28.03.2023	09:31	24°00.03	67°00.08	CTD, IKMT-S
014	28.03.2023	17:49	23°29.94	67°00.00	CTD, IKMT-S
015	29.03.2023	00:29	23°00.31	67°00.34	CTD, IKMT-S
016	29.03.2023	07:17	22°30.60	66°59.72	CTD, IKMT-S
017	29.03.2023	14:00	22°03.16	67°00.14	CTD, IKMT-S
018	30.03.2023	04:44	20°29.97	65°26.88	CTD, IKMT-S
019	30.03.2023	19:26	19°00.26	63°59.90	CTD, IKMT-S
020	31.03.2023	01:11	19°29.95	63°59.88	CTD, IKMT-S
021	31.03.2023	06:37	20°00.20	64°00.06	CTD, IKMT-S
022	31.03.2023	12:00	20°30.01	63°59.76	CTD, IKMT-S
023	31.03.2023	17:34	21°00.21	64°00.06	CTD, IKMT-S
024	31.03.2023	23:12	21°30.39	63°59.69	CTD, IKMT-S
025	01.04.2023	04:31	22°00.10	64°00.25	CTD, IKMT-S
026	01.04.2023	10:16	22°31.19	63°59.43	CTD, IKMT-S
027	01.04.2023	17:10	23°00.41	64°00.07	CTD, IKMT-S
028	01.04.2023	23:01	23°29.75	63°59.53	CTD, IKMT-S
029	02.04.2023	04:27	24°00.08	63°59.80	CTD, IKMT-S
030	02.04.2023	09:47	24°30.22	64°00.50	CTD, IKMT-S
031	02.04.2023	15:11	25°00.13	64°00.05	CTD, IKMT-S
032	02.04.2023	20:39	25°30.32	63°59.81	CTD, IKMT-S
033	03.04.2023	02:35	25°59.89	63°59.61	CTD, IKMT-S
034	03.04.2023	08:27	26°30.13	63°59.79	CTD, IKMT-S, IKMT-MN
035	04.04.2023	00:14	26°59.84	64°00.08	CTD, IKMT-S
036	04.04.2023	06:12	27°30.39	64°00.09	CTD, IKMT-S
037	04.04.2023	11:39	28°00.17	64°00.05	CTD, IKMT-S
038	04.04.2023	17:29	28°29.91	63°59.75	CTD, IKMT-S
039	05.04.2023	00:01	29°00.38	63°59.93	CTD, IKMT-S, IKMT-L
040	05.04.2023	11:23	29°30.66	64°00.18	CTD, IKMT-S
041	06.04.2023	00:10	29°00.57	64°00.22	CTD, IKMT-S

042	06.04.2023	05:28	29°30.00	64°00.20	CTD, IKMT-S
043	06.04.2023	10:49	30°00.57	64°00.00	CTD, IKMT-S
044	06.04.2023	16:09	30°30.33	64°00.00	CTD, IKMT-S
045	06.04.2023	21:12	31°00.03	63°59.82	CTD, IKMT-S
046	14.04.2023	12:12	38°08.00	38°39.16	IKMT-L
047	17.04.2023	03:57	43°01.90	24°49.50	IKMT-L