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RESEARCH ARTICLE

A scuba diving direct sediment sampling methodology on benthic transects in glacial lakes: procedure description, safety measures, and tests results

Alfonso Pardo

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Abstract This work presents an in situ sediment sampling method on benthic transects, specifically intended for scientific scuba diver teams. It was originally designed and developed to sample benthic surface and subsurface sediments and subaqueous soils in glacial lakes up to a maximum depth of 25 m. Tests were conducted on the Sabocos and Baños tarns (i.*e*., cirque glacial lakes) in the Spanish Pyrenees. Two 100 m transects, ranging from 24.5 to 0 m of depth in Sabocos and 14 m to 0 m deep in Baños, were conducted. In each test, 10 sediment samples of 1 kg each were successfully collected and transported to the surface. This sampling method proved operative even in low visibility conditions (<2 m). Additional ice diving sampling tests were conducted in Sabocos and Truchas tarns. This sampling methodology can be easily adapted to accomplish underwater sampling campaigns in nonglacial lakes and other continental water or marine environments.

Keywords Sampling methodology · Sediments · Subaqueous soils · Benthic zone · Transect · Glacial lake · Tarn · Scuba diver · Underwater · Altitude diving · *Banquise* · Ice cover · Ice diving · Hypothermia · Diving safety · Sabocos · Baños · Truchas

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A. Pardo (✉)
Departamento de Ciencias Agrarias y del Medio Natural, Área de Edafología y Química Agrícola, Escuela Politécnica Superior de Huesca, Universidad de Zaragoza, Carretera de Cuarte, s/n, 22071 Huesca, Spain
e-mail: pardo@unizar.es

Introduction

The physical properties of lake beds are essential in environmental studies and habitat descriptions, since the lake substrate provides key biotopic conditions to benthic organisms. Sediment features also reflect many physical processes acting on the benthos; thus, grain size and physicochemical analyses become essential tools when analyzing and classifying sedimentary environments.

Consequently, benthic surface and subsurface sediments and subaqueous soils sampling techniques in lakes are essential to different scientific disciplines, as they provide the physical matter for analysis to obtain the data on the actual composition of the lake floor. They are a part of the field work routine that should be carefully adapted to each particular environment and research objectives.

Benthic samples are commonly classified according to the type of information they can provide after analysis (MESH 2010):

- A sample of benthic sediment and rock fragments that can be analyzed in terms of its mineralogy, sedimentology, structure, pollutants, etc., regularly taken for geological, sedimentological, and geochemical analysis, is known as a *physical sample*;
- A sample providing a collection of the organisms living on or in the benthos that can be identified and counted to give information of the biological assemblage or ecological community present at a point is known as a *biological sample*.

There is a range of sampling devices regularly used for benthic sampling, each of them designed to provide a certain kind of sample on a specific ground type (cf., Coggan et al. 2007; Eleftheriou 2013). Grab samplers and corers are probably the most common sampling devices regularly used for sampling in soft, unconsolidated sediments (Eleftheriou, and Holme 1984; Blomqvist 1985; MESH 2010; Xu et al. 2011).

Grab samplers produce disturbed sediment samples (cf., WHOI 2006a). To obtain undisturbed or minimally disturbed samples, a coring device is needed (cf., Blomqvist 1991; WHOI 2006b). Cores can give a wide array of endobenthic information such as sediment composition variations, texture changes, and bedding structures below the sea or lake floor surface, thus adding time as a new variable to the study, according to well-known Steno's law of superposition (cf., WHOI 2007). Such coring devices include box corers, megacorers, gravity or corers, and vibro-corers (WHOI 2007; MESH 2010).

As with water sampling devices (Pardo and Rodríguez 2007, Fig. 1), most benthic sediment sampling techniques and instruments, such as the Van Veen, Smith and McIntyre, Day, Ekman, Shipek, Hamon, or Ponar type grab samplers, were originally developed to sample ocean floor sediments (e.g., Van Veen 1933, 1936; Smith and McIntyre 1954; Oele 1978; Eleftheriou, and Holme 1984; Blomqvist 1990), and later some of them have been successfully adapted to their use in lakes.

All these sediment sampling devices share some common features. They have to be remotely operated from a boat (Fig. 2). The size of the sampling apparatus will define the size of vessel needed to deploy it and the number of people necessary to handle it, and its size depends on the type of study to be performed (MESH 2010). For instance, when performing representative particle size analysis, greater volumes are required from gravel-rich sediments than for muds. Grabs and corers yield random and isolated samples, which may or may not be representative of the benthic environment and its sedimentological features. Researchers have little or no control to select the specific spot where the samples will be taken. Moreover, unless such sampling devices are equipped with some kind of photographic or video camera, scientists are deprived of any other contextual information of the sampling area that may be crucial to the sample analysis and data interpretation.

Fig. 1 *CRALF* water sampling bottle was specifically designed to allow scuba divers collecting water samples in situ in glacial lakes (Source: Pardo and Rodríguez 2007)

Fig. 2 A Hamon type grab sampler operated on board of an oceanographic ship (Source: Modified from Wessex Archeology)

Random isolated samples produce limited data that should not be used for general or average characterizations or descriptions of the areas of study. Only when samples are collected in high enough densities they can be used to establish sedimentation patterns and define physicochemical distributions and variations (MESH 2010). To get such collections of samples, transect or grid configurations have to be used in the sampling design. Those multi-sampling campaigns are feasible in oceanographic studies that cover wide areas, on board of large ships used as field campaign mobile bases (Fig. 2).

But when dealing with studies of benthic sediments and subaqueous soils in tarns (i.e., lakes located in high mountain cirques; cf., Arruebo 2014; Santolaria et al. Hydrochemistry dynamics in remote mountain lakes and its relation to catchment features: the case study of Sabocos Tarn, Pyrenees, Unpublished) usually that kind of approach is not possible. In those remote and restricted aquatic environments, scuba divers can be successfully employed in a variety of tasks (cf., Flemming and Max 1988, 1996; Joiner 2001; U.S. Navy 2008).

Properly trained scuba divers are able to navigate over long distances per dive, which allows underwater exploration at a wide range of scales, from micro observation to broader scale over transects of hundreds of meters, being visibility and water temperature the main limiting factors. Well-trained scientific divers, in addition to direct hand sampling, can use a number of tools for sample collection, such as push-cores, or other supplementary tasks like photographic and video recording (cf., Rabeni and Gibbs 1978; Brazier 2001; Mitchell and Golding 2007; Fig. 3). Moreover, with the exception of destructive sampling procedures, the environmental impact of skilled scientific divers upon the aquatic environment should be minimal.

In this work, we present an in situ sediment sampling method on benthic transects, specifically designed to be executed by scientific scuba diver teams with sound experience in cold water and low visibility dives. It has been originally designed and successfully tested to collect benthic sediment cores and subaqueous soil samples in high mountain glacial lakes up to a maximum depth of 25 m.

Fig. 3 Scientific scuba divers can provide valuable photographs and video recordings from the benthic aquatic ecosystems (photograph Paloma Gacías)

Below, we thoroughly describe the underwater sampling methodology, paying special attention to the main safety equipment and measures that should be taken into account.

Although the sampling and safety procedures described herein were originally designed and tested to collect physical samples (i.e., sediment cores and subaqueous soils) in cold water lacustrine environments, they can be easily adapted to warmer water environments and marine shelf sampling campaigns by simply adjusting the recommended scuba diving equipment, as described in Appendix 1, and adapting the safety measures to the different aquatic environments.

Methodology

A transect sampling methodology should be used to study, analyze, and describe variability in composition, texture, and physicochemical properties of benthic biotopes in lakes. A number of transects arranged in a grid pattern can be used for an exhaustive characterization of the whole lake benthos or can provide a detailed inventory and description of the different biotopes within a given study area.

The sampling procedure described here can be used to collect different samples types (e.g., sediment cores, surface sediment, subaqueous soils, rock fragments, sessile benthic organisms) according to the needs of the researchers in their field campaigns. Thus, the varieties of specific sampling techniques that may be used to obtain the different types of samples while carrying out this transect sampling methodology are not the purpose of this article, and therefore are not described herein.

Scuba divers team organization

As a main and basic safety measure, scuba divers should be organized following the "*buddy pair*" system (Flemming and Max 1988, 1996; Parham 2006), where the two divers operate as a unit, both dive partners are always in visual contact with one another, can always communicate with one another, and can always render prompt and effective assistance either in response to an emergency or to prevent an emergency (AAUS 2013). A sampling diving team should be organized as follows:

- One scientific diver coordinator on surface, who acts as lifeline tender if the operations require deploying a lifeline,
- One sample-collecting couple, with one of the divers acting as the underwater team leader and being responsible for the actual sampling procedure,
- Several sample carrier couples (depending on the amount, type, and weight of the samples to be collected),
- One measuring tape roll up+safety couple.
- In case of ice diving, at least one equipped rescue scuba diver couple on the surface, near the lifeline tender.

Regarding the divers certification system, we follow the CMAS[1] Standard for Scientific Diver (Norro 2000; Appendixes 2 and 3) in this work. CMAS diving qualifications are recognized by the UNESCO and can be easily compared and adapted by chief divers and diving scientific coordinators to the standards of other scuba diving organizations that issue scientific diving certifications (cf., Appendix 7 in Flemming and Max 1996; Phillips 2012; AAUS 2013; NOAA 2013).

Prior to the sampling operation, all underwater and surface participating fellows should attend a briefing where the scientific diver coordinator will revise the sampling procedures and safety measures that should be followed (Fig. 4).

Laying the transect measuring tape

Our underwater research in Pyrenean tarns requires sampling transects starting at the lake shore and reaching its maximum depth. The starting position on the shore should be taken by GPS coordinates, and an azimuth should be specified for laying the sampling transect. All participating divers should mark that azimuth with their compass bezel. The measuring

[1] *La Confédération Mondiale des Activités Subaquatiques*/World Underwater Federation.

Fig. 4 The briefing is an essential part of the safety protocol, which ensures that every team member knows what to do, both underwater and on the surface, during the sampling operation or in the event of an emergency (photograph: Tomás Arruebo)

tape has to be secured with a piton, an ice screw, or a metal pole to the ground on the lake shore. A carabiner should be used to fasten the measuring tape to the piton.

With good water visibility, the measuring tape that marks the sampling transect can be laid directly underwater, following the agreed azimuth bearing (Fig. 5). The location of the divers can be followed from the shore by means of a surface marker buoy (SMB) attached with a string to one partner of the safety scuba diver couple that closes the underwater formation (Fig. 5).

With low water visibility, the measuring tape should be unrolled on the lake surface (following an azimuth+180° bearing) and, later, all divers should plunge vertically to lay it on the lake bottom (Fig. 6).

The transect layout equipment consists of a 50 to 100 m tape measure, a piton, an ice screw or a metal pole, and a carabiner.

Sampling

Once the diving team has reached the end of the measured transect at the bottom, the survey and sampling process starts by gradually navigating back, following an azimuth+180° bearing (Fig. 7). At the selected spots—that might be chosen according to depth variations, distance to the shore, or any other set of features—samples are carefully collected. Sampler scuba diver can collect either undisturbed push-cores up to 50 cm deep into the sediment or disturbed surface to 20 cm deep sediments. In both cases, screw-top containers should be used if possible, since pressure tops are not easily closed and sealed with 5 mm neoprene gloves, necessary to avoid hypothermia and hands numbness while diving in waters ranging 6 to 4 °C in a glacial lake bottom.

Thus, the diver team should be organized in couples as shown in Fig. 7. The sampler and his *buddy* navigate back at the head of the group (Fig. 7(a)). The sampler hands the filled container to his partner and receives from him a fresh empty container to collect the next sample. The sampler's partner hands the filled containers to the sample carrier couple, who navigate right after them (Fig. 7(b)) taking care of the collected samples up to the end of the dive. Closing the group, the safety couple retrieves the measuring tape, and checks the lake floor for missing samples or other objects (Fig. 7(c)). With good visibility conditions, the safety couple might navigate 1 or 2 m above the other divers to have a panoramic view of the operation area. With low visibility caused by turbidity, all divers should keep visual contact with the pair ahead and the measuring tape.

The sediment sampling process invariably produces turbidity in the lake bottom (Fig. 8). Therefore, the sampler must achieve a very good control of buoyancy and have sound sampling technique experience to minimize the visibility decrease due to sediment turbulences.

The number of samples that a carrier diver should be able to manage and transport depends both of their kind and weight. For example, when dealing with 1 kg samples of disturbed sediment sealed in screw-top containers, a diver should be able to carry up to five samples, since an extra 5 kg load can be effectively managed by using the buoyancy compensator device (BCD). However, when samples consist of push-cores, each sample has to be carefully carried, without losing its original vertical orientation during the whole dive and when the core is handled to the surface attendants (Fig. 9). Otherwise, fine laminated structures within the sediment

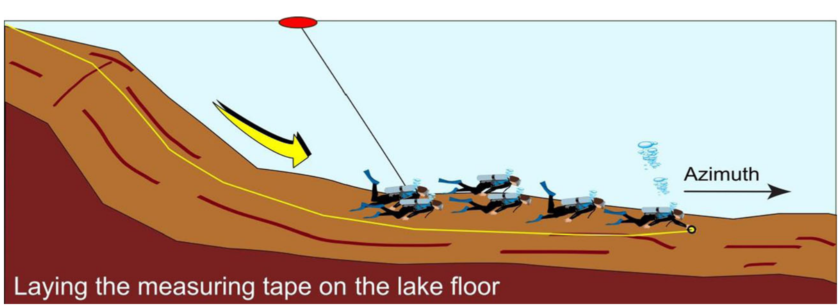

Fig. 5 With clear water, divers would prefer laying the transect measuring tape following the azimuth on the lake floor. Underwater operation allows them to perform a first visual inspection of the benthos, take photographs before the sediments are disturbed by sampling, and preview or select some possible sampling points (some modified symbols courtesy of IAN/UMCES, 2013).

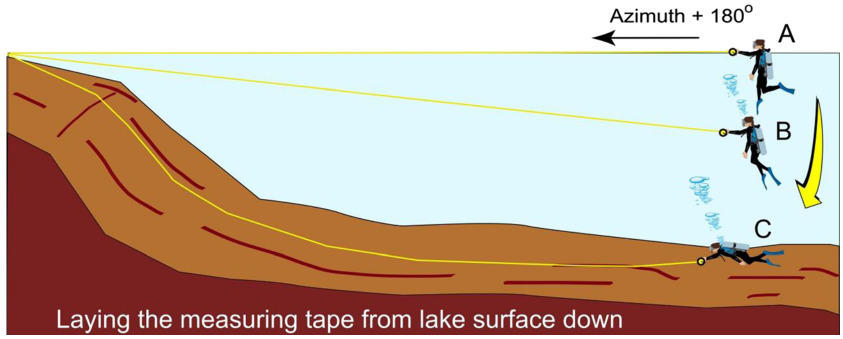

Fig. 6 With poor water visibility conditions, divers should unwind the transect measuring tape on the lake surface following the azimuth+180° bearing. Once completely unrolled, the divers' team should dive vertically to the lake floor (some modified symbols courtesy of IAN/UMCES, 2013).

might be disturbed or obliterated, rendering a completely useless core for certain analyses (Somerfield and Clarke 1997). Therefore, only very skilled divers should handle more than one core at a time, with a maximum of two cores per diver.

Sampling under the banquise

Winter transect sampling underwater operations, when a lake is covered with an ice *banquise,* should be avoided if possible. The ice cover transforms the lake in an enclosed space, and as such, extra safety measures must be taken and additional staff has to participate during the sampling operation (Fig. 10).

Those minimum additional crew members are the following:

- A lifeline tender (Fig. 10(a)) who has to keep contact with the diving team by means of a lifeline, and by using a line-pull signal code (Appendix 4) that should be known by everyone who participates in the operation,
- At least a rescue diver couple has to be equipped and ready to assist the underwater team in the event of an emergency (FEDAS 2005, U.S. Navy 2008; Figs. 10(b)).

When a dive takes place in confined environments, the main safety device is a lifeline (Fig. 10(c)) that connects all divers to the surface. The lifeline is also employed for communication between the scuba diver leader and the lifeline tender, who is in charge to deploy any rescue action if needed. Although all divers while operating underwater must be attached to the principal lifeline using secondary lifelines and carabiners (Fig. 10(d)), only the dive leader should use the line-pull code to communicate with the tender. Other special ice diving equipment includes a dry suit and redundant cold water regulators (Appendix 1B; cf., U.S. Navy 2008).

In this specific working situation, the measuring tape used to mark the sampling transect should be attached to a pole driven on the lake floor, right beneath the ice cap entrance opening (Fig. 10(e)).

Having a single point of entry and exit to the water implies that the number of divers must be reduced, so that an eventual rescue operation can be operative and successful. Thus, for ice diving sampling campaigns, we designed and tested a trio diving team, composed by a sampler, a sample carrier, and a measuring tape retriever and safety diver (Fig. 11).

Safety and health considerations

Health and safety considerations regarding the physiological effects of high altitude and cold water dives impose important restrictions upon time spent underwater and maximum working depth. Therefore, a set of safety rules must be followed:

- Prior to a dive in high altitudes, divers should acclimatize themselves to that lower pressure environment, for at least a 16 h interval (Flemming and Max 1988, 1996; Parham 2006). This means that, in the case of sampling campaigns

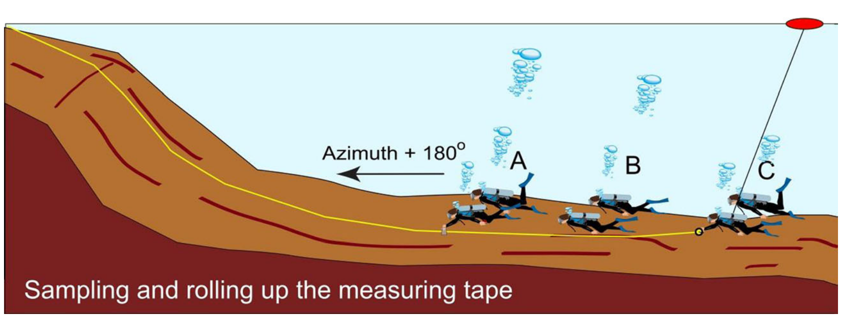

Fig. 7 Sampling underwater teams operating in a glacial lake transect sampling campaign should be arranged as follows: (*A*) samplers, (*B*) sample carriers, and (*C*) measuring tape retrievers and safety divers. Their position is marked by surface marker buoy (SMB) attached to one partner of the safety scuba diver couple (some modified symbols courtesy of IAN/UMCES, 2013).

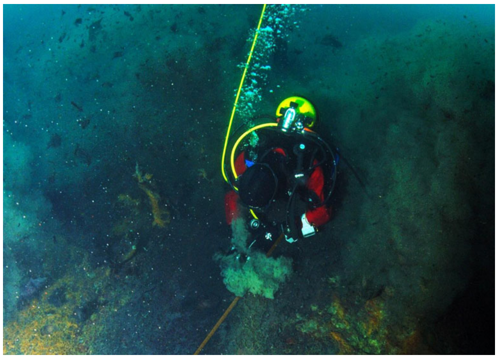

Fig. 8 Sediment sampling results in an increase in turbidity at the bottom of the lake. Divers progressing behind the sampler must be familiar with security protocols for diving in low visibility conditions. Note the yellow lifeline attached to the scuba diver, which indicates a winter dive under an ice cover (photograph José Manuel Cruz)

in high altitude lakes (>2,500 m) and operational depths >15 m, the divers should stay overnight at the lake altitude before the actual dive.

- The dive plan has to be adjusted to the existing altitude and pressure (U.S. Navy 2008; FEDAS 2005; Appendix 5).
- Decompression dives should be avoided (Flemming and Max 1988, 1996; Parham 2006).

The second main risk factor is cold water, which may cause a lowering of the core temperature of the body. Hypothermia is a potential hazard whenever a dive takes place in cold water environments and is triggered when the difference between the water and body temperature is large enough for the body to lose more heat than it produces. Paradoxically, exercise in cold water may cause the temperature to fall more rapidly, since movements stir the water in contact with the diver creating turbulences that carry off the body heat (U.S. Navy 2008). Mild hypothermia symptoms include shivering, poor judgment, imbalance, and slurred speech (Hayes 1991; FEDAS 2005; U.S. Navy 2008). Any of those symptoms by themselves increase a risk for any underwater operation and could cause a diving accident. A loss in core temperature of as little as 0.5 to 0.8 °C may result in a loss of mental capacity of 10–20 % and as much as 40 % in memory, and similar deterioration is observed in muscular strength and dexterity (Flemming and Max 1988). Therefore:

Fig. 9 Push-cores handling must be careful during the sampling and transport processes. At no time during the underwater carriage or its surface collection, a core has to lose its original orientation. Turning it over or even a mild shake would cause disturbance or destruction of the sediment lamination rendering a useless sample for certain paleolimnological and sedimentary analyses (photograph Tomás Arruebo)

- Long exposures to cold water even with dry suits should be avoided. A practical method to minimize hypothermia risk is to plan dives lasting a maximum of 20 minutes (FEDAS 2005).
- At any moment, if any diver experiences shivering or any other hypothermia symptom, the operation should be called off immediately.

In the case of diving operations under an ice cover, air supply is another important limiting factor, adding risk to the underwater sampling action. In such overhead environment, a careful gas consumption planning known as "*Rule of Thirds*", in which the diver reserves two thirds of their breathing gas supply for returning from the maximum penetration point to the *banquise* exit hole, or a more conservative substitute, should be followed by all divers (cf., Bozanic 1997; Graullera et al. 2012). If a direct sampling procedure of long transects (>50 m) in an ice-covered lake should be accomplished, use of emergency gas cylinders and gas supply endurance calculations, such as those recommended by the IMCA (2014) using 40 l/min as a minimum consumption rate, should be planned and deployed.

Tests

Underwater transect sampling tests were conducted in the Sabocos and Baños tarns, both located in the Tena Valley (province of Huesca, Spain). Additional ice diving sampling tests were conducted in Sabocos and Truchas tarns, covering a wide range of altitudes and diving work depths in Pyrenean glacial lakes.

Sabocos is located at an altitude of 1,905 m. Its geographical location is UTM Zone 30, 724800-4730500. This 25 m deep lake can be reached either by foot or driving a four-wheel drive vehicle through two different dust tracks, one that starts at the base of the Panticosa ski resort, and the second starting from Hoz de Jaca.

With a maximum depth of 14 m, Baños, also known as "*lago del Balneario de Panticosa*", is situated at an altitude of 1,630 m in the *Balneario de Panticosa* hotel resort. Its geographical location is UTM Zone 30, 726200-4738000. It can be reached from Panticosa, driving the A-2606 local road some 10 km to its end at the hotel resort.

Truchas is located at 2,115 m of altitude in the Aragón river Valley (province of Huesca). Its geographical location is UTM Zone 30, 705400-4743500. This 4.6 m deep tarn can be reached

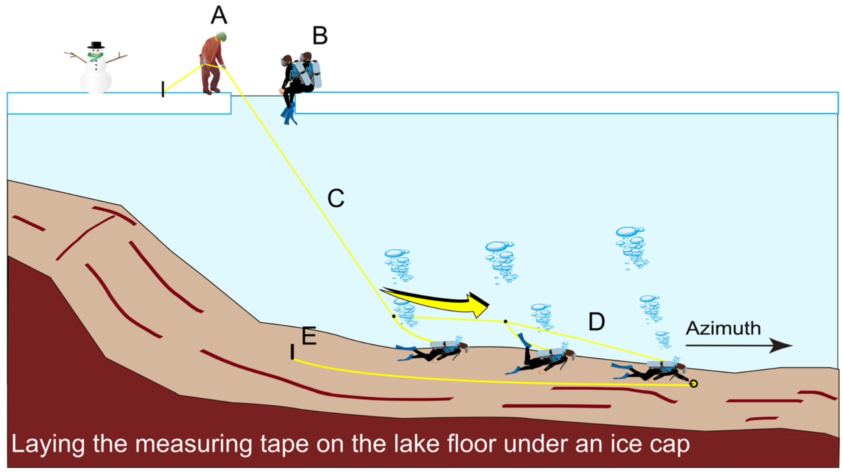

Fig. 10 If a transect sampling should be performed under a *banquise*, additional staff has to participate and specific safety measures must be taken: (*A*) a lifeline tender, (*B*) rescue divers, (*C*) a lifeline that must be attached (*D*) to all divers underwater, and (*E*) the measuring tape should be attached to a pole on the lake floor, right beneath the entrance opening in the ice cap (some modified symbols courtesy of IAN/UMCES, 2013)

either by foot or driving a four-wheel drive vehicle through a dust track starting at the base of the Astún ski resort. In winter, a chair lift of the ski resort can be used to reach the Truchas tarn.

In our tests in Sabocos and Baños, a 100 m transect running from the lake shore to its maximum depth was laid on the lake floor and sampled thereafter. A total of 10 1 kg disturbed sediment samples of the surface soft substrate (from 0 cm to 20 cm deep) were retrieved.

In Sabocos, water had a fair visibility of about 6 m and a bottom temperature of 6.2 °C. Thus, the measuring tape was laid directly underwater, in a 90° azimuth bearing, ranging from 0 to 24.5 m deep, and yielding an East-West benthic transect (Fig. 12). In contrast, visibility in Baños was <2 m, but recorded a similar bottom temperature of 6.0 °C. Hence, in this test, the measuring tape was completely unrolled on the lake surface (in a 180° azimuth bearing). Then, the diver team submerged vertically to the lake floor, laying a North-South transect on the benthic sediment, ranging from 0 to 14 m deep (Fig. 13).

Six divers participated in each sampling operation: one sample-collecting couple, one sample carrier couple (who carried five samples each back to the lake shore), and one measuring tape roll up+safety couple. All divers were equipped with redundant—principal and emergency stages—complete cold water regulators, dry suits, and 5 mm gloves to cope with cold water conditions.

Additional ice diving sampling tests were conducted by three-member underwater teams at Sabocos and Truchas tarns in winter, with 3.8 and 4.1 °C bottom water, and 1.6 and 2.1 °C surface water, respectively, and *banquises* thickness ranging 0.6 to 1.2 m. Sample collecting depths ranged from 3 to 24 m in Sabocos (Fig. 8), and 2 to 4.3 m in Truchas.

In all tests, the sampling procedure took place without incidents. Transects were fully deployed, all samples were collected as planned; all team members developed their tasks with ease and precision. It is important to note that the briefing, previous to the actual sampling operation, proved crucial to clarify doubts and systematize the working method and the role of each one underwater and in the lake shore.

Discussion

Election of a sampling method is mainly based on the kind of research and its required analyses. Remote sampling grabs mount jaws which close in a semicircular mode, and thus, sediment layers are inevitably semiquantitatively sampled (Blomqvist 1991). Moreover, sampling performance and efficiency of different remote-operated devices vary among different bottom substrates.

Truly quantitative soft-bottom sediment sampling must involve remote-operated open-barrel gravity corers (*sensu* Emery and Hülsemann 1964), box corers, piston corers (Blomqvist 1991), or direct sampling by scuba divers. Most of the times, logistics of remote-operated sampling devices make them unsuitable to use them in high altitude glacial lakes. Generally, the higher the lake is located, the more remote is the area, which imposes restrictions carrying such heavy pieces of equipment. Moreover, direct sampling by scuba divers is considered to be the best method for sampling benthic sediment, as the core tube or box can be carefully and slowly inserted, and the sample quality can be checked right away (Fleeger et al. 1988). Furthermore, although time-consuming, in situ benthic sampling by scuba divers yields quantitative data, apt for statistical analysis and interpretation, and offer a common standard among

Fig. 11 When working under a *banquise*, a trio configuration is preferred. This minimizes potential problems in the event of an underwater emergency (photograph by Tomás Arruebo)

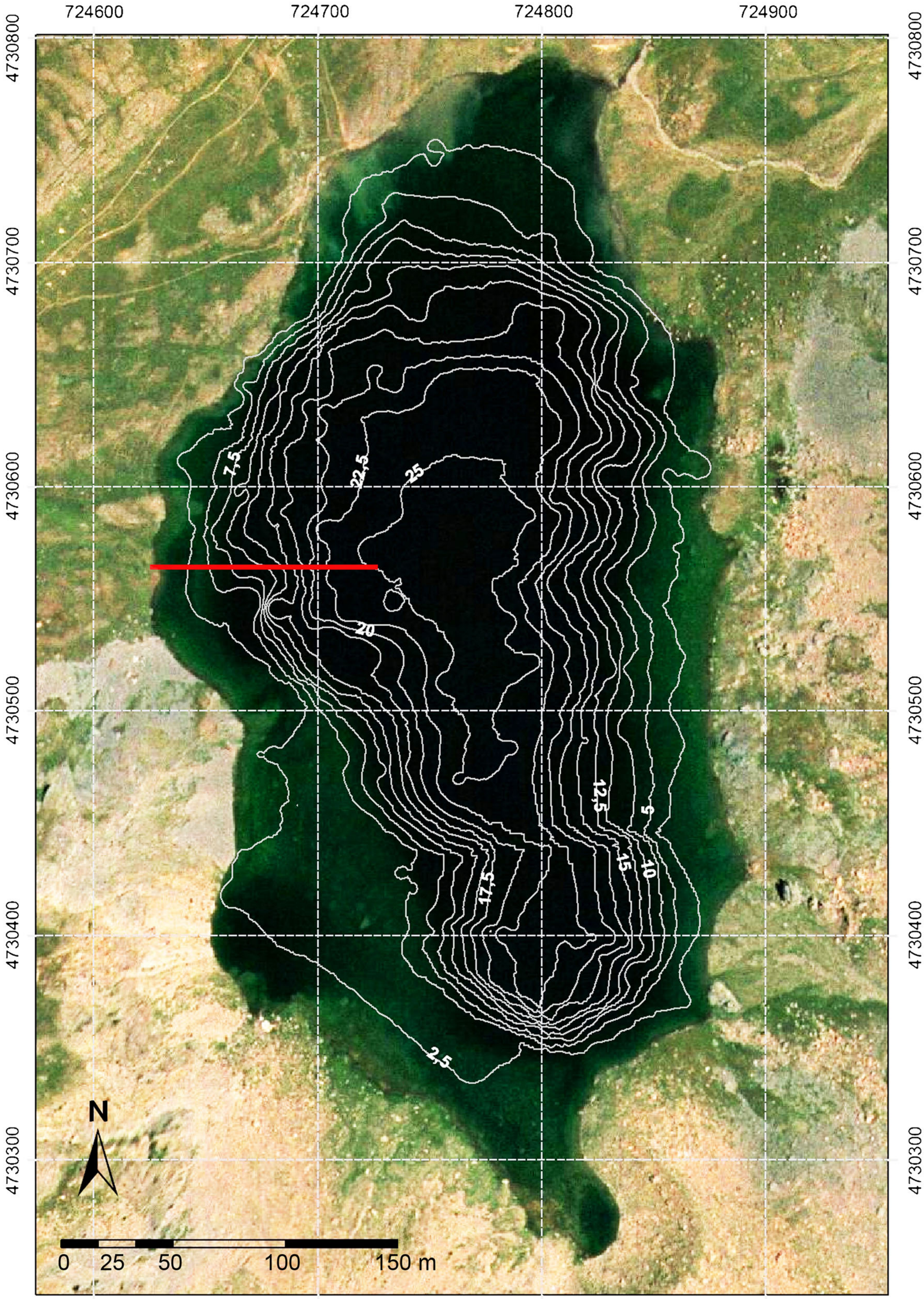

Fig. 12 East-West 100 m transect laid and sampled on the Sabocos tarn floor (modified from Arruebo 2014)

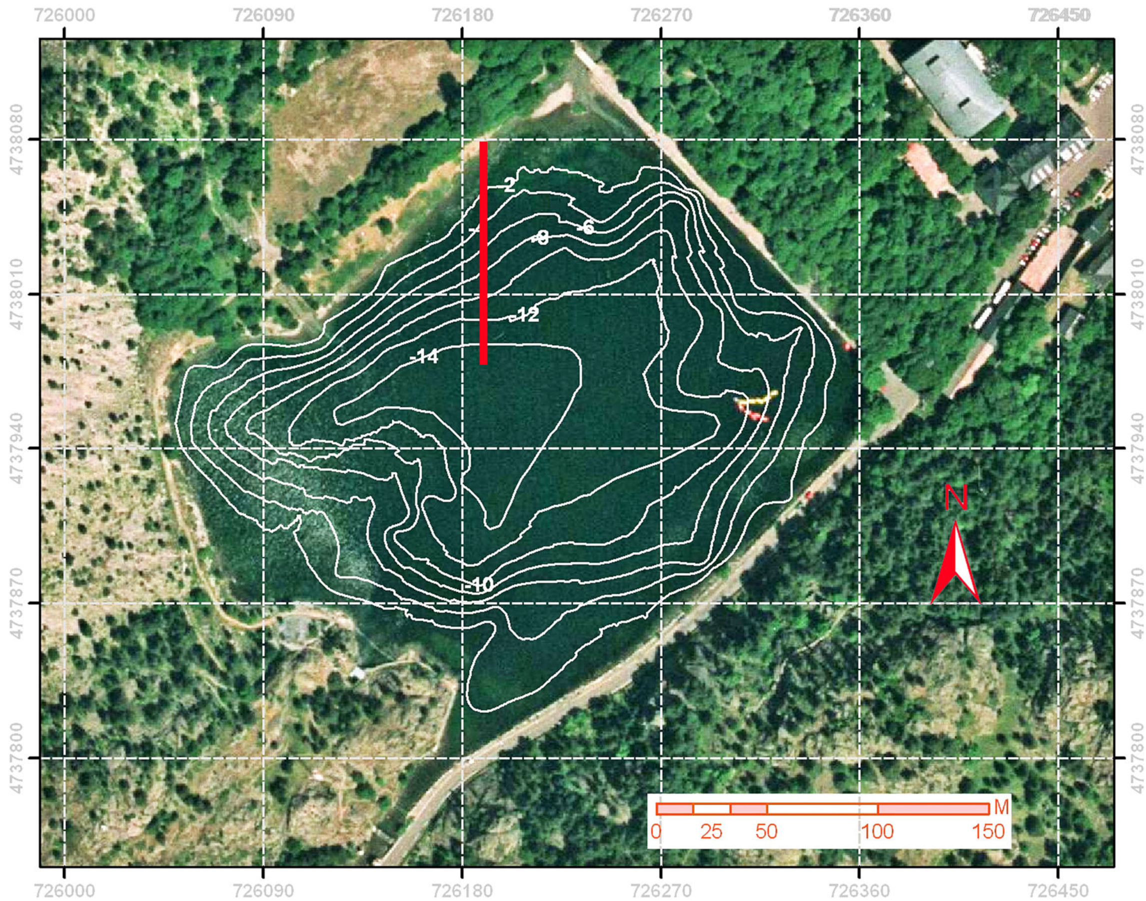

Fig. 13 North-South 100 m transect laid and sampled on the Baños tarn floor (modified from Arruebo 2014)

datasets (Brazier 2001). Thus, scuba diver direct sampling seems the best solution to collect samples in glacial lakes if both safety and sampling protocols are properly adapted and applied.

It is known that scientific dives often take place in extreme conditions or environments, since the research requirements control most of the dive variables. Underwater field research in high-altitude glacial lakes combines several of the most hazardous diving scenarios: high altitude, cold waters, low visibility, and even under ice diving. Each of those diving conditions are considered as potentially dangerous in all technical and scientific diving manuals (e.g., Flemming and Max 1988; Joiner 2001; U.S. Navy 2008). Nevertheless, ISO 31000:2009 *Risk Management* standard should always be considered. Therefore, following the ISO 31000 hierarchy to deal with risk, direct scuba diver sampling should never be the first option if there is a viable alternative to obtain the samples needed for a particular research.

The equipment and protocols described herein are adapted from well-tested air scuba diving techniques and material configurations, since those are the most affordable and easy to transport and implement. One of our main objectives was to design an underwater sampling method and safety protocol that could be deployed using scientifically trained scuba divers and equipment easily available in most universities and research institutions, Army and Navy units, or underwater federations and associations. Other diving practices and equipment (e.g., surface-supplied diving, full face masks, voice communication devices, or rebreathers) originally developed for military diving or professional high-risk deep diving used by offshore oil and gas production companies would increase considerably the logistics and budget of the field campaigns and, at the same time, reduce the mobility of the field team both in the surface and underwater. However, adding to the basic equipment described here, certain professional pieces of equipment like full face masks or communication systems might increase security during the underwater work, especially if new divers are trained or participate in potentially risky sampling operations for the first time.

Although scientific divers trained and certified in the use of rebreathers remain a minority, their use might be particularly beneficial in cold water under an ice cover since these pieces of equipment deliver a large reserve of recycled, and therefore warm, breathing gas, minimizing both hypothermia and the risk of gas supply shortness. Nevertheless, both closed circuit (CCR) and semiclosed circuit (SCR) rebreather equipment need highly specialized generic and specific training for the model of rebreather used, which makes difficult to replace out of order pieces of equipment on the field. Moreover, compared with open circuit scuba, besides gear expense, rebreathers have some disadvantages such as their complex operation and maintenance routine, with more critical parts prone to failure and usually difficult to repair on the spot. A malfunctioning rebreather can supply a hypoxic gas mixture, or it may cause oxygen or carbon dioxide toxicity (Goble 2003), which can endanger seriously and promptly the diver's life.

In our tests, due to the length of the sampled transect and thus the maximum distance to the shore, we did not use an inflatable boat to add security to the underwater sampling operation. Nor did we use sample container racks and variable volume parachute lift bags to retrieve samples to the surface. Nevertheless, if longer transects and higher number of samples had to be collected; the combination of a safety raft near the SMB and a sample rack attached to an open lifting bag might be useful supplements to the whole underwater operation.

Following this same principle of simplicity, we use standard U.S. Navy (2008) air decompression tables in this proposal since they are used by most military, governmental, scientific, and federative scuba divers organizations (e.g., NOAA, CMAS, NAUI[2], FEDAS[3], Spanish Army and Navy), widely accessible, and well-known by most scuba divers. Nevertheless, this sampling method can be deployed and adapted to other more conservative air decompression tables.

We found particularly critical and useful the preliminary briefing to define roles and tasks for each underwater and surface participant. In this regard, although certain sampling techniques may increase the sampler's air consumption, in our dive tests, we decided not to switch roles between the sampler diver and his partner during the entire dive, since that might have caused some uncertainties among the divers, especially in low visibility conditions. Certainly, such operative decisions have to be agreed depending on the specific conditions of the sampling area, the samples type, and the scientific needs.

Location, altitude, accessibility, maximum and average diving depths, existence of a *banquise*, distance to the nearest hyperbaric chamber medical facility, and other particular geographical and logistic factors are crucial to design and organize the specific emergency and evacuation plan for each lake sampling campaign. Thus, due to the high caseload, a generic emergency and evacuation plan for the scuba diving direct sediment sampling methodology proposed herein would be of little usefulness, and therefore is not the purpose of this article. In any case, this transect direct sampling method requires similar emergency and evacuation procedures to other similar scuba diving campaigns; therefore, chief divers and diving scientific coordinators should not have special difficulty to design an emergency plan adapted to each particular underwater field campaign when using this methodology.

[2] National Association of Underwater Instructors.

[3] *Federación Española de Actividades Subacuáticas* (Spanish Underwater Activities Federation).

Conclusions

The in situ sediment sampling method on benthic transects, specifically designed for scientific scuba divers, presented herein has been successfully tested in a set of high mountain glacial lakes, with substantial differences in altitude, maximum depth, and water visibility. After analyzing the tests results, we reached the following conclusions:

- This sampling methodology proved to be a useful tool to collect underwater multi-sample transects up to 100 m in high altitude, cold water, and low visibility environments.
- With good water visibility conditions, the measuring tape that marks the sampling transect can be laid directly underwater. This allows a first benthic survey that could help the selection of the sampling spots.
- With low water visibility, the measuring tape should be unrolled on the lake surface and then laid on the lake bottom.
- Winter transect sampling operations, when a lake is covered with a winter ice *banquise*, should be avoided.
- If an underwater sampling operation should take place in an ice-covered lake, specific safety measures, as described herein, should be met.
- When ice diving, all divers should wear dry suits and redundant cold water regulators (U.S. Navy 2008).
- Stage decompression should be avoided in cold water, since peripheral vasoconstriction will slow nitrogen elimination and render the decompression ineffective (Flemming and Max 1988; Parham 2006).
- Dive time should be adjusted to altitude, cold water, and stress conditions (U.S. Navy 2008; Appendix 5). As a rule of thumb, to minimize hypothermia risk, dives lasting more than 20 min should be avoided (FEDAS 2005).
- We recommend following all the high altitude and cold water dives safety and health described herein, and in standard Diving Manuals for Scientific, Military, and Professional diving (e.g., Flemming and Max 1988; Joiner 2001; U.S. Navy 2008).
- Screw-top containers should be used for sampling, since pressure tops are not easily closed with thick neoprene gloves.
- A careless handling of samples may result in serious disturbance during or after their collection (Somerfield and Clarke 1997).
- We recommend that all participating scientific scuba divers have a sound experience in cold water and low visibility dives, outstanding buoyancy control, and are specifically trained in the different sampling procedures.
- We emphasize to follow the "*buddy pair*" system in all underwater operations (Flemming and Max 1988, 1996; Parham 2006; AAUS 2013) except for ice diving operations where we recommend a trio formation (Fig. 11) plus a

surface rescue diver pair fully equipped and ready to dive in the event of an emergency (FEDAS 2005, U.S. Navy 2008).
- All divers and at least the tender must be familiar with a standard line-pull communication signal code (cf., Appendix 4).
- The scuba divers team organization and working procedures described herein and tested on the field proved to be excellent for this kind of work.

Caveat lector This article does not attempt to provide all of the information necessary to safely collect direct underwater samples. Prior to participating in such an activity, the reader should engage in specialized scientific and technical scuba diving training under the direction of a qualified scientific-technical diving instructor.

Acknowledgments Tests of the in situ sampling method presented in this paper were performed in collaboration with military scuba divers from the *Unidad Militar de Emergencias* [4](UME) based in Zaragoza (Spain), and codirected by Jorge Burgos, 1 Star CMAS instructor, Dry Suit and Ice diver FEDAS[5] instructor. Ice diving sampling tests were performed in collaboration with scuba divers from the *Grupo Especial de Actividades Subacuáticas*[6] (GEAS) based in Huesca (Spain). Underwater photographs were taken by José Manuel Cruz, 3 Star CMAS instructor. Surface photographs were taken by Tomás Arruebo and Paloma Gacías. Field tests surface assistance was provided by Tomás Arruebo, Zoe Santolaria, Carlos Rodríguez, Javier Lanaja, and José Urieta, from the "*Ibones Research Group*" of the Universidad de Zaragoza. I thank three anonymous reviewers for insightful comments that helped to improve this manuscript. This paper is dedicated to the loving memory of my father, Alfonso Pardo Zubiri (1934-2014).

Appendix 1 Scuba diver's equipment checklists for scientific dives in cold waters and ice-covered glacial lakes

A) Regular scientific dives in glacial lakes

Documents	Check ✓
Diving certification	
Diving insurance	
Emergency and Evacuation Plan	
Logbook	
Medical certificate	

Diving Equipment	Check ✓
Mask	
Hood	
Dry suit/ semi drysuit/ wet suit	
Dry suit low pressure air hose (if used)	
Weight Belt	
Weights (kg=____________)	
Diving boots	
Neoprene gloves (5 mm)	
Fins	
Regulator	
Octopus	
Pressure gauge	
Buoyancy compensator (BC)	
BC low pressure air hose	
Tank (*V*=__ *L*, Load Pressure=_____atm)	
Knife	
Depth gauge	
Watch	
Dive table (US Navy)	
Dive computer	
Compass	
Wrist writing board	
Snorkel	
surface marker buoy (SMB)	

B) Scientific ice dives in glacial lakes (to be added to the A checklist)

Documents	Check ✓
Ice diving certification	
Dry suit diving certification	

Diving Equipment	Check ✓
Dry suit	
Dry suit low pressure air hose	
Extra weights (kg=____________)	
1st complete nonfreezing regulator (EN250)	
2nd complete nonfreezing regulator (EN250)	
Tank with 2 independently closable valves (*V*=__ *L*, Load Pressure=_____atm)	
Extra Weights (kg=____________)	
Cave Diving rope (_____ m)	
Spool (20 m)	
1st Flashlight	
2nd Flashlight	
Carabiners (#_____)	
Ice screws (#_____)	
Snow shovel	
Ice saw	

[4] Emergency Military Unit, Spanish Army.
[5] *Federación Española de Actividades Subacuáticas* (Spanish Underwater Activities Federation).
[6] Underwater Activities Special Group, *Guardia Civil*.

Diving Equipment	Check ✓
Chain saw	
Ice auger	

Appendix 2 CMAS Standard for Scientific Divers (Norro 2000)

There are two different levels of standard, both of which are professional:

- A *CMAS Advanced Scientific Diver* (CASD) is a diver capable of organizing a scientific diving team,
- A *CMAS Scientific Diver* (CSD) is a diver capable of acting as a member of a scientific diving team,

Both the CSD and CASD certificates will be issued to members of the permanent staff, contract staff, research students, technicians, and trainees or students of nationally recognized research institutions.

Additionally:

- An *Amateur Scientific Diver* is a diver participating in a scientific project but who receives no compensation at all and who has no employment type relationship with the organization responsible for the work.

Appendix 3 Proposed CMAS diving certifications required for the underwater transect sampling methodology described herein

Table 1 CMAS diving certifications required for underwater transect sampling

Diver Role	Cold water dives	Ice dives
Scientific diver coordinator	• Advanced Scientific Diver	• Advanced Scientific Diver • Dry Suit diver • Ice Diver • Rescue Diver
Sample collecting diver (in command of the underwater team)	• Advanced Scientific Diver or • Scientific Diver	• Advanced Scientific Diver or • Scientific Diver • Dry Suit diver • Ice Diver
Sample carrier diver	• Scientific Diver or • Amateur Scientific Diver	• Scientific Diver or • Amateur Scientific Diver • Dry Suit diver • Ice Diver
Measuring tape retriever+safety diver	• Scientific Diver	• Scientific Diver • Dry Suit diver • Ice Diver • Rescue Diver
Rescue diver	• Rescue Diver	• Rescue Diver • Dry Suit diver • Ice Diver

Appendix 4 Line-pull signals for surface-diver communication

When performing ice dives, scuba divers are required to operate with lifelines that tether them to the ice opening and the surface, attended by a surface line tender. Both diver and tender must be familiar with a standard communication signal code used in this situation. A line-pull signal consists of one pull or a series of sharp, distinct pulls on the lifeline (of at least 0.5 m) that are strong enough to be felt at either sides of the line.

Originally, line-pull signals were developed for use by commercial and military divers, and they can be complex and difficult to use. There are a number of line-pull signal codes that military, rescue, professional, or scientific divers use while working in dark, low visibility, or confined environments (cf., Flemming and Max 1988; Joiner 2001; U.S. Navy 2008; Phillips 2012), but all of them follow the same basic criteria.

In ice dives, the line-pull communication code should be kept as simple as possible, with few pull combinations that can be understood and felt even with thick neoprene gloves in a stressful situation. Line-pull signals may be given either by the tender to the diver or by the diver to the tender through the lifeline. For that reason we have chosen the FEDAS (2005) Ice Dive signals code. It is widely tested in ice dives, and known by all ice divers that got their Ice Diving certification under the CMAS/FEDAS standards (Tables 2 and 3):

Dive team leader in charge of communication and tender should agree upon who should send regular OK signals, to keep contact between underwater and surface teams. A single pull should be sent every minute for the entire dive. Special signals may be arranged between the divers and Scientific Diving Supervisor to meet particular mission requirements.

Most signals are acknowledged as soon as they are received. This acknowledgment consists of a quick reply with the same signal. If a signal is not properly replied by the diver, the surface signal is sent again. A continued absence of signal confirmation is assumed to mean one of the following three things: the line has tangled, there is too much slack in the line, or the diver is in trouble. In any case, the emergency and rescue procedure should be initiated.

Table 2 Diver to tender (tender has to answer with the same number of pulls)

1 pull: I am OK
2 pulls: I need more line
3 pulls: Take up line slowly
5 or more pulls: Emergency: bring me to the surface/send rescue divers (no reply required)

Table 3 Tender to diver (diver has to answer with the same number of pulls)

1 pull: Are you OK?
2 pulls: Do you need more line?
3 pulls: There is no more line available.

Appendix 5 Altitude correction for U.S. Navy (2008) decompression tables

The standard US Navy air decompression tables may be used without correction for dives conducted at altitudes between sea level and 100 m. At altitudes between 100 and 300 m, correction is required for dives deeper than 44 m of actual depth. At altitudes above 300 m, correction is required for all dives.

In addition to altitude, atmospheric pressure is affected by air temperature, local weather conditions, and other variables to a lesser extent. However, for computation purposes, just the average atmospheric pressure at a given altitude should be used (Table 4).

To determine the no-decompression time limit with the standard US Navy decompression tables, for a dive at a certain altitude above 300 m and given a specific maximum depth, the following formula should be used (U.S. Navy 2008):

$$\frac{D_s}{D_l} = \frac{P_s}{P_l} \tag{1}$$

Thus,

$$D_s = \frac{P_s \times D_l}{P_l} \tag{2}$$

Where:

D_s is the equivalent sea depth,
D_l is the actual lake depth,
P_s is the average atmospheric pressure at sea level,
P_l is the average atmospheric pressure at lake altitude.

D_s is the depth value to be used in the standard US Navy Air Decompression Table to find the no-decompression time limit. For instance, at the Sabocos tarn, located at 1,905 m of altitude (with an average atmospheric pressure of 603,7 mm Hg), a 24.5-m dive, equals a 30-m dive at sea level. According to standard U.S. Navy (2008; Table 5) air decompression tables, no-decompression time limit at 30 m depth is 25 minutes.

If atmospheric pressures are not available, a simple rule can be applied to obtain a safe equivalent sea depth to operate with tables (BSAC-RNPL 1987; Flemming and Max 1988, 1996, Table 6):

For cold or demanding dives, the next longer duration for the dive schedule should be used. If it is not possible to use the next longer duration because it places the diver into decompression, use the next less available bottom time (Joiner 2001; U.S. Navy 2008). For instance, in the case of an ice dive in Sabocos glacial lake, a maximum no-decompression time of 20 minutes (at 33.5 m), instead of 25 minutes (at 30.5 m), should be used to plan the operative dive time.

Table 4 Altitude and Average Air Pressure Chart

Altidue		Barometer		Atmospheric pressure	
Feet	Meter	in Hg	mm Hg	psi	kPa
-5,000	-1,524	35.58	903.7	17.48	120.5
-4,500	-1,372	35	889	17.19	118.5
-4,000	-1,219	34.42	874.3	16.9	116.50
-3,500	-1,067	33.84	859.5	16.62	114.6
-3,000	-914	33.27	845.1	16.34	112.7
-2,500	-762	32.7	830.6	16.06	110.7
-2,000	-610	32.14	816.4	15.78	108.8
-1,500	-457	31.58	802.1	15.51	106.9
-1,000	-305	31.02	787.9	15.23	105
-500	-152	30.47	773.9	14.96	103.1
0	0	29.92	760	14.7	101.3
500	152	29.38	746.3	14.43	99.49
1,000	305	28.86	733	14.16	97.63
1,500	457	28.33	719.6	13.91	95.91
2,000	610	27.82	706.6	13.66	94.19
2,500	762	27.32	693.9	13.41	92.46
3,000	914	26.82	681.2	13.17	90.81
3,500	1,067	23.33	668.8	12.93	89.15
4,000	1,219	28.84	656.3	12.69	87.49
4,500	1,372	25.37	644.4	12.46	85.91
5,000	1,524	24.9	632.5	12.23	84.33
6,000	1,829	23.99	609.3	11.78	81.22
7,000	2,134	23.1	586.7	11.34	78.19
8,000	2,438	22.23	564.6	10.91	75.22
9,000	2,743	21.39	543.3	10.5	72.4
10,000	3,048	20.58	522.7	10.1	69.64
15,000	4,572	16.89	429	8.3	57.16
20,000	6,096	13.76	349.5	6.76	46.61
25,000	7,620	11.12	282.4	5.46	37.65
30,000	9,144	8.9	226.1	4.37	30.13
35,000	10,668	7.06	179.3	3.47	23.93
40,000	12,192	5.56	141.2	2.73	18.82
45,000	13,716	4.37	111.1	2.15	14.82
50,000	15,240	3.44	87.5	1.69	11.65
55,000	16,764	2.71	68.9	1.33	9.17
60,000	18,288	2.14	54.2	1.05	7024
70,000	21,336	1.33	33.7	0.651	4.49
80,000	24,384	0.827	21	0.406	2.8
90,000	27,432	0.52	13.2	0.255	1.76
100,000	30,480	0.329	8.36	0.162	1.12

Table 5 No-decompression time limits for first air dives (time in minutes)

Maximum depth (m)	U.S. Navy 2008
6.1	595
9.1	371
10.7	232
12.2	163
15.2	92
18.2	60
21.3	48
24.4	39
27.4	30
30.5	25
33.5	20
36.6	15
39.6	10
42.7	10
45.7	5
54.8	5
61	-

Table 6 Depth adjustments in altitude dives

Altitude	Depth adjustment
Under 100 m	No adjustment
100–300 m	Add 1/4 actual depth to obtain table depth
300–2,000 m	Add 1/3 actual depth to obtain table depth
2,000–3,000 m	Add 1/2 actual depth to obtain table depth

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