

A detailed underwater and on-board workflow for marine ecological research, using a subtidal kelp forest study

Un detallado flujo de trabajo subacuático y en superficie para investigaciones en ecología marina: el caso de la evaluación de bosques submareales

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ABSTRACT

Compared to its terrestrial counterpart, work at sea to collect scientific data calls for an efficient workflow and particular skills from personnel, particularly when the study involves several observation variables. Therefore, a procedure that incorporates both the underwater and on-board phases in a coordinated manner is required. Although there exist published and validated sampling protocols for benthic habitats, in the existing literature it is more difficult to find workflows or recommendations for use prior to data collection. Using a kelp forest ecological assessment study as a model, we present a detailed workflow for sampling, both on-board and under hyperbaric conditions, so that it can be used as an example for subsequent evaluations, as a standardized method for systematic study in blue carbon ecosystems.

Keywords: work at sea, marine forests, kelp beds, hyperbaric conditions, scientific diving, protocol

RESUMEN

En comparación con su contraparte terrestre, el trabajo en mar para colecta de datos científicos demanda un flujo de trabajo eficiente, así como habilidades particulares del personal, más aún al tratarse de estudios ecológicos que incluyen diferentes variables a ser evaluadas. Por ello, se requiere un procedimiento que integre las fases de trabajo subacuático y en superficie de manera coordinada. Si bien existen protocolos de muestreo publicados y validados para hábitats bentónicos, en la literatura es más difícil encontrar los mencionados flujos de trabajo o recomendaciones para tener en cuenta previo a la colecta. Utilizando una evaluación ecológica en huirales (bosques de algas pardas) como modelo, presentamos un flujo de trabajo detallado para el muestreo tanto en superficie como bajo condiciones hiperbáricas. Esto con el fin de que sirva como antecedente para evaluaciones posteriores, y existan métodos estandarizados para el estudio sistematizado en ecosistemas de carbono azul.

Palabras clave: trabajo en mar, huirales, condiciones hiperbáricas, buceo científico, protocolo



Compared to land-based studies, work at sea often requires additional planning and particular skills. It is essential to take into consideration not only environmental conditions, but also the adaptations required from the workforce to the (dynamic) workspace and the need to meet all established goals within a reasonable timeframe, while prioritizing safety at all levels. In this study, we offer a detailed workflow and procedures for use both at the surface and underwater, employing the assessment of kelp forest biogenic habitats as an example for integrative ecological study.

Well established methodologies facilitate reliable and trustworthy data collection. In this sense, protocols are very useful for standardizing ecological surveys in different marine ecosystems. However, no data, whether collected underwater or on-board, are secure unless properly backed up or digitalised. Moreover, both data collection and sample processing on-site must be undertaken carefully and systematically, focusing on the importance of recording any occurrence or event as metadata for consideration during subsequent data curation, analysis or interpretation.

Kelp forest surveying was one of the first applications of scientific diving by means of the diving bell, c.1934 (Kitching *et al.*, 1934) and subsequently using scuba equipment (self-contained underwater breathing apparatus), c.1952 (Dayton, 1985; Hanauer, 2003). Today, multiple studies in kelp forests describe their methodologies while focusing on aspects of possible relevance to the interpretation of results in accordance with their own specific objectives (Zavala *et al.*, 2015; Tejada *et al.*, 2018; Aller-Rojas *et al.*, 2020), but many details, such as planning, workflow, equipment, and other relevant human stressors are often overlooked (Moreno, 2020a). In this sense, the aim of this article is to present some of the principal methodological details that determine the success of a marine survey operation, both at the surface and underwater, based on the experience of surveying *Lessonia trabeculata* kelp forests in the rocky subtidal areas of Nasca (Peru), during the winter of 2021.

Planning, remote reconnaissance and environmental limitations

Much of the success of any scientific endeavor is dependent upon the planning phase. Mission planning is fundamental to the success of operations, particularly when work must be conducted in isolated, confined and extreme (ICE) environments. Therefore, weather forecasts must be monitored weeks and days before fieldwork, since both tidal and wind regimes can influence the duration of surface activities, with pauses required until environmental conditions make it possible for work to continue safely. Days characterized by low values for these variables offer the promise of optimal performance by crew members. Remote reconnaissance of the study area can also be achieved by viewing satellite images (remote

sensing) or, if possible, in a more empirical manner, by asking local fishermen to provide updated information on conditions.

Subscriptions to applications such as Magicseaweed (MSW Pro) (Table S2) enable access to reliable 16-day advance weather forecasts. Details of daily conditions such as surf height, wind speed and direction are shown in Fig. S2. It is imperative that access be available to detailed information regarding the environmental conditions of the study area, such as geography and weather. On-board crew movement, material integrity, data recording, and on-site sample processing can be affected by local environmental conditions. Additionally, efficiency is fundamental to the conducting of research, and this can be achieved by creating templates and simulating data collection prior to the commencement of fieldwork. While at sea, the main factor hindering the execution of any on-board task is the movement of the vessel resulting from surface currents and oscillation, which may impede the free movement of the crew and, consequently, activities related to data-recording, including photography, biometric measurements and sample processing. In addition to sea conditions, another limiting factor is wind, which can result in the blowing away of any light material, beyond the crew's reach.

Vessel characteristics and navigation

Given the extension and frequently complex topography of the shallow rocky shores of southern Peru's coasts, small to medium-sized boats are most often used for ecosystem assessment. Although inflatable boats are appropriate and versatile platforms for scientific diving operations, work is mostly done together with, or in coordination with, artisanal fishermen (divers), using their own boats. It is in this context that the materials, steps and recommendations described below are provided, considering data collection engaged in from a small wooden vessel, with a length (8.1 meters), width (2.92 meters), gross tonnage (3.8 tons) and beam commonly associated with those of Peruvian Pisco-style shellfish boats (Fig. 1, Fig. S4). This type of vessel falls within the Class II small-boat category (i.e., 26 to <40 feet length) (National Oceanic and Atmospheric Administration, 2021) and within the Peruvian category of '*embarcación de cerco menor*' (DICAPI, 2021). Although it is not the fastest option, this type of boat is appropriate for surveying study sites, due to its sturdiness, resistance to (wave) impact and stability.

Each day, the crew set sail from a fishing cove ("Caleta San Nicolás», Fig. S1) in the early morning for the scheduled sampling stations, returning to the cove in the late afternoon, depending on environmental conditions. It is also possible to stay aboard overnight by anchoring in a sheltered cove («Dormidero», Fig. S1). This enables greater efficiency by reducing navigation time and giving more daylight hours in which to work. Time spent at sea

is partly determined by environmental conditions, but the human factor can also exert a strong influence.

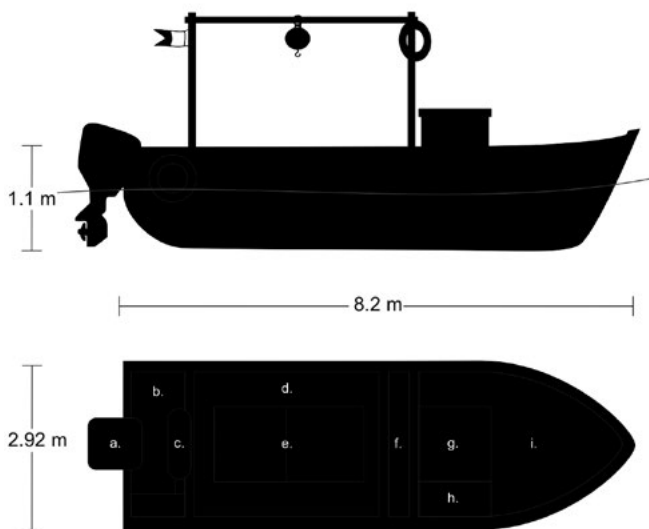


Figure 1. Artisanal boat showing measurements and areas for on-board activities. Top: side view. Bottom: overhead view. The top panel shows the frame of the artisanal boat with raised alpha flag (*diver down*) and hanging scale (max. 25 kg) for weighing kelp sporophytes. a) 40 HP engine, b) artisanal diver area for preparation, boarding and rest; c) low-pressure compressor for semi-autonomous diving (tethered, *hookah*); d) sides of the boat; e) roof storage; d and e) on-board workspace; f) dry section for diving cylinders and other devices, and scientific diver area for preparation and rest; g) rest chamber (overnight); h) galley; i) bow, space for anchor and shot line. See Fig. S4 for additional information.

Figura 1. Medidas de la embarcación artesanal mostrando espacios utilizados durante la actividad a bordo, superior: vista lateral; inferior: vista cenital. El panel superior muestra el armazón del barco artesanal con bandera alfa en alto (buzo abajo) y balanza colgante (25 kg) para pesar esporofitos de algas pardas. a) motor de 40 HP, b) área de buceo artesanal para preparación, embarque y descanso; c) compresor de baja presión para buceo semiautónomo (atado, *hookah*); d) laterales de la embarcación; e) tapa de bodega, d+e = espacio de trabajo a bordo; f) franja seca para cilindros de buceo y otros artefactos, y área de buzo científico para preparación y descanso; g) cámara de descanso (durante la noche); h) cocina; i) proa, lugar para ancla y línea de tiro. Ver Figura S4 para más información.

On-board (OB) and underwater (UW) workflow

Details of materials, equipment and software are shown in Table S1 and Table S2.

[OB]

The logbook manager at surface fulfils one of the most important roles during fieldwork. Their duties include recording any event, noting the time of the occurrence, observations, decisions taken, any failings, etc. Ideally, a log-form structure should be prepared prior to any survey, incorporating columns containing variables of interest. This not only facilitates the filling out and ordering of the data, it also avoids the possibility of forgetting about any measurement or observation that should be recorded. In

addition to surface data logging, workspace preparation and resource allocation is another important activity, as it reduces the response time to events such as those mentioned above. Preparation of the workspace must prioritize the crew's safe on-board movement, while protecting work materials and ensuring the processing of samples and the quality and time-efficient gathering of data. The availability of work material, ranging from measuring tapes and supplies (markers, bags, containers, etc.) to electronic devices such as cameras, GPS receivers or CTD (conductivity-temperature-depth) instruments, must be synchronized and checked at least once after anchoring, and before leaving each sampling station. It is advisable not to place objects near the gunwales: instead, materials should be stored in an orderly manner in closed containers or buckets while not in use. This keeps them dry and also readily available for immediate use. In order to gather data as accurately as possible, workflow must be systematic, efficient and coordinated. Adherence to the mind-set of *no data left behind* (Granzow-de la Cerda & Beach, 2010), while obtaining as much information as possible from the extracted organisms in a time efficient manner, is recommended.

Once the area of the sampling station has been reached, a HawkEye handheld depth finder or other device (Table S2) can be used to fix the location more precisely, in accordance with the requirements of the study (e.g., depth strata), before dropping anchor. Also, deploying a compact handheld CTD instrument (Table S2) can provide useful information on the water column temperature and conductivity of each sampling site. The sampling station is defined based on these readings, and the logbook manager proceeds to save the data, using a GPS receiver to establish the location.

Before the divers get in the water, it is recommended that the «SciDive Record» proposed by Moreno (2020a) is filled out, as it contributes to the logging activity while also serving as a checklist of materials and diving procedures. Five random numbers are dictated to the scientific diver (D1), who writes them down on the submersible slate. D1 must annotate on his/her slate the basic information for the sampling station and random numbers for replicates on the transect (Fig.2 and 3). After each immersion, the slate with annotations is photographed to save the data, and then erased with a mildly abrasive sponge, in preparation for the next transect. Also, either a clapperboard or an initial video of the area can be used to differentiate and sort the multimedia during the data download. All the materials required should be distributed among divers in accordance with the workload of each individual. A quadrat can be used to reel the transect line, in order to avoid chaotic uncoiling (Fig. S3A). The artisanal diver (or scientific diver #2) (D2) can carry the sampling bags, the modified axe («*barreta*»), the dismantlable circumference, and any other equipment needed for the underwater work (Fig. 2).

[UW]

Once underwater, the 20-meter transect is reeled out, ensuring that it is as taut as possible and at the same depth (isobath) (Fig. 3). The idea is to maintain a taut line, so both the start and the end of the transect can be tied to either a rock or kelp stipes (Fig. S3B). If the rugosity (Friedman *et al.*, 2012) of the substrate increases (e.g., large boulders or abrupt slopes), the direction of the transect should either be slightly changed (without altering the depth), or the natural obstacle should be contoured. Any of these events must be annotated on the slate. Once the transect is set and tightly deployed, D1 should register the depth and the compass bearing (azimuth) of the line on the submersible slate and start the measurements, according to the random numbers defined at the surface. Along the transect, at each predetermined random point, the 2m² circumference is assembled and, immediately next to it, the 0.25m² square frame is placed to record the photo/video-quadrat, using the *in situ* labeling approach recommended by Moreno (2020b). The density of sporophytes found within the circumference is annotated and then the morphometrics and meristics are conducted using a flexible measuring tape. This procedure is then repeated in each replicate (random number). Knowing the maximum holdfast diameters, two adult (DMR > 20cm) and two young sporophytes (DMR < 20cm) (Zavala *et al.*, 2015) are extracted in each transect using a «barreta» (modified axe, Fig. S3D). Immediately after its extraction, each sporophyte is placed in a collection bag made of 500µm mesh (Fig. S3E) and transported to the surface for processing (see next section). Once each transect is completed, a video is recorded in order to provide an overview of the kelp forest environment, then the transect is reeled in and, before surfacing, a safety stop is made at 5m for three minutes.

A short video has been edited and uploaded to YouTube, demonstrating some of the underwater procedures (https://youtu.be/zzhOBGybP_A).

[OB]

Data recording and sample processing

Photographic records

Photographic records gather details and information from the specific moment of extraction and can be revised later to reconstruct the workflow sequence. The photographic station can be installed even with rudimentary materials. It is recommended that a white contrasting surface be used, properly secured to the deck of the boat (e.g., a white rigid board or cloth, with weights on the edges) (Fig. S4C). Also, next to each sample, a labeled flask can be positioned, so that the time and location where the samples were collected can be readily identified. Another key advantage of the photographic record is the obtaining of detailed information that can be used to easily distinguish samples from each other (e.g.,

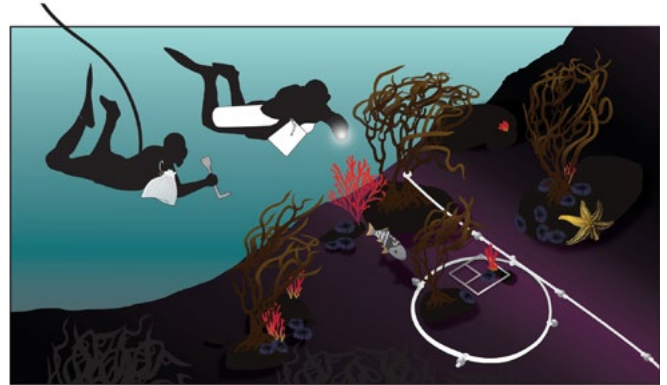


Figure 2. A depiction of the underwater ecological assessment work in *Lessonia trabeculata* kelp forests. First, the 20m transect line is deployed at a defined depth and secured at both ends. Direction (bearing) and depth are annotated prior to the evaluation. Then, the sand-filled dismountable circumference is placed next to the randomly selected knots for kelp density evaluation, while the stainless steel quadrat is positioned to establish area cover of macrobenthos (both fauna and algae). The artisanal diver (left) carries the modified axe («barreta») and the collection bags (500µm); the scientific diver (right), using a scuba sidemount configuration, carries the acrylic plate for *in situ* annotations (attached to a harness by stainless steel rings) and the camera system (action-cam with macro adaptor and video lights) for visual recording (photo/video quadrats, video-transects, additional sightings). Digital illustration by Bruno Cevallos.

Figura 2. Representación gráfica del trabajo subacuático para el estudio ecológico en huirales de *Lessonia trabeculata*. Primero, la línea-transecto de 20 m se despliega a un estrato de profundidad definido y se asegura en ambos extremos, la dirección y la profundidad son anotadas antes de iniciar la evaluación. Luego, la circunferencia desmontable rellena con arena (lastre) es colocada a la altura de los nudos seleccionados aleatoriamente para la evaluación de densidad, mientras que el marco cuadrado de acero inoxidable se coloca para la cuantificación de la cobertura del macrobentos (tanto de fauna como algas). El buzo artesanal (izquierda) lleva la barreta y las bolsas tamiz (500 µm); el buzo científico (derecha), en configuración lateral SCUBA, lleva la tabla de acrílico para anotaciones *in situ* (asegurada al arnés mediante llaveros de acero inoxidable) y el sistema de cámara (cámara de acción con adaptador macro y luces continuas de video) para los registros visuales (foto/video-cuadratas, video-transectos, observaciones adicionales). Dibujo digital realizado por Bruno Cevallos.

coalescent organisms, reproductive structures, evident epibionts, etc.).

Biometric records

Graphite pencils are ideal for writing down biometric measurements on acrylic boards. Both materials resist work under wet conditions very well. It is important to prioritize the integrity of these materials, remaining aware of the movements of the boat and wind, to ensure that all annotations are recorded correctly. The sporophyte should be accommodated maximizing its extension when recording the main measurements. For this, a water-resistant measuring tape is recommended. Measurements are taken of the maximum holdfast diameter (DMR), the minimum holdfast diameter (dmr), holdfast perimeter and elevation (from the basal end to the beginning of the cauloid). Then, the total length (TL), number of stipes, average length of the kelp blades

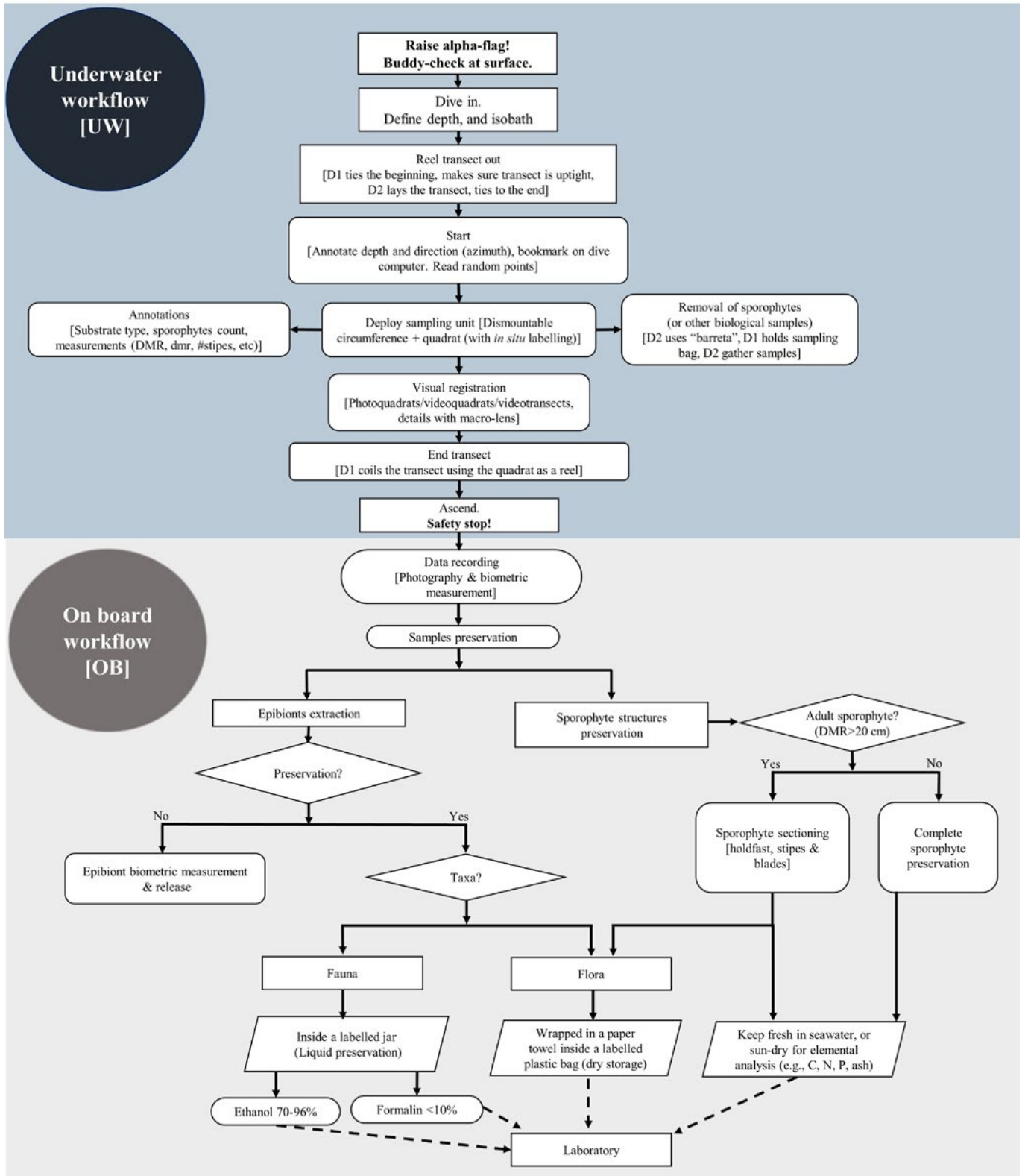


Figure 3. Flowchart showing workflow both underwater (top) and on-board (bottom), from diving safety measures to the preservation of sporophytes and associated organisms.

Figura 3. Flujograma que muestra el flujo de trabajo tanto subacuático como a bordo. Desde las medidas de seguridad para el buceo hasta la preservación de los esporofitos y organismos asociados.

(depending on the number of blades, between five to ten should be measured), and qualitative characteristics such as coalescent organisms and reproductive structures are recorded. Following meristic (counting features) and morphometric (measurements) recording (e.g., Atoche-Suclupe *et al.*, 2021), the total weight (fresh biomass) of the sporophyte is recorded using a hanging scale (Fig. S4D). To avoid damage or loss of the organisms associated with the plant, this final procedure is conducted only after the collecting of all seaweeds and fauna.

After biometric measurements and photographic records have been obtained, the sporophyte is sectioned along its main parts. Using the «barreta», the holdfast is separated from the rest of the sporophyte, thus facilitating manipulation and access to cavities from which the epibionts are extracted. For the extraction of invertebrates, the use of knives, stylets and tweezers facilitates optimal manipulation while avoiding damage, particularly in the case of small and hard sessile organisms, and other seaweeds. Each individual and associated representative structure can be stored in labeled plastic jars with seawater or ethanol/formaldehyde, pending proper preservation on land.

The human factor

Human behavior is a determinant factor in the success of the mission, given the different stressors associated with on-board work during scientific surveys in marine environments. While the human factor forms the basis of work at sea, it can also be the principal disruptive cause of accidents (Paua, 1999; Corovic & Djurovic, 2013; Apostol-Mates & Barbu, 2019).

Human adaptation to stressful conditions has been a central interest for researchers and those involved in the planning of expeditions to isolated, confined and extreme (ICE) environments, such as those found at sea, in the polar regions, and in outer space (Sandal *et al.*, 2006; Čorović & Djurovic, 2013; Groemer *et al.*, 2020). Although distinct in many aspects, fieldwork engaged in during the study of subtidal environments in relatively remote locations presents similar challenges, primarily associated with physical conditions, life support, crew attributes and operational goals. The main ICE factors affecting human performance at sea during the present study are described in Table S3.

Rohrer (1961) described three stages of crewmember response with respect to mission time, based on observations from Antarctic and submarine missions: anxiety at the start of the mission, mid-mission monotony and depression caused by routine, and late-mission euphoria as completion is anticipated. These three stages can be expected to manifest themselves if the assessment period is extended considerably in ICE environments (Sandal *et al.*, 2006).

Further recommendations

The workflow described and the recommendations included in this article can be considered in the design and planning of future surveys in the shallow subtidal environments of the region. Safety must be prioritized at all levels. All dives were planned taking into account no-decompression limits (NDL). The diving mode of choice will depend on logistics and facilities. This research was conducted using an artisanal diver's boat (Fig. 1, S3, S4), and therefore a hybrid diving mode was employed: the artisanal diver used surface-supply diving (SfS), and the scientific diver used either SfS or scuba (Fig. 2), or both (with the scuba sidemount configuration as a bail-out system). The scientific diver used a Suunto Vyper Novo dive computer for the decompression procedure (Table S2), and the artisanal diver followed the same suggested dive profile (this computer incorporates a RGBM -reduced gradient bubble model- algorithm). However, the artisanal diver may also use other available decompression tables for professional divers (DIRECTEMAR, 2022). NDL can be omitted if the scientific divers are trained in technical diving and if gas and cylinder management is conducted correctly and safely. However, for this kind of fieldwork this is not recommended, unless a portable hyperbaric chamber is available on-board. We recommend mandatory inclusion of a well-stocked first-aid kit and 100%-oxygen cylinder (both for underwater and on-board accidents) (Moreno, *In press*); the development of emergency contingency plans (for on-board, navigation and hyperbaric accidents), in which Very High Frequency (VHF) radios and Emergency Position Indicating Radio Beacon (EPIRB) devices are mandatory, as well as emergency numbers (coastguard, medical centers, etc.).

Low to null impact on the natural environment must be prioritized and supported by innovation and technology transfer, taking into account the traditional knowledge and on-field experience of artisanal fishermen. Parity is encouraged. Further studies should focus on psychological responses and human stressors, in order to improve crew performance during complex and risky scientific endeavors on the rocky subtidal reefs of the Humboldt Current Large Marine Ecosystem (HCLME).

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Conflicts of interest

The authors declare no conflicts of interest.

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