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ECOSYSTEMS

# A new ROV storage device for deep-sea sampling

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**Abstract:** Sampling deep-sea biota is a significant challenge because of the logistics required, in terms of vessels and equipment, to obtain minimally preserved specimens. Traditional methods (trawls, nets, and dredges) cause physical damage, stress, and even contamination during the process of removal from the seabed and their displacement through the water column to the surface. Preserving conditions similar to those found *in situ* is particularly important when the sampling strives to maintain living organisms and for analyses where contamination or degradation by stress or damage may interfere with the results. Therefore, for the sampling and storage of this biota with less interference, a polypropylene box was designed based on the model of Kellogg et al. (2009) incorporating adaptations to be used by a Remotely Operated Vehicle (ROV). This new device has been successfully used in eight oceanographic campaigns, adequately performing for sediment and biota sampling, including coral reef forming or framework species (Scleractinia), octocorals, associated fauna, and rhodoliths, at depths between 50 and 900 m.

**Key words:** Associated fauna, sampling box, corals, octocorals, rhodoliths.

## INTRODUCTION

Deep-sea research is intrinsically challenging. The sampling of deep-sea biota requires complex logistics and extra care to obtain preserved specimens (Przeslawski & Foster 2020) and to avoid or reduce stress and contamination. Stress and physical damage, which are more likely to occur using traditional sampling methods such as trawls, nets, or dredges, are particularly unfavorable when preserving the organisms alive is a priority. Scleractinian corals, which were the main focus of our sampling, are susceptible to temperature variations (Brooke et al. 2013). Other direct and indirect effects may be observed depending on the sampling method, such as tissue damage that causes loss of coloniality because of the dissociation of polyps (the basic modular units of the colony) from their connective tissue (coenosarc) (Kvitt et al. 2015). In addition, corals become more prone to fouling as they lose the protection and antifouling properties of the coenosarc (Orejas & Jiménez 2019). Corals also present lower skeletal growth with reduced coenosarc cover (Mortensen 2001). Additionally, with traditional methods, the organisms are collected in batches and come into contact with each other. This may impair, for example, microbiological studies because the microbial community of one organism could contaminate that of the other. Similarly, contact with sediment, other invertebrates, mobile fauna, or even with different water masses during transportation between the sampling point and the surface could contaminate coral samples (Kellogg et al. 2009). Therefore, preserving conditions similar to those found *in situ* is a major challenge, particularly when the sampling procedure must both maintain the organisms alive and minimize degradation (produced by damage or stress) and contamination. Both degradation and contamination may interfere with subsequent analysis results.

Deep-sea data acquisition requires significant financial investment and specialized logistical resources. Consequently, in Brazil, as well as in many other countries, oil and gas industry along continental margins provides significant deep-sea coral data. For example, the Campos Basin has the largest volume of deep-sea coral data compared to other sedimentary Brazilian coastal basins, since it concentrates major oil and gas production fields (Rocha et al. 2021). Since 2004, the Brazilian Energy Company PETROBRAS has been leading research projects on deep-sea corals in addition to the acquisition of data during its offshore operations. These initiatives have mapped and characterized deep-sea coral ecosystems in the Campos Basin by analyzing physical (geological and oceanographic) and biological (biodiversity) aspects (Cavalcanti et al. 2017, 2019). From 2016 to date, PETROBRAS' "Marine Sensitive Environments - SENSIMAR Project" is mapping and characterizing deep-sea coral environments in addition to evaluating the impacts of oil and gas industry activities through laboratory and field experiments.

In Brazil, deep-sea corals can be found at depths varying from 200 to 1,200 m, off the coast between Northeastern and Southern regions, from 9 to 35° S, which includes the Brazilian Exclusive Economic Zone (Pires 2007, Pires et al. 2015, Cordeiro et al. 2020). As a result of PETROBRAS and other oil and gas companies' efforts, the knowledge of deep-sea environments and ecosystems is increasing. These ecosystems are structurally complex environments that provide habitats for many invertebrate and fish species and offer ideal conditions for their settlement, growth, and reproduction (Freiwald et al. 2004).

The bathymetric distribution of deep-sea biota implies sampling difficulties, which includes the stress of removing the organisms from the seabed and their displacement through the water column to the surface, and the risk of sample contamination due to the contact between organisms and sediment, water masses, other fauna, etc. (Kellogg et al. 2009). It is also important to consider that temperature may vary drastically between the sampling site and surface. According to Foloni Neto et al. (2010), this variation can reach more than 25 °C in the Campos Basin for corals sampled at 1,200 m depth (Upper Circumpolar Water = 2.5 °C and the surface (Tropical Water = 26.8 °C). Corals are especially susceptible to temperature variations (Brooke et al. 2013) and, depending on the region, there may also be other stress factors, including the salinity gradient, as reported by Kellogg et al. (2009) in the Gulf of Mexico.

Remotely Operated Vehicles (ROVs) are routinely used as part of offshore oil and gas operations (Dalhatu et al. 2021). ROVs for subsea activities have been seeing a lot of development since the 1990s, when highly capable ROVs started being used. In a simplified way, they are subdivided into: work-class ROVs (powered electrically or hydraulically with manipulators and grabbers to perform a range of subsea operations) and observational-class ROVs (which have visual and recording capabilities, used for surveying and inspections). They have *per se*, as a key advantage in a monitoring context, the ability to be dynamically controlled in 'real time' across a range of depths and habitats (Przeslawski & Foster 2020). While ROVs can be used for deploying a variety of sensors as well as taking samples of substrata and organisms, they are also used to generate spatially accurate photomosaics and fine-scale digital elevation models. Moreover, ROVs can also provide color information (via photomosaics), which is crucial for morphotyping or identification of species and evaluation of conditions (e.g., live vs. dead coral). These tools are also essential for guiding the sampling of deep-sea biota (Przeslawski & Foster 2020).

Until 2018, the SENSIMAR Project oceanographic campaigns used a sampling box operated by ROVs composed of ten uninsulated compartments made of 3 × 3 cm gridded mesh plates, which did not retain water (Figure 1 a, c, e). After packing the organisms in each compartment and moving the box through the water column to the surface, the water passed through the fragments/colonies and washed them. As a consequence, it caused the loss of associated fauna (previously attached to the fragments), physical damage (such as coenosarc loss in corals and octocorals), and stress (such as mucus production and reduced activity). Therefore, corals reached the surface without water in the sampling box, being in direct contact with air and subject to temperatures much higher than that of the sampling site. Coral samples collected with this box were observed to be affected. It was common to observe polyps retracted and copious mucus production as clear signs of stress due to using non-insulated sample containers (Kellogg et al. 2009).


**Figure 1. Previous sampling box with ten uninsulated storage chambers employed for deep-sea biota sampling until 2018 (left images), and new sampling box designed with six insulated storage chambers, employed for deep-sea sampling from 2019 to date (right images). a: Previous sampling box in detail, b: new sampling box in detail, c: previous sampling box allocated in the ROV skid/tool drawer before sampling, d: new sampling box allocated in the ROV skid/tool drawer, e: previous sampling box held by the ROV through its strap after sampling, f: new sampling box located on the seabed.**

Such conditions may hinder the subsequent maintenance of these organisms alive in the laboratory (because stress and coenosarc loss/damage compromise coral health) and analysis, such as gene expression and transcriptome. Also, these conditions may cause contamination, limiting certain analyses such as metagenomics and microbiological.

Soft sediments are also widely sampled for biology and geology studies using box corers and multicorers, where the water/sediment interface is needed and information on vertical structure is important (Eleftheriou & Holme 1984). However, they present various disadvantages due to their large size and weight, requiring particular conditions for deployment on the seabed. This sampling is also challenging for studies in sensitive areas, such as deep-sea coral and rhodolith bed environments. Deep-sea sediment samplers began to diversify from the 1980s onwards, seeking low cost, light weight, compactness, easy operation, and high adaptability to sea conditions. In addition, another prerequisite for investigations of deep-sea sediment is providing sampling techniques capable of preventing distortion during recovery (He et al. 2020).

Motivated to identify solutions regarding the observed deleterious condition particularly of the biota, we designed a polypropylene box to be used by a ROV, based on the Kellogg et al. (2009) model with adaptations for the collection and storage of deep-sea samples with less external interference. This sampling device has six removable storage chambers that function as individual insulated compartments, protecting samples from drastic changes in parameters such as temperature or salinity (Figure 1b, d, f). Here, we intend to describe this device in detail so that others can reproduce it for using in deep-sea sampling.

## MATERIALS AND METHODS

### Design and dimensions

The new device is made of polypropylene and composed of six flanged storage chambers fitted by turning and coupling between two parallel plates with stainless steel fasteners and butterfly wing nuts. The parallel plates are 73 cm long, 50 cm wide, and 1 cm thick, held 34 cm apart by 10 tubes at the edges (43 cm long and 4 cm in diameter) and two central tubes (39 cm long and 4 cm in diameter) with eyebolts at the ends for lifting, where the ropes are attached for support by the ROV. Each storage chamber has an external diameter of 16 cm, an internal diameter of 15.5 cm, a depth of 35 cm (total volume of approximately 6 L), and is closed by a 5.2 cm high lid. The lid has a taper that guide the fitting, an O-ring for sealing, two pressure relief valves, and a cylindrical rod (15 cm long and 3.3 cm in diameter) as a handle for the ROV. Each empty storage chamber weighs almost 1 kg, and about 7 kg when full of water/samples. The lid also weighs almost 1 kg. All measurements of the sampling box and its parts are presented in detail in Figure 2 and in the AutoCAD project (Supplementary Material - BoxS1).

As the material used to make the sampling box is lightweight, six ballasts of 2 kg each (total of 12 kg) were fixed to the central hollow tubes of the device so that it would not float when on the seabed. Nylon zip ties were used to fix the ballasts to the central hollow tubes of the sampling box by passing them through the holes of each ballast. The final weight of the sampling box (with ballasts) is 33 kg, when empty. However, it can reach 70 kg when full of water/samples. The total cost of the sampling box (built in 2019, with values corrected according to inflation index and dollar exchange rate since then), with two extra storage chambers, did not exceed US$ 1,400.00.

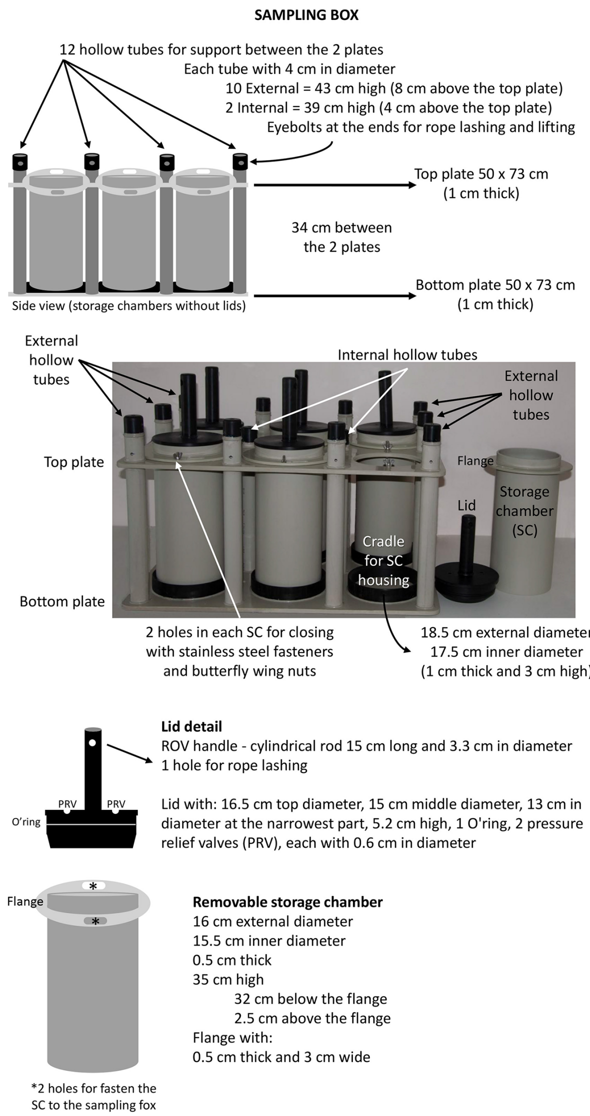


**Figure 2. New sampling box scheme with detail regarding all its components and associated dimensions in detail.**

## Deployment and retrieval

The sampling box was designed with dimensions and characteristics that facilitate both its transportation in the ROV skid/tool drawer and manipulation by the ROV arms, especially when opening and closing the storage chambers (as shown in Figure 3). In our experience, the ROV's 5-function manipulator arm carries the sampling box while the 7-function operates the box, capping and uncapping the storage chambers and pouring samples into them. The 7-function manipulator arm can also carry the sampling box, but the 5-function might not be able to operate the box due to movement limitations.

Once the ROV is on the seafloor, the sampling box is placed on the seabed close to the sampling sites, and its geographical coordinates are recorded to prevent it from being lost. Next, biota sampling is performed with the aid of a 5 L steel cup attached to a rod that allows it to be held by the ROV's 7-function manipulator arm. The coral fragments are gently broken up with the tip of the steel cup and packed in small quantities into the cup. Returning to the sampling box spot, the storage chamber is uncapped, and the samples are poured into it (see Supplementary Material - Video S1). When the sampling box arrives at the vessel, each storage chamber can be unscrewed and removed from the box separately, simplifying sample processing.

The size of the storage chambers was defined to accommodate medium-sized organisms and colonies (up to 25 cm in height). For proper functioning and to reduce the risk of sample contamination, the storage chambers must be filled with osmosis or deionized water before deployment. If there is no need to mitigate contamination effects, it is possible to fill the storage chambers with just seawater. This procedure is essential to prevent the storage chambers from collapsing from the pressure of the overlaying water column during the displacement to the seabed.

Additionally, soft sediment collection is also possible using a small acrylic push-core (6 cm external diameter, 5.4 cm inner diameter and 30 cm long), open at both ends. A braided nylon rope handle was attached to the corer to allow for gripping by the ROV. For sediment collection, the ROV drives the corer into the sediment by pushing it against the seafloor. To remove

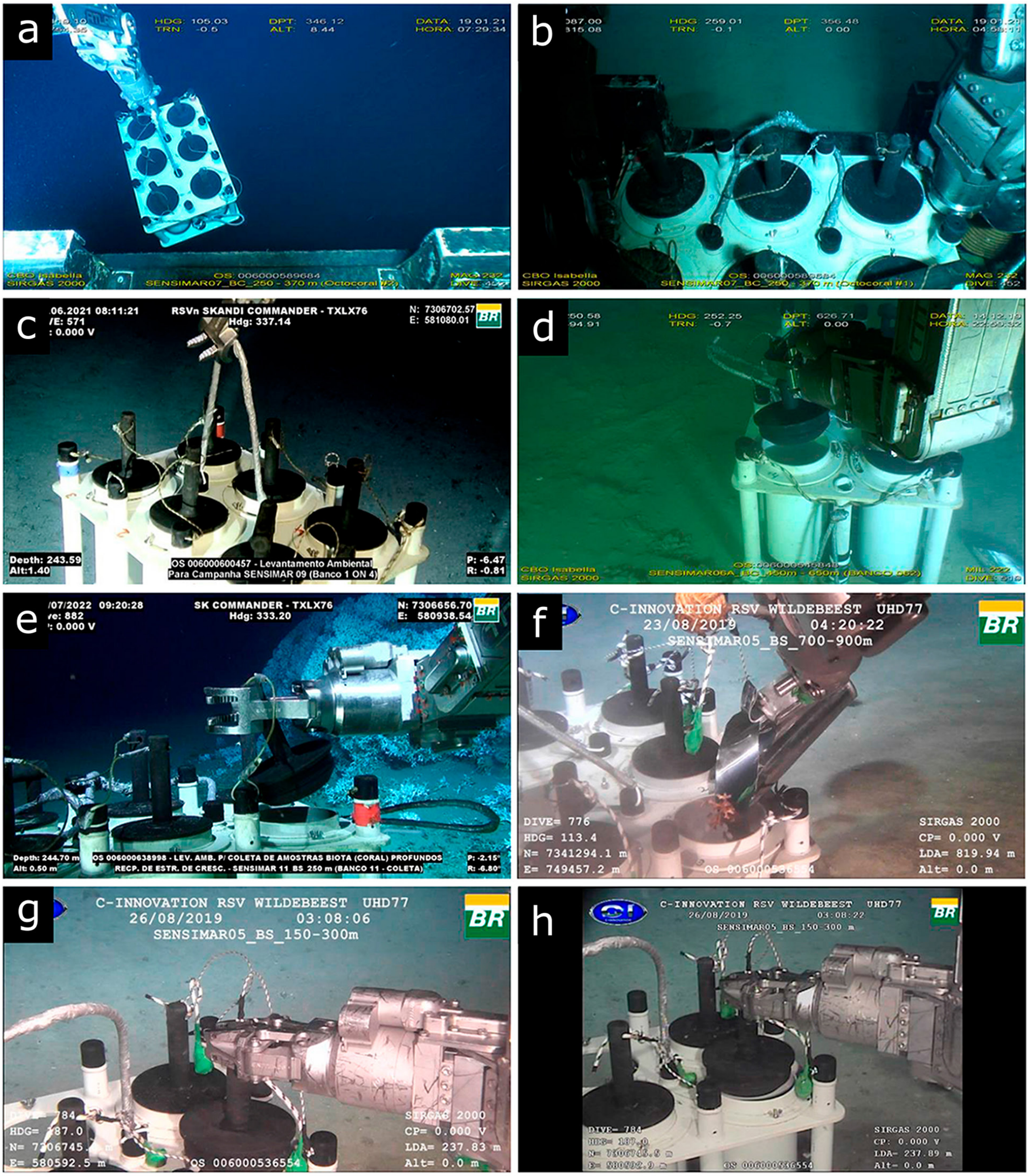


**Figure 3.** New sampling box being operated by different ROVs. a: Sampling box being carried by ROV's 5-function manipulator arm, b: sampling box being carried in the ROV skid/tool drawer, c: sampling box being placed on the seabed by ROV's 7-function manipulator arm, d: ROV uncapping the storage chamber, e: ROV uncapping the storage chamber (in detail), f: ROV pouring biota sampling into the storage chamber, g: ROV capping the storage chamber, h: ROV capping the storage chamber (in detail).

the corer, the ROV pulls it by the handle. Once containing sediment, the corer is deposited into the storage chamber of the sampling box that is capped, which also avoids sample contamination during the displacement to the surface (Figure 4). Then, a PVC plunger (composed of a circular base of 5.4 cm in diameter and 1 cm in thickness, and a rod of 35 cm) is used to push the sediment towards one end. The openings at both ends allow the collection of sediment in the upper and lower portions without mixing, i.e., preserving its vertical structure.

## RESULTS

Originally, this sampling box was designed to sample fragments of *Desmophyllum pertusum* (Linnaeus, 1758) and to keep them alive for subsequent laboratory maintenance and experimentation. However, since its fabrication,

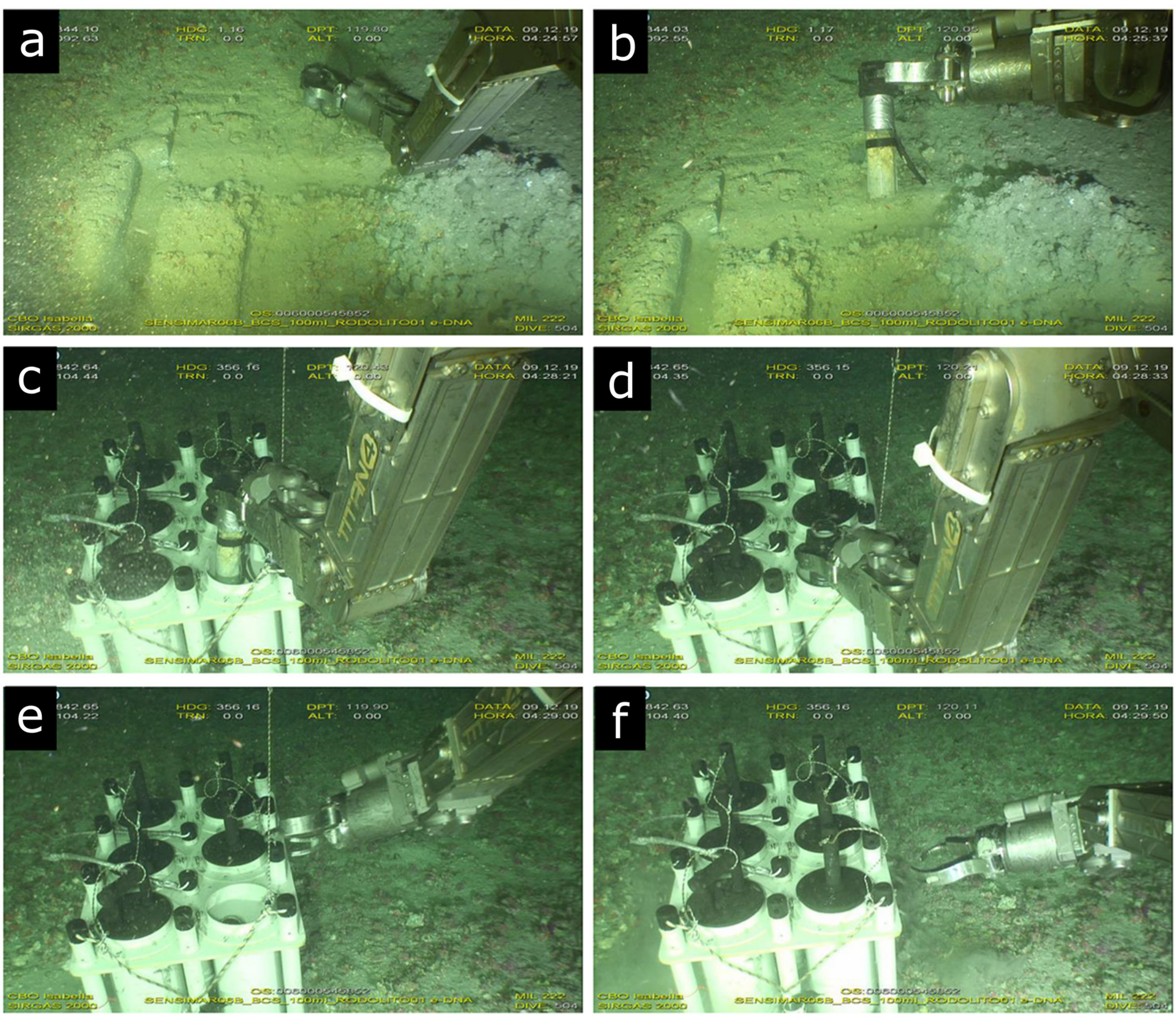


**Figure 4. Small acrylic push-core being operated by ROV for soft sediment collection. a: ROV pushing the corer against the seafloor, b: ROV pulling the push-core, c-d: push-core being deposited by the ROV into the storage chamber of the sampling box, e: push-core inside the storage chamber, f: storage chamber recently capped by the ROV.**

this device has been used to collect other species of reef-forming corals, octocorals, rhodoliths, and their associated fauna, enlarging its use for mesophotic environments. Sampling was performed under IBAMA/MMA (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis/Ministério do Meio Ambiente) authorization (nº 921/2018), when required.

Our sampling device has already been successfully used in eight oceanographic campaigns of the SENSIMAR Project: SENSIMAR 5 (August/2019), 6 (December/2019), 7 (January/2021), 8 (September/2021), 9 (June/2021), 10 (March/2022), 11 (June/2022), and 12 (September/2022), adequately performing biota sampling between depths of 50 and 900 m, which included the main species of reef forming corals: *Desmophyllum pertusum*, *Solenosmilia variabilis*, *Enallopsammia rostrata*, and *Madrepora oculata*. In addition, sampling included calcareous algae and entire colonies of octocorals (up to 20 cm), such as specimens from the Paragorgiidae and Plexauridae families. In these campaigns, visible damage to the organisms was not observed (Figure 5).

Sediment collection was satisfactory when performed on soft sediment, obtaining a sample volume of up to 350 mL. The structure of the sediment in the corer was preserved and allowed the analysis of granulometry, total organic carbon (TOC) and metagenomics, since the displacement to the surface inside the storage chamber of the sampling box kept the sample free of contamination.

## DISCUSSION

This sampling box has been deployed using a work-class ROV, but smaller or larger versions can be adjusted for use with different ROV models. ROVs are available in a range of sizes and configurations, from smaller observation-class vehicles (~3-20 kg for mini and ~30-120 kg for regular-sized models) to larger work-class systems (100-1,500 kg for light- and up to 5,000 kg for heavy-duty models) (Przeslawski & Foster 2020). The observation ROV model might not be suitable for operating this sampling box due to its weight, however work-class models are able to do so.

In contrast to the model of Kellogg et al. (2009), in which the sampling device is fixed to the ROV, our sampling box is a standalone device, and it can be carried by any ROV with its robotic manipulator arm. However, the 7-function manipulator arm is the most recommended to operate the box (sampling biota, capping and uncapping the storage chambers, and pouring samples into them) since it has higher dexterity and range of motion to perform tasks with more accuracy. Therefore, our device does not require ROV adjustments, which facilitates its use by different models in distinct vessels and oceanographic campaigns. Along with the eight SENSIMAR Project campaigns in which our sampling box has already been used, it was operated by ROVs from Oceaneering, DOF Subsea, and C-Innovation, in different vessels: RSV Isabella, Wildebeest, Skandi Commander and Skandi Chieftain. Additionally, in all of these oceanographic campaigns, even involving different vessels, ROVs and pilots, the sampling box has in all cases been operated safely, quickly and efficiently.

Additionally, considering that each storage chamber weighs around 7 kg when full of water/samples (while the whole sampling box can reach 70 kg), it is clear that the removable storage chamber greatly facilitates the manipulation of the collected organisms/samples, which allows more agility during sample processing.

The designed sampling box operated by ROV resulted in the successful collection of deep-sea

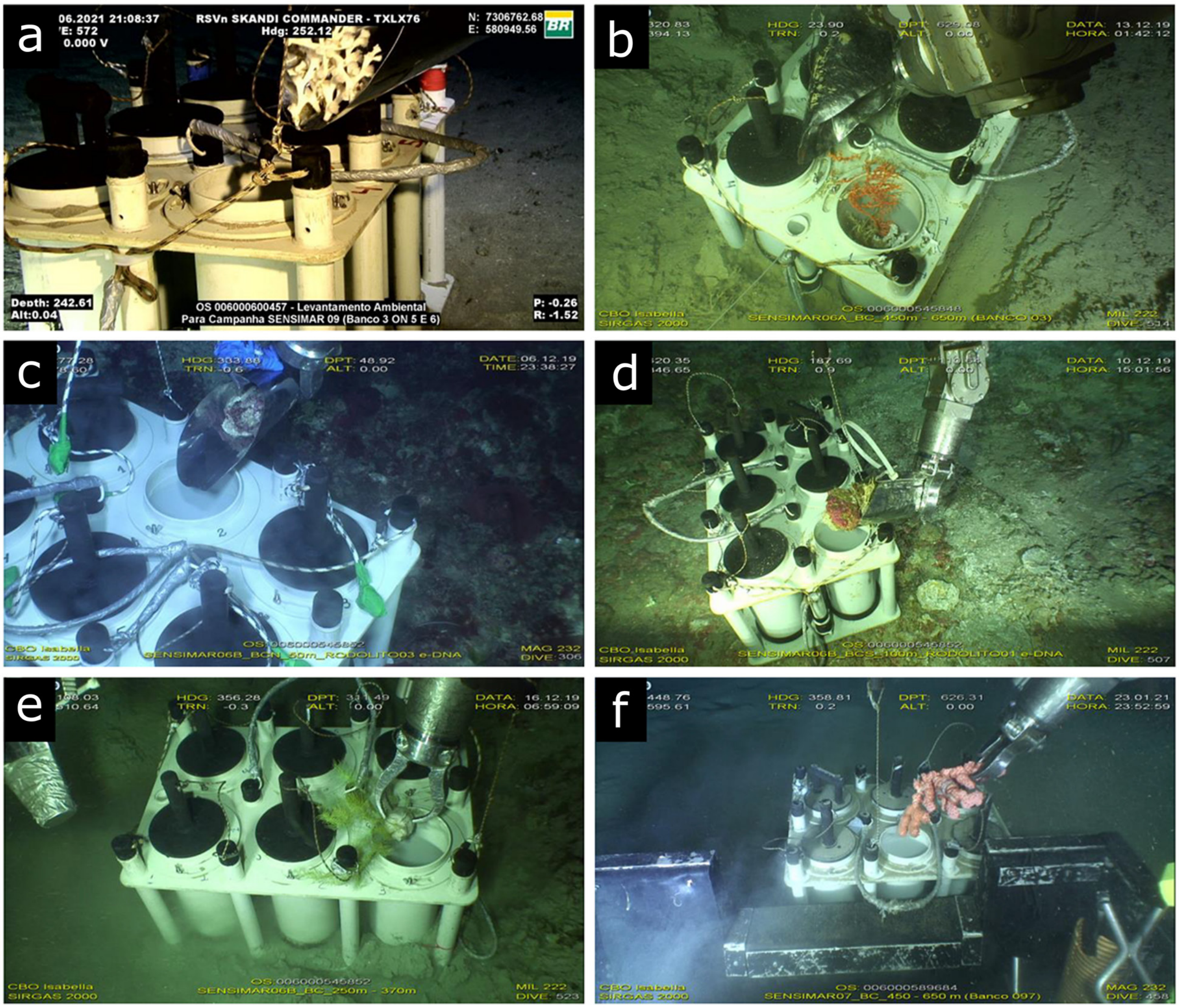


**Figure 5. Examples of the application of the new sampling box for the collection of different species of reef forming corals, octocorals, and calcareous algae. a: *Desmophyllum pertusum*, b: *Madrepora oculata*, c-d: calcareous algae, e: octocoral (Family Primnoidea), f: octocoral (*Paragorgia* sp.).**

biota in good health conditions. Examples of their subsequent maintenance in laboratory includes six months for *Desmophyllum pertusum* fragments (until their use in experiments), four months for octocorals, and more than two years for calcareous algae. In all cases, the organisms were kept alive and in good health status: with high percentage of live polyps, polyp activity and tissue-cover skeleton for corals and octocorals (e.g., health criteria described by DeLeo et al. 2016 and Weinnig et al. 2020), and high percentage of pigmentation and no reduction in fluorescence rates as Fv/Fm', attesting the health of the photosystem II in calcareous algae (e.g., Ludlow 1987 and Demmig & Bjorkman 1987). Among the factors directly involved in stress reduction, temperature stabilization (i.e., maintaining the temperature of the storage chamber as close as possible to the temperature of the sampling site) is the most important one for corals. With the new sampling box, water temperatures varied from 2.6 to 4 °C (mean = 3.4, SD = 0.5) between the seafloor (sampling sites) and the surface (as soon as the sampling box arrived at the vessel) in Santos and Campos basins. In contrast, the previous box had a temperature variation of approximately 15-20 °C (reaching 25 °C in some instances).

In terms of pressure, our sampling box contains pressure relief valves in its lids, which equalize the pressure inside and outside the storage chambers as the device moves through the water column. Pressure-retaining samplers may be useful to study deep-sea microbes under *in situ* conditions (Garel et al. 2019) and sediment history (Huang et al. 2019), for example. However, for deep-sea corals, pressure variation is not a great concern, since evidence suggests that adaptation to high pressure is most pronounced in organisms found deeper than 1,000 m. Therefore, for organisms inhabiting depths shallower than 1,000 m, which is the case for many deep-sea coral species, temperature is the primary physical factor to consider when replicating the physical environment (Lunden et al. 2014).

Regarding sediment sampling, although there are several corer-type samplers available today (e.g., ROPOS 2016, Garel et al. 2019, Huang et al. 2019, Tsuchiya et al. 2019, He et al. 2020), the one described here shows as advantages: compatibility to be used together with the sampling box, low cost, ability to keep the sediment stratification, and protection against contamination, since the corer is coupled inside the storage chamber.

Advantages of our sampling box are summarized in Table I. It is particularly useful on-board vessels not dedicated to scientific purposes, so it can be easily operated using different ROVs (often dedicated to oil and gas industry activities, for example), without requiring any adjustments. Based on our experience, the only disadvantage of this device is the limitation in size or number of specimens that can be sampled during a single deployment, since the storage chambers have limited space (Table I).

Therefore, considering the lack of specialized deep-sea sampling commercial devices, the sampling device presented in this study is functional, with low manufacturing cost, and can be used by all models of work-class ROVs without the need for adjustments. Being able to maintain comparable *in situ* conditions, especially temperature, is a key advantage, because deep-sea corals are particularly sensitive to temperature variations. Reducing stress during sampling implies fewer chances of tissue damage and permits maintaining these organisms alive in laboratory conditions for a longer amount of time. Likewise, the use of this device reduces the risk of contamination, enabling studies such as metagenomics and microbiology. Furthermore, it protects against

**Table I.** **Advantages and disadvantages of the new sampling box for deep-sea sampling.**

<table>
<tr><th>ADVANTAGES</th><th>DISADVANTAGES</th></tr>
<tr><td>Low manufacturing cost;</td><td rowspan="8">Limitation in size and/or number of specimens to be sampled, since the storage chambers have limited space</td></tr>
<tr><td>Usable by all models of work-class ROVs without the need for adjustments;</td></tr>
<tr><td>Maintenance of sampling site conditions, particularly in relation to temperature;</td></tr>
<tr><td>Reduced induced stress on sampled organisms;</td></tr>
<tr><td>Reduced risks of sample contamination;</td></tr>
<tr><td>Keeping associated fauna;</td></tr>
<tr><td>More agility during sample processing since the storage chambers are removable;</td></tr>
<tr><td>Joint use with the sediment sampler (push-core).</td></tr>
</table>

the loss of minor organisms from the associated fauna. Thus, this device is versatile for the use both in dedicated scientific expeditions, and also opportunistically, in vessels dedicated to offshore oil and gas operations. We believe that this new sampling device can be of great utility to deep-sea community and beyond.

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## SUPPLEMENTARY MATERIAL

**BoxS1 – Sampling box project in AutoCAD.**
**VideoS1 – Video showing the sampling box operated by ROV during biota sampling.**

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## Author contributions

ECR, IVR and MPCF designed the sampling box and wrote the paper. PRS and MVR helped to design the sampling box and reviewed the manuscript.