



A Test in a High Altitude Lake of a Multi-Parametric Rapid Methodology for Assessing Life in Liquid Environments on Planetary Bodies: A Potential New Freshwater Polychaete Tubeworm Community

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On our planet, aqueous environments such as deep sea or high-altitude aphotic lakes, subject to present or past volcanic activity and active deglaciation, may provide analogs to the aqueous environments found on such planetary bodies as Europa, Titan or Enceladus. We report here on the methodologies and technologies tested in Laguna Negra, a high altitude lake in the Central Andes, Chile, for exploring and assessing the presence of life within planetary lakes or interior oceans. We adopted a multi-parametric Rapid Ecological Assessment (REA) approach centered around collecting video imagery (by an Underwater Imaging System) and sampling benthic sediments (for sedimentological, biological and geochemical analysis) to depths of 272 m, to complement physico-chemical sampling of the water column and collection of shallow sediments for microbiological analysis (reported in separate publications). This enabled us to classify and assess the apparent status of benthic habitats, based on substrata and environmental characteristics, together with floral and faunal community characteristics and bioturbation artifacts. Video imagery showed that the lower water column was characterized by a variably intense sestonic flux of particles and debris, among which were planktonic organisms such as ostracods, copepods, and possibly cladocerans. Sediment analysis revealed at all depths abundant diatom frustules, mainly of an acidophile pennate diatom *Pinnularia acidicola*, amid vegetal debris likely originating

from littoral macrophytes. Video imagery showed that the lakebed was partly covered by microbial mats and depositional matter and harbored an unexpectedly rich assortment of macrofauna, including sponges, tubificid worms, flatworms, bivalves and crustaceans. Various forms of bioturbation were also encountered, some with the animals in the tracks. Most notably, at the deepest site, a previously undescribed faunal feature was evident, apparently formed by a mat-like community of several layers of what appeared to be polychaete tubeworms, possibly of the family Siboglinidae. It is hypothesized that the hydrothermal activity observed in the region may supply the compounds able to support the deep-water microorganisms from which such tubeworms typically gain sustenance. Such processes could be present on other deep and aphotic liquid-water-bearing planetary bodies.

Keywords: planetary exploration, rapid environmental assessment, life detection, macrophytes, diatoms, high altitude lakes, andes environment, tubeworm communities

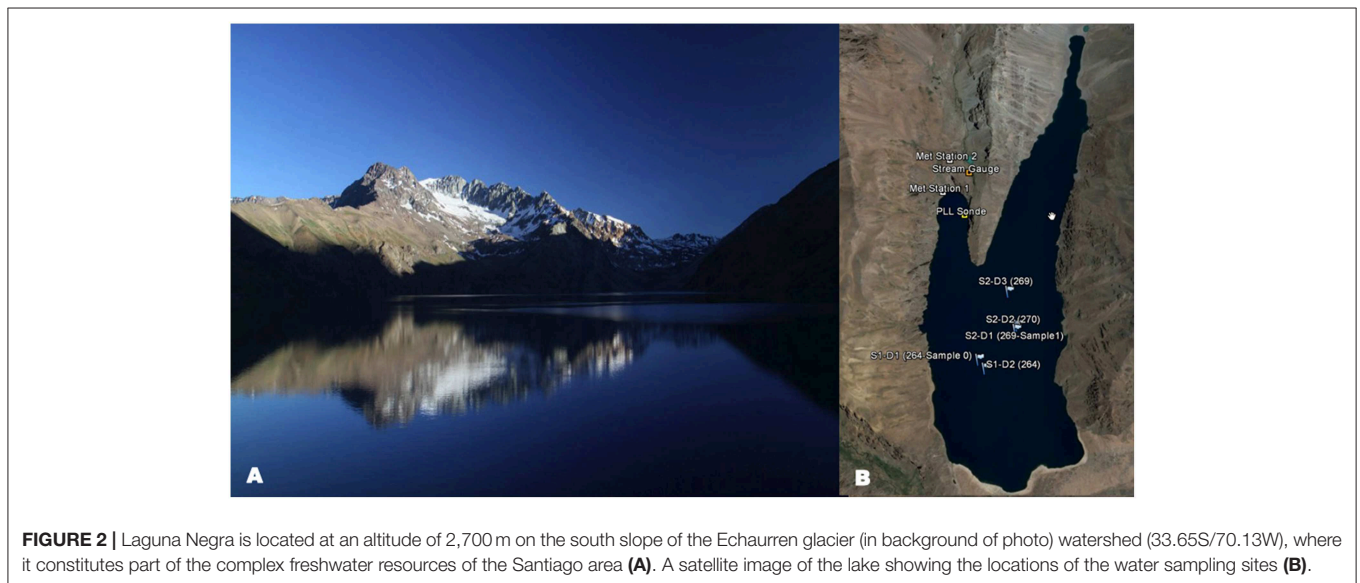
INTRODUCTION

Interpretation of images taken by the Hubble Space Telescope would suggest that plumes of water erupt from Jupiter's icy moon *Europa* (Roth et al., 2014) and from *Enceladus*, one of Saturn's moons (Postberg et al., 2011; Thomas et al., 2016). These plumes are thought to be material ejected from internal oceans or lakes existing beneath an external ice crust as a result of hydrothermal activity, as suggested by Hsu et al. (2015), Postberg et al. (2011) and Thomas et al. (2016) for *Enceladus* and Vance et al. (2007) for small ocean planets. Consequently other authors have envisioned that *Europa's* inner oceans might contain a variety of potentially life-supporting environments (Chyba and Phillips, 2002; Pappalardo et al., 2013; Phillips and Pappalardo, 2014), ranging from hydrothermal vents to cold liquid brines (Lowell and DuBose, 2005; Russell et al., 2017). In addition, an internal ocean of salty water is thought to exist on *Titan*, as revealed by its rotation (Lorenz et al., 2008), while the presence of liquid methane lakes on its surface is confirmed by data received by the Cassini-Huygens spacecraft (Sagan and Dermott, 1982; Lorenz et al., 2008; Lunine and Atreya, 2008; Moriconi et al., 2010).

NASA's *Europa Lander Project* has as its aim the detection of life or its fingerprints in the internal ocean system of *Europa*, through exploration and sampling of potential ocean life-forms or of biotic material that might have accumulated through time (Pappalardo et al., 2013; Russell et al., 2017). With this purpose in mind the Planetary Lake Lander (PLL) project (Cabrol et al., 2012, 2014; Pedersen et al., 2015) selected the extreme environment of Laguna Negra, a glacier lake in the southern volcanic zone of the central Chilean Andes, in order to test methods and technologies suitable for work in extreme planetary aqueous environments. The PLL campaign undertaken in 2013 involved a complete investigation of the lake ecosystem, including (i) collection of data on bathymetry (Cabrol et al., 2015), temperature and light distribution levels through the whole water column (Rose et al., 2014; Cabrol et al., 2015), (ii) sampling for water chemistry and the distribution

of organics (Sobron et al., 2013), and (iii) investigation of the microbiology of both benthic sediments (Parro et al., 2018) and the water column (Echeverría-Vega et al., 2018). Complementary work involved the analysis, using a Rapid Ecological Assessment (REA) approach, of video imagery collected from all depths, supplemented by the collection of water-sediment samples from the bottom of the lake for sedimentological, biological and geochemical analysis (Tilot, 2014a). The latter supplemented previous microbiological analysis of bottom sediments using (a) DNA extraction, cloning and sequencing, (b) ammonia oxidizing activity and nitrate and sulfate evolution testing in separate mesocosm experiments, and (c) microarray immunoassays with an immunological Life Detector Chip (LDChip) with a 300-antibody microarray biosensor specifically developed for planetary exploration and environmental monitoring (Parro et al., 2018, 2019). Here we present the main results of the REA work directed at to a multi-parametric assessment of the lake ecosystem directed at understanding the structure and functioning of the lake ecosystem.

A separate issue that it was intended for the PLL to address was the concern about the capacity of high-altitude lake systems to buffer the potential effects of nutrient addition anticipated as a consequence of either periodic deglaciation or the deglaciation associated with current climate change CONAMA (2007). Glacier surface areas have diminished by more than 30% in the past 50 years, a trend that has accelerated recently (Rabatel et al., 2011), including for the Echaurren Glacier close to Laguna Negra (Escobar et al., 2000; Carrasco et al., 2005; Rabatel et al., 2011). Increased nutrient input to high altitude lakes has been anticipated as a result of either increased input of water as a result of glacial melt (Roig et al., 2001; Le Quesne et al., 2009), or as a consequence of melting of the upstream permafrost within the catchment (Rogora et al., 2008; Salerno et al., 2016). Such alterations may lead to oligotrophic lakes becoming more eutrophic (Lyons et al., 2006; Modenutti et al., 2013) and to linked changes in trophic chains and benthic habitats (Modenutti et al., 1998, 2013; Uehlinger et al., 2010).

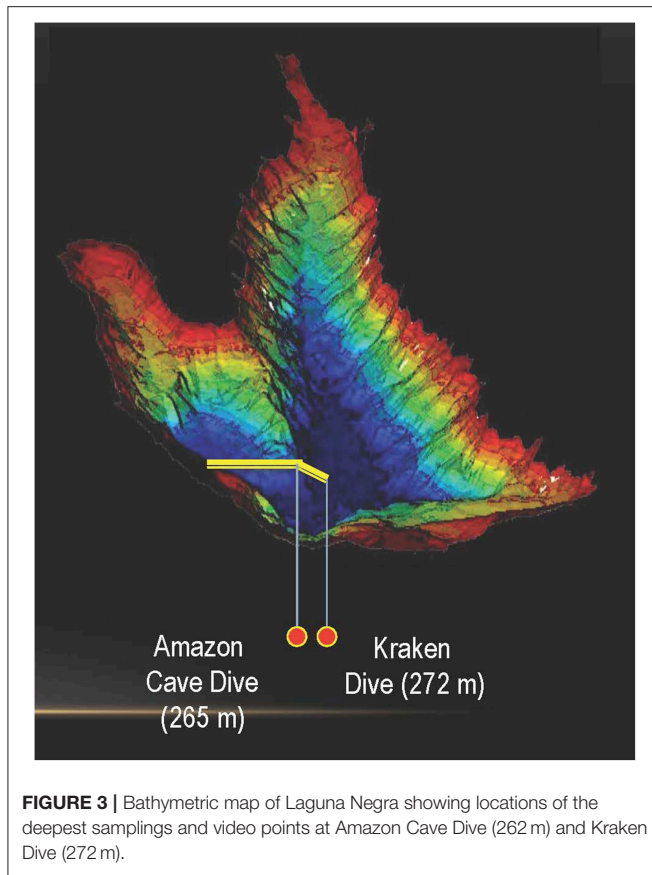


MATERIALS AND METHODS

General Description of the Lake

Laguna Negra is located at an altitude of 2,700 m on the south slope of the Echaurren Glacier watershed (33.65S/70.13W), where it constitutes part of the complex freshwater resources of the Santiago area (Figures 1, 2A). It has a maximum depth of 272 m, its size (6.1 × 1.7 km) and form being the result of glacial carving of a deep narrow valley combined with local tectonic deformation of bedrock (Farias, 2007) (Figures 2B, 3).

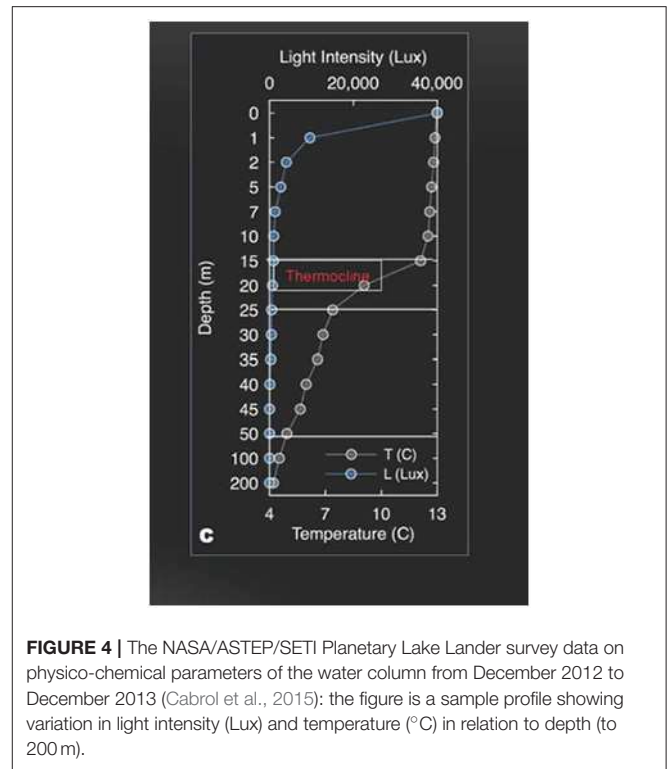
During the austral summer, December–May, the lake becomes stratified, with water temperatures in the upper euphotic zone, down to 20 m, reaching 12–20°C (Figure 4), whereas during July–October there is a winter overturn with temperatures falling to 4–5°C throughout the water column (Cabrol et al., 2015). The lake's water is relatively transparent, with turbidity declining from closet to 1 NTU near the surface (0–5 m) to 0.9 NTU in deeper water (>20 m) (Cabrol et al., 2015); thus there is a relatively deep euphotic layer (Figure 4), but below this a large aphotic zone, so that the lake's overall mean primary production



is relatively low, resulting in its classification as a cold (4°C), deep, essentially oligotrophic lacustrine ecosystem (Montencino, 1991; Soto, 2002). Laguna Negra is one of at least 84 closed-basin lakes present in this part of Chile and adjacent Bolivia, most of which are saline in nature (Risacher et al., 2001; Alvial et al., 2008), but the water column of Laguna Negra has been recorded as having a low conductivity (130–139 uS/cm) (Echeverría-Vega et al., 2018) indicating that, at least in its upper parts, it is not at all saline. Likewise, as reported in Echeverría-Vega et al. (2018), the pH of the upper portion of the lake appears to be slightly alkaline (pH 7.1–7.4), although, as discussed further below, faunal evidence suggests that the conditions near the lakebed are more acidic.

Multi-Parametric Rapid Ecological Assessment (REA)

Ecological assessment of an ecosystem is undertaken to evaluate relationships among organisms and their natural habitat and biophysical environment (Chapman, 1996; Hill and Wilkinson, 2004; Spellerberg, 2005; Hering et al., 2006; Tilot et al., 2008). Rapid Ecological Assessment (REA) is a flexible, accelerated and targeted biodiversity and environmental survey methodology, based on a collection of semi-quantitative field data, or of quantitative data on selected indicator variables. The approach addresses the lack of detailed data that necessarily results when survey time or resources are very limited, or the area to be



surveyed is unexplored and/or very extensive. Nevertheless, it is intended to preserve spatially explicit information and facilitate integration of data obtained from different sources and at different scales. Ideally it can be used to generate useful and visually appealing products that can be used to facilitate conservation and management. The REA methodology thus bridges the gap between collection of high volumes of quantitative data and purely qualitative assessments of aquatic or other habitats, while nevertheless allowing statistical comparisons in a manner that makes it a useful ecosystem management and decision-making tool (Tilot, 2014b; Tilot et al., 2018a). The approach serves to provide as close to a quantitative approach as practicable in situations where collection of samples for quantitative analysis is not possible.

In the present case the REA approach was adapted to provide a simple, yet robust, means for obtaining semi-quantitative data from video imagery that would achieve the characterization of distinct habitats or sub-habitats, together with their associated fauna and flora, and an assessment of environmental state. For this purpose, a video camera and sediment sample collection equipment were attached, along with a YSI 6600 multi-parameter probe, to a “lake-lander” platform (Sobron et al., 2013; Rose et al., 2014; Cabrol et al., 2015), which was lowered or towed from a small inflatable boat (Figures 5A–D).

Imagery Collection and Analysis

For video imagery collection the lander, fitted with a GoPro Hero3 camera (resolution of 1,920 × 1,080) with a housing and lights rated for 300 m depth, was guided along “video transects” that covered an area extending from the western shoreline

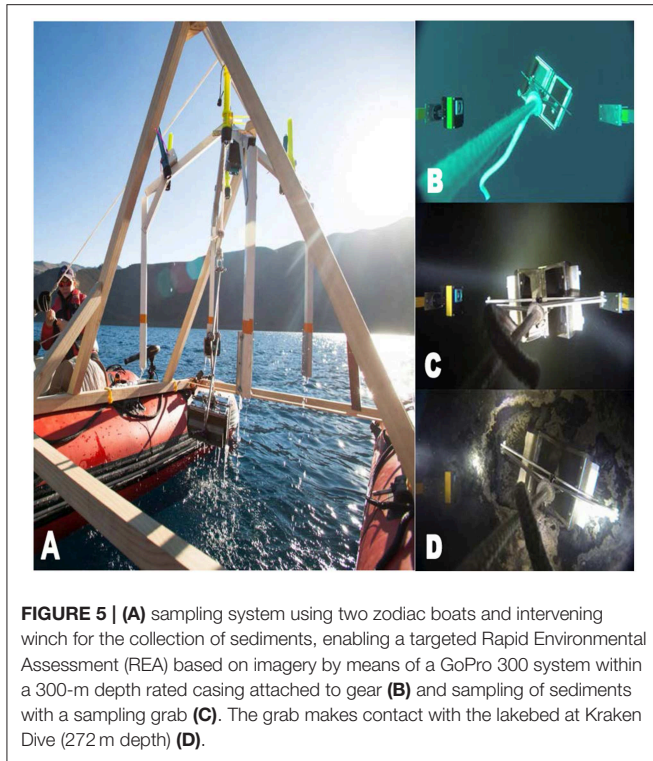


FIGURE 5 | (A) sampling system using two zodiac boats and intervening winch for the collection of sediments, enabling a targeted Rapid Environmental Assessment (REA) based on imagery by means of a GoPro 300 system within a 300-m depth rated casing attached to gear (B) and sampling of sediments with a sampling grab (C). The grab makes contact with the lakebed at Kraken Dive (272 m depth) (D).

to the deepest part of the lake. The lander with camera was deployed for a total of more than 210 h, during which video imagery was recorded on traverses at two sites, from shallow to deeper waters, at Amazon Caves (AC) up to approximately 90–100 m depth and at Peninsula West Shore (PSW) up to approximately 40–50 m depth. In addition, a series of vertical “jump” or “spot” dives (lowering the lander directly to the lake bed and lifting it again) were performed with the lander and video cameras to reach landing sights at target depths of 50, 60, 70, 80, 90 and 100 m. Jump dives were also achieved at the greatest depth obtainable on the lakebed at 4 separate sites, “Amazon Caves” (AC) (2 sites at 262 m), and 2 sites at Kraken Dive (KD) (272 m) (Cabrol et al., 2015) (Figures 2B, 3). Video material was analyzed through visual assessments by an expert observer related to each of a series of timed portions of each bottom transect or jump dive. The observer recorded habitat, and floral and faunal characteristics, along with the presence of evident biota or signs of bioturbation. So far as practicable, features were graded on a semi-quantitative scale of 1–3 or 1–5, with the assistance of written criteria used to aid the recognition of each grade. Where sufficient data was available, scaled values were analyzed statistically to assist recognition of characteristic habitats or biological communities and to assess the apparent status of benthic habitats (Tilot, 2014a).

Sediment Collection and Analysis

Using samplers attached to the Lake Lander, sediments were sampled for sedimentology, biogeochemical, and biological analysis at a depth of 262 m at site AC and a depth of 272 m at KD. The first samples were collected at KD during 2013 (sample

S0) (Figure 5D) and two additional samples at AC in April 2015 (samples S1). The sediment samples were collected in 50 mL sterile Falcon tubes, kept refrigerated during shipment, and then stored at 4°C (the temperature measured on the lakebed) pending further treatment (Cabrol et al., 2015).

Grain-size analysis was carried out in the Sedimentology Laboratory of the Spanish Institute of Oceanography (Málaga, Spain). Replicate samples (1 g each) were pre-treated with 1N HCl to remove carbonate particles and 10% H₂O₂ to oxidize organic matter, 1% sodium polyphosphate was added to prevent particle flocculation, and particle size-distribution data obtained using a laser diffraction method in a Malvern Mastersizer 3000 (grain-size range from 0.01 to 3,000 μm). Grain-size statistics (mean particle size, sorting, skewness and kurtosis) were calculated (leptokurtic distributions having more values toward the mean than in a normal mesokurtic, distribution) and samples classified according to the sorting classes of Folk and Ward (1957).

Biological examination of the water-sediment interface samples was carried out at the University of Málaga, Department of Ecology and Geology, using an optical microscope with 200× enlargement. Examination of phytoplankton was undertaken using 1 ml sub-samples within sedimentation chambers viewed with an inverted microscope, Protozoa and copepod nauplii were classified under a binocular microscope with 200× magnification, and larger organisms, such as copepods and cladocerans, determined in 1 ml samples under a binocular microscope with 100× magnification.

Data concerning the physical, biogeochemical and microbiological foundation of this region has been published previously (Montencino, 1991; Catalán et al., 1993; Mermillod-Blondin et al., 2001; Van der Vijver et al., 2004; Alvia et al., 2008; Modenutti et al., 2013; Echeverría-Vega et al., 2018; Parro et al., 2018) and will not be reviewed here, but referred to when appropriate.

RESULTS

Benthic Sediments

The texture of the total and the carbonate-free fractions of sample “Lake S1” (collected at AC) was silt with median grain-size values of 20 and 16 μm, respectively, with the sediment being poorly sorted (sorting of 3.0 and 2.4, respectively) but finely skewed (skewness of ca. –0.1), which indicates a relatively high proportion of fine material, and leptokurtic (kurtosis of ca. 1.1) (Figure 6A) which means less particle scattering and a more homogeneous distribution.

The texture of both total and carbonate-free fractions of sample “Lake S0” (collected at KD) was sandy silt with median grain-size values of 20 and 15 μm, respectively. In general, the sediment was poorly sorted (sorting of 3.8 and 3.4, respectively) and displayed symmetrical (skewness close to 0) and mesokurtic (kurtosis of ca. 1) distributions (Figure 6B), indicating a large admixture of medium to fine particles.

Water-Sediment Interface

The biological analysis of the sediment samples revealed a large quantity of vegetal debris which most likely originated from the

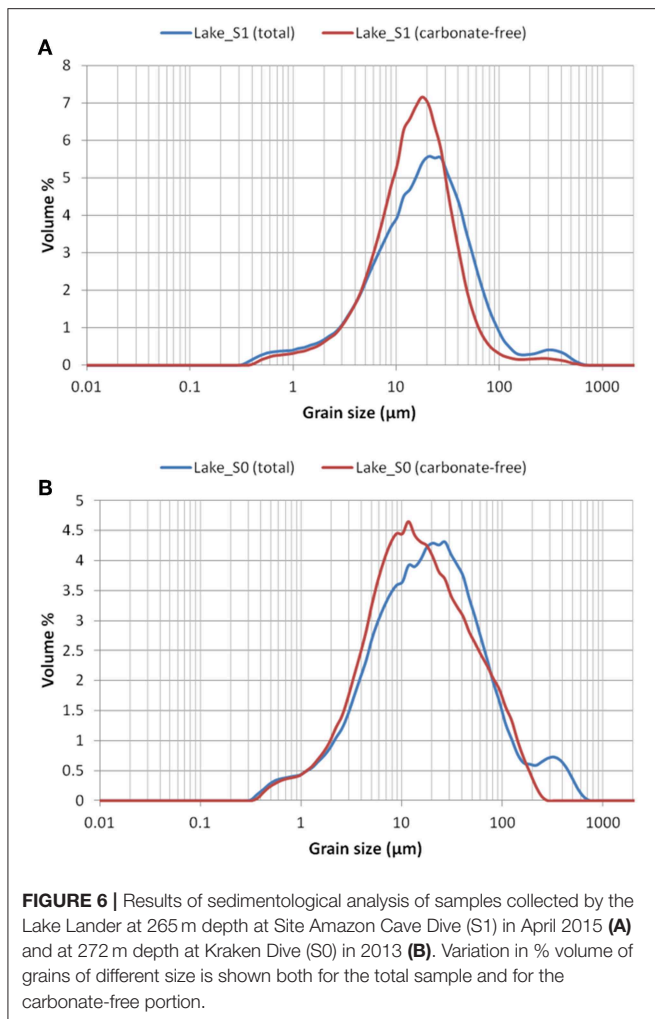


FIGURE 6 | Results of sedimentological analysis of samples collected by the Lake Lander at 265 m depth at Site Amazon Cave Dive (S1) in April 2015 (A) and at 272 m depth at Kraken Dive (S0) in 2013 (B). Variation in % volume of grains of different size is shown both for the total sample and for the carbonate-free portion.

littoral macrophytes observed in the videotransects along the shoreline and deeper (at AC and PWS), such as *Myriophyllum* sp., *Chara* sp., *Nitella* sp., *Potamogeton* sp.

In addition, abundant diatom frustules were present (Rivera, 2006), mainly of the pennate diatom *Pinnularia acidicola* along with deceased zooplankton (Ostracoda *Cladocera* sp.) (Villalobos, 2006) and fecal pellets and inorganic particles. Both macrophyte debris and diatom frustules appeared to have drifted down through the water column to settle on the lakebed. There was however no evidence of benthic macroinvertebrates within the sediment samples.

Video Imagery

The imagery recorded an abundance of life in the water column and on the benthic substrate (Figure 7) and also revealed various forms of life and bioturbation associated with the faunal communities down to the maximum depths of 262 m at AC (Figures 8A,B) and 272 m at KD (Figure 9). Details of the observations made on rapid ecological assessment of this imagery are shown for the towed video transects at the shoreline at AC and PWS in Table 1, and for the jump dives in Table 2. These data was compared to produce generalized descriptions of the

main sub-habitats observed, for the towed video transects in Table 3 and for the jump-dive imagery in Table 4. The presence of benthic macroinvertebrates was notable on the videos, as was a marked heterogeneity of microhabitats and patchiness of epifaunal communities, together with abundant bioturbation activity. Apart from the biota and the biosignatures, the video imagery indicated significant amounts of organic matter drifting downwards within the water column. For supplementary information justifying the above identifications, as well as for data on depth, substrate, habitat associations, faunal and floral assemblages, and a general assessment of the apparent health or naturalness of the study sites, see Tilot (2014a).

Macrophyta and Algae

The evident macrophyte communities appeared surprisingly lush and green, especially in shallow waters where both macrophyta and algae were present (Table 1). This can be related to the high illuminance recorded in shallow water down 10–20 m (see Figure 4). The dominant species of macrophyta were *Myriophyllum* sp. (*M. quitense?*) (Figures 7A,D), *Chara* sp. (*globularis?* *rusbyana?*) (Figures 7C,E), *Nitella* sp. (*N. acuminata?*) (Figure 7E). Some macrophyta extended to great depth such as *Nitella* sp. (*N. acuminata?* *N. flexilis?*) (Figures 7G,H at 60 m), and *Potamogeton* sp. (*P. pectinatus?*), both species were still seen at 100 m. Bright green filamentous algae *Cladophora* sp. (*C. vagabunda?*) were also quite abundant, often covering in thick layers the substrate and large communities of *Chara* sp. (*Ch. rusbyana?*, *Ch. globularis?*) and *Nitella* sp. (*N. acuminata?*, *N. flexilis?*) (Figures 7B–E). On sequences of macro-imagery video, charophytes could easily be identified, especially at blooming season in spring, such as *Chara globularis* by its corticated branchlets and stems, its needle shaped stipulodes and elongate black oospores, and *Nitella acuminata* by its crown oospores. The smooth stonewort, *Nitella flexilis* had very long stems (more than 100 cm) and often formed dense mats, frequently hanging over cliffs in deeper darker waters (Figures 7E,G). It appeared on the imagery that once maturity is reached, the oldest parts of these algae die and decompose. Another observation was that charophytes appeared morphologically different with increasing depth and reducing light availability, their stems appearing more elongated, leaves larger and pigments lighter.

Macrophytes were present in abundance (100% cover) in shallower water, although quite often their growth appeared constrained by the presence of the green algal mats described previously. Colonies of *Myriophyllum* sp. (most probably *Myriophyllum quitenses*) were generally found in more sheltered places such as behind rocks, in small grooves, within gulleys, and along the sides of ridges (Figure 7A). These macrophytes often trapped particles of sediment and planktonic debris and appeared to serve as nurseries for some faunal species (such as fish larvae seen on close-up in videos).

On the basis of the assessments of the video-transects recorded at Amazon Caves and Peninsula West Shore, a series of communities could be distinguished, characterized by the following macrophytes at different levels of abundance (Tables 1, 3):

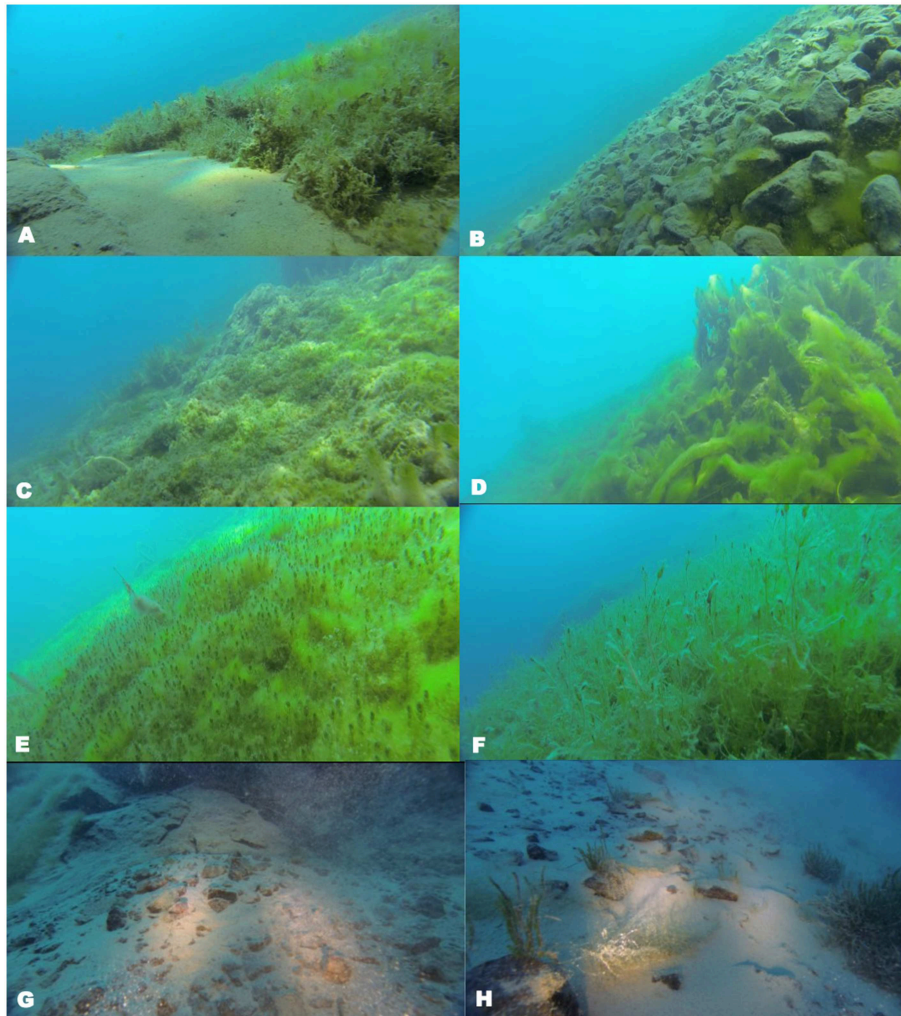


FIGURE 7 | Images from the 2013 video surveys and jump dives illustrating representative habitats and associated biotic communities observed at different depths. (Timings refer to time lapsed on video recordings). **(A)** Amazon Cave video transect part 1 (08:02): slope 20–40°, 5–10 m depth, rock and sediment, *Myriophyllum* sp. (*M. quitense?*) and small *Nitella* sp. (*N. acuminata?*, *N. flexilis?*), *Chara* sp. (*C. globularis?*, *C. rusbyana?*). **(B)** Dive peninsula west shore profile (11:42): slope 40–55°, 10–20 m depth, stony mass of fallen rocks with a thin layer of filamentous bright green algae *Cladophora* sp. and oligochaete worm. **(C)** Dive peninsula west shore profile (13:09): slope 40–55°, 10–20 m depth, 100% coverage of small *Chara* sp. (*C. globularis?*, *C. rusbyana?*) densely covered by bright green algae *Cladophora* sp., in foreground (and background), *Myriophyllum* sp. (*M. quitenses?*) covered by extensive algal mat. **(D)** Dive peninsula west shore profile (12:04): slope 40–55°, 10–20 m depth, extensive bright green algal mat of *Cladophora* sp. covering large colonies of *Myriophyllum* sp. (*M. quitenses?*). **(E)** Dive peninsula west shore profile (15:07): slope 40–55°, 10–20 m depth, 100% *Charas* sp. (*C. globularis?*, *C. rusbyana?*) and *Nitella* sp. (*N. acuminata?*) covered by a thick layer of bright green algae *Cladophora* sp., old dead stems of *Myriophyllum* sp. (*M. quitenses?*) covered by algal mat and sponges Spongiilidae? **(F)** Amazon Cave (13:40): slope 40–60°, 30–40 m depth, close up of 100% cover of *Nitella* sp. (*N. acuminata?*) green algal flocculates on flora, most apex still free. **(G)** Amazon Caves transect 60 m depth (9:15): slope 40–60°, sediment surface with rocks, some boulders, close to a thick strip of algae *Nitella* sp. (*N. acuminata?* *N. flexilis?*) **(H)** Jump Dive 60 m (20:00): rocks and thick layer of sediments. One *Nitella* sp. strip and several bushes of Charophytes *Potamogeton* sp. (*P. pectinatus?*).

- At shallow depths (1–10 m), the general coverage of macrophytes was about 30–40%. There were large patches of tall stemmed old *Myriophyllum* sp. (*M. quitense?*), which at very shallow depths bordering the shoreline was partly covered by small colonies of algal mat. On slopes there were also a few small *Nitella* sp. (*acuminata?*),
- At 10–30 m depth, on slopes of 20–40°, there was mostly 100% coverage of macrophyta *Nitella* sp. (*N. acuminata?* *N. flexilis?*) and lesser amounts of *Chara globularis?* covered by a thick mat of bright green filamentous algae *Cladophora* sp., with

patches of long stalked *Myriophyllum* sp. (*M. quitense?*). On the video transects at Peninsula West Shore the total coverage at this depth was only 30–40%, since there were some areas covered by an extensive algal mat with abundant but mostly dead macrophytes.

- At 30–40 m depth, there was about 60–80% coverage of macrophyta on the steep slopes and vertical cliffs, interspersed with small shelves and gentler slopes. At the bottom of the cliffs there was typically 100% cover of *Nitella acuminata?*, with some *Chara globularis?*, covered by a dense bright green mat of

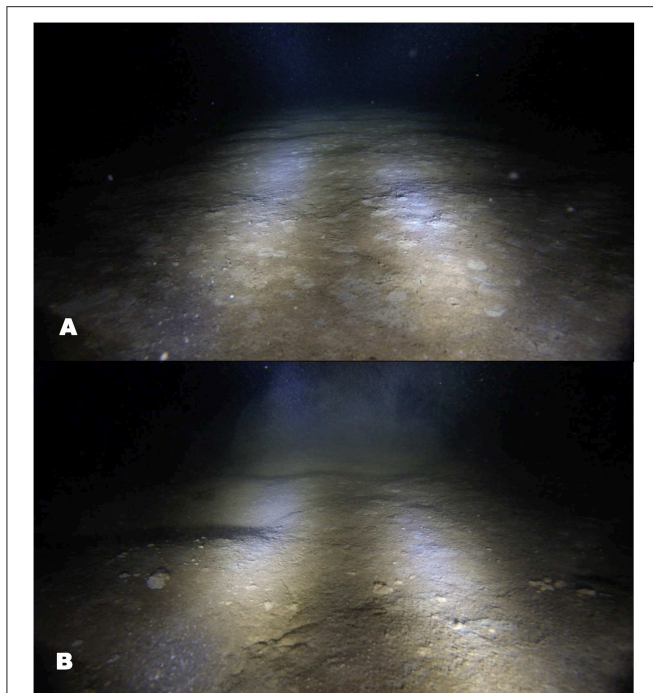


FIGURE 8 | Images from videos taken in 2013 during jump dives to 262 m at the Amazon Caves sites. **(A)** Amazon Caves Jump dive site 1 (23:24): brownish gray sediment with a layer of deposited particles with numerous (40–50% of area) white circular marks and traces of bioturbation including many small faunal features possibly produced by burrowing bivalves, crustaceans, worms etc. The large white circular marks could also be fluid seeping features (fsf) considering the thermal activity in the area. **(B)** Amazon Caves Jump dive site 2 (00:20): slightly hummocky lakebed covered by sediment with flocculate detritus matter conglomerated in whitish masses and traces of bioturbation (5–10%) and small grooves. Above the sediment surface, one can see the flow of particles and plankton.

Cladophora sp., among which *Nitella flexilis*? could also be seen in macro-images. On the small shelves and gentler slopes there was often 100% coverage of *Chara globularis*? under a thick bright green layer of filamentous *Cladophora* sp., with *Nitella flexilis* and *Myriophyllum quitense*? sometimes also visible. On the cliffs themselves, there were hangings of *Nitella* sp. (*N. acuminata*? *N. flexilis*?), with a dense algal mat and occasional strips of bright green filamentous algae *Cladophora* sp.

- At 50–70 m, there was about 50% coverage of macrophyta on very steep slopes and cliffs, with dense algal mat and long strips of *Nitella* sp. (*acuminata*? *obscura*? *flexilis*?) also present.
- At more than 70 m, there was only a small coverage of macrophyte, the precise amounts being difficult to assess due to the limited visibility. *Nitella* sp. and *Potamogeton* sp. were still present down to 100 m. *Potamogeton* sp. (*P. pectinatus*?, *P. illinoensis*?) was conspicuous between 50 and 100 m; it may have been present in shallower waters, but escaped detection because of the dense cover of macrophytes.

On the basis of the video sequences from jump dives, the main communities were characterized by the following macrophytes at different levels of abundance (**Tables 2, 4**):



FIGURE 9 | Image from videos taken in 2013 during jump dives to 272 m at Kraken site (14:20). A low mat-like structure is evident that appears to consist of several layers of tubular structures (~10 cm long) with one upright extremity with pinkish moving features. These are possibly polychaete tubeworms, Siboglinidae. Sponges and molluscs are present on top of the tubeworm mat and small planktonic forms, ostracods, shrimps are apparent above the lakebed. The flow of particles seems to move slowly upwards with simmering water.

- at 50 m, on slopes of 40–60°, there was 100% cover of macrophytes, *Nitella* sp. (*N. acuminata*? *N. flexilis*?), possibly with filamentous algae *Cladophora* sp.? and *Potamogeton* sp. (*P. pectinatus*?).
- at 60 and 70 m depth, on slopes of 40–60°, there was 20–40% cover of the green algae *Nitella* sp. (*N. acuminata*? *N. flexilis*?) forming mats and long strips following the slope. There were also some small bushes of *Potamogeton* sp. (*P. pectinatus*?) anchored in the sediment.
- at 80, 90 and 100 m depth, on slopes of 60–80°, there was <5% cover of macrophytes, only a few small strips of *Nitella* sp. and *Potamogeton* sp. (*P. pectinatus*?) both present in the twilight zone.

Benthic Invertebrates

A wide variety of benthic and epibenthic invertebrates were also visible in the video sequences, especially in bottom transects, including: crustaceans (amphipods), molluscs (bivalves, Pisidiidae? *Pisidium* sp.?, Sphaeriidae? Hyriidae? *Diplodon chilensis*?), gastropods (Ampullariidae?, Tateidae?, Lymnaeidae?, Melanopsidae?, Physidae?), limpets (Planorbidae?, Thiaridae?), segmented annelid worms (Naididae), oligochaete worms (Tubificidae? Branchiura?), flatworms or plathyhelminths (Dendrocoelidae? Turbellaria?), hydroids, sponges (Spongillidae?), Planariidae (Turbellaria) and Oligochaeta, although because of the limited lightning and resolution, rarely could invertebrates be identified to genus level. Also visible were animal tracks, mounds, burrows, and other forms of bioturbation (see below).

Sponges belonging to the family Spongillidae, were recorded, peaking at about 50 m depth. They were typically observed emerging from the dense algal mats, where they were often attached to the dead stems of *Myriophyllum* sp., generally in shallow areas that might have been affected by

TABLE 1 | Results of analysis of the video sequences taken on bottom transects at two sites, Amazon Caves and Peninsula West Shore, using a GoPro 300 camera system, showing the geomorphological characteristics of the substrate and the most evident flora and fauna.

Video Sequence	Time	Geomorphology						Flora						Fauna	
		Approx. depth	Habitat code	Slope	Rugosity	Sediment	Algal mat	Macrophytes	Myriophyllum	Chara	Nitella	Cladophora	Comments	Fish	Fish species
Max value		5		5	5	5	5	5	5	5	5	5		3	
Amazon Caves															
1A	6.38	1~1-5 m	AC1	2	2	2	0	3	3	0	0	0	M	3	Pt, usf
1B	7.56	1~5-10 m	AC2	4	4	1	0	1	1	0	1	0	M, few Na		
1C	9.34	2~10-20 m	AC3	2	2	3	4	5	1	4	5	4?	Na, NcCla, CcCla, thick fluo Cla mat, Nf, M		
1D	11:26	3~20-30 m	AC4	3	NV	3	5	5	1	5	4	4?	Na, NcCla, Cg, CgcCla, thick fluo Cla mat, Nf, few M		
1E	13.35	4~30-40 m	AC5	3	4	NV	3	5	0	5	3	4?	Cg, CcCla, fluo mat, Na, Nf	1	
1F	16.33	4~40 m	AC6	2	4	3	4	5	0	4	5	4?	Na, NcCla, Cg, dense mat		
1G	18.55	4~30-40 m	AC7	5	5	0	4	3	0	3	3	2?	Na, Nf, NcCla hangings		
1H	21.24	3~30 m	AC8	4	5	1	5	4	1	4	4	4?	CgcCla fluo, fewM		
1I	22.30	4~30-40 m	AC9	5	5	2	5	5	NV	5	5	5	Cg, CgcCla fluo dense mat with Nf on shelf, hangings of Na	3	Sd? At? Az?
1J	25-26.05	4~40 m	AC10	5	5	1	4	5	0	5	5	5	NacCla hangings, CgcCla fluo dense mat with Nf on shelves	1	
1K	0.15-1.01	4~40 m	AC11	4	4	NV	4	5	0	5	5	5	CgcCla fluo dense mat with Nf on shelf and soft slope		
1L	7.02-7.57	6~50-60 m	AC12	4	4	2	NV	4	0	NV	4	4?	Thick mat Na, Na hangings, Cl?PV		
1M	9.02	6~60 m	AC13	5	5	3	NV	4	0	NV	4	4?	Na hangings and some mats, Cl? PV		
1N	12.18-13.11	6~50-60 m	AC14	5	5	4	Poss3	4	0	NV	4	4?	Thick mat Na, Na hangings Cl?		
1O	16.44-17.27	6~60 m	AC15	4	4	3	NV	4	0	NV	4	NV	Thick mat Na, Na hangings		
1P	21.41	8~70-80 m	AC16	4	4	4	NV, algal film	2	0	0	2	NV	Na thick mat strip following slope, No?		
1Q	24.17-25	7~70 m	AC17	4	4	4	NV	2	0	0	2	NV	Na thick mat long strips, No?		
1R	6.58-7.50	10~90-100 m	AC18	0-1?	3	4	Algal film	0	0	0	0	0	Red rocks Bioturbation?		
WS Peninsula															
2A	4.34	1~5-10 m	PWS1	2	2	1	1	2	2	NV	2	3?	M, NaCla? fluo mat, PV	1	
2B	5.3	2~10-20 m	PWS2	3	4	1	4	4	3	NV	3	4?	NacCla with Nf dense fluo mat, McCla, PV	3	Gs
2C	8.30	2~10-20 m	PWS3	3	4	1	2	1	1	1	1	2?	NacCl?, M, fluo patches McN	3	Usf, Oh?
2D	9.20	2~10-20 m	PWS4	3	3	2	3	1	1	NV	1	1?	EAM, NacCl? CgcCla? M	PV	
2E	10.05	2~10-20 m	PWS5	3	3	3	5	2	2	NV	2	2?	EAM, MMD, NacCla? Cla patches, fluo, McCla	1	Oh? Gs?
2F	11.43	2~10-20 m	PWS6	3	5	0	2	0	0	0	0	1?	Cla	1	Gs
2G	11.53	2~10-20 m	PWS7	3	4	2	4	4	4	0	NV	4	EAM, McCla, fluo columns and mats	3	At? Az? Sd?
2H	12:09	2~10-20 m	PWS8	3	3	2	4	2	2	NV	NV	2	EAM, MMD, McCla, some very tall M + branchlets with sponges, PV	3	At? Az? Gs, usd
2I	12:53	2~10-20 m	PWS9	3	2	2	4	4	2	4	NV	3?	EAM, NacCla?, CgcCla? CrcCl? McCla, sparse thin fluo mat	3	At

(Continued)

TABLE 1 | Continued

Video Sequence	Time	Approx. depth	Geomorphology							Flora			Fauna			
			Habitat code	Slope	Rugosity	Sediment	Algal mat	Macrophytes	Myriophyllum	Chara	Nitella	Cladophora	Comments	Fish species	Fish	
Max value	5	5	5	5	5	5	5	5	5	5	5	5	5	3	3	
2J	13:17	2~10-20 m	PWS10	3	4	2	2	4	4	3	4	4	3?	EAM NacCla, McCla, fluo mats+ Nf	3	AI? usf
2K	13:35-15:4	2~10-20 m	PWS11	3	3	2	2	2	5	3	3	3	4	EAM especially on M, NacCla, McCla, bright green mats thick 100% with Nf, CgcCla? Some tall long stem M with EAM in shallows	3	AI? Gp? Sd? usf
2L	17:55-20:44	4~40 m50 m	PWS12	3	NV	1	1	NV	5	0	NV	5	NV	Na, Nf?		PV

On detailed study the video recordings were sub-divided into a series of sequences (labeled 1A-27) within each of which the substrate remained relatively consistent. The times given show the time during the video at which a distinction was drawn between one sequence and the next. The habitat codes were assigned for reference within the text. e.g., AC1, Video transect Amazon Cave station number1. The abundance of plants and animals were assessed using a semi-quantitative Abundance Code as follows: 0 = none or absent, 1 = single specimen, 2 = a few specimens, 3 = several/10-30% of substrate, 4 = 30-70% of substrate. Mean slope of the lake bed within a sequence was assessed using a similar semi-quantitative scale as follows: 0 = none or absent, 1 = gentle slope, 2 = 20-40 degrees, 3 = 40-60 degrees, 4 = 60-80 degrees, 5 = vertical or sub-vertical. Depth from 1 (0 to 10m), 2 (10 to 20m), 3 (20 to 30m), 4 (30 to 40m), 5 (40 to 50m), 6 (50 to 60m), 7 (60 to 70m), 8 (70 to 80m), 9 (80 to 90m), 10 (90 to 100 m). Other information is provided via a series of abbreviations as follows: NV, none visible but not certain (e.g., when camera further from substrate); poss, possible; PV, poor visibility; Flora (in comments by order of dominance) M, Myriophyllum sp. (Mq, M. quitense?); C, Chara sp. (Cg, C. globularis; Cr, C. rusbyana?); N, Nitella sp. (Na, Nitella acuminata; Nf, Nitella flexilis); Nm, Nitella sp. Mat; Cla, Cladophora sp. (C. vagebunda?) or Ulva sp.?, reticulated filamentous bright green (flu) algae in strips or thick mat. When Cla covers other macrophytes: McCla, Myriophyllum sp. covered by Cla; CcCla, Chara sp. covered by Cla; NcCla, Nitella sp. covered by Cla. Algal mat, Cyanobacteria and sediments; EAM, Extensive Algal Mat (overgrowing substrate and other macrophyta). Generally Cla covers the algal mat when present. MMD, Macrophytes mostly dead; FD, flocculent deposits; WCM, whitish conglomerate masses (can be mistaken with sponges in deepest sites).

eutrophication. There were indications that here, as in other extreme environments, they adapt to seasonal change by forming “gemmales,” resistant buds that can survive dormant after the mother sponge has died until conditions improve.

Bioturbation

Besides direct observations of living animals, signs of their activity on or within the bottom sediment (bioturbation) were also visible at the bottom of the lake, especially in its deepest parts on the 2 jump dives sites at Kraken (272 m) and the 2 sites at Amazon caves (262 m). Bioturbation was evident not only where there was physical disturbance, but where nutrient cycling has been accelerated as a result of bivalves, crustaceans or worms mixing the sediment. Otherwise, a large number of bioturbation features were identified and described, readily recognizable tracks and characteristically shaped burrows such as rosette-shaped ones, usually found to be caused by worms (Figures 8A,B). Vermiform animals (principally annelids) typically create vertical burrows, while crustaceans primarily excavate more open burrow systems. Horizontal traces were also present, including a range of branching and variably sinuous to meandering burrows made by worms. Based on such signs, bioturbation was observed that was ascribed to sponges, annelids (naidid oligochaetes and tentaculate polychaetes), nematodes, arthropods including crustaceans, isopods, amphipods, pericarids and decapods, molluscs (sphaericean and unionid bivalves) and also fish.

In places, the benthos was covered with white marks on the brownish gray mat of deposited organic particles and sediment (Figures 8A,B). Elsewhere were other features characterized by a central burrow with a polychaete tube, or a mound or series of mounds. In particular, a “rosette shaped mound” was recorded, probably created by a benthic worm dwelling under the tumulus, the “rosette” markings being formed where the worm’s proboscis is “licking” the surface sediment in search of nutritive particles. Other markings noted resembled those created by pelecipod molluscs, taking the form of a series of small burrows, some with part of a shell apparent. There was also a remarkable funnel-shaped burrow that had an unidentified type of worm protruding from the center. At 90 and 100 m on videos of the jump dives at Amazon caves, animals that were probably relatively big flatworms of around 5-7 cm (Dendrocoelidae?) were also observed. Generally planarians measure only a few mm in length, but some giant species (up to 40 cm long) have been found in greater depths in Lake Baikal. In Laguna negra their tracks resembled the long furrows made by sipunculids on the deep sea benthos (Tilot, 2006).

At both deepest sites, REA of jump dive imagery identified zooplankton (crustacean *Ostracods*, and possibly *Cladocera* spp.) and, in the lower water column, copepods, amid an intense sestonic flux of plankton, fecal pellets, inorganic particles and debris, which on descent and ascent appeared present through the entire water column. At AC the benthos was covered by a microbial mat and deposited matter. Several indications of biological activity were recorded, such as different artifacts, mounds, funnel-shaped burrows and rosette-shaped mounds, probably caused by benthic fauna such as annelids, crustaceans, fish (Figures 8A,B). Other biological activity covering 30-40%

TABLE 2 | Results of analysis of the video sequences on drop-down dives using the deep-water lander, showing the geomorphological characteristics of the substrate and the most evident flora and fauna.

	Max or units	Habitat code	Habitat code	Habitat code	Habitat code	Habitat code	Habitat code	Habitat code	Habitat code	Habitat code	Habitat code
Station number		1	2	3	4	5	6	7	8	9	10
Station name time		JAC 1 GOPR9006 (07:52)	JAC 2 GOPR9006 (17:55)	JAC 3 GOPR9006 (03:39)	JAC 4 GOPR9006 (13:52)	JAC 5 GOPR9006 (01:35)	JAC 6 GOPR9006 (17:08)	Kraken LB1 GOPR9007 (14:25)	Kraken LB2 GOPR9007 (03:35)	JAC LB1 GP019005 (14.36)	JAC LB2 GP019005 (00.36)
Depth	m	50	60	70	80	90	100	272	272	262	262
Slope	5	3	3	3	4	4	NV 1?	0	0	0	0
Rugosity	5	4	2	3	3	2	3	1	1	1	0
Sediment	5	1	3	4	3	4	3	NV	5	5	5
Lake bed details		Sim VT AC12, 14, PWS12	Sim VT AC13,15	Redish rocks Sim VT AC16	Redish rocks Sim VT AC16, 17	Sedim+few boulders Sim VT AC18	Red rock Sim VT AC18	100% cover of tubeworms over several layers	Sediment mixed with small rock fragments slight undulations	100% sediment 50–60% whiter circular marks in WCirMk sediment Bio?FSF? +a few WCM	100% Sediment slightly hummocky FD (whitish conglomerate masses WCM), some small grooves
Algal mat	5	NV	0	0	0	0	0	0	0	0	0
Macrophytes	5	5	3	1	1	1	0	0	0	0	0
<i>Muriophyllum</i> sp.	5	0	0	0	0	0	0	0	0	0	0
<i>Chara</i> sp.	5	0	0	0	0	0	0	0	0	0	0
<i>Nitella</i> sp.	5	5	3	1	1	0	1	0	0	0	0
<i>Potamogeton</i> sp.	5	1 Pp NV more?	2 Pp	1Pp	0	1	1	0	0	0	0
<i>Cladophora</i> sp. (fluo)	5	5poss	3poss	NV	0	0		0	0	0	0
Bacterial film	5	NV	1	2	2	1	3	NV	NV	5	4
Flora details		Nm and 100% cover from alt, FD	Nm, 20–30% Cover Particle flux lateral (slope)	N small strips Part Flux slow	N small strips Part flux slow	Few N and P Particl flux lateral (slope)med	N few, small P one leaf close up Part flux high	Polychaete ~10 cm tubewormmat with upright extremity with pinkish moving features, siboglinids? FD covering TB mat Particle flux high, simmer FSF?	Great amount of FD, flux high	FD high, particle flux not as high as 272 m sites	FD high, particle not as high as 272 m sites
Sponges	5	3	0	0	Poss	2	3	2	2 or WCM	Poss or WCM	Poss or WCM

(Continued)

TABLE 2 | Continued

	Max or units	Habitat code	Habitat code	Habitat code	Habitat code	Habitat code	Habitat code	Habitat code	Habitat code	Habitat code	Habitat code
Station number	1	2	3	4	5	6	7	8	9	10	
Station name time	JAC 1 GOPR9006 (07:52)	JAC 2 GOPR9006 (17:55)	JAC 3 GOPR9006 (03:39)	JAC 4 GOPR9006 (13:52)	JAC 5 GOPR9006 (01:35)	JAC 6 GOPR9006 (17:08)	Kraken LB1 GOPR9007 (14:25)	Kraken LB2 GOPR9007 (03:35)	JAC LB1 GP019005 (14.36)	JAC LB2 GP019005 (00.36)	
Molluscs	5	NV	NV	NV	Poss	2	2	Poss	Poss	2 poss	1 poss more
Plankton crustacea	5	3	2	2	2	2	2	3	2	3	3
Larger crustacea	5	NV	0	1	0	2	2	2	1	1	1
W worms	5	NV	0	0	1	2	2	1	2 poss	2 or more	1
FW Flatworms	5	NV	0	0	0	2	3	2 poss	NV	1 poss	1 poss
Bioturb-ation	5	NV	0	1	0	2	2	TM	1	5 or FSF+BT	2 tracks, grooves
Fish	5	poss	NV	NV	NV	NV	NV	0	0	0	0
Fauna details		NV Nmat		W		W	W	TM BT (100%)	BT (5–10%)	BT	BT

The habitat codes were assigned for reference within the text, e.g., JAC1, Jump dive Amazon Cave station number1. The abundance of plants and animals were assessed using a semi-quantitative Abundance Code as follows: 0 = none or absent, 1 = single specimen, 2 = a few specimens, 3 = several / 10–30% of substrate, 4 = 30–70% of substrate. Mean slope of the lake bed within each image sequence was assessed using a similar semi-quantitative scale as follows: 0 = none or absent, 1 = gentle slope, 2 = 20–40 degrees, 3 = 40–60 degrees, 4 = 60–80 degrees, 5 = vertical or sub-vertical. Lake bed (LB) details: when similarity with habitat identified on video transects (VT), noted as example: sim(ilar) VT JAC13.

Other information is provided via a series of abbreviations as follows: FD, flocculant deposits, PV, poor visibility; poss, possible; NV, none visible but not certain (e.g., when camera further from substrate); FSF?, possible fluid seeping features. Flora: M, Myriophyllum sp (Mq, M. quitense?); C, Chara sp. (Cg, C. globularis; Cr, C. rusbyana?); N, Nitella sp. (Na, Nitella acuminata; Nf, Nitella flexilis); Nm, Nitella sp. Mat; Potamogeton sp. (Pp, P. pectinatus?; Pi, P. illinoensis?); Cl, Cladophora sp. (C. vagabunda?) or Ulva sp.? reticulated filamentous bright green (fluo) algae in strips or thick mat. Fauna: BT, bioturbation; W, worm (oligochete), track; FW, flat worm (planarian); TM, tubeworms mat (possibly polychaetes, Siboglinids?); FD, flocculant deposits; WCM, whitish conglomerate masses (can be mistaken with sponges in deepest sites); WCirMk, whiter circular marks in sediment (BT or FSF).

Depths are in bold at the different jumpdive stations are characterized by their different depth zones.

TABLE 3 | Summary of distinct habitats and associated species of macrophytes and fish observed on the video transects at Amazon Caves (5 to 90–100 m) and Peninsula West Shore (5 to 40–50 m).

Habitat Code	Habitat Description	Flora	Fish	Environmental State*
Amazon Caves (AC)				
GOPR9003.MP4				
AC 1, 2	~1–10 m slope 20–40°	- 40% cover of macrophyta with large patches of tall stemmed old <i>Myriophyllum</i> sp. (<i>M. quitense</i> ?) powdered by algal mat. On slopes, few small <i>Nitella</i> sp. (<i>acuminata</i> ?)	- In water column: large fish, <i>Percichthys trucha</i> ? unidentified small fish	- Thin algal mat on <i>M.</i> sp.
AC 3, 4	~10–30 m above vertical cliff	- 100% cover of macrophyta <i>Nitella</i> sp. (<i>N. acuminata</i> ? <i>N. flexilis</i> ?) and lesser proportion <i>Chara globularis</i> ? covered by a thick mat of green fluo algae <i>Cladophora</i> sp. and patches of long stalks of <i>Myriophyllum</i> sp. (<i>M. quitense</i> ?)	- Salmonid fish or salmoniform (native fish), unidentified small fish	- 40–50% strips of <i>Cla</i> fluo mat on 100% cover of flora - Dense green algal flocculates on flora, (more abundant with depth)
AC 5	~30–40 m Slope 40–60° before vertical cliff	- 100% cover of macrophyta (<i>Chara globularis</i> ?) covered by a thick mat of green fluo algae <i>Cladophora</i> sp.	Unidentified small fish	- At depth 100% <i>Cla</i> fluo mat on 100% cover of flora - Smaller algal flocculates on flora - Bacterial film on rocks
AC 6, 7, 8, 9, 10, 11, 12	Deeper ~30–60 m and slopes >40° vertical cliffs, with small shelves and softer slopes between cliffs	- 60–80% cover of macrophyta - At bottom of cliffs: 100% cover of <i>Nitella acuminata</i> ? with some <i>Chara globularis</i> ? visible covered by dense fluo mat of <i>Cladophora</i> sp. in which <i>Nitella flexilis</i> ? - On small shelves and soft slopes above drop offs: 100% cover of <i>Chara globularis</i> ? covered by thick bright green mat of <i>Cladophora</i> sp. with sometimes visible <i>Nitella flexilis</i> and a few <i>Myriophyllum quitense</i> ? - On cliffs, hangings of <i>Nitella</i> sp. (<i>acuminata</i> ?) with dense algal mat and some strips of green fluo algae <i>Cladophora</i> sp. hangings	- Salmonidae: <i>Salmo trutta</i> ? <i>Salvenilus fontinalis</i> ? <i>Oncorhynchus mykiss</i> ? or native salmoniform <i>Aplochiton taeniatus</i> ? <i>Aplochiton zebra</i> ?	- 100% grayish algal mat moss-like on cliffs with long strips hanging, underlayer pale green slimy aspect - 40–50% <i>Cla</i> fluo mat on 100% cover of flora (Na) - 100% thick <i>Cla</i> mat + Nf on Cg - Deposition of large algal flocculates on top of cliffs + shelves
AC 13, 14, 15, 16, 17	~50–70 m, very steep slopes, cliffs, relatively a lot of sediment and flux of particles	- 60% cover of macrophyta On slopes, dense algal mat and long strips - On cliffs, hangings of <i>Nitella</i> sp. (<i>acuminata</i> ? <i>flexilis</i> ?) - Less flora at 70–80 m but algal film present	- No fish seen, poor visibility	- Green algal mat of <i>N.</i> moss-like on cliffs with long strips hanging, (not fluo but also PV, more obscure)
AC 18	~90–100 m Nearly no slope, on a flat? a lot of sediment, obscurity	- No fauna - Bioturbation, algal film important, no fauna		- No flora
Peninsula West Shore (PWS)				
GOPR8971.MP4				
PWS 1, 2, 3, 6, similar to AC 1, 2	~5–20 m Shoreline habitats+ beginning of slope 30–40°, turbid water, some bare sediment and rocks (landslides)	- 30–40% cover of macrophyta - Big patches of macrophyta, upper depth a border line of tall stemmed <i>Myriophyllum</i> sp. (<i>quitense</i> ?), covered by algal mat - Deeper, on slopes, 10–20% patches of <i>Nitella acuminata</i> , patches of <i>Chara</i> sp. (<i>globularis</i> ?), patches of both with <i>Myriophyllum</i> sp. All covered by thick green fluo mat of <i>Cladophora</i> sp. - Bare rock covered by thin algal mat	- Small <i>Galaxias</i> sp. - Bigger fish: <i>Odontesthes hatcheri</i> ? - <i>Aplochiton taeniatus</i> ?	- Thin layer of encrusting algae on <i>M.</i> - About 50% <i>Cla</i> fluo mat on all substrate - ~100% thick <i>Cla</i> fluo mat +Nf on Nc. (<i>Cla</i> mat covers underlying algal mat on all substrate and plants)

(Continued)

TABLE 3 | Continued

Habitat Code	Habitat Description	Flora	Fish	Environmental State*
PWS 4, 5,7, 8, 9,10	~10–20 m Shoreline habitats, and beginning of slope 30–40°, with sediment, boulders, patches of landslides of rocks turbid water, PV	<ul style="list-style-type: none"> - 40–50% cover of macrophyta - Extensive Algal Mat - Patches of thick mat of green fluo <i>Cladophora</i> sp., - patches of extensive Macrophyte death, - Patches of both <i>N. acuminata?</i> and <i>C. globulosa?</i>, and small <i>Myriophyllum</i> sp. fully covered by fluo algal mat <i>Cladophora</i> sp., - Close to shoreline, tall stemmed old thick stemmed <i>Myriophyllum</i> sp. (<i>quitense?</i>), covered by algal mat and sponges <i>Spongilla</i> sp.? 	<ul style="list-style-type: none"> - Unidentified small fish (<i>Galaxias</i> sp.?) - Bigger fish: <i>Odontesthes hatcheri?</i> <i>Aplochiton taenitus?</i> <i>Aplochiton zebra?</i> <i>Salmonidae?</i> 	<ul style="list-style-type: none"> - EAM on all substrate - Some areas of MMD (lumps) or tubes (M)) - Long strips and patches of fluo Cla mat + N - Some areas 60–80% Cla covers the EAM + patches of 100% cover of small Na (or Cg?) and M - Some patches of thick Cla fluo coating of M (net likemat) - Old M with algal mat and sponges
PWS11	~10–20 m Slope 30–40°	<ul style="list-style-type: none"> - 100% cover macrophyta, lush mats of <i>Nitella acuminata?</i> (close ups) and small <i>Myriophyllum</i> sp. covered by a thick green fluo mat of <i>Cladophora</i> sp. with <i>N. flexilis</i>, - Patches of <i>Chara globulosa?</i> covered by a thick green fluo mat of <i>Cladophora</i> sp. - At shallower parts, a lane of <i>Myriophyllum</i> sp. with longer stems cover by algal mat - longer and thicker stems of old <i>Myriophyllum</i> sp. « bushes » covered by algal mat and sponges <i>Spongilla</i> sp. 	<ul style="list-style-type: none"> - Unidentified small fish <i>Galaxias</i> sp. (<i>Galaxias platei?</i>) Bigger fish: <i>Aplochiton taenitus?</i> <i>A. zebra?</i> <i>Salmonidae?</i> Several fish <i>Aplochiton taenitus?</i>, Salmonids, <i>Salmo trutta?</i> 	<ul style="list-style-type: none"> - EAM especially on M and upper slope, - Strips or patches of fluo Cla, - 100% Ca fluo when patch Na or Cg - Old long M coated by algal mat +Spongiidae
PWS12 Similar to AC4,AC5	~40–50 m, semi obscure Slope 30–40°, substrate non visible	<ul style="list-style-type: none"> - 100% cover of macrophyta: <i>Nitella acuminata?</i> <i>Nitella flexilis?</i> long branchelets, free of algal mat 	- PV	<ul style="list-style-type: none"> - Thin algal mat on each branchlet of Na - 100% cover of Na with long stems and branchlets and no visible fluo mat of Cla (maybe underneath?) - No flocculant deposits either (occurred at same depth at AC)

* Assessment of the Environmental State concerning the cover of algal mat and *Cladophora* sp. layer on substrate or macrophyte (Cla, green fluo *Cladophora* sp. Cover; EAM, Extensive Algal mat (small gray, brown small algae) which can be accompanied by MMD, Macrophyte mainly dead).

NV, none visible but not certain (e.g. when camera further from substrate); PV, poor visibility. Flora (in comments by order of dominance) M, *Myriophyllum* sp. (Mq, *M. quitense?*); C, *Chara* sp. (Cg, *C. globularis?*; Cr, *C. rusbyana?*); N, *Nitella* sp. (Na, *Nitella acuminata?*; Nf, *Nitella flexilis?*; Nm, *Nitella* sp. Mat; Cla, *Cladophora* sp. (*C. vagabunda?*) or *Ulva* sp.?, reticulated filamentous bright green (fluo) algae in strips or thick mat. When Cla covers other macrophytes: McCla, *Myriophyllum* covered by Cla; CcCla, *Chara* sp. covered by Cla; NcCla, *Nitella* sp. covered by Cla. Algal mat, Cyanobacteria and sediments; EAM, Extensive Algal Mat (overgrowing substrate and other macrophyta). Generally Cla covers the algal mat when present. MMD, Macrophytes mostly dead; FD, flocculant deposits; WCM, whitish conglomerate masses (can be mistaken with sponges in deepest sites).

of the benthos was probably produced by burrowing bivalves, crustaceans, tubificid worms, or lophophorate bryozoans, and probably again large flatworms (planarians), like those described above. On one video sequence from the Amazon Cave jump dive site (Figure 8A), the brownish gray sediment showed numerous (40–50% of area) white circular marks (about 5 cm in diameter) which could be bioturbation artifacts or fluid seeping features considering the thermal activity in the area.

The most notable observation however was made on the jump dive at site KD, where, at the deepest point in the lake, there was evidence of a low mat-like structure, covering 100% of the substrate. The mat consisted of several layers of tubular structures, each with one upright extremity showing pinkish moving features. These appear to be polychaete tubeworms of the family Siboglinidae moving their ciliated tentacles (Figure 9). Sponges and molluscs were present on top of this potential

polychaete tubeworm reef, apparently having colonized the structure (see also Cabrol et al., 2015; Tilot et al., 2018b). The sediment plume caused by the landing of the camera indicated the texture of these tubular structures; they were not agglomerated cohering structures since it was noted that large amounts of soft tubes and independent elements were lifted into the water column by the lander before subsequently redepositing. These artifacts thus responded quite differently than the very fine benthic particles that formed finer sediment plumes when it was disturbed by the lander. It appeared that many of the tubes were hollow, suggesting that the worms that formed them were no longer alive, while those worms that were alive appeared to inhabit tubes that were more erect than the others, possibly with the living worms extending out of the tube to catch food with tentacle-like antennae. Interestingly, the water column close to the lakebed appeared to be simmering, with particles moving

TABLE 4 | Summary of the main habitats and associated species of macrophytes and macrofauna observed on the videos taken during the jump dives.

Habitat code, depth, similar habitats	Habitat description	Flora	Fauna, bioturbation, other special features on sediment and above
Jump Dives			
GOPR9006.MP4			
50 m JAC1 Similar to AC12, 14, PWS12	Rocky bottom quite irregular, slope (40–60°), thin layer of sediments, slow particle flux	- 100% cover of macrophyte, <i>Nitella</i> sp. (<i>Na?</i> <i>Nf?</i>) maybe with an algal mat of <i>Cla?</i> FD + <i>Potamogeton</i> sp. (<i>P. pectinatus?</i>) hard to see if more with thick cover of <i>Nitella</i> sp.	- Ostracods, copepods, - Several whitish-yellow sponges (Spongillidae?) on the benthos - BT not visible since N. mat
60 m, 70 m JAC2, JAC3 Similar to AC13, AC15, AC16	Quite irregular, Slope (40–60°), rocks mostly covered by sediment, some boulders	- 20–40% green algae <i>Nitella</i> sp. (<i>Na?</i> , <i>Nf?</i>) long strips with maybe algal <i>Cla?</i> + <i>Potamogeton</i> sp. (<i>P. pectinatus?</i>) on sediment	- Ostracods, copepods - No visible bioturbation on benthos, no tracks or mounds made by fauna - Bacterial film
80 m, 90 m, 100 m JAC4, JAC5, JAC6 Similar to AC16, AC17, AC18	Slope (60–80°), current, Gray-whitish sediment, in some areas, a lot of rock fragments and boulders	On close up, <5% macrophytes, a few small strips of <i>Nitella</i> sp. + one leaf of <i>Potamogeton</i> sp. (<i>P. pectinatus?</i>) upto 100 m depth	- Ostracods, copepods - Bioturbation on benthos, yellowish-orange sponges?, flatworms (planarians), worms (oligochetes?), molluscs? Bacterial mat on sediment
262 m JACLB1	Flat lakebed 100% sediments mixed with small rock fragments Slight undulations Particular gray-white circular marks on brownish sediment	No Flora Important Bacterial film	- Cladocera? ostracods? - Most remarkable (50–60%) white circular marks (WCirMk) in sediment. Bioturbation (BT)? Fluid seeping features (FSF)? Bacterial mat, flocculant deposits, - BT: spherical or more complex burrows, mounds probably caused by benthic epifauna (annelids, worms, molluscs), - Flatworm?
262 m JACLB2	Flat lakebed 100% Sediment Slightly hummocky FD (whitish conglomerate masses WCM), some small grooves, mounds, tracks	No Flora Important Bacterial film	- Abundance of plankton Cladocera? ostracods? - A few WCirMk Bio?FSF? - BT 5–10% probably caused by benthic epifauna (annelids, worms, molluscs) - A few WCM or sponges?
272 m Kraken LB1	Flat lake floor covered by quite conspicuous mat of tubular artifacts, polychaete tubeworms? over layers, coated by FD No sediment apparent. Water simmering? Particles upwards? strong particle flux	- No flora visible	- Zooplankton: shrimps, cladocera? ostracods? - 100% polychaete tubeworm mat over several layers with one upright extremity with pinkish moving features, Siboglinids moving their ciliated tentacles? Sponges and molluscs are present on top of the tubeworm mat, water appears simmering. To be associated to a possible seeping of fluids or other process (see microbiology)
272 m Kraken LB2	Sediment mixed with small rock fragments slight undulations, great amount of FD, strong particle flux	- No flora visible - Great amount of FD	- Cladocera, ostracods? - A few WCM or sponges? - Worms? - BT (5–10%)

Other information is provided via a series of abbreviations as follows: FD, flocculent deposits; PV, poor visibility; poss, possible; NV, none visible but not certain (e.g., when camera further from substrate); FSF?, possible fluid seeping features. Flora: M, *Myriophyllum* sp. (*Mq*, *M. quitense?*); C, *Chara* sp. (*Cg*, *C. globularis?*; *Cr*, *C. rusbyana?*); N, *Nitella* sp. (*Na*, *Nitella acuminata?*; *Nf*, *Nitella flexilis?*); Nm, *Nitella* sp. Mat; *Potamogeton* sp. (*Pp*, *P. pectinatus?*; *Pi*, *P. illinoensis?*); *Cla*, *Cladophora* sp. (*C. vagabunda?*) or *Ulva* sp.? reticulated filamentous bright green (fluo) algae in strips or thick mat. Fauna: BT, bioturbation; W, worm (oligochete) track; FW, flat worm (planarian); TM, tubeworms mat (possibly polychaetes, Siboglinids?); FD, flocculant deposits; WCM, whitish conglomerate masses (can be mistaken with sponges in deepest sites); WCirMk, whiter circular marks in sediment (BT or FSF).

upwards. Unfortunately however, given the aims and scope of the study, gear was not available with which these organisms could be sampled.

Vertebrates

Fish were clearly present on the video recordings and it was anticipated that the common native fish, *Galaxias platei*, which

inhabits a wide range of lake habitats might be present (Dyer, 2000; Arismendi and Penaluna, 2009). However, its presence could not be confirmed while other species present proved very hard to determine under the prevailing conditions. A particular salmonid (*Salmo trutta* or *Salvenilus fontinalis* or *Oncorhynchus mykiss*) could be distinguished by a characteristic silhouette. In addition, a native salmoniform (*Aplochiton taeniatus* or *A. zebra*), usually reported (Habit et al., 2006, 2012; Arismendi and Penaluna, 2009) from more southern latitudes (37–56°C) than Laguna Negra, could be recognized by its smaller size, thinner body, and smaller head with relatively larger eyes, and on some videos was relatively common. Other native fish species, tentatively identified, were *Odontesthes hatcheri*, mostly recorded in shallow water, and a larger species with a characteristically rounded back, determined as the perch *Percichthys trucha*. One or more small benthic fish species also appeared to be present close to the lake bed in its deepest parts.

Assessment of Environmental Status

Based on the species present and their relative abundances, estimated by REA from the recorded imagery, and on the apparent naturalness of each area based on the principal observer's experience, the environmental status of each area was assessed. In comparison with other oligotrophic deep water lakes (Hoffmann et al., 1997; Hauenstein, 2006; Ramírez and San Martín, 2006; Zuloaga et al., 2008), the biota appeared somewhat abnormal, with large areas of the shallow parts of the lake bed overgrown by macroalgae.

In particular, a large part of the substrate at 5–20 m depth at Peninsula West Shore was covered by a thick layer of extensive brownish gray algal mat, with any macrophytes mainly dead (PWS habitats 4, 5, 7, 8, 9, 10, 11, **Table 3**). Also many areas, especially between 10 and 40 m depth, were covered by a thick mat of filamentous green algae *Cladophora* sp., often mixed with *Nitella fragilis* and extending over thick patches of *Chara* sp. (*C. globularis*?) and *Nitella* sp. (*N. acuminata*?) and on *Myriophyllum* sp. stems. In deeper parts of the lake, a grayish algal moss-like mat covered the submerged cliffs.

The excessive overgrowth by green algae and the abundance of macrophytes suggested higher than usual amounts of inorganic nutrients.

DISCUSSION

Water Physico-Chemistry and Microbiology of Sediments

Previously, the water of Laguna Negra had been described as transparent, with a deep euphotic layer and low primary productivity (Montencino, 1991). The microbial diversity of water samples was considered consistent with the oligotrophic and photic characteristics of the upper water column (Echeverría-Vega et al., 2018). Taken together, the studies were thought to confirm that Laguna Negra is an oligotrophic, cold water (4°C) lake, typical of the region (Modenutti et al.,

2013). However, the water near the lake bed differed from the water column in containing relatively higher but variable levels of ammonium (up to 2.8 ppm) and nitrate (up to 1.7 ppm) (Parro et al., 2019), while as described above, the large amounts of macrophyte and algae in many shallower areas suggest parts of the lake may be mesotrophic, rather than oligotrophic.

Other observations from the PPL study of the water temperature of the lake down to 40 m depth (under the thermocline) over 2 years showed that the melting season and resulting fluctuations in discharge influenced the physico-chemical parameters of the environment, its pattern of productivity and the depth distribution of flora and fauna. The thermocline depth varied according to season; during the austral summer it varied from –15 to –35 m with the water temperature beneath the thermocline being at 4.3–6°C. Then, at the beginning of the austral winter, the lake overturned bringing nutrients from the lake bed toward the surface, resulting a period of high productivity. Subsequent to this overturn there was little variation in temperature with depth.

Top sediments from the deep lake bed of Laguna Negra contained diatom species indicative of a low-pH environment, including *Pinnularia acidicola* which has been found previously in Andean lakes when associated with a pyritic environment (Alvial et al., 2008). On the video sequences of the lakebed, freshwater tubificids oligochaete worms were present. These macroinvertebrates are also acid-tolerant (Ilyashunk, 1999). Such species feed mainly within the sediment selectively ingesting fine organic particles with attached microflora and are able to survive in low oxygen environments (Chekanovskaya, 1962; Brinkhurst et al., 1972; Ilyashunk, 1999; Mermillod-Blondin et al., 2001).

In addition, the microbiological composition of the sediments based on an immunological life detection chip (LDChip) revealed the presence of iron and sulfur oxidizing acidophiles similar to *Leptospirillum* spp., *Acidothiobacillus* spp. or *Halothiobacillus* spp. (Parro et al., 2019), which support the idea of acidic niches with certain amounts of iron-sulfur minerals. While we did not sequence the sediments from the current study, here we provide a brief summary of the microbiology of Laguna Negra sediments (272 m depth, but at a different site and year), which is fully described in Parro et al. (2019). Diverse Proteobacteria, dominated by Epsilonproteobacteria, comprised ca. 30% of recovered 16S rRNA sequence data. While groups commonly associated with sediments and soils were recovered (*Bacterioidetes* and *Verrucomicrobia*), the nitrite-oxidizing Bacteria *Nitrospirae* comprised >5% of the recovered sequences. These organisms are commonly associated with marine environments, though they have also been found in freshwaters (Beaton et al., 2016). The potential for sulfide oxidation in these sediments is indicated by the presence of sulfide-oxidizing *Sulfurimonas* and *Sulfuricurvum* species.

The Archaeal 16S rRNA data recovered in the sediments indicated links to carbon and nitrogen cycling (Parro et al., 2018, 2019), while > 30% of the recovered Archaeal 16S rRNA data was affiliated with the Marine Group I Archaea (*Nitrosoarchaeum*), suggesting active ammonia oxidation in this region. *Methanomicrobia*, a methanogen, comprised most of

the Archaeal sequence data. The remaining classified groups of organisms were also both extremophiles. *Halobacteriales* are typically halophilic, while *Thermoplasmatales* are often acidophilic and thermophilic.

Flora and Fauna

The benthic plant species observed may also be indicative of an alternating oligotrophic / eutrophic regime, since *Chara rusbyana*, *Chara globularis*, and *Nitella acuminatus* are usually described as indicative of oligotrophic conditions, while *Ceratophyllum demersum* and *Myriophyllum quitense* are considered indicative of eutrophic conditions, based on observations of similar high-altitude lakes in Ecuador (Kiersch et al., 2004; Aranguren-Riaño et al., 2018). The native *Nitella* sp. (*acuminata* or *flexilis*) reported here has also been found in other of the region's high altitude lakes (Parra et al., 2003).

The impression that the charophytes in the video sequences varied morphologically according to depth and light availability is substantiated by the literature (Andrews et al., 1984a; Casanova, 1994; Casanova and Brock, 1999; Blindow and Schütte, 2007; Wang et al., 2008, 2015). The squatter form is considered an adaptation to protect the gametangia from damage by high light intensity and low water levels (Schneider et al., 2006, 2015; Wang et al., 2008). Conversely, charophytes adapt to depth and low light availability by developing elongated stems and branchlets as seen on the videotransects of Laguna Negra.

Our observations of shifting abundances and species specific zonation in relation to depth and light availability are supported by the literature (Schwarz et al., 1996, 2002). To this picture we would add topography and nature of substrate as additional parameters structuring macrophytic communities. These distribution patterns can be explained by inter-specific differences in photosynthetic responses and intracellular partitioning patterns of newly fixed organic carbon in response to light (Sorrell et al., 2001). Concerning our observation that pigments appear to change according to depth, this can be explained by the fact that charophytes photosynthetically acclimate to different amounts of light by regulating pigment content and pattern (Andrews et al., 1984b; Czeczuga, 1986; Howard-Williams et al., 1995; Küster et al., 2000, 2004, 2005; Schagerl and Pichler, 2000; Wang et al., 2008).

Although there was no evidence of benthic macroinvertebrates in the sediment samples, this was probably due to the low sediment volumes collected in the samples. In contrast, macro-invertebrates were conspicuous on the video imagery and the REA methodology enabled both determination to an acceptable level of most of the taxa observed and characterization of the separable habitats encountered. Broadly speaking, almost all the macro-invertebrates identified on screen appeared compatible with previous ecological studies of the region's lakes and freshwater systems (Kong Urbina and Castro Fuentes, 2002; Figueroa et al., 2003; Valdovinos, 2006; Prat et al., 2009; Thorp and Rogers, 2015).

One question that may arise in connection with the distribution of these invertebrates is how they manage to colonize these remote locations. However, it is now widely accepted that besides being distributed downstream within watersheds most

invertebrates may be dispersed by occasional accidental transport within the beaks or plumage of wildfowl and other birdlife (Green and Figuerola, 2005; Frisch et al., 2007). In addition, the possible salmonid fish species observed on videotransects have been introduced from the northern hemisphere to many South American lakes to provide improved fishing opportunities (Arismendi et al., 2009; Cussac et al., 2009; Penaluna et al., 2009; Escobar et al., 2017). However, besides presenting a serious challenge to the survival of native fish species (Macchi et al., 1999; Soto et al., 2006, 2007; Pascual and Cianco, 2007; Habit et al., 2010, 2012), they also effect the abundance of planktonic and benthic invertebrates (Mouillet et al., 2018), and, on liberation into lakes may have with them other species.

Bioturbation

As indicated by the data, the video camera recordings revealed a great variety of different forms of bioturbation evident on the lakebed. Besides indicating the presence of a wide range of macro-invertebrates, many forms of bioturbation contribute to mixing of sediment and so increase the rates of recycling of macro- and micronutrients (Mermillod-Blondin et al., 2001).

Polychaete Tubeworm Mats

The most remarkable faunal community feature revealed by the video recordings was the low mat-like structure consisting of several layers of tubular structures (~10 cm long), provisionally identified as a polychaete tubeworm mat discovered at one site at Kraken in the deepest part of the lake (272 m depth). The visual identification of the mat as composed of clusters of polychaete tubeworms was very convincing to those authors with experience of encountering similar species elsewhere (VT, JR, pers.com.), since in a few places there appeared on the videos to be pinkish moving features on upright tubular structures which could be interpreted as polychaete tubeworms moving their ciliated tentacles (**Figure 9**). Alternatively, the structure could represent some sort of periphyton configuration resulting from the degradation of organic matter that, in turn, may support the reported microbial ammonium oxidation and nitrification processes, but this would not be possible at that depth in an aphotic zone. It is more plausible that it is a mat of chitinous tubular structures supporting a large colony of tubeworms.

This tubeworm community mat, living in what can be identified on videos as an organic-rich sediment habitat, presents many similarities to deep seafloor counterparts found living in vent and seep habitats (Tilot et al., 2010) and mud volcanoes (Paul et al., 2015). The worms observed in the present study appear to be polychaetes belonging to the family Siboglinidae. Their presence in freshwater systems is unrecorded in the literature. However, although different morphologically and ecologically, communities of a biomineralizing tube-building polychaete, identified as a serpulids, *Marifugia cavatica*, have been described from freshwater cave systems distributed through ground waters of the Dinaric Karst of the northeastern part of the Adriatic sea (Kupriyanova et al., 2009). Interestingly, phylogenetic analyses of the freshwater serpulids revealed that they are most closely related to brackish water tubeworms (*Ficopomatus* spp.), it being hypothesized that the freshwater

species evolved from this ancestral estuarine species after it penetrated into freshwater lakes (Kupriyanova et al., 2009). Communities of serpulids have also been identified during surveys performed in 2008–2010 by the deep-water manned submersible Mir in Lake Baikal, a unique freshwater lake of tectonic origin, characterized by strikingly heterogeneous physico-chemical conditions (increased heat fluxes, discharges of mineralised and oil-and gas-bearing liquids, methane seeps and mud volcanoes). In the literature there is evidence of similarities between the vent/seep ecosystems of Lake Baikal and its counterparts in the marine realm (Zemskaya et al., 2012). However, no siboglinid tubeworm communities have been recorded in Lake Baikal.

Sulfide is the most common electron donor for known siboglinid symbionts and can support high carbon fixation activities (Girguis and Childress, 2006). Active hydrothermal venting may lead to sulfide emissions in the study area, as previously described within the Andean volcanic belt (Vergara et al., 2004). The benthic diatom taxa observed in the lake point to the presence of sulphuric acid (Alvial et al., 2008), while the benthic microbiology associated in Laguna Negra suggests an active sulfur cycle. The high organic carbon load supplied by macroalgae and related phytodetritus supports sulfate reducing communities in the sediments (Parro et al., 2019), and the active sulfate production measured in incubated waters points to high rates of sulfide oxidation in the Laguna Negra benthos (Parro et al., 2018, 2019).

Thus, our PLL observations suggest the presence within Laguna Negra of a novel ecosystem centered upon a possible siboglinid tubeworm community and associated symbiotic microbes. Its presence could be associated with past or present hydrothermal activity or gas-emissions described as occurring within the Andean volcanic belt (Vergara et al., 2004), with, as in other cases, the siboglinids supported by microbial communities that are dependent on compounds released through the vents and other outlets. In fact in some of the video imagery the water just above the mat appeared to be simmering, with some suspended particles being carried upwards, an effect not noted elsewhere on the lake bed. Also, the white circular marks observed in video sequences of the lakebed near Amazon Caves (Figure 8A), while possibly some form of bioturbation, could also be fluid seeping features, a sign of hydrothermal activity.

The primary nutrition of known siboglinids associated with hydrothermal vents is derived from the sulfide-rich fluids emanating from the proximate vents. These sulfides are metabolized by symbiotic hydrogen sulfide-oxidizing bacteria living in an internal organ, the trophosome (adult siboglinids lack the complete digestive track).

The possibility of active chemoautotrophy in the Laguna Negra sediments was indirectly supported by the microbiological work undertaken in association with the present work (Parro et al., 2018, 2019). Specifically, DNA sequencing revealed a relatively large proportion of ammonia-oxidizing archaea (AOA), very similar (97%) both to *Nitrosopumilus maritimus* (Thaumarchaeota), a species associated with deep marine environments, hydrothermal vents, and in particular with sponges (Lazar et al., 2010; Kennedy et al., 2014), and also

to (99% similarity) a Yellowstone Lake vent archaea. Also detected were nitrite oxidizing bacteria (NOB) from the *Nitrospira* group at relevant proportions, suggesting a syntrophic relationship between ammonia and nitrite oxidation. A similar co-occurrence of AOA and NOB has been described in the literature for marine environments (Ward, 2008), but not for the benthos of a freshwater oligotrophic system. However, the few eukaryotic sequences retrieved from the PLL microbiology analysis corresponded to golden algae, diatoms, some amoeba, various fungi and yeasts as well as copepods and worms, but not tubeworms. Additionally, some sequences corresponded with >98% similarity to methanogenic archaea (97% identity to *Methanosaeta concilii*) (Parro et al., 2018, 2019).

The lack of definitive evidence of tubeworms and sponges in the massive 16/18 S rRNA gene sequencing can be explained by the extreme difficulty of accurate sampling at 272 m depth without a remotely operated vehicle (ROV), or autonomous underwater vehicle (AUV). Thus, for the time-being the identification of the mat as composed of siboglinid polychaetes must be regarded as provisional. Hence further investigations with dedicated technology will be necessary to confirm the presence of tubeworm communities that is suggested by the video and microbiological data.

There is now a scientific consensus that the family of Siboglinidae has Mesozoic or Cenozoic origins and that they include four distinct clades: vestimentiferans, *Sclerolinum*, frenulates, and *Osedax*, which represent four evolutionary tracks and different habitat associations. Vestimentiferans live in vent and seep habitats and *Sclerolinum* is a monogeneric clade living on organic-rich remains. Frenulates live in often oxic organic-rich sediments habitats and *Osedax* is a monogeneric clade specialized in living on whale and fish bones. Hydrothermal vent communities have survived in diverse sulphidic refugia in the ocean (McArthur and Tunnicliffe, 1998), and it may be that ancestral communities colonized Laguna Negra or neighboring saline lakes and were able to persist there due to the availability of sulfide to support their chemoautotrophic symbionts.

Effect of Deglaciation and Climate Change

The excessive overgrowth by green algae and the abundance of macrophytes suggested that the ecology of shallower areas had been impacted by the input of higher than usual amounts of inorganic nutrient. However, while this might reflect long-term anthropogenic influences (Adams, 2002; Hauenstein et al., 2011), it might only be a seasonal phenomenon. The signs of eutrophication evident in the upper portion of the lake, including notably extensive overgrowth by green algal mats and in particular the spread of the stonewort *Nitella* sp. at depths down to 100 m, suggest recent input of nutrients. Increased input of nutrients could be a consequence of slow deglaciation and/or thawing of the thermofrost occurring as a consequence of climate change (see Introduction). The present survey took place during the earlier part of the austral summer (i.e., November 26–December 19, 2013), shortly after the period when a Spring algal bloom might have been anticipated, following the return of warmth and light to the photic zone after a winter overturn. Thus, some increase in nutrient availability and plant growth might

occur naturally at that time of year. However, algal blooms are uncommon in predominantly oligotrophic high-altitude lakes (Acha et al., 2018).

In addition to seasonal and pluri-annual patterns (El Niño, la Niña and general climate change), human impacts could increase the phenomenon of eutrophication. Glacier surface area changes over the last decades, in particular of the Echaurren Glacier close to Laguna Negra, have been primarily driven by the observed decreasing trend in precipitation (Santibanez, 1997; Quintana, 2004; Carrasco et al., 2005; CONAMA, 2007). An additional threat lies with the continuing increase of Persistent Organic Pollutants (POPs) and Poly Chlorinated Biphenyls (PCBs) inputs that may reach remote Chilean lakes by Long Range Atmospheric Transport (LRAT) and deposition (Wania and Mackay, 1995; Poster and Baker, 1996; Schulz-Bull et al., 1998; Pozo et al., 2007). Remote altitudinal lakes seem also to be more vulnerable to this kind of pollution because climatic conditions (cool environments) may enhance the residence time of POPs in these abiotic compartments (Grimalt et al., 2004). Moreover, most of the urban settlements are located on the western side of the Andes (Laguna Negra is not too distant to Santiago di Chile), which might support the regional background concentrations or influence atmospheric transport of contaminants toward remote zones (Pozo et al., 2007). The orographic effect (mountain range acting as barrier) impedes atmospheric transport of contaminants from the western to the eastern region. Contaminants absorbed on particles and dissolved in rain droplets are washed out of the atmosphere during rainfall mainly onto the western side of the mountain, enhancing deposition, and most likely accounts for the significantly higher levels of PCBs found in the western and eastern region of Chile (Pozo et al., 2007).

Methods and Instrumentation for Planetary Exploration

A Rapid Environmental Assessment (REA) approach, based on semi-quantitative but statistically valid assessment of video imagery obtained by a latest generation high resolution miniature video has been successful here when combined with monitoring of basic physico-chemical parameters of the water column, biological and ecological sediment analysis and in depth advanced life detection technologies in geochemistry, immunological (with Life detector LDChip) and 16S rRNA gene sequencing. It has proved effective in overviewing the biota and ecosystem elements of an extreme

aquatic environment represented by a high altitude oligotrophic lake within the Andean volcanic belt. In the present study this approach permitted analysis of over 200 h of video recording and resulted both in the documentation of life forms and signs of bioturbation, and in the classification of distinct habitats, community associations, environmental variations and indicators over very much larger areas than would be possible using fully quantitative methods. Such a multi-layered methodology can be successful in exploration of very large terrestrial and ocean areas, such as the deep slopes of coral reefs or continental slopes or the deep ocean floor (Tilot, 2006; Tilot et al., 2010). This same approach should prove of value in the exploration of alien non-liquid and liquid environments such as seas and lakes elsewhere in the solar system.

AUTHOR CONTRIBUTIONS

NC, VP, and AF designed the project. VT conceived an REA methodology to analyse the video imagery and assess environmental parameters, identify biological species, associated communities, and habitats. VT worked on the ecological analysis with RO. NL-G performed sedimentological analysis. EM-O and FF achieved the limnological analysis of the sediments. CT, ES, and PS performed sampling (sediments and imagery) campaign and *in situ* measurements. VP, IG-C, AF, and CD undertook the microbiological work and considered the astrobiological implications. JR made the interpretation of the microbiology in reference to the possible discovery of a tubeworm community. VT wrote the manuscript. RO and JR helped with the editing. All authors read and approved the final manuscript.

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