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CASE REPORT

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Deep sea ecosystem exploration and 'health check': sampling strategy and methods applied during the iAtlantic_BR10_Petrobras cruise in the Santos Basin, Southwest Atlantic

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ABSTRACT

The iAtlantic Project has established an international collaborative strategy to improve mapping and characterization of deep and open ocean ecosystems in understudied regions of the Atlantic and evaluate their health. In December 2022, the first iAtlantic expedition in the South Atlantic set off to map and explore seafloor ecosystems in the Santos Basin slope (200-1,000 m depths) in collaboration with the Petrobras 'Santos Basin - Regional Characterization Project.' The 17-day 'iAtlantic_BR10-Petrobras' cruise was conducted on board the research vessel NPqHOc Vital de Oliveira (Brazilian Navy) and performed (a) water column structure characterization, (b) seafloor morphology mapping, (c) description of benthic habitats and communities by seafloor imagery and biological/ geological sampling, and (d) ex-situ experiments to assess the functioning of sedimentary ecosystems and their responses to climate-related environmental changes. This study describes the rationale behind the iAtlantic_BR10-Petrobras cruise science plan, reports its sampling strategy and methods, and summarizes its collected data and preliminary results.

Descriptors: iAtlantic Project, Deep-sea Ecosystems, Seafloor Mapping

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INTRODUCTION

Global warming and escalating marine economic activities have placed the Atlantic Ocean under intense pressure (Halpern et al., 2019). As a result, ocean conditions have changed across depth layers and geographic regions, potentially affecting marine ecosystems at several spatial scales (Sweetman et al., 2017). Changes in ecosystem structure and functions are expected consequences of such effects, which may threaten the provision of services to societies around the Atlantic. These are key elements for current and future ocean management and governance, which seem insufficiently and unevenly assessed, partly due to North-South disparities in research capacity (Perez et al., 2023; Roberts et al., 2023). The iAtlantic Project (Integrated Assessment of Atlantic Marine Ecosystems in Space and Time - European Union's Horizon 2020 - grant agreement no. 818123) has established a collaborative strategy to compensate for such disparities, improving mapping and characterization of deep and open ocean ecosystems in understudied regions of the Atlantic and conducting ocean "health checks" (Roberts et al., 2023).

The Santos Basin (SW Atlantic) is one of the target regions of the iAtlantic project (study region 10; https://www.iatlantic.eu/our-work/study-regions/). Located in the Brazilian Meridional Margin center (sensu Alberoni et al., 2019) and near the Brazilian demographic and economic core, the Santos Basin is one of the South Atlantic regions most impacted by humans. For over five decades, the region has concentrated most Brazilian fishing and oil and gas exploration activities (Perez et al., 2020) and it has been part of a major marine 'hotspot', i.e., a region in which sea temperatures have increased at rates above the average for the Atlantic Ocean due to the effects of global climate change (Hobday and Pecl, 2013; Franco et al., 2020).

In December 2022, the first iAtlantic expedition in the South Atlantic set out to map and explore seafloor ecosystems in the Santos Basin slope (200-1000 m depths). The explored area lies in the Santos deep-water hydrocarbon province, which spans over 350,000 km^2 up to 3,000 m depths (Mahiques et al., 2017). Seafloor is characterized by extensive sedimentary terraces and prominent geomorphological features, including abundant crater-like depressions, known as 'pockmarks,' carbonate mounds and ridges, and salt diapirs (Mahiques et al., 2017; Santos et al., 2022). These features contribute to the regional diversity of seascapes that may sustain different deep-sea benthic communities, including cold-water coral aggregations (Sumida et al., 2004; Kitahara et al., 2020). The interest in describing these communities has increased in the past 20 years as the region is associated with the extension of pre-salt oil fields (Cavalcanti et al., 2017) and was, from 2000 to 2008, subjected to intense bottom fishing (Perez et al., 2009). Significant advances in our understanding of the biological, geological, and geochemical aspects of these benthic ecosystems have derived from recent initiatives of the oil and gas industry, especially focused on exploring potential gas seepages in the region and its associated chemosynthetic communities (Bendia and Carrerette, 2022; Carrerette et al., 2022; Sumida et al., 2022, and others). Adding to these efforts and to the need to provide environmental baselines for the sustainable development of oil and gas exploration in the region, the 'iAtlantic_BR10-Petrobras' expedition was a joint iAtlantic – Petrobras enterprise conducted on board the research vessel 'NPqHOc Vital de Oliveira' of the Brazilian Navy, which focused on mapping the local seafloor and characterizing its deep ecosystem structure and functioning. This study describes the rationale behind the iAtlantic_BR10-Petrobras expedition science plan, reports its sampling strategy and methods, and summarizes its collected data and preliminary results.

SCIENCE PLAN AND SAMPLING STRATEGY

The 'iAtlantic_BR10-Petrobras' expedition stemmed from the overarching goals of the iAtlantic Project, which fundamentally involve improving scientific knowledge on factors that control the distribution, stability, and vulnerability of the deep and open ocean ecosystems of the Atlantic (Roberts et al., 2023). Its cruise science plan included activities focused on two of the ‘working packages’ (WPs) of the project: Mapping Atlantic Ecosystems (WP2) and Impact of Multiple Stressors (WP4). The Santos Basin comprises the iAtlantic study region 10 (‘Deep-sea continental slope, banks, and cold seep ecosystems off Brazil’).

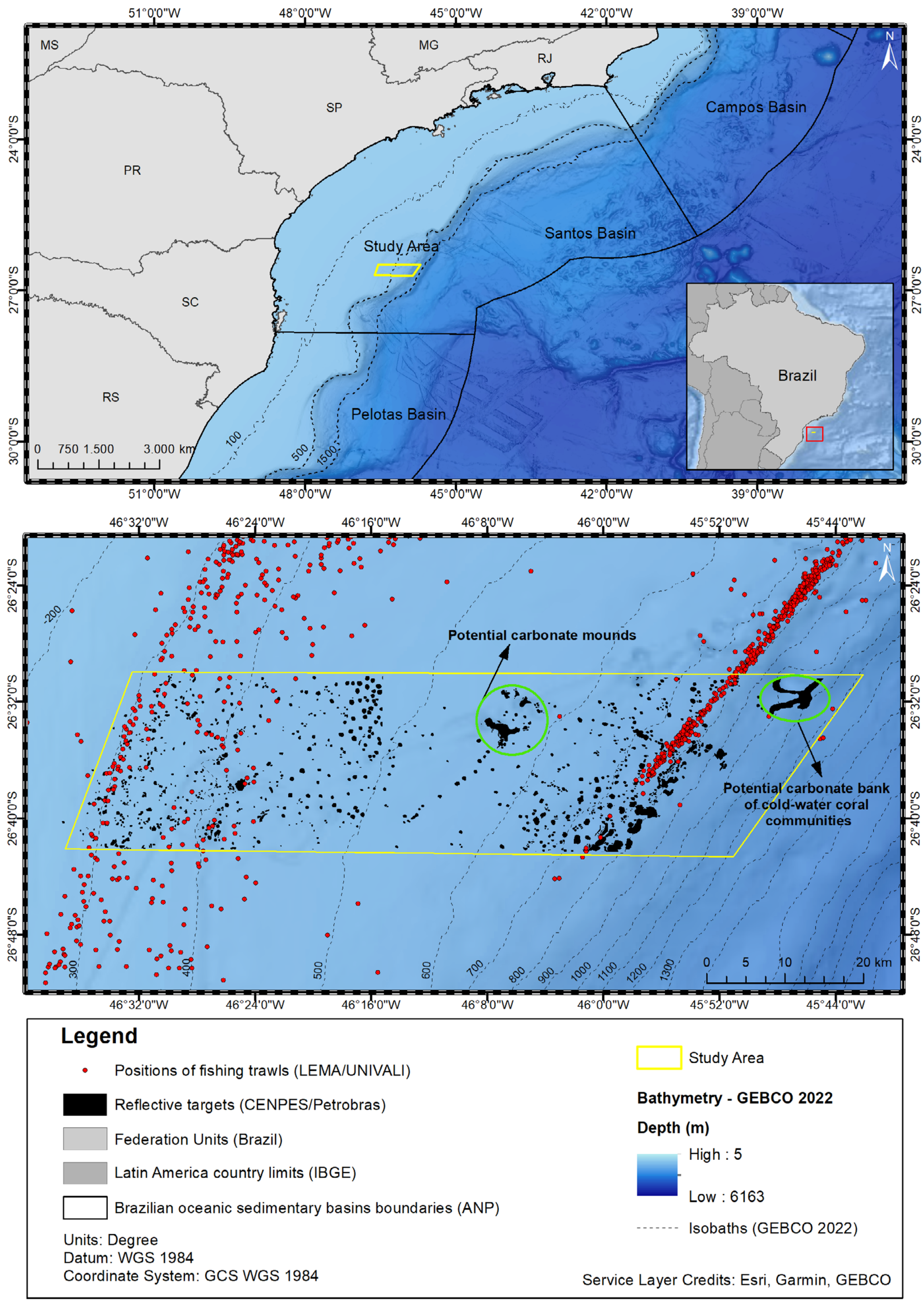

Figure 1. Cruise iAtlantic_BR10-Petrobras study area (yellow box) in the Brazilian Meridional Margin (upper panel) and in detail (lower panel), including reflective targets (in black, data from CENPES – Petrobras) and positions of fishing trawls (in red, data from UNIVALI). Green circles indicate large reflective targets of specific interest.

The cruise explored a cross-section of the Brazilian Meridional Margin at the southern end of the Santos Basin, including sectors of the local shelf break and upper slope (Figure 1). This area encompasses a dense pockmark field whose origin has been associated with salt diapirism (Mahiques et al., 2017). Previous seafloor surveys conducted by Petrobras indicated that these features coincided with reflective targets in seismic profiles, which suggested the occurrence of carbonate banks potentially associated with cold-water coral communities (Figure 1). Some reflective targets also largely exceeded the sizes of pockmarks in the region and suggested the occurrence of larger geological features (e.g., carbonate mounds or ridges) (Figure 1). Additionally, studies conducted by UNIVALI showed that commercial fishing sought profitable fish and crustacean stocks in the area between 2000 and 2008 (Perez et al., 2009). Historical records show particular concentrations of trawl, gillnet and pot fishing operations in the upper slope around the margins of the shelf-break (~300 m depths) and of a slope terrace (~700 m depths). The latter was also associated with a dense pockmark field and reflective targets (Figure 1). This piece of evidence jointly suggested that the selected region contained unique ecosystems with elevated diversity and productivity, and scarcely described or understood structure and functioning patterns. The science plan was designed to describe and characterize these ecosystems, assessing elements of their functioning and potential signs of impacts produced by past bottom fishing activities. Combining the general goals of the iAtlantic project with background datasets and previous knowledge, the iAtlanticBR10-Petrobras cruise aimed to:

a. conduct high-resolution bathymetry mapping of the selected area, with special emphasis on the regions with the highest concentrations of pockmarks and fishing records,
b. characterize habitats and communities by seafloor imagery and biological/ geological sampling,
c. obtain cold-water coral samples for geochemical and taxonomic studies, and
d. conduct on-board ex-situ experiments to assess the functioning and responses of sedimentary ecosystems to climate-related environmental changes.

The cruise lasted 17 days at sea. It began in the Santos Harbour (São Paulo) on December 6, 2022, and ended in Niterói Harbour (Rio de Janeiro) on December 21, 2022. Table 1 describes the sampling activities during the iAtlantic_BR10-Petrobras cruise.

Table 1. Summary of data acquisition and sampling activities during the iAtlantic_BR10-Petrobras.

Activity		**Description**
CTD/ L-ADCP casts	CTD	Continuous measurements of temperature, salinity, dissolved oxygen concentration, fluorescence, turbidity, and current speed/ direction along the water column. Maximum depth with data acquisition was 1,000 m. The rosette carrying CTD and L-ADCP was lowered to a minimum altitude of 80 m above the seafloor. Some casts supported the laboratory experiments by providing bottom temperatures and seawater used in sediment incubation.
Box Corer deployments	BCS	Sediment and benthic fauna sampling were conducted to (a) support on-board laboratory experiments with live organisms, (b) assess environmental DNA, (c) characterize substrate type (grain size and organic matter), and (e) describe benthic infauna and epifauna. Deployments that supported laboratory experiments were conducted in 1,000- and 700-m-deep areas.
Towcam transects	Tcam	The towcam system was towed over the seafloor to produce continuous images of the benthic environment. Sites of the towcam profiles were defined according to the bathymetric survey and followed areas in which cold-water coral banks were likely to occur.
Bathymetry mapping	MBES	A bathymetric survey was conducted with a multibeam echosounder to delineate seafloor surface morphology. The bathymetric survey was conducted along pre-determined parallel track lines.

On-board scientific activities were sequentially conducted according to an initial strategy that included 1) sediment sampling in the deepest sectors of the study area to support experiments with live organisms (Table 1) and 2) bathymetry mapping to define the path of the towcam transects. Unfortunately, the towcam system became inoperable after the first video profile (see below). Based on these circumstances, the original strategy was altered, intensifying the bathymetric survey, adding a CTD cast transect across the slope bathymetric gradient, and increasing the number of box corer stations to cover all regions in which cold-water coral communities were likely to occur. The following section details the sampling methods and preliminary results.

METHODS

Seafloor mapping

The seafloor was mapped using hull-mounted Kongsberg EM 120 and EM 122 multibeam echosounders (MBES). Multibeam bathymetry was planned to detail seafloor morphology in two areas with a dense concentration of pockmarks and fishing records available near the 300 and 700 m isobaths (Figure 1), and define the towcam transects. As the camera system became inoperable, bathymetric mapping was intensified to fully describe the study area. Multibeam echo sounding was conducted along 1,046 NM-long track lines, covering an area of 2,565 km^2 (Figure 2). The distance between track lines varied between 400 and 600 m according to depth and maintaining a 20% overlap between the covered area. Along all the MBES track lines, sub-bottom acoustic data were acquired using a Kongsberg SBP 20 sub-bottom profiler (Kongsberg SBP 20).

The surveyed area partially coincided with the southern portion of the area in de Mahiques et al. (2017). The processed data had a pixel size of 13 × 13 m, enabling a detailed representation of the pockmark field with denser pockmark concentrations between 450 and 500 m and 600 and 800 m (Figure 3). In total, two prominent geological features (4-11 km long) were described in areas in which data from Petrobras showed exceptionally large reflective targets (Figure 3). The geological nature of these features remains unclear but they may configure large salt diapirs or carbonate mounds. Additionally, the high-resolution bathymetric map showed long linear grooves about 40 m wide that were potentially made by bottom trawls for deep sea fish and shrimps, frequently conducted in 200-750 m deep areas in the Santos Basin from 2001 to 2008 (Perez et al., 2009) (Figure 3).

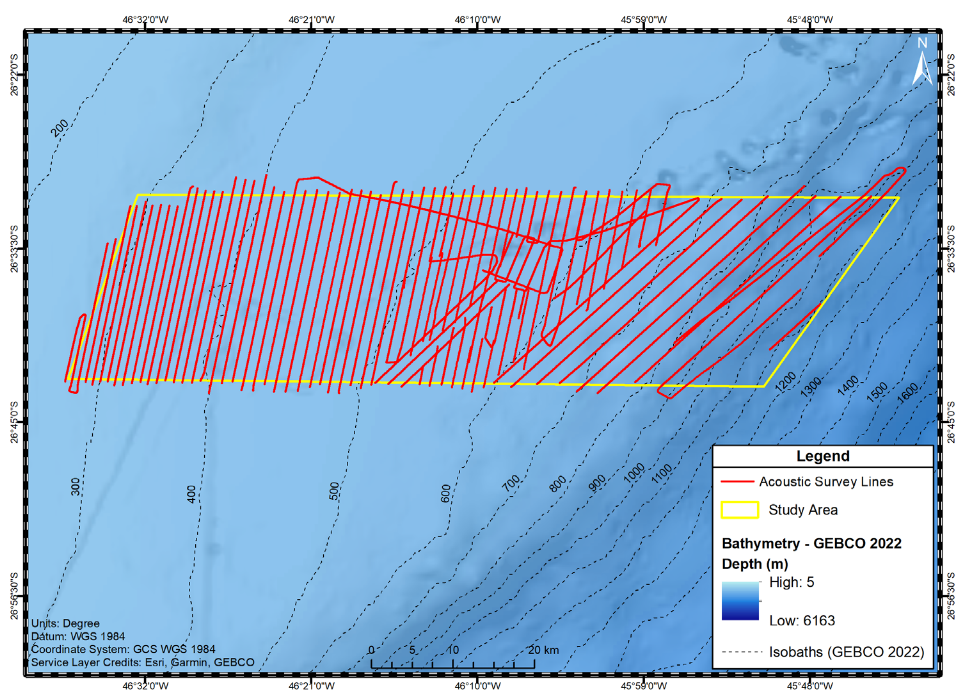

Figure 2. Multibeam echosounder and sub-bottom profiler track lines of the iAtlantic_BR10-Petrobras cruise.

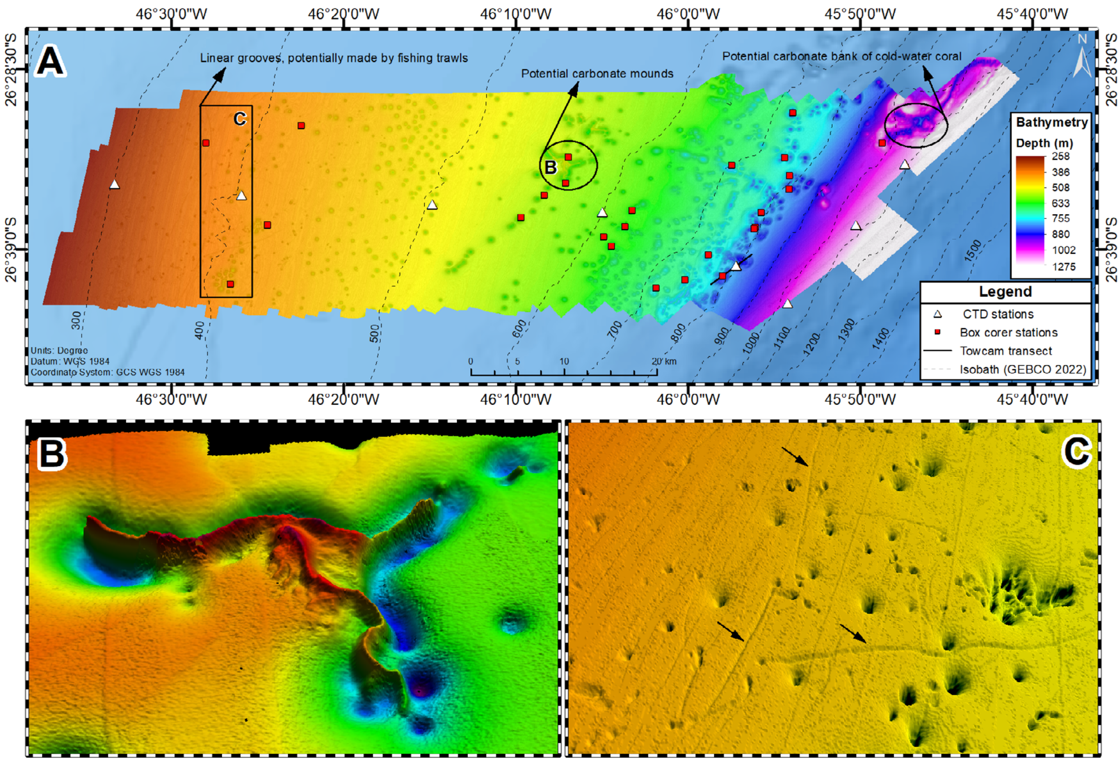

Figure 3. Bathymetric map of the study area produced during the iAtlantic_BR10-Petrobras expedition (A). Red squares indicate stations with box corer deployments. White triangles indicate stations with CTD casts. A black line indicates the towcam transect position. B) 3-D view of the seafloor showing a large geological feature identified as potential carbonate mounds (Figure 1) and pockmarks. C) 3-D view of seafloor showing linear grooves (~400 m-deep) possibly made by fishing trawls.

Characterization of Benthic Habitats and Communities

Seafloor imaging

The towcam system included a towed vehicle ('fish') carrying one HD camera, two light sources, and an altimeter sensor. The fish is towed by a 1,500 m long optic fiber cable operated by a dedicated winch exclusively installed on the ship for this cruise (Figure 4). The fish can be operated up to 2 m above the seafloor between 250 and 1,500 m depths. A pilot towcam system operation was conducted in a 200-m deep area along the route from Santos to the study area. This trial enabled the iAtlantic team and crew members to define safe routine procedures to deploy and retrieve the fish, control fish altitude, check the best settings for camera and light sources, and familiarize themselves with the camera software and image acquisition system during the video profile (Figure 4).

Based on the first seafloor maps produced by MBES in the deepest regions of the study area (~850 m), the first towcam profile was planned to move on a 2-NM long straight line over the rims of two pockmarks (Figure 3). The fish was lowered for 45 minutes as the vessel moved at approximately one knot until the seafloor was visible and then kept at 1-2 m over the seafloor on average for the best seascape visualization (see details of the operation in the Supplementary Material (Table S1). The towcam was towed for two hours, producing a fairly good visualization of the sedimented substrate and numerous components of megafauna (Figure 4). After this period, communication with the camera system was lost, and the fish was immediately retrieved to the deck. The camera builders were contacted from the ship and recommended that the navy crew conducted a system check-up. An irreparable rupture in the optic fiber cable was finally detected. The towcam system could no longer operate, and seafloor imaging was canceled.

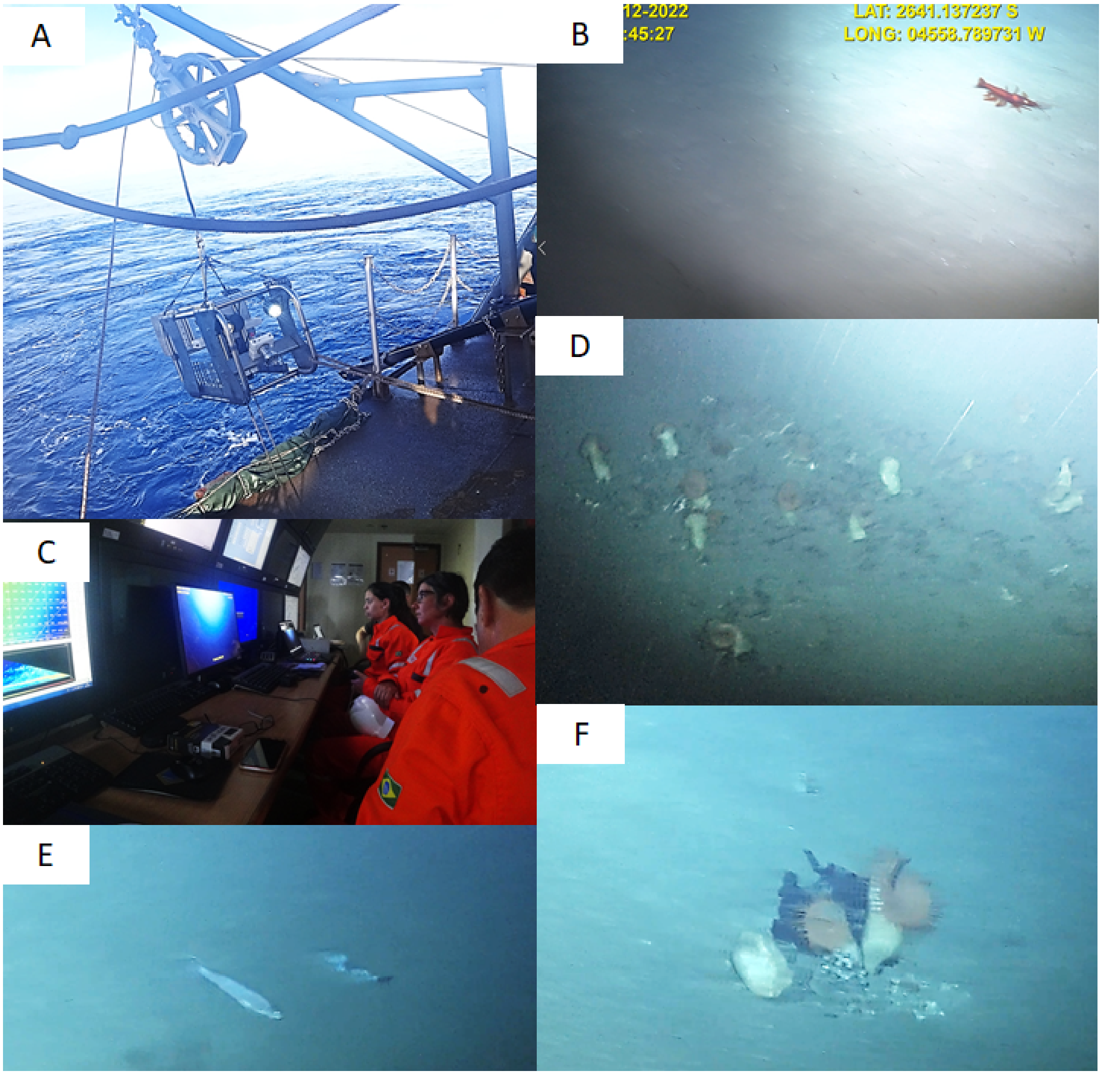

Figure 4. Towcam system operated during the iAtlantic_BR10-Petrobras cruise. A) stern deployment of the towcam 'fish' C) towcam control center in the RV Vital de Oliveira dry lab; B, D, E, F) seafloor images produced by the towcam system on ~800 m deep areas. B) deep sea shrimp Family Aristeidae; D and F) anemones and sponges; E) fish Order Ophidiiformes.

Characterization of the substrate, infauna, and epifauna

A total of 46 box corer deployments (34 valid) were carried out in the study area, covering pockmark rims and ridges detected during the previous bathymetry survey (Table S2 Supplementary Material, Figure 3). This strategy aimed at describing substrate types and characterizing epifauna diversity (specially octocorals) and infauna associated with the local geological features.

The sediment collected in 23 box-corer samples were completely sieved using 1,000 and 500 µm mesh sieves to retain the epifauna and large macrofauna (Figure 5). Specimens were preserved in ethanol and sediment subsamples from each box-corer were frozen for subsequent granulometric and organic matter content analysis. Coral rubble, mostly formed by fragments of stony corals (*Desmophyllum pertusum, Madrepora sp., Solenosmilia variabilis,* and *Enallopsammia rostrata*), was found in 24 box corer samples. In two stations, named "Coral 17" and "Coral 24" (Table S2), coral rubble dominated the substrate. These samples were collected over ridges between pockmarks, suggesting they could be coral banks (Figure 5). At station "Coral 24," a living black coral (*Bathypathes* sp.) colony was collected.

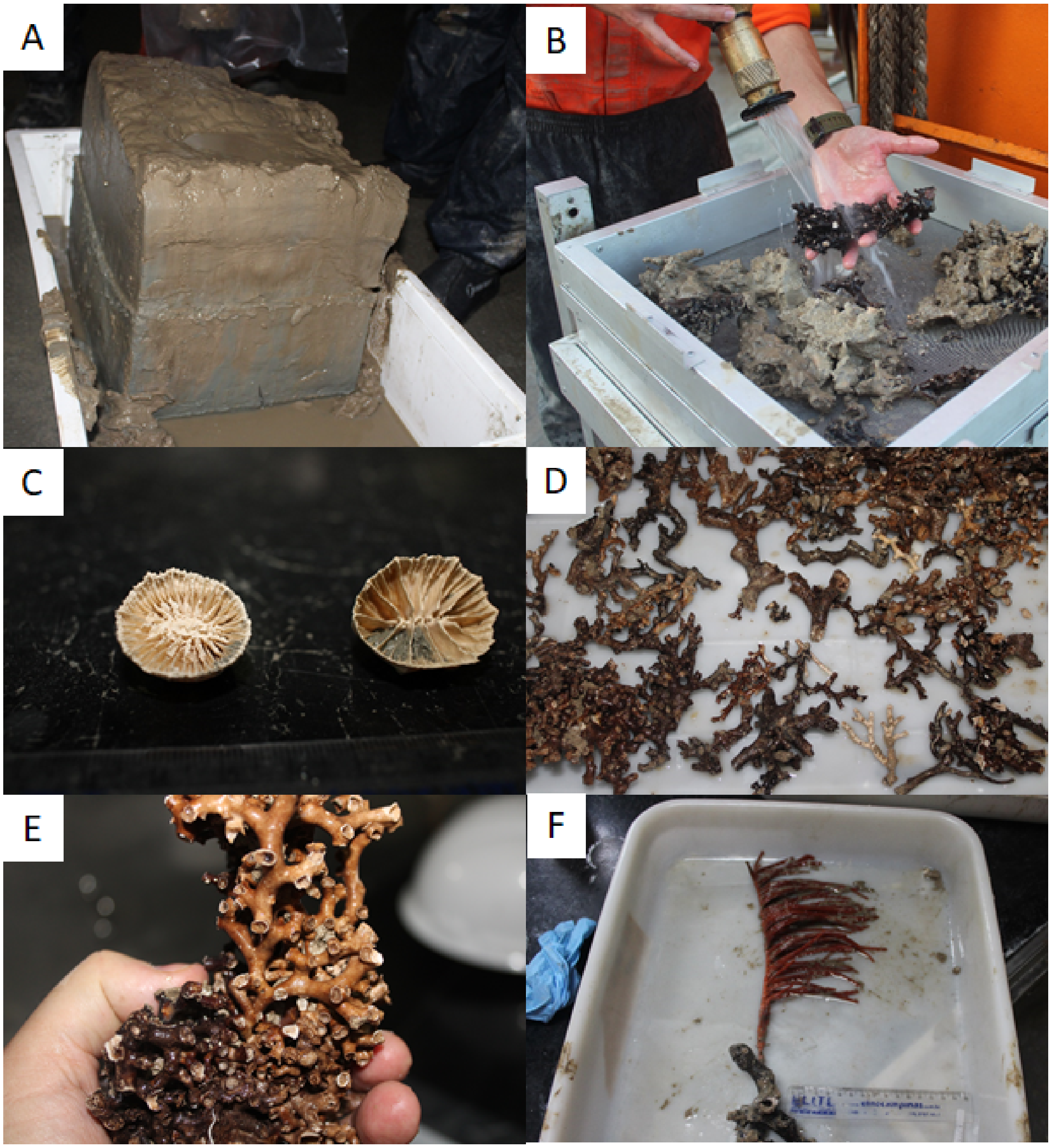

Figure 5. Seafloor sediment sampling with box corers during the iAtlantic_BR10-Petrobras cruise. A) total sediment sample collected in one box corer deployment; B) on-deck sediment processing with sieves; C) solitary corals found in the sediment; D) coral rubble with different Scleractinia species; E) ancient colony fragment of *Solenosmilia variabilis*; F) live black coral colony (*Bathypathes* sp.) captured in a box corer deployment.

Sediment was collected from the 11 remaining box corer samples for background characterization of the smaller benthic organisms (macrofauna, meiofauna, and microorganisms). Macrofauna samples were collected with a 10 cm diameter core, sieved through a 300 µm mesh, and fixed in 10 % formalin, whereas the meiofauna samples were collected with a 3 cm diameter core and immediately fixed in a 10% formalin solution. Sediment samples for benthic microorganisms were collected using a spoon to fill a 60 mL falcon tube and stored frozen (Table S2). The remaining sediment was sieved following the methods for the epifauna and large macrofauna as described above. Additionally, sediment subsamples for environmental DNA (eDNA) were also collected using a spoon. These samples were stored in Whirl-pak and frozen according to specific protocols for successive analysis on land.

Water column profiles

A CTD/L-ADCP transect was conducted across a depth profile with deployments over the 300-, 400-, 500-, and 700-m isobaths (Table S3 Supplementary Material, Figure 3). In the deepest regions of the study areas (~1,000 m) and at 700-m depths, CTD casts provided information on environmental temperatures and deep-water samples to support the experiments with living organisms in the wet lab of the vessel (see below). The combination of CTD and L-ADCP enabled a comprehensive representation of water mass characteristics and ocean currents influencing marine habitats. CTD casts provided 24 Hz sampling and a resolution of 0.0002 ºC for temperature, 0.00004 S/m for conductivity, and 0.001% of the full-scale range for pressure. The L-ADCP was configured to record 20 bins with a bin size of 10 m, a blank distance of 1.76 m, and a frequency of 1 Hz.

Study on the functioning of sedimented benthic ecosystems

In order to determine the trophic webs and assess and quantify the effect of POC quality decline on the benthic assemblages, two incubation enrichment experiments with ^{13}C and ^{15}N (labelled diatoms) and *Phaeodactylum tricornutum* as organic matter were developed on board. Seafloor sediment was collected during five valid box corer deployments at 600-1,000 m depths (Table S2). Before each deployment, CTD casts recorded environmental temperature and collected deep-water samples. The upper 10 cm sediment of each box-core was collected with 10 cm diameter cores, which functioned as benthic chambers in the incubation experiments (eight from 1,000 m depths and four from 600 m depths). These subsamples were transferred to freezers in the wet laboratory of the vessel, minimizing sediment disturbance in the cores and topped off with seawater at *in-situ* temperature (8°C for 600 m and 4°C for 1,000 m) (Figure 6). After sediment stabilization (12 hours under aeration), 30.4 mg of algae (corresponding to approximately 24% of the site-specific annual POC flux) were hydrated in filtered seawater at the experimental temperature and gently injected into the top water of the cores. The algae were of two different qualities: fresh and artificially degraded diatoms (by removing low molecular-weight compounds – LMWCs) that were previously grown and isotopically labelled in laboratory following the (adjusted) procedure in Aspetsberger et al. (2007). Half of the 1,000 m deep sediment cores were injected with fresh algae and the other half with degraded algae, whereas the cores with sediments from 600 m depths were all injected with fresh algae. The experiments continued for 48 hours and were constantly monitored. Then, the sediment was homogenized and sediment samples for microorganisms were taken with the help of a spoon by filling a 60 mL falcon tube and kept frozen. The remaining sediment from each core was sieved through a 300 µm mesh to retain macrofauna organisms. These organisms were fixed in 10% formalin for future taxonomical identification, biomass analysis by dry weight, and C-uptake by isotopic analysis, which will enable the assessment of the trophic network.

CONCLUSIONS

The iAtlantic_BR10-Petrobras cruise was the first initiative to apply the iAtlantic Project approach in the South Atlantic (Roberts et al., 2023). It included a chance to image the seafloor which would enable the visualization of habitats and megafauna communities, adding new elements to the characterization of benthic communities from previous studies (e.g., Carrerette et al., 2022; Santos et al., 2022 and other). Thus, the sampling plan that was originally developed for the expedition was severely impacted by the inoperability of the towcam system. However, the sole obtained video transect contains unprecedented information about the sedimentary habitats dominating the Santos Basin slope and its fauna.

The contingency plan conducted throughout the expedition produced important complementary results, especially the bathymetric map of the entire study region with a 13 m × 13 m pixel resolution. This high spatial resolution enabled a refined interpretation of the seafloor morphology, showing remarkable geological features and tracks produced by fishing trawls. Combined with substrate description from the extensive sediment sampling, the seafloor morphological description will improve understanding of the deep habitats in the Santos Basin and guide future studies. It may also show the spatial and temporal extent of fishing pressure in the area and the vulnerability/resilience of the benthic ecosystem to this type of impact, considering that trawl fishing in the slope of Santos Basin nearly ceased in 2009.

Seafloor sediment sampling with box corer produced a large set of biological samples that can support qualitative studies on the local fauna diversity, adding information to previous studies (e.g., Carrerette et al., 2022). Also, the collected coral fragments supported earlier inferences about the distribution of coral banks (Sumida et al., 2004). The exploration of new tools such as eDNA will offer a more complete baseline of the biodiversity of the sedimentary ecosystems of the Santos Basin and may even offer new biological records for the area.

The incubation experiments that addressed the functioning of deep sedimentary ecosystems were successfully finalized and expanded to shallower areas to study secondary production in an area historically impacted by trawling. Considering that biodiversity and ecosystem functioning can change in response to a variety of environmental and human stressors and that predicted climate change scenarios show a decline in the POC quality to the seafloor over the next century (Sweetman et al., 2017), these experiments will produce a first assessment on the potential impacts to the deep-sea benthic ecosystems of the South Atlantic Ocean. Results from this expedition will be compared with the results obtained by iAtlantic's *iMirabilis2* expedition to deep regions around the Cabo Verde archipelago (https://www.iatlantic.eu/imirabilis2-expedition/).

Despite unforeseen drawbacks in developing the science plan, the iAtlantic_BR10-Petrobras cruise provided valuable data for benthic ecosystem characterization in the upper slope of the Santos Basin, including a preview of benthic habitats and communities around pockmarks. The ongoing in-depth analysis of the collected data is expected to show more about seafloor morphology, habitat and fauna diversity, and the water mass structure and dynamics in the explored region. Clearly, iAtlantic goals have been only tackled during the iAtlantic_BR10-Petrobras cruise and additional efforts are greatly needed to improve our understanding of Santos Basin deep ecosystems. The collected baseline data and derived interpretations constitute, nonetheless, important steps in those directions and will contribute to the success of future expeditions in the region.

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AUTHOR CONTRIBUTIONS

J.A.A.P.: Conceptualization; Supervision; Investigation; Writing – original draft; Writing – review & editing;

R.C.M.A.; H.M.C.B.N.: Supervision; Investigation; Writing – review & editing;

D.Y.G.; C.F.S.: Investigation; Supervision; Resources; Writing – review & editing;

F.M.M.A.; J.A.C.; P.D.C.E.; T.S.F.; I.S.L.; R.U.N.; P.O.N.; J.V.M.R.; A.L.F.S.; E.C.S.; R.S.; L.C.S.; F.S.S.S.: Investigation; Writing – review & editing;

L.G.: Investigation; Visualization; Writing – review & editing;

A.F.B.; G.H.C.; A.L.; M.M.M.; C.M.; P.R.; A.K.S.: Conceptualization; Supervision; Writing – review & editing;

J.M.R.: Conceptualization; Project Administration; Funding Acquisition; Writing – review & editing.

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