

Article

Design and Evaluation of Sequential Near-Bottom Mobile Organism Sampling Equipment Intended for Deep-Sea Mining Plume-Affected Zones

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Abstract

Deep-sea mining generates plumes containing heavy metals harmful to marine ecosystems. Assessing their impact on benthic organisms is essential. Traditional monitoring methods commonly employ acoustic, optical, and electrical approaches to investigate collective behaviors of organisms, while direct acquisition and in situ preservation of biological specimens during deployment remain challenging. To address this limitation, a novel sequential near-bottom mobile organisms sampling equipment equipped with multiple chambers was developed. Sequential sampling of near-bottom mobile organisms was conducted to obtain samples at different time points for individual-level biological assessment. This study investigates the alcohol diffusion dynamics at various injection volumes. The results show that during injection, alcohol is concentrated in the upper section of the chamber. When 5.0 L of alcohol is introduced, the alcohol mass fraction within the sampling container rapidly reaches and stabilizes at 87%. The sampling equipment's performance was evaluated through dock experiments and deep-sea trials at a depth of 1571 m in the South China Sea. Field tests successfully captured five jellyfish, with alcohol mass fractions of 85%, 87%, and 87% achieved in the respective chambers. These results demonstrate the feasibility of the equipment for sequential biological sampling and in situ preservation in deep-sea environments.

Keywords: ecological environmental monitoring; near-bottom mobile organisms sampling; alcohol injection; sampling equipment



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1. Introduction

In the context of carbon neutrality, the accelerating global transition to clean energy has led to a soaring demand for strategic metals such as cobalt and nickel, thereby intensifying interest in deep-sea commercial mining [1]. Nevertheless, mining activities disturb seafloor surface sediments, generating plumes enriched in iron and manganese ions [2,3]. Propelled by ocean currents, these plumes can disperse over scales of ~100 km and remain suspended for extended periods [4,5]. Such processes induce light attenuation and heavy-metal ion contamination, resulting in substantial disruption of deep-sea ecosystems [6,7]. Therefore, ecological and environmental monitoring during mining operations is imperative to mitigate potential adverse impacts.

Monitoring of mining-induced plumes has primarily focused on the physico-chemical characteristics of the surrounding seawater by using acoustic, electrical, and optical sensors. Measurements of parameters such as turbidity, suspended sediment concentration, and dissolved oxygen have been widely employed to quantitatively characterize plume geometry, spatial extent, and temporal persistence [8–14]. For example, a recent field experiment in the Clarion–Clipperton Zone deployed calibrated sensor platforms and autonomous vehicles to monitor mining-induced plumes at 4500 m depth. The study revealed sediment concentrations up to 10,000 times background levels near the source, with fine-particle plumes dispersing up to 4.5 km from the mining site [5].

Monitoring the ecological impacts of mining-induced plumes is often conducted by acoustic, electrical, and optical approaches. These approaches are typically employed to assess the effects of plume dispersion on the behavioral responses of biological communities. For instance, direct in situ video and imaging surveys conducted by Remotely Operated Vehicles and towed cameras provide valuable observations of behavioral responses, including avoidance, altered swimming patterns, or attraction to disturbed sediments [15–17]. Furthermore, acoustic monitoring has been employed to detect changes in fish and crustacean distributions in relation to plume dispersion, with quantitative analyses of backscatter intensity revealing shifts in animal density within affected zones [18,19]. Moreover, long-term seafloor observatories equipped with environmental sensors and imaging modules enable continuous assessment of faunal activity and habitat use before, during, and after plume events, thereby linking plume persistence to ecosystem-level responses [20,21].

Nevertheless, these methods primarily capture community-level ecological responses and seldom investigate the physiological or molecular pathways through which individual organisms cope with plume-induced stimuli. The effects of heavy metals in mining-induced plumes on the proteins, metabolism, and DeoxyriboNucleic Acid (DNA) of organisms are non-negligible. For example, excessive copper uptake has been shown to induce oxidative stress in organisms by increasing the presence of reactive oxygen species [22]. However, the underlying mechanisms of these impacts remain insufficiently understood owing to their complexity [23–25]. Therefore, studies targeting individual-level processes—such as DNA sequence variations and population genetics—are required to elucidate the pathways of adaptation and resilience in near-bottom mobile organisms exposed to mining-induced plume.

Therefore, a long-term sequential near-bottom mobile organisms sampling equipment (hereafter referred to as the “sampling equipment”) has been developed. The sampling equipment enables the collection of near-bottom mobile organisms at three distinct time points, thereby providing biological specimens for subsequent individual-level analysis of mining-induced stress responses and post-disturbance recovery dynamics. The remainder of this paper is organized as follows. Section 2 describes the structural design, sampling procedure and electronic control system. Section 3 introduces the investigation of the diffusion of alcohol and seawater through numerical simulations and experiments. Section 4 explores the diffusion of alcohol and seawater under different injection volumes and the results of the dock experiment as well deep-sea trials. Finally, Section 5 presents the conclusions.

2. Design of Sequential Near-Bottom Mobile Organisms Sampling Equipment

Near-bottom mobile organisms refer to near-bottom mobile fauna, including amphipods, crabs, benthic shrimps, near-bottom jellyfish, and juvenile demersal fish. During mining trials, sampling equipment was deployed near mining areas to collect samples once a month over a three-month period, alcohol injected autonomously to prevent degradation

of samples. After retrieval of the equipment, the biological samples are analyzed to assess genetic structures and morphological variations in near-bottom mobile organisms exposed to elevated concentrations of mining-related heavy metals. Because the equipment operates autonomously on the seafloor, deployment requires only a short amount of time, and retrieval can be conducted during a subsequent research cruise, eliminating the need for the vessel to remain at the site.

2.1. Structural Design

The sampler was designed for a water depth of 6000 m. Its length is 1080 mm, and the sampling tube has a diameter of 108 mm with an opening size of 162 mm in length and 70 mm in width. The volume of the sampling tube is 2.2 L, and the minimum mass fraction of alcohol is 70%.

As shown in Figure 1, the sampling equipment consists of an alcohol injection device, a channel switching device, and several samplers. The alcohol injection device uses a deep-sea motor (Model SDGA-02C11BD, Tode Technologies Co., Ltd., Shenzhen, China) to drive a piston downward, compressing a bladder containing 15 L of alcohol to release it. The bladder's compressibility maintains equal internal and external pressures at depth, eliminating the need for a pressure-resistant housing. The channel switching device, composed of a valve core and valve body, directs alcohol to different sampling tubes and can be rotated to Sample Positions 1, 2, or 3, or to an idle position that seals the outlet. Each sampler is arranged horizontally and includes a deep-sea motor, transmission mechanism, and sampling chamber, which comprises a cylinder and tube. The front end of the tube is fitted with bait and a cutter, with a backstop structure in the middle section. Bait is selected according to the target species—for example, rotten chicken is used when targeting amphipods due to its strong diffusion and attractant range. The deep-sea motor drives the transmission mechanism to convert rotary motion into linear motion of the tube, extending and retracting it. When fully extended, the cutter opens the vacuum-sealed bait. The top of the sampling cylinder is connected to a one-way valve allowing inflow, and the bottom to another one-way valve for outflow, enabling sample collection and alcohol injection.

2.2. Sampling Procedure

Preparation and deployment: The sequential sampling system was prepared by placing the vacuum-packed rotten chicken bait between the cutter and piston, slightly extending each sampling tube, and switching the channel device to the idle position. The equipment was then deployed via ROV or mounted on a lander. During deployment, the tubes were extended to achieve internal–external pressure equilibrium.

Sampling and fixation: As shown in Figure 2, the deep-sea motor of Sampler 1 drives the transmission mechanism, extending the sampling tube. Once the sampling tube has been fully extended, the cutter will cut the bait to expose it. After the predefined sampling duration, the sampling tube is retracted. The channel switching device is then switched to Sample Position 1. The alcohol injection device begins to inject 5 L of alcohol specifically into the respective sampling chamber, and the channel switching device is then switched to the idle position. After one month, Sampler 2 begins sampling, repeating the same sampling procedure. The channel switching device is then switched to Sample Position 2. The alcohol injection device begins to inject the alcohol. After one month, Sampler 3 repeated the same actions.

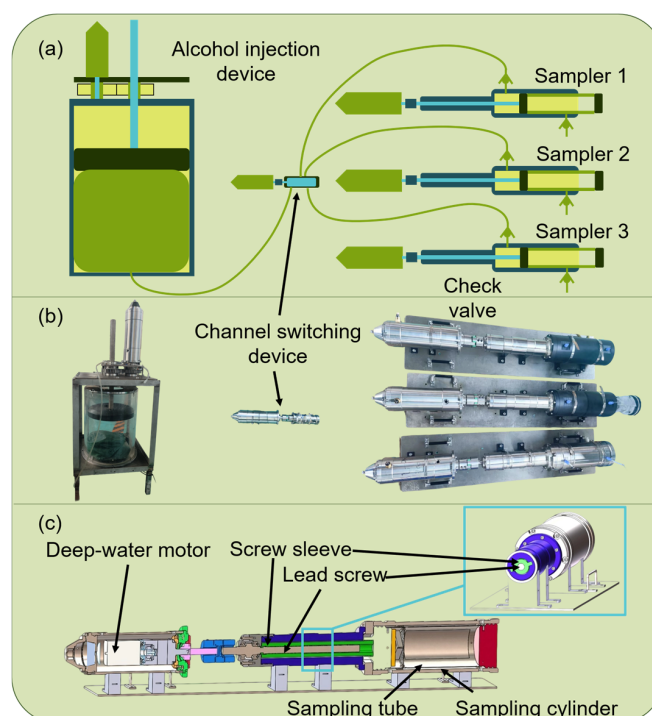


Figure 1. Diagram of the structure. (a) Schematic of the sequential near-bottom mobile organisms sampling equipment system. It shows the relationships between the various devices. The green lines connecting the devices represent polyurethane hoses. (b) Photographs of the physical devices, positioned corresponding to the schematic shown in (a). (c) Longitudinal cross-sectional view of the sampler.

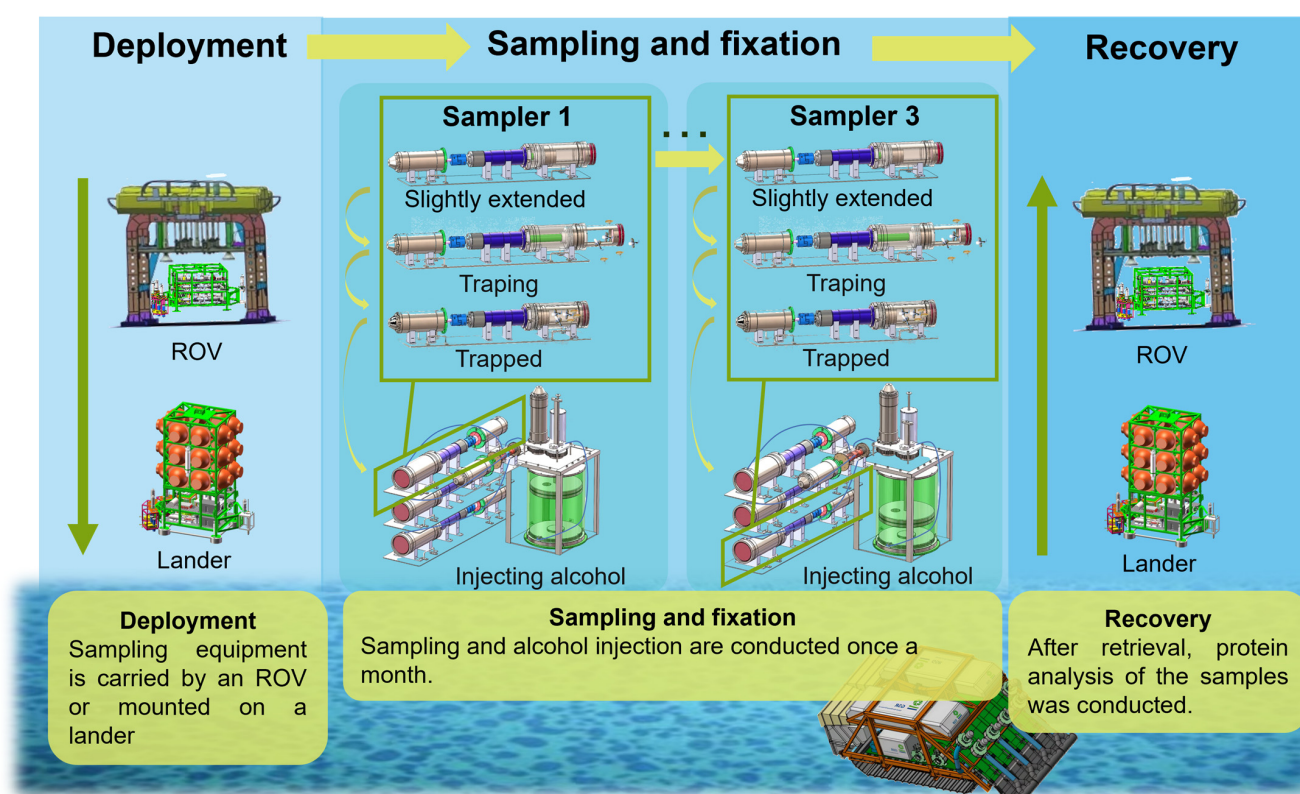


Figure 2. Workflow diagram of the sampling equipment.

Recovery: The sampling equipment is then retrieved, and near-bottom mobile organism samples are obtained at different times within a three-month period. Subsequently, the organisms within the various sample tubes are analyzed. The digestive glands, gills, and

other tissues of benthic organisms were dissected to examine genetic sequence integrity and morphological traits under metal ion exposure over time.

2.3. Electronic Control System Design

Given the inherent challenges associated with real-time communication at depths exceeding 6000 m, the system was designed to operate autonomously. As shown in Figure 3, the electronic control system employs a highly efficient dual-cabin power management architecture to physically decouple standby energy from heavy-load consumption. The dry cabin houses the main control board MCU-M, a video recorder, and one or two dedicated 3.7 V, 14,000 mAh rechargeable lithium battery packs. For a 12-month deployment, two packs connected in parallel provide a total capacity of 103.6 Wh. Utilizing an ultra-low-power 32-bit STM32L496RGT3 microcontroller and a wide-voltage input low-power DC-DC chip, the MCU-M operates independently with a standby power consumption of ~11 mW. This mathematically ensures continuous standby operation for over 13 months, based on 103.6 Wh divided by 0.011 W equating to approximately 9418 h. The pressure-compensated oil cabin includes a communication board MCU-C and a power distribution board Power-CP, which are powered by a separate, high-capacity 24 V, 4 kWh power battery pack for active-duty cycles. During standby, the power cabin is completely powered off. Upon a scheduled sampling event, the MCU-M wakes up the MCU-C via an isolated RS-485 bus, which then activates the Power-CP using MOS transistors to switch on the 24 V supply. The active-duty cycle per sampling event is precisely quantified: three 1.2 W deep-sea cameras and two 36 W LED lights operate for 30 min, consuming 1.8 Wh and 36.0 Wh respectively, to attract organisms and record the process. Concurrently, the 400 W sampling servo motor extends for 5 min and retracts for 5 min, consuming 66.7 Wh. The 400 W channel switching motor completes two 20 s rotations, consuming 4.4 Wh to seal the idle position. The 400 W injection motor operates for 5 min, utilizing 33.3 Wh to release 5 L of absolute ethanol. Consequently, the theoretical energy consumed per complete active sampling event is approximately 142.2 Wh. For the entire mission comprising three sequential sampling events, the theoretical total consumption is roughly 426.6 Wh. In actual sea trials and dock experiments, field measurements confirmed that the total energy consumption of the entire lander, including the sampling equipment and all other co-deployed instruments, was only 1.2 kWh, leaving an overwhelming margin within the 4 kWh capacity of the power battery. Furthermore, encoders integrated into the motors record the number of rotations, enabling accurate correction and closed-loop control of motor activity.

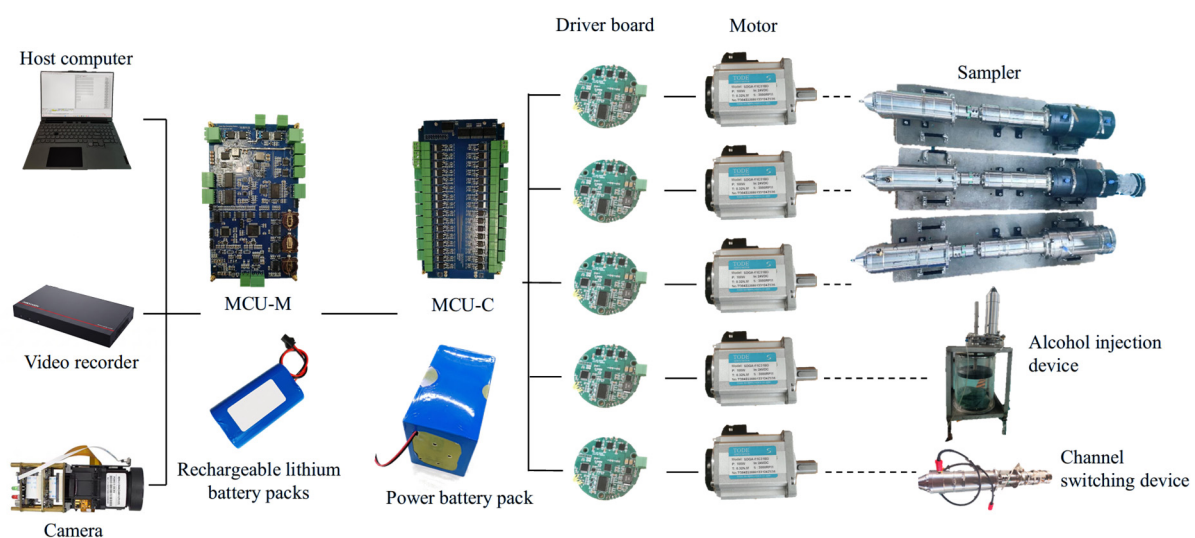


Figure 3. Electronic control system.

3. Methods

Due to prolonged deployment, microbial degradation of samples is likely, making high proportions of in situ fixation essential. Near-bottom mobile organisms are commonly preserved using 70% alcohol [26–29]. Efficient in situ alcohol injection is challenging due to miscibility with seawater, unknown outlet concentrations, and potential loss, requiring experiments to optimize diffusion, mixing, and injection volumes.

3.1. Numerical Simulation

The diffusion of alcohol in seawater was simulated using the software of computational fluid dynamics (CFD), FLUENT 2020 (ANSYS, Inc., Canonsburg, PA, USA). The mixture of alcohol and water is classified as a multicomponent flow problem [30]. The species transport model was applied to simulate flow and mixing behavior, with the configuration shown in Figure 4. Water (L_1) was assigned a numerical tracer to distinguish it from alcohol (L_2), enabling the determination of local concentrations of L_1 and L_2 at any location within the tube. The governing equations were formulated under steady-state conditions, assuming incompressible Newtonian flow. The continuity equation is expressed as follows:

$$\frac{\partial u_i}{\partial x_i} = 0. \quad (1)$$

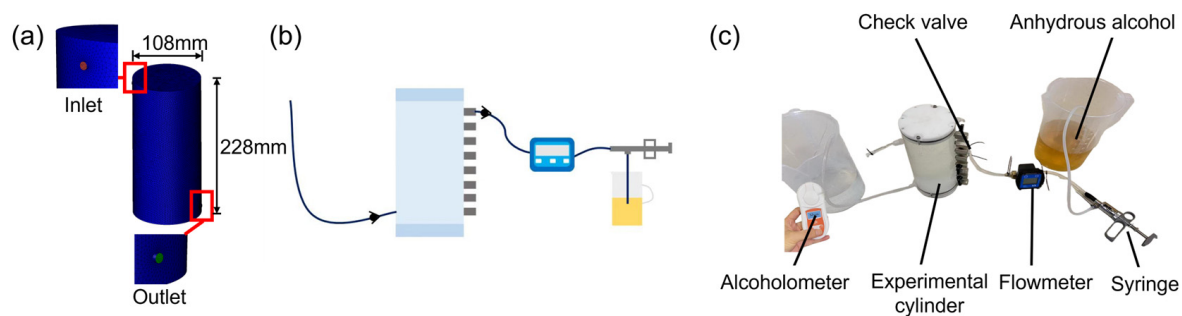


Figure 4. Experimental setup for alcohol injection. (a) Numerical simulation model and corresponding boundary conditions. (b) Schematic diagram of the system. (c) Photograph of the experimental setup.

The momentum equation is given by

$$\frac{\partial}{\partial x_j} \left[\mu \left(\frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i} \right) - \rho u_j u_i - \delta_{ij} p \right] = 0. \quad (2)$$

The scalar transport equation is given by

$$\frac{\partial}{\partial x_j} \left[\Gamma \left(\frac{\partial \Phi}{\partial x_j} \right) - \rho u_j \Phi \right] = 0, \quad (3)$$

where u_i and u_j denote the fluid velocity components in the spatial coordinate directions x_i and x_j , respectively, μ denotes the dynamic viscosity, ρ denotes the fluid density, δ_{ij} is the Kronecker delta, p denotes the fluid pressure, Γ denotes the diffusion coefficient from L_1 to L_2 (identical to those from L_2 into L_1 due to symmetry), and Φ denotes a scalar quantity, corresponding to the mass fraction.

The internal diameter of the sampling tube is 108 mm with a length of 228 mm. As shown in Figure 4a, the boundary conditions are defined as follows: the velocity inlet is the alcohol injection port, with a constant velocity of $v_1 = 0.1$ m/s. The set of constant velocity is based on empirical data obtained from the downward injection of alcohol. The constant pressure outlet represents the discharge port for both alcohol and seawater at

a constant pressure of 0.0221 MPa. It encompasses the opening pressure of the one-way valve (0.02 MPa) and the static water pressure prior to the one-way valve (0.0021 MPa). The diffusion coefficient between the alcohol and seawater was set to $6 \times 10^{-10} \text{ m}^2/\text{s}$. This value was adjusted for deep-sea ambient temperatures ($\sim 2\text{--}4^\circ\text{C}$), which significantly lowers the mutual diffusivity compared to room temperature [31].

To investigate the impact of cyclonic flow on turbulence, the RNG $k\text{--}\varepsilon$ turbulence model was used by solving RANS Equations (4) and (5) [32]:

$$\frac{\partial \rho}{\partial t} + \frac{\partial}{\partial x_i}(\rho u_i) = 0, \quad (4)$$

$$\begin{aligned} & \frac{\partial}{\partial t}(\rho u_i) + \frac{\partial}{\partial x_j}(\rho u_i u_j) \\ &= \frac{\partial p}{\partial x_i} + \frac{\partial}{\partial x_j} \left[\mu \left(\frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i} - \frac{2}{3} \delta_{ij} \frac{\partial u_l}{\partial x_l} \right) \right] + \frac{\partial}{\partial x_j} \left(-\rho \overline{u_i' u_j'} \right), \end{aligned} \quad (5)$$

where u_i and x_i represent the velocity components and spatial coordinates (acting as dummy indices for the velocity divergence), and $-\rho \overline{u_i' u_j'}$ represents the Reynolds stress tensor. The SIMPLE algorithm was used to couple the velocity and pressure, and the segregated solver was used to solve the steady flow. The solution was deemed convergent when the normalized residuals of the conservation equations were less than 10^{-5} .

3.2. Experiment

As shown in Figure 4b,c, the experimental setup consisted of an experimental cylinder, anhydrous alcohol, a flowmeter (Model K24, Shanghai Lanbao Sensing Technology Co., Ltd., Shanghai, China), a syringe, and an alcohol meter. The cylinder matched the dimensions of the sampling tube and was filled with seawater. Eight lateral switching valves, spaced 30 mm apart, allowed extraction of 2–3 drops of solution for alcohol mass fraction measurement using a calibrated dropper and alcoholometer (Model TY-SP02, Shandong Tianyan Instrument Co., Ltd., Weifang, China). The flowmeter measured the alcohol injection rate, and an iodine reagent was added to the alcohol to produce a stable brown color for visualizing its distribution within the cylinder. The iodine reagent was added in trace amounts, having a negligible effect on the bulk density and diffusion properties of the mixture. An injection syringe affixed to the upper terminus of the experimental cylinder was fluidically coupled to the cylinder through an inward-oriented check valve. Conversely, the cylinder's basal port terminated at an outlet check valve connected to a vented conduit exposed to ambient conditions. In an actual seafloor environment, the seawater at in situ pressure is situated outside the outlet check valve. However, during the alcohol injection experiment, atmospheric air surrounded the outlet check valve. To replicate the hydrostatic pressure conditions encountered during deep-sea sampling, the outlet check valve was positioned at the same height as the liquid level inside the experimental cylinder. It is acknowledged that simulating hydrostatic pressure via a liquid column at atmospheric conditions is a simplified approach. This setup primarily validates density-driven stratification; real bottom-layer hydrodynamics will be evaluated in future high-pressure flow analyses.

Anhydrous alcohol was injected into the experimental cylinder using the syringe. After each 200 mL injection of anhydrous alcohol, the mass fraction of alcohol in the experimental cylinder was measured. To do so the switching valves on the lateral side of the cylinder were opened to collect several drops of the mixture. These samples were then analyzed with the alcohol meter to determine the concentration of alcohol in the alcohol–seawater mixture. The alcoholometer initially recorded the volume fraction of the mixture. To ensure consistency with the CFD mass fraction outputs and the targeted 70% mass fraction specification, these readings were subsequently converted to mass fractions

based on standard ethanol-seawater density tables. The conversion was calculated using the equation:

$$\omega = \frac{V \times \rho_{eth}}{V \times \rho_{eth} + (1 - V) \times \rho_{sea}} \quad (6)$$

where ω is the measured mass fraction, V is the measured volume fraction, ρ_{eth} is the density of anhydrous alcohol (789 kg/m³), and ρ_{sea} is the density of seawater (1024 kg/m³) at the experimental temperature.

To investigate the effects of cylinder placement on the measured alcohol mass fraction, two experimental cases were conducted. Case 1: The cylinder was placed vertically. Case 2: The cylinder was placed horizontally.

4. Results and Discussion

4.1. Discussion of the Mixing and Diffusion of Alcohol and Seawater

To explore the effects of alcohol injection under different placements of experimental cylinders, experiments were conducted with the tube positioned horizontally and vertically. Figure 5 shows the distribution of alcohol for various injection volumes.

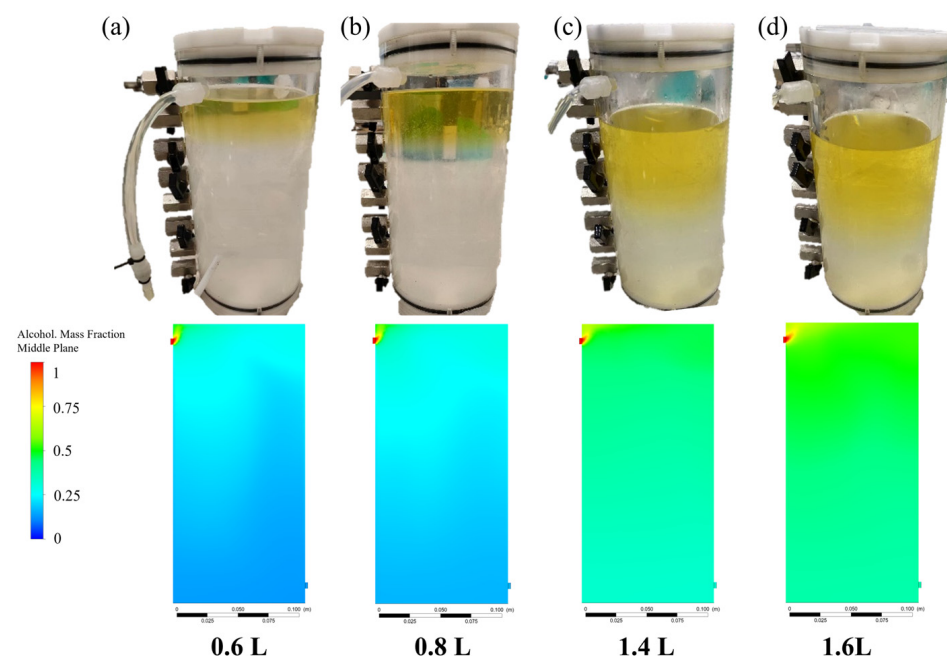


Figure 5. Distribution of alcohol in the experimental cylindrical barrel placed vertically without fish inside (Case 1) for different alcohol injection volumes. (a) Alcohol distribution in experiments and numerical simulations after injecting 0.6 L of alcohol. (b) Alcohol distribution in experiments and numerical simulations after injecting 0.8 L of alcohol. (c) Alcohol distribution in experiments and numerical simulations after injecting 1.4 L of alcohol. (d) Alcohol distribution in experiments and numerical simulations after injecting 1.6 L of alcohol.

The experiment revealed that when alcohol was injected from the top of the tube, the brown liquid swiftly ascended toward the top and stabilized there. It indicated that the alcohol rapidly ascended and spread laterally at the injection site, resulting in a consistent mass fraction of alcohol at each level. This rapid ascent was attributed to the lower density of alcohol compared with seawater. As the volume of the anhydrous alcohol injection increased, the amount of brown liquid at the top gradually increased, while the color faded toward the bottom. Notably, no clear interface was observed between the brown and transparent liquids, suggesting that the two liquids were miscible. Further data analysis was warranted to examine the distribution of the alcohol mass fraction in more detail.

In the experiment where the experimental cylinder was oriented vertically, the alcohol distribution across various depths, influenced by diverse volumes of alcohol injection, were clearly depicted in Figure 6a,b, corresponding to an infusion of 1.8 L of alcohol into the system. After the introduction of 0.2 L of alcohol, a rapid surge in alcohol concentration to 39% was observed at the uppermost section of the sampling tube. Further into the depth from the interface of the alcohol–water mixture, a sharp decline from 28% to 10% was recorded within the depth range of 55–85 mm, eventually tapering off to 0% at a depth of 115 mm. With a subsequent injection of 0.4 L, the concentration peaked at approximately 43% at the top, only to diminish rapidly along the depth gradient from 43% to 10% between 25 and 115 mm. Similarly, as the injected volume gradually increased, the mass fraction of alcohol at the topmost level also rose, along with an expansion in the volume of liquid with a high alcohol mass fraction. Furthermore, when the alcohol injection volume reached 1.6 L, the alcohol mass fraction at the upper portion of the sampling tube reached 72%. Even with continued injections, it proved challenging to further increase the mass fraction of alcohol. Quantitative comparison between the numerical simulation and experimental data yielded a Root Mean Square Error of 4.2%, indicating a reliable correlation and mathematically validating the CFD model. Furthermore, although high in situ hydrostatic pressures can slightly alter fluid properties and reduce molecular diffusivity, the rapid mixing process in this device is heavily dominated by injection momentum and macroscopic buoyancy. Because alcohol remains significantly less dense than seawater even at 60 MPa, the buoyancy-driven stratification and the 87% mass fraction are expected to hold firmly under deep-sea conditions. It should be noted that while the bulk mass fraction ultimately stabilizes at 87%, the relatively low passive diffusion rate in cold deep-sea water implies that local concentrations around the organisms might initially be uneven. However, the preservation process in this system does not rely on slow passive diffusion. The entire 5 L of absolute ethanol, more than twice the 2.2 L chamber volume, is aggressively injected into the chamber within 3 min. This rapid, high-volume injection forcefully flushes and physically displaces the residual seawater. Consequently, the organisms' exposure time to intermediate, low-concentration alcohol is under 3 min, effectively minimizing potential osmotic shock and ensuring the swift chemical fixation required for the robust preservation of macromolecules.

Figure 6c,d show the distribution of the mass fraction of alcohol along the depth direction when the sampling tube was placed horizontally, for different alcohol injection volumes, with a total of 4.2 L. The experimental results showed that as the injected volume of alcohol increased, the mass fraction of alcohol at the bottom and top of the sampling tube increased, with the rate of increase gradually slowing. When the injection volume reached 3.0 L, the rate of increase began to decline, ultimately reaching a plateau at approximately 87%. Additionally, the mass fraction of alcohol increased gradually with depth. At an injection volume of 2.0 L, the total mass fraction of alcohol in the experimental cylinder exceeded 70%. When the experimental cylinder was placed vertically, the total mass fraction of alcohol exceeded 70% only when the injection volume reached 1.6 L. This difference could be attributed to the position of the unidirectional valve, which was located at a depth of 0.108 m when the tube was placed horizontally and at 0.235 m when it was placed vertically.

Several trends regarding alcohol injection into the sampling tube were observed. (1) As the injected volume increased, the mass fraction of alcohol at the top gradually increased, with the rate of increase progressively diminishing. (2) Along the depth direction from shallow to deep, the mass fraction of alcohol sharply decreased within a certain range, rather than declining gradually. (3) When positioned vertically, the increase in the mass fraction of alcohol slowed markedly after reaching 72%, whereas when placed horizontally,

the mass fraction continued to increase until it reached 87%, after which further increases became difficult.

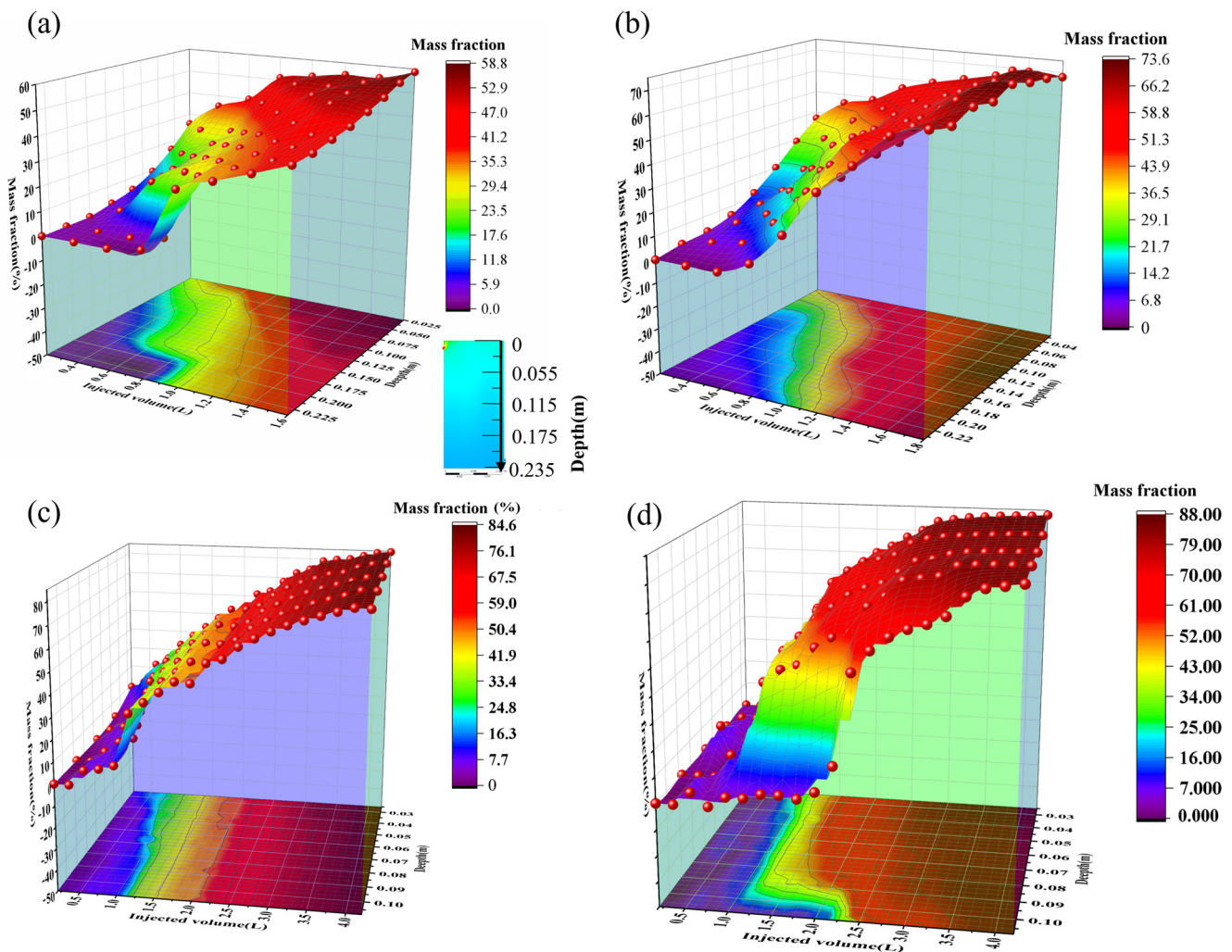


Figure 6. Relationship between the mass fraction of the alcohol and the injected volume when the sampling tube was positioned vertically and horizontally. (a) numerical simulation, Case 1; (b) physical experiment, Case 1; (c) numerical simulation, Case 2; (d) physical experiment, Case 2. “Injected volume” refers to the alcohol volume introduced into the tube, “Depth” to the distance from the alcohol–water interface, and the Z-axis represents the alcohol mass fraction at each depth.

Accordingly, the horizontal placement method was selected for the sampling equipment design. Notably, achieving an alcohol mass fraction of 87% in the sampling tube was more feasible with horizontal placement as the injected volume increased. Additionally, horizontal placement allowed for a more convenient layout, reducing the overall height of the sampling equipment. Experimental data and numerical simulations showed that an injected volume of approximately 3.0 L is the minimum threshold to reach an alcohol mass fraction plateau of 87%. However, to provide a substantial safety margin and ensure the rapid physical displacement of residual seawater, the actual engineering injection volume per chamber was determined to be 5.0 L, utilizing a 15 L bladder for the three samplers.

4.2. High-Pressure Experiment

To ascertain the dependability and pressure-holding capability of the sampling equipment, high-pressure tests were conducted in a high-pressure chamber, as illustrated in Figure 7, to simulate the deep-sea environment. The high-pressure chamber, with a max-

imum diameter of 0.8 m and a length of 2.7 m, was a cylindrical apparatus capable of withstanding a maximum pressure of 160 MPa. During the test, a pressure of 70 MPa was applied to the high-pressure chamber. The pressurization rate was 10 MPa/min, and after each 10 MPa increment, the pressure was maintained for 9 min to facilitate stress relaxation resulting from metal deformation. Once the pressure reached 70 MPa, it was maintained for 3 h. During this period, the extension and retraction of the sample tubes from the three samplers, the rotation of the valve core, and the injection of alcohol were performed sequentially. The pressure was then released at a rate of 20 MPa/min, and after each 10 MPa reduction, the pressure was maintained for 9.5 min to allow the device to adapt to the low-pressure environment. The evolution of the chamber pressure over time is illustrated in Figure 7.

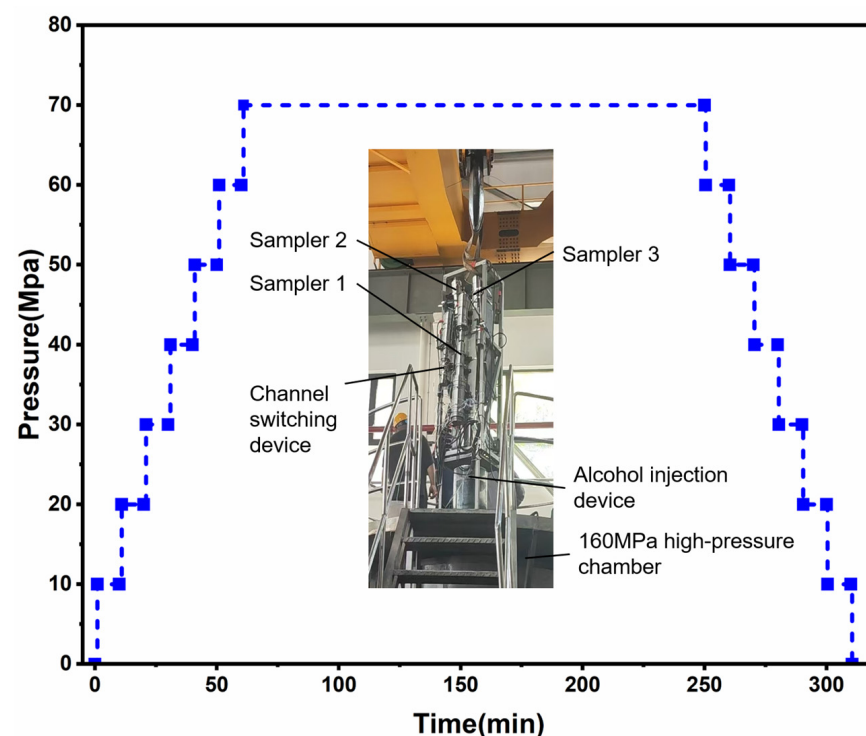


Figure 7. Pressure within the high-pressure chamber over time. At 0 min, the pressure in the pressure drum began to rise.

After full pressure release, the sampling equipment was retrieved for evaluation. The sampler successfully performed extension and retraction inside the high-pressure chamber. Rotation of the channel switching device and downward alcohol injection were also completed without issue. No damage was observed after removal from the high-pressure simulation setup. The device was thus demonstrated to withstand pressures up to 70 MPa.

4.3. Investigation of the Backstop

Prior to the formal sea trials, we conducted dock experiments to guide local structural optimization, improve sampling efficiency during sea deployment, fully utilize the opportunity of sea trials, and simultaneously verify the feasibility of the sampling equipment.

Two types of backstops were tested: rod and net. The rod backstop rotates around a shaft, allowing near-bottom mobile organisms to enter the sampling tube when pushed aside, but blocking their exit via a counter shaft. The net backstop uses a mesh layer with openings, permitting entry while preventing escape once inside. Sequential near-bottom mobile organism samplers were mounted on a lander with three platforms on one side

and two on the other. As shown in Figure 8a,c,d, to isolate the variables, Sampler 1 (net backstop) and Sampler 2 (rod backstop) were mounted at identical heights on opposite sides of the lander and operated simultaneously. Additionally, Sampler 3 (rod backstop) was mounted directly above Sampler 2 on the second tier to isolate the height variable. Alcohol injection and channel switching devices were positioned at the lander center. Dock experiments used rotten chicken bait to attract shrimp, crabs, and juvenile fish. Each session lasted six hours, and four trials were conducted to evaluate backstop effectiveness and trapping success.

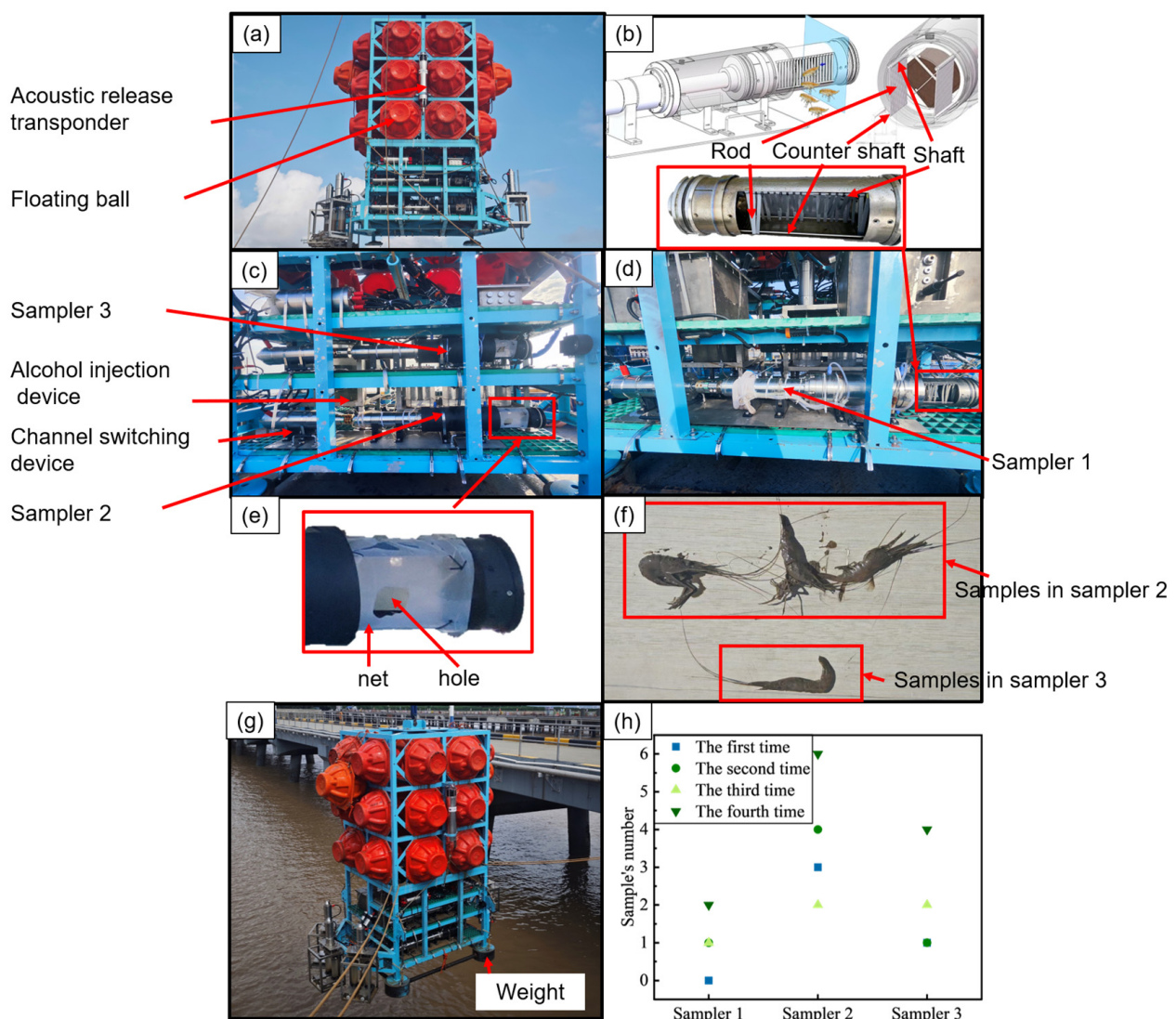


Figure 8. Dock test of the backstop design. (a) Photograph of the sampling equipment mounted on the lander. (b) Structural diagram of the rod backstop. The top left panel shows the side and cross-sectional views of the sampling tube with the rod backstop. The top right panel presents a detailed sectional view of the left cross-section. The bottom panels display a photograph of the actual sampling tube. (c) Samplers 2 and 3 were placed on one side of the lander. Both samplers used a rod backstop design. Sampler 2 was positioned on the first platform, whereas Sampler 3 was placed on the second level. (d) Sampler 1 was placed on the opposite side of the lander. Sampler 1 was equipped with a net backstop. (e) Photograph of the net backstop. (f) Samples inside each sampler during the first sampling. (g) Photograph of the dock experiment. (h) Number of samples in different sampling tubes across four test trials.

Figure 8f,h show the trapping results. Captured near-bottom mobile organisms were mainly shrimps, with a few juvenile fish. Average captures were 1, 3.75, and 2 for Samplers

1, 2, and 3, respectively. Sampler 2, using the rod backstop, captured 3.75 times more organisms than Sampler 1, indicating higher trapping efficiency. This may be due to the rod's opaque structure, reducing the propensity of near-bottom mobile organisms to enter the sampling tube, and its weight, making it harder to push. The net backstop's open mesh allows easier passage, lowering trapping success. Sampler 2, placed lower than Sampler 3, captured 1.875 times more organisms, suggesting higher efficiency near the seabed where near-bottom mobile organisms are more active. Overall, combining a rod backstop with low sampler placement enhances trapping efficiency for near-bottom mobile organisms. However, it must be explicitly stated that given the small sample size, these comparative results remain qualitative and warrant further statistical validation.

4.4. Sea Trials and Results

To ensure the proper functioning of the sampling equipment under deep-sea conditions, a one-day deep-sea experiment was conducted in the South China Sea. The sea trial site was located at 115°28.6973' E, 19°51.7436' N with a water depth of 1571 m. The sampling equipment was installed on a self-powered lander equipped with several cameras and deployed aboard the research vessel Da Yang Hao in the northern South China Sea. Due to the short duration of the experiment, the buoyancy materials were removed from the lander, and an electrooptical cable was used for deployment and recovery. During deployment, the ship activated dynamic positioning to prevent drift, and video signals were transmitted to the vessel via an electrooptical cable. When the lander reached 50 m above the seabed, it was slowly lowered until it made contact with the bottom. After landing, the sampling tube of Sampler 1 was extended. For long-term deployment, the standard sampling duration is set to 6 h. However, due to the operational constraints of the electrooptical cable used during the sea trial, the sampling duration was temporarily adjusted to 1.5 h. After sampling, the sampling tube was retracted, followed by alcohol injection.

Due to the high cost and logistical constraints of deep-sea operations, this single deployment serves primarily as a functional validation of the mechanical and operational workflow rather than a quantitative ecological baseline survey. As shown in Figure 9, the experimental results confirmed that all three samplers successfully completed the programmed extension and retraction operations. The channel switching device effectively performed precise sample tube transitions, and the alcohol injection device consistently administered the actions of 5 L alcohol injection. Each sampling tube contained a well-preserved mixture of the collected specimens and a preservative solution. The field data show that five morphologically intact near-bottom jellyfish (measuring approximately 3–5 cm in bell diameter) and several benthic shrimps were captured across the three sampling units. These samples were well-preserved, remaining highly viable for subsequent laboratory molecular analyses rather than being discarded. Compared to traditional unpreserved baited traps or ROV-operated suction samplers, this autonomous sequential injection method ensures rapid *in situ* chemical fixation, mitigating the risk of tissue degradation [33]. The alcohol concentration measurements in the sampling cylinders reached 85%, 87%, and 87%—considerably exceeding the 70% threshold required for effective biological tissue preservation in near-bottom mobile organisms [26]. Moreover, this result is consistent with the findings from numerical simulations and experiments on the diffusion of alcohol and seawater mixtures. These sea trial results demonstrated the system's operational reliability.

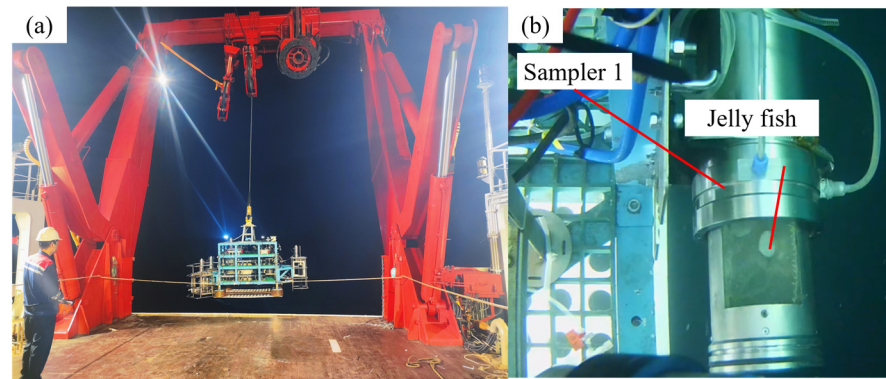


Figure 9. Sea trial in the South China Sea. (a) Sea trial site. (b) Research vessel used for deployment.

It should be noted that while the chamber tests validated mechanical integrity up to 70 MPa, equivalent to 6000 m, this field validation occurred at 1571 m, which is well below the maximum design depth. Operations at the 6000 m design depth await further field verification. In future study, experiments will be carried out in polymetallic nodule areas, during which a mining vehicle will conduct extraction, and the sampling equipment will perform sequential near-bottom mobile organisms collection.

5. Conclusions

This study developed a novel sequential near-bottom mobile organisms sampling equipment that consists of three sets of benthic samplers, an alcohol injection device, and a channel switching device. The sampling equipment enables the collection of near-bottom mobile organisms at three distinct time points, thereby providing biological specimens for subsequent individual-level analysis of mining-induced stress responses and post-disturbance recovery dynamics. This addresses the limitations of current biomonitoring approaches that predominantly focus on community-level behaviors.

To investigate the spatial distribution of the alcohol mass fraction within the experimental cylinders as the injected volume increased, numerical simulations and experimental tests were conducted under different placements. The result showed, as the injected volume increased, the mass fraction of alcohol at the top gradually increased, with the rate of increase progressively diminishing. When the sampler is positioned horizontally and the injection volume reaches 3 L, the mass fraction of alcohol will attain 87%.

Next, 70 MPa high-pressure and dock experiments were conducted to evaluate the sampling equipment's pressure resistance and feasibility. A deep-sea trial conducted at 1571 m depth in the South China Sea successfully captured five jellyfish using the sampling equipment. The alcohol concentration measurements in the sampling cylinders reached 85%, 87%, and 87%, demonstrating the sampling equipment's capability to both collect near-bottom mobile organisms and maintain alcohol concentrations exceeding 70%. A limitation of this current study is that the field trial was conducted in undisturbed waters, and the system's performance in turbid, heavy-metal-laden plume environments was not directly evaluated. Furthermore, the deployment and recovery of the lander-based system are inherently constrained by weather and surface sea conditions, typically requiring a Sea State of 4 or below for safe operation. While the energy system supports multi-month endurance, extended field parameters such as biofouling tolerance remain to be validated in future long-term deployments. Future research will focus on deploying this device directly in polymetallic nodule mining areas synchronized with mining vehicle operations. To mitigate the systematic detritophagy bias observed in current dock trials, multiple samplers with varied, taxon-specific chemo-attractants will be deployed alongside standard benthic trawl comparisons. Furthermore, the equipment will be co-deployed

on a benthic lander equipped with turbidity sensors, optical cameras, and heavy-metal water samplers to directly correlate the captured specimens with precise plume metadata, enabling individual-level physiological evaluations of near-bottom fauna exposed to actual mining plumes.

Author Contributions: K.W. designed the study and drafted the manuscript. J.G. reviewed the manuscript. H.Y. drafted the manuscript. J.C. designed the study and drafted the manuscript. X.T. and J.W. assisted with the experiment. B.H. designed the study. J.Y. helped with the experiment. C.H. processed the data. All authors have read and agreed to the published version of the manuscript.

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