TWInning Laboratory for an Innovative Global Hub To Explore the Deep

Deliverable 3.3
TWILIGHTED Expedition Report I

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1. Overview

This TWILIGHTED expedition report summarizes the outcome of the hands-on cruise-based training via the participation of ARDITI team members in GEOMAR-led research expeditions in the Baltic Sea and the Atlantic Ocean, with a focus on the time period until the end of 2025. The expeditions took place in the context of ongoing GEOMAR projects funded from other sources, and as such offered immersion in "real-world" seagoing research, including unique opportunities for hands-on training, but also for the initiation of new collaborative research lines.

1.1 Project introduction

TWILIGHTED is a Horizon Europe Twinning project that seeks to revolutionize deep-sea research, development and innovation (RD&I) in Portugal, ultimately benefiting all of Europe. Collaborating closely with GEOMAR (Germany) and NTNU (Norway), TWILIGHTED aims to transform ARDITI (Madeira, Portugal) into a global hub for deep-sea RD&I. Focusing on the mesophotic zone (40-200m) and the twilight zone (200-1,000m), and capitalizing on Madeira's unique proximity to deep waters, TWILIGHTED will help accelerate our global understanding of the ocean and its essential role in sustaining life on earth.

TWILIGHTED's key objectives are to: (1) Collaborate across research institutes in Europe, (2) Elevate the research profile of Portugal and especially the European Outermost Region of Madeira, (3) Innovate low-cost alternatives to state-of-the-art deep-sea research technologies, (4) Democratize deep-sea research, (5) Globalize deep-sea RD&I and (6) Share ocean science across stakeholders.

To achieve its objectives, TWILIGHTED will adopt state-of-the-art approaches to training and networking. Development activities include staff exchanges, expert visits, training schools, joint research missions, the International Twilighted Conference and novel cross-sector workshops stimulating creativity in solving the ocean's greatest challenges (the Impossible Things Workshops). Such capacity-building will not only catalyze deep-sea RD&I in Madeira but also ensure a lasting impact on the deep-sea scientific landscape, sustainable ocean policy, and Madeira's socioeconomic development.

2. Expeditions training report

2.1 TWILIGHTED expeditions training plan

This section gives an overview of the specific scientific training via expeditions planned in TWILIGHTED, as well as the timeline for their realization in the project (Table 1).

Baltic Sea research cruises in 2025 and 2026

GEOMAR has been carrying out integrative multi-week research cruises (hydrography, plankton surveys, scientific trawl fisheries) in the Baltic Sea region since the 1980s, to maintain and

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expand one of the best available long-term data series of the rapidly changing Baltic Sea ecosystems under climate change and anthropogenic pressures ("BILTS" - Baltic Sea Integrative Long-term Data Series). While the Baltic Sea is comparatively shallow and does not harbor deep-sea ecosystems, the cruises contributing to this time-series data are characterized by a wide range of deployed gear and sample types taken in a short time period. This includes zooplankton sampling with various nets, water sampling with a Rosette water sampler (with subsequent phytoplankton, microbe and eDNA sampling), fish sampling (with subsequent single fish measurements and sub-sampling for genetic and dietary tracer analyses), and physical measurement with CTD-probes. Work covers a large standardized station grid and is conducted in a 24-hour shift system. Participation in the cruises therefore also provides opportunities to obtain new methodological as well as (cruise) organizational skills.

ARDITI team member Rodrigo Silva participated in the GEOMAR expedition AL630 from April 5 – 16, 2025 **(this report)**, and one berth on board has been reserved for an ARDITI team member for a 2026 expedition with the midsize federal German research vessel RV ALKOR **(to be reported in D4.4)**.

Cabo Verde deep-sea expedition M209 (March-April 2025)

ARDITI scientist Dr. Sonia KM Gueroun joined the expedition M209 in March-April 2025 **(this report)** with the large German research vessel RV METEOR. The expedition started in Mindelo, Cabo Verde, and ended in Ponta Delgada, Azores. This cruise investigated the coastal deep seas of Cabo Verde (part of Macaronesia, with Madeira, the Azores, and the Canary Islands) with a multitude of oceanographic equipment, including the remotely operated vehicle ROV KIEL6000, GIRONA500 AUV, towed cameras XOFOS and PELAGIOS and various nets. Dr. Gueroun was involved in the midwater food web sampling with the ROV and nets, and additionally trained in imaging (e.g., deployment of the various camera systems, observation of midwater and benthic fauna) and laboratory techniques on board. The participation will result in subsequent joint publications following the cruise, as well as additional professional skills development during a guest scientist stay at GEOMAR by Sonia Gueroun in February 2026 to jointly work on cruise zooplankton samples and analyses.

Cabo Verde workshop (March 2026)

This GEOMAR-led workshop in Cabo Verde will leverage ongoing GEOMAR fieldwork in the COAST-project to train ARDITI scientists in field and laboratory methods. The workshop was shifted from November 2025 to March 2026 after tropical storm Erin caused large-scale destruction in Fall 2025 (link). The workshop will include eDNA collections, megafauna observations and soundtrap deployments to record passively acoustic whale sounds. Course lectures and data processing will take place at the Ocean Science Centre Mindelo (OSCM) (https://www.oscm.cv). We expect involvement of GEOMAR, NIOZ, NTNU and ARDITI, with an envisioned number of up to five ARDITI team members. The workshop will be coupled with a symposium at OSCM to foster Macaronesian Science exchange **(to be reported in D4.4)**.

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Table 1. Scientific training during GEOMAR expeditions - timeline and milestones

Milestone/ deliverable	Deadline	Time of event	Description
M3.2, D3.3	May 2025	April 2025	Baltic cruise I: expedition AL630
(D3.3)	Opportunity arose short-notice, not promised originally (☐ no deadline)	March-April 2025	Cabo Verde expedition: M209
D3.3	Dec 2025		Expedition report I
M3.3	Shifted to 2026	March 2026	Cabo Verde workshop
M4.4	May 2026	April 2026 (tentative)	Baltic cruise II
D4.4	July 2027		Expedition report II

2.2 Report on training during Baltic expedition AL630

AL630 scientific program

The multidisciplinary cruise AL630 extended the "Baltic Sea Integrative Long-term Data Series" (BILTS), constituting one of the best available time series (1987 to the present) on ecosystem composition and functioning of the deeper basins of the Baltic Sea. This time series is characterized by the integration of biological and oceanographic data, and thus provides the foundation for assessments of recent rapid changes in planktonic communities, fish populations and food webs in response to anthropogenic pressures as well as climate change. The specific work during AL630 included depth-resolved hydrographic measurements (temperature, salinity, oxygen concentrations), water and plankton sampling carried out on standardized station grids in the central (Bornholm Basin, ICES subdivision 25) and western (Kiel and Mecklenburg Bight, ICES subdivision 22) Baltic Sea, as well as scientific fishery trawls. All planned stations and main cruise objectives were successfully covered. The resulting data- and sample sets support ongoing projects in the Research Unit Marine Evolutionary Ecology at GEOMAR and multiple national and international collaborations, including the German Alliance Marine Science funded project SpaCeParti, and the EU project TWILIGHTED, with the participation of ARDITI team member Rodrigo Silva for training purposes (Figure 1).

ARDITI participant training summary

ARDITI team member Rodrigo Silva was part of the regular teams carrying out the AL630 work (fish sampling: all 12 scientists in one team; plankton and CTD station grids: teams of four scientists and technicians conducting 24-hour operations in shifts), and therefore received hand-on training in all aspects of the cruise and work with a wide range of gears and sample types. This included zooplankton sampling with various nets (multinet for depth-resolved

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sampling; Bongo-, WP3, WP2, Apstein nets for sampling of a range of plankton size fractions; water sampling with a Rosette water sampler with subsequent phytoplankton, microbe and eDNA sampling; fish sampling with subsequent single fish measurements and sub-sampling for genetic and dietary tracer analyses), and physical measurement with CTD-probes. Training also included background on cruise logistical planning, establishment of station and work plans, sample labeling schemes and shift-work organization.
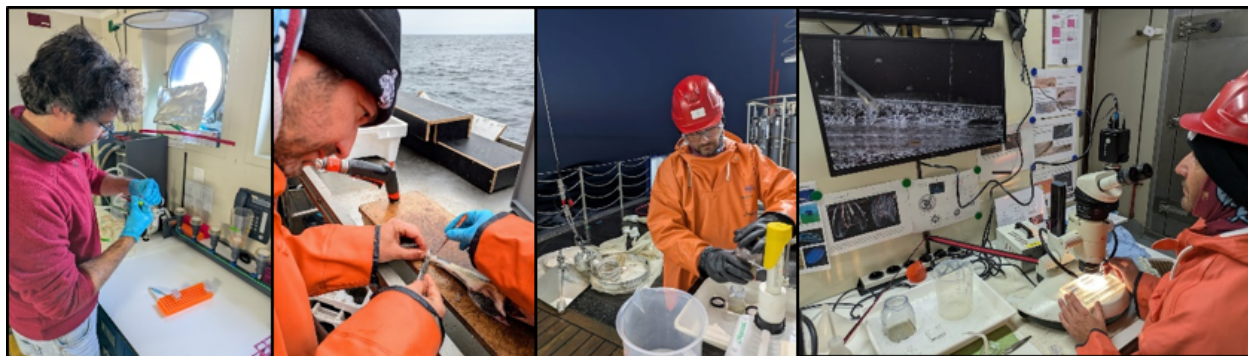
Figure 1. Participation of ARDITI team member Rodrigo Silva in cruise AL630, contributing to knowledge exchange and capacity building in the EU TWILIGHTED project (Photos: Dierking)

ARDITI participant Rodrigo Silva's first-hand training report

"I am a research technician and scientific diver at MARE-Madeira/ARDITI, Madeira Island, Portugal. I am here reporting on my training during expedition AL630 with RV ALKOR, in the context of the EU TWILIGHTED project - a freezing, sunny, and fascinating experience being aboard! My expedition participation in the context of the EU TWILIGHTED project provided the opportunity to gain experience, scientific knowledge, and understanding of sampling methodologies done by GEOMAR since 1987, with the perspective to adapt to deep-sea research in Madeira Island, a different oceanic environment when compared with the Baltic Sea.

Below is a short description of some distinct and interesting aspects between a volcanic Island in the middle of the Atlantic Ocean versus the temperate Baltic Sea, with only a narrow connection to the open ocean, and on the importance of monitoring programs to increase scientific knowledge and provide concrete time series data that supports the creation of good policies to preserve the marine environment.

Madeira is a volcanic archipelago on the North East Atlantic, comprising the Macaronesia ecoregion together with the Azores, Canary Islands, and Cabo Verde, 600 km from the African continent. As Dr. João Canning-Clode would say, Madeira Island is "a research vessel in the middle of the Atlantic." Its privileged location allows us to reach great depths a few nautical miles (nm) from the shore (in certain locations, the 2000 m depth is at less than 10 nm from shore). Their oligotrophic waters, with oceanic salinity (34 psu - 37 psu), with SST (sea surface temperature) of 18°C in the winter and 25°C in summer, are home to a wide diversity of pelagic fish (several Tuna species, Atlantic horse mackerel, Atlantic mackerel, among others), marine mammals (32 species recorded), and a rich and diverse plankton species that migrates vertically from abyssal regions, during the night, and from shallow depths during the day.

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Evaluating my experience aboard RV ALKOR, I found three interesting factors about the Baltic Sea that broadened my understanding of ecosystems: first, the maximum depth reached on all sampling stations during expedition AL630 was only around 90 m. Secondly, the salinity gradient in the Baltic Sea was very strong, with marine salinities in the north-west (due to the location exposure to the seawater entrance from the North Sea) to freshwater in the north-east (where streams and rivers are the main water input vectors). Finally, salinity, water temperature, and oxygen conditions changed strongly in deeper water layers in the deeper basins (i.e., Bornholm basin) due to water column stratification. Higher salinities are found in deeper water when compared to shallow water, and oxygen concentration starts to reach hypoxic (< 2 ml/l) to anoxic (0 ml/l) values at depths below 65-75 m. These characteristics really highlighted the difference between oceanic systems and regional seas.

The focus of the expedition also showed me the importance of implementing long-term monitoring programs to increase scientific knowledge to support sustainable fisheries management plans and conservation programs for marine biodiversity. In Madeira, it will be challenging to implement such systematic and detailed sampling, due to the challenging weather conditions, and difficulties in operating heavy-duty instruments at great depths. On the other hand, adapting the methodologies used at GEOMAR cruises will be one of the main goals retrieved from this experience. This may be possible by creating low-cost devices and sensors together with our IT team for sample and data collection, with a focus on instruments that can be handled from a rib speed boat during daily campaigns instead of a large research vessel. The use of the Multinet would be very interesting due to its capacity of sampling different depths for each deployment, but it is a complex and heavy apparatus, and it would not be an easy task to operate from a small rib boat.

Even coming from a subtropical mild climate to the Baltic spring weather, all the experience was worth it. All the learning on board, brainstorming, and healthy discussions with other researchers strengthened the partnership for future projects. In my view. The TWILIGHTED project goals, such as sharing scientific knowledge, leaving our comfort zones to open our horizons, and having the chance to collaborate with other institutions, have been fulfilled.

To end, I would like to express my sincere gratitude to Dr. João Canning-Clode for selecting me to participate in this cruise, to Dr. Jan Dierking for all the support, welcome, and good vibes during the entire process, particularly during the cruise, and to all my cruise colleagues. It was a pleasure to share these 10 days with you aboard! Hope to see you soon, who knows, in Madeira…"

Links to training related output

Social media:
- Blog with first-hand account by ARDITI participant Rodrigo Silva:
  https://twilighted.eu/it-was-baltic/

Blogs:
- GEOMAR RV ALKOR time series cruise blog:
  https://www.oceanblogs.org/baltic-rvalkor/2025/04/19/al630-successfully-completed-but/

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- TWILIGHTED blog:
  https://twilighted.eu/we-be-cruisin-in-cabo-verde/

Cruise report:
Dierking, Jan (2025) Long-term monitoring of biodiversity changes and their functional consequences in the pelagic ecosystems of the central Baltic Sea: Cruise No. AL630. Open Access. Alkor-Berichte, AL630. GEOMAR Helmholtz Centre for Ocean Research Kiel, Kiel, Germany, 22 pp. DOI 10.3289/CR_AL630.

Figure 2. Team of expedition AL630. Rodrigo Silva (ARDITI) 2nd from right. Photo: Neugebauer

2.3 Report on training during Cabo Verde expedition M209

M209 scientific program

Expedition M209 on RV METEOR took place from 21.3.2025-25.4.2026 in the Exclusive Economic Zone (EEZ) of Cabo Verde. The EEZ of Cabo Verde is a hotspot for biodiversity, with the presence of high numbers of endemic species and endangered megafauna such as whales and sharks. It consists of more than 90% of deep-sea habitats (> 200 m water depth) most of which remains unexplored and unmapped. Biodiversity in these deep waters also remains poorly known, although past cruises have repeatedly documented novel species and records. The high island topography provides sheltered conditions in the leeway of the islands, and the deep waters close to shore allow deep-sea research to take place under calm conditions. The

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cruise M209 aimed to better understand the deep-sea ecosystems in the vicinity of the Cape Verde islands by mapping the seafloor on island and seamount slopes from shallow to deep water depths and by observing, documenting and sampling seafloor habitats, their zonation and their communities. Additionally, we investigated organisms and communities in the middle of the water column (the mesopelagic zone) and above the seafloor (the benthopelagic zone).

The scientific objectives of the cruise included:

Objective 1: *Habitat mapping of the Cabo Verde Exclusive Economic Zone.*

Objective 2: *Characterize and sample benthic and benthopelagic macro and megafauna for biodiversity and food web analysis.*

Objective 3: *Characterize, observe, measure and sample pelagic fauna for biodiversity and food web analysis.*

Objective 4: *Document the interaction between migration behaviour, physical oceanography and topography (island/seamount slope).*

The cruise started in Mindelo, Cabo Verde, and focused on the islands Santa Antao, Fogo and Santiago, with a comparable cruise program performed at each island (Figure 3).
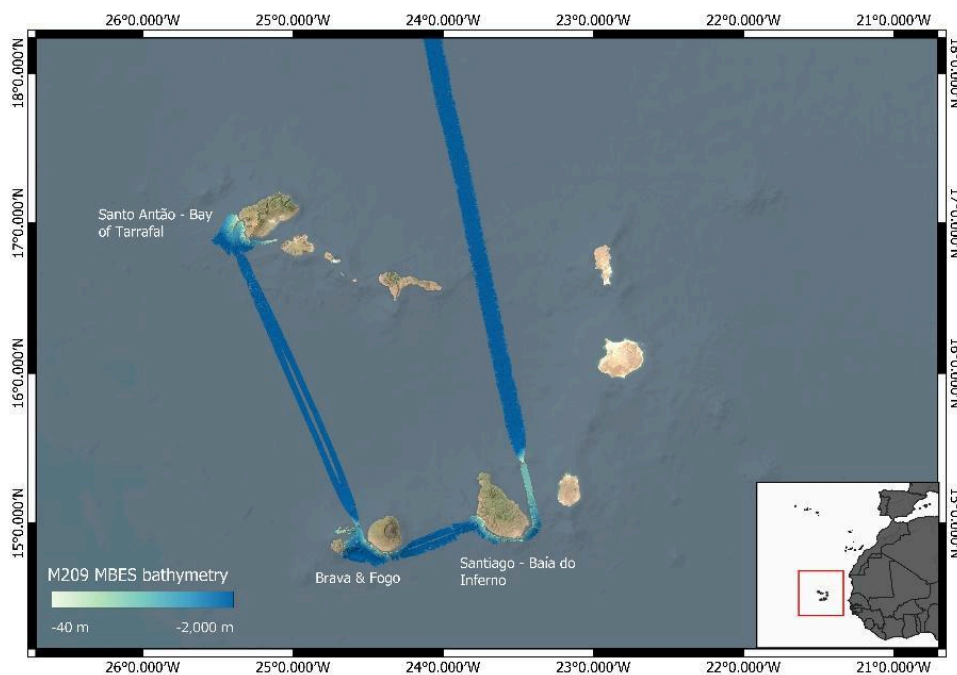

Figure 3. The cruise track of cruise M209.

ARDITI participant training summary

Dr. Sonia KM Gueroun was part of the M209 scientific teams investigating pelagic communities with nets and with the remotely operated vehicle (ROV) KIEL 6000, with particular focus on gelatinous zooplankton. These taxa were sampled with a multinet maxi, a WP3 net and a Tetranet. Additionally, large delicate gelatinous zooplankton was observed and then sampled with the ROV. The latter method is particularly suitable for the collection of siphonophores, which typically fall apart in nets. In contrast, the strength of net sampling is that higher numbers of smaller and sturdy gelatinous zooplankton can be captured. The multinet maxi is able to sample nine discrete depths during a single deployment and the tetranet has a wider mouth opening, allowing it to sample more volume per unit of time.

Dr. Gueroun contributed with her taxonomic expertise to the identification of sampled and observed gelatinous fauna. ROV surveys consisted of 12-hour dives which started at 8:00 AM and ended at 6:00 PM. One scientist joined the ROV in the ROV container and the other scientists communicated with the scientists via intercom in another room where the ROV footage could be watched via live stream. The day before each ROV dive, the dive had to be planned, documented and discussed between the ROV dive lead and the ROV team. After being part of the pelagic ROV team for several dives, Dr. Gueroun also led a ROV dive during the expedition. The experience of working with a ROV was a unique opportunity and the samples that were obtained are very rare. Dr. Gueroun also contributed to the pelagic ROV dive section of the official cruise report with text and figures (e.g. Figure 4).

M209 sampling efforts resulted in a large sample set that will be analyzed at GEOMAR in Kiel in 2026. Dr. Gueroun secured a travel grant to conduct sample analysis at GEOMAR jointly with the GEOMAR team in February 2026, providing the opportunity for scientific exchange and further training. She will be co-author on resulting publications that involve gelatinous zooplankton diversity, distribution and food web ecology and will be leading a paper on zooplankton day and night distribution from the multinet maxi samples, and leading one or multiple papers on the diversity of ROV sampled gelatinous zooplankton.

ARDITI participant Sonia Gueroun first-hand training report

"My participation in the M209 expedition was an immensely valuable experience that greatly enriched my professional development and expertise. As part of the ROV team, I had the unique opportunity to contribute directly to the planning (one dive) and execution of ROV dives. I learned the intricate process of conducting these dives, which involved not only technical preparation but also active collaboration with ROV pilots and fellow scientists. We made real-time decisions to optimize observations and sampling, which significantly enhanced my ability to think critically under pressure and work as part of a multidisciplinary team.

In addition to my role during the dives, I was responsible for handling the samples post-dive. This involved making decisions regarding the processing sequence, prioritizing tasks based on the needs of the various scientific groups on board—whether it was for 3D imaging, stable isotope analysis, diversity studies, or genetic research. This aspect of the role enhanced my organizational skills, my ability to coordinate with multiple teams, and my capacity to prioritize effectively in a fast-paced environment.

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From a scientific standpoint, working with the ROV samples provided my first extensive experience with the diversity of ctenophores—an organism group I had encountered only sparingly in previous work. The data we collected (ROV and net sampling) and analyzed will contribute to the growing body of knowledge on these underexplored organisms and their interaction with their environment.

Overall, the M209 expedition significantly bolstered my communication, leadership, and organizational skills while deepening my expertise in marine biodiversity. The experience also allowed me to gain new insights into deep-sea ecosystems, particularly in relation to gelatinous zooplankton and their role in marine food webs. I look forward to continuing to analyze the samples and contribute to the scientific output resulting from the expedition."
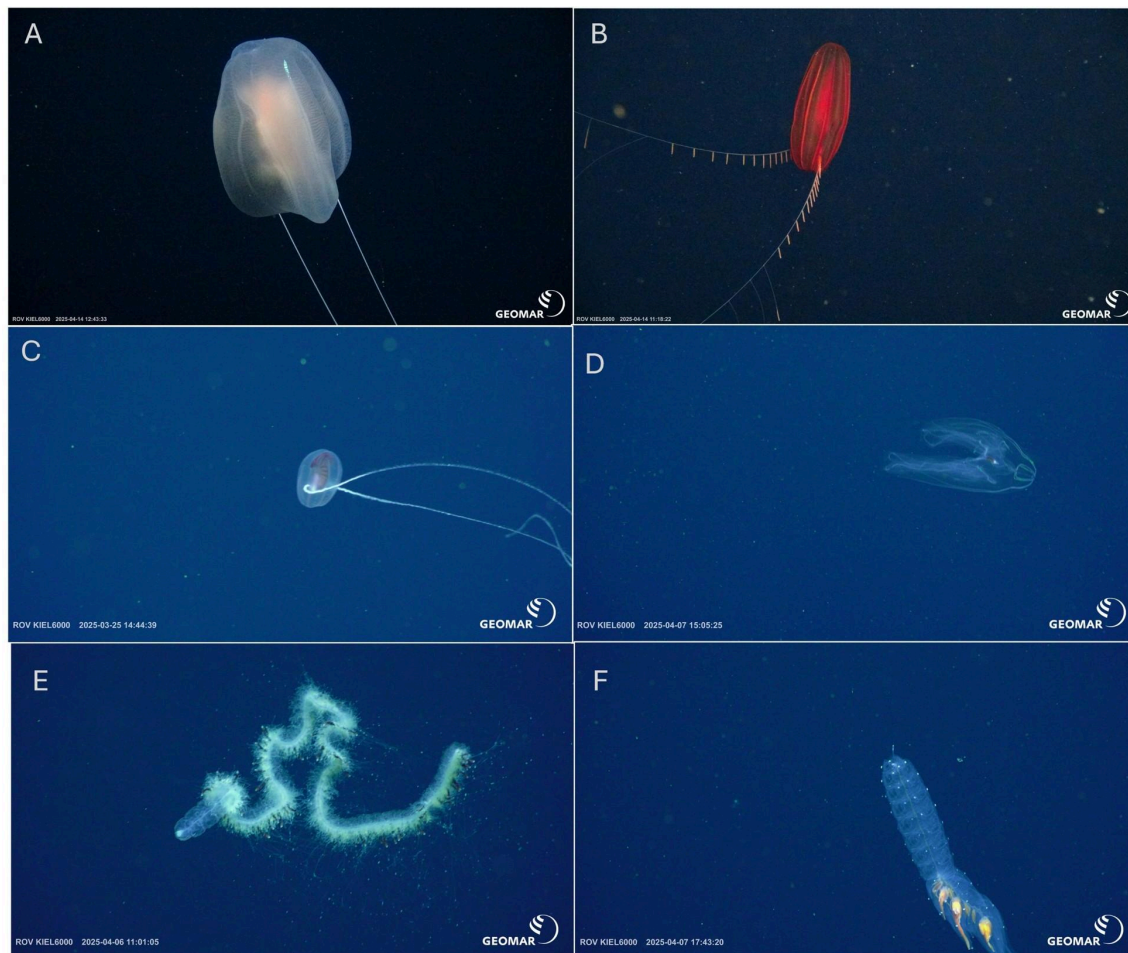

Figure 4 Example of gelatinous specimens encountered during the M209 pelagic ROV dives. A-D: Ctenophores: A. Undescribed Aulacoctenidae; B. Undescribed cydippid; C. Bathyctena *sp.; D.* Bathocyroe *sp.; E-H: Hydrozoa: E. Erenna richardi physonect; F. Physonect; G.* Aeginura *sp.; H.* Halicreas *sp. I-L: Scyphozoa: I.* Atolla wyvillei*; J.* Periphyllopsis braueri*; K.* Deepstaria enigmatica*; L.* Poralia rufescens

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Links to training related output

Social Media:
- Instagram:https://www.instagram.com/p/DJWZgPepWDN/?utm_source=ig_web_copy_link&igsh=MzRlODBiNWFIZA
- X: https://x.com/MARE_Madeira/status/1920071282231525383

Blog:
- Blog with first-hand account by ARDITI participant Sonia Gueroun:
  https://twilighted.eu/we-be-cruisin-in-cabo-verde/

Cruise report:
The cruise report for this expedition will be published on the PANGAE repository shortly. In the meantime, weekly reports and a short cruise summary report are available under the link: https://www.ldf.uni-hamburg.de/en/meteor/wochenberichte.html (keyword "M209").

Figure 5. Team of expedition M2090. Sonia Gueroun (ARDITI) 1st from left, front row.