JOURNAL OF CRUSTACEAN BIOLOGY, 28(1): 185–188, 2008

IMPROVEMENTS TO THE "SKET BOTTLE": A SIMPLE MANUAL DEVICE FOR SAMPLING SMALL CRUSTACEANS FROM MARINE CAVES AND OTHER CRYPTIC HABITATS

Pierre Chevaldonné, Boris Sket, Christian Marschal, Christophe Lejeusne, and Ricardo Calado

(PC, CM, CL) UMR CNRS 6540 «DIMAR», Centre d'Océanologie de Marseille, Aix-Marseille Université, Station Marine d'Endoume, Rue de la Batterie des Lions, 13007 Marseille, France;
(BS) Oddelek za biologijo, Biotehniska fakulteta, Univerza v Ljubljani, pp. 2995, SI-1001 Ljubljana, Slovenia (boris.sket@bf.uni-lj.si);
(RC) CCMAR, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal (rjcalado@hotmail.com);
(PC, corresponding author: pierre.chevaldonne@univmed.fr)

ABSTRACT

Dark littoral submarine caves can act as enclaves of the deep aphotic zone in shallow coastal areas, and their survey has revealed the existence of a very particular fauna of specialized and poorly known organisms among which crustaceans are particularly well represented. In these particular habitats, the use of conventional sampling techniques, such as hand nets, is often not recommended since they disturb bottom sediments causing hazardous situations to scientific divers. The use of baited traps, while technically possible, is not always practical is such remote habitats. The present work describes a simple and inexpensive manual device that can be operated by divers in submarine caves and other cryptic habitats to recurrently catch small motile organisms such as mysid crustaceans, caridean shrimps, or even gobiid fishes. This small suction bottle derived and improved from the original "Sket bottle" design considerably reduces the risks of disturbing the cave's bottom sediment and can be easily operated using a single hand. The described sampling device can also be easily used outside caves, in a variety of particular habitats, e.g., rubble filled bottoms, branching coral reefs, cracks, and small holes on rocky surfaces, in which small motile organisms usually escape from traditional sampling gears, e.g., fishnets and traps, or simply go unnoticed by researchers during sampling.

KEY WORDS: caves, field biology, Mysida, natant decapods, Peracarida, Sket bottle

INTRODUCTION

In recent decades, the systematic survey of dark littoral submarine caves has had special attention from the scientific community (Ott and Svoboda, 1976; Hart et al., 1985; Iliffe et al., 1985; Harmelin, 1997; Vacelet and Boury-Esnault, 1995; Bianchi et al., 1996; Boxshall and Jaume, 2000; Bussotti et al., 2002; Chevaldonné and Lejeusne, 2003). The particular environmental conditions of these habitats (absence of light, oligotrophy, and reduced hydrodynamic action) make dark submarine cave enclaves of the deep aphotic zone in shallow coastal areas (Harmelin et al., 1985). This particular aspect has been highlighted by several studies addressing their environmental conditions and also by the record of bathyal or abyssal organisms in some caves that conveniently became natural laboratories, presenting unique opportunities for researchers to access organisms and processes otherwise very difficult to study (Vacelet et al., 1994; Harmelin and Vacelet, 1997; Calado et al., 2004; Bakran-Petricioli et al., 2007). Due to their relatively small size, accessibility, environmental stability, and presence of poorly resilient communities of endemic and specialized species (Harmelin et al., 1985), dark submarine caves are excellent model habitats to address important ecological and evolutionary questions such as the influence of life cycle and habitat fragmentation on gene flow (Lejeusne and Chevaldonné, 2005, 2006). In addition, all information gathered on such habitats can be interpreted in the context of global climate changes and can help researchers to increase the worldwide awareness of these issues (Chevaldonné and Lejeusne, 2003).

However, sample collection of such extreme environments is far from being a simple task. Besides all general constraints that must be considered for safety reasons when using SCUBA gear underwater, researchers working in dark marine caves face even greater challenges. Cave size and morphology, together with redundant safety gear commonly decrease the freedom of movements needed to perform adequate sampling, especially when attempting to collect motile fauna. Another serious problem that must be addressed by marine cave researchers is the resuspension of the fine sediment commonly deposited at the bottom of the caves. In these particular habitats, the use of conventional sampling techniques such as hand nets is not recommended since they disturb bottom sediments increasing an already hazardous situation. The use of baited traps, while technically possible, is not always practical (repeated visits required) in such remote habitats; further, some animals are not attracted.

We describe in detail a simple and inexpensive manual suction bottle that can be easily used in submarine caves to sample small motile organisms, such as mysid crustaceans, caridean shrimps or gobiid fishes. This equipment reduces the risks of disturbing the fine sediment in the bottom of marine caves and can be easily employed by divers using a single hand. Its design was inspired by a sampling device long known by speleobiologists as the "Sket bottle," which has been widely used since Boris Sket made his original idea available to colleagues more than 30 years ago. This idea was never published, but on several occasions the bottle has been quoted, briefly described, or even shown on

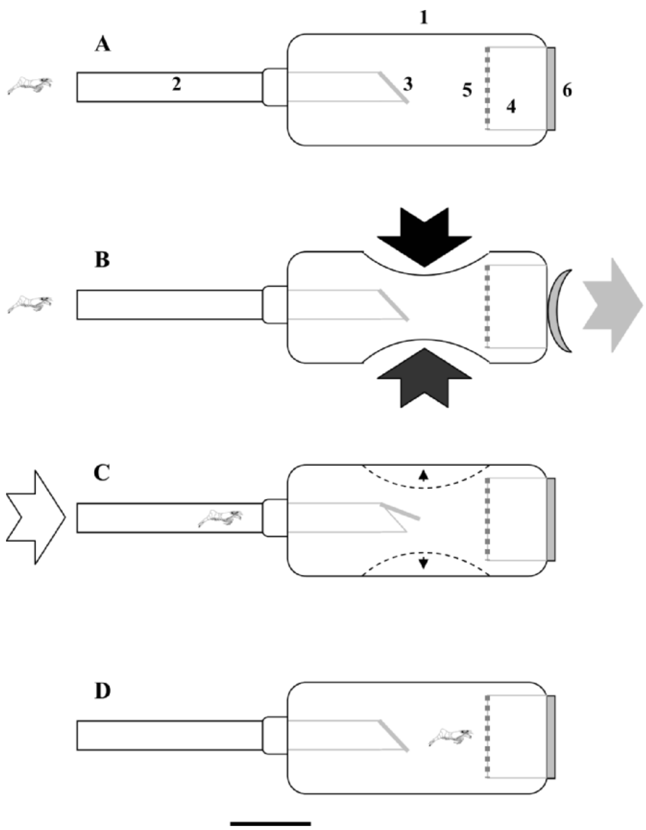

Fig. 1. Schematic representation of the manual suction bottle used to sample motile organisms. Components: 1) flexible plastic bottle; 2) plexiglass pipe; 3) front one-way rubber valve; 4) PVC pipe; 5) 400 μm mesh screen; 6) rear one-way rubber valve. Operating procedure during sampling: A) resting position, bottle filled with water, both valves closed; B) hand pressure on the bottle forces water out the rear valve; C) going back to its original shape, the bottle sucks water inside through the front pipe, pushing the front valve open (3); D) back to resting position, specimens are trapped and intact inside the bottle. Large black arrows represent the diver's hand pressure on the bottle; large grey arrow represents the water pushed outside the bottle through the valve; white arrow represents the inflow of water being sucked in the device; small black arrows represent the bottle returning to its original shape. Scale bar: 50 mm.

pictures (Bozanic, 1985; Kensley and Williams, 1986). The design presented here, however, significantly improves this original design, essentially by the addition of a unidirectional valve at the rear end. A detailed list of all different taxa already collected using this sampling device is provided and the suitability of this sampling method in marine environments where cryptic crustaceans are commonly overlooked when using traditional sampling techniques is also discussed.

Materials and Methods

A flexible plastic bottle (ca. 200 × 70 mm) is used as the main body of the sampling device (Fig. 1). A plexiglass pipe (250 × 20 mm) is fitted and secured to the opening; semi-rigid rubber can be used as a more flexible alternative to easily obtain a tight fit. The fit must be tight but not too much so, since the pipe must be removed each time samples are recovered. Most (ca. 180 mm) of the pipe is left outside the bottle opening, with the remaining 70 mm resting inside the bottle. The posterior (inside) end of the pipe is bevelled and a piece of rubber is glued to act as a one-way valve. A PVC pipe (50 × 50 mm) is fitted to the bottom of the bottle with a 400 μm mesh screen at the anterior part (inside the bottle) and a one-way rubber valve, e.g., from dust masks, at the posterior end (outside the bottle) (Fig. 1). Both pipes are fitted to ensure that the entire device is water tight when the rubber valves are closed. A wrist-strap or loop of any sort (not shown) can be fitted to the bottleneck to prevent accidental drop or to allow hands-free manipulations.

The operating procedure of the sampling device is illustrated in Fig. 1 and can be briefly described as follows: using a single hand, the diver points the device towards the specimen(s) to be collected; the diver presses the lateral walls of the flexible plastic bottle; the water inside is forced out only through the rear rubber valve; releasing the hand pressure on the bottle creates a suction of outside water in the bottle; the suction force will depend on the plastic material used for the bottle, but usual material, e.g., 0.5 L laboratory squirt bottles, industrial soft drink bottles, etc., creates enough suction to drag most motile fauna inside the bottle. The organisms trapped inside the bottle are retained by the 400 μm mesh and are kept intact. The procedure can be repeated if needed, as long as captured animals can be mixed.

Discussion

This improved sampling device has been successfully used for more than six years all around the Mediterranean and Eastern Atlantic, mostly in marine caves. We have been able to collect a wide range of motile organisms within littoral dark marine caves. Examples of the main taxa collected by the sampling device is provided in Table I. Sampling efforts have been purposely biased towards mysids and caridean shrimps, with remaining animal groups commonly being a "by-catch." The disturbance caused to the thin sediment commonly covering the bottom of submarine caves is reduced to a minimum. Nevertheless, it is important that the diver avoids positioning the rear of the bottle (where water flows out) too close to the sediment. This could be improved if the outlet valve were on the side rather than the rear of the bottle, but a trade-off must be found between efficiency and simplicity. Also, when recovering samples, the bottle must be oriented with the rear up until it is emptied otherwise inside water is lost through the rear outlet valve and samples are damaged. Alternatively, the bottle may rest vertically in a vessel with some water. With the bottle in this position, captured organisms are recovered by removing the pipe over a collection bucket. The original "Sket bottle" with both inlet and outlet on the tube is, with this respect, easier to handle, allowing bottles to stand and to be emptied the regular way. However, the main improvement described here over the original "Sket bottle," viz., the rear valve, makes hunting for highly mobile animals much easier since pressing and depressing the bottle can be achieved while still aiming at the animal target; the old design only allowed one suction movement at a time. There is an interesting parallel with fluid biomechanics: such a unidirectional water flow system (from the mouth through gill slits) has been found to be better suited for catching aquatic prey in neotenic newts and salamanders, while their metamorphosed counterparts (water in and out of the mouth - as in old version of Sket bottle) are performing better with terrestrial preys (Denoël et al., 2005). For very fragile animals such as polynoid polychaetes, or to reduce stress to otherwise intact organisms, the diameter of the rear pipe (4; Fig. 1) can be decreased, so that it will be less likely that animals will be damaged by suction against the filter. The general design is, therefore, highly adaptable to specific needs.

Table I. Significant examples of taxa collected from cryptic habitats using the manual suction device described in the present work.

Order or high taxonomic rank	Family	Taxon	Location, habitat, substrate, depth below seawater
Calanoida	Ridgewayiidae	*Ridgewayia marki minorcaensis*	Marseille, France, marine cave, 17 m
	Stephidae	*Stephos* sp. nov.	La Ciotat, France, on cave mud, 25 m
Mysida	Mysidae	*Hemimysis* spp. (more than 10 different species	Mediterranean and E. Atlantic, all sorts of cryptic habitats, 0-40 m
		Siriella gracilipes	id., 0-30 m
		Harmelinella mariannae	Marseille, France, marine cave, 17 m
Thermosbaenacea	Monodellidae	*Tethysbaena* sp. nov.	Cassis, France, underground river with brackish water, on mud, 20 m
Amphipoda	Niphargidae	*Niphargus* sp. nov.	Cassis, France, underground river with brackish water, 20 m
Decapoda	Alpheidae	*Athanas nitescens* forma *laevirhynchus*	Marseille, France and Monaco, caves, 10-35 m
Decapoda	Bresiliidae	*Bresilia saldanhai*	Madeira, Portugal, marine cave, 15 m
Decapoda	Hippolytidae	*Caridion* sp.	Marseille, France and Monaco, caves, 15-40 m
		Eualus sp.	Marseille, France and Monaco, caves, 25-35 m
		Lysmata nilita	Marseille, France, marine cave, 17 m
		L. seticaudata	Marseille, France, marine caves and other cryptic habitats, 15-25 m
Decapoda	Galatheidae	*Galathea strigosa* (juvenile)	Monaco, marine cave, 25 m
Chaetognatha	Spadellidae	*Spadella ledoyeri*	Marseille, France, marine cave, 17 m
Teleostei	Gobiidae	*Gammogobius steinitzi*	Marseille, France and Monaco, caves, 17-30 m

This device has been a useful tool for researchers working on small motile organisms in dark marine caves, particularly mysids and carideans. The use of this sampling device has already allowed the collection of several crustaceans new to science (Table 1), including the small bresiliid, *Bresilia saldanhai* Calado, Chevaldonné, and dos Santos, 2004, or species new to some geographic areas, e.g., the caridean shrimp genus *Caridion* having only recently been collected in significant numbers in the Mediterranean Sea from marine caves (unpublished data). Marine cave mysid research has benefited from use of this device in recent years: time-series of mysid samples suitable for systematic, molecular, and biometric analyses, as well as experiments on the live specimens have been recovered by this method (Chevaldonné and Lejeusne, 2003; Lejeusne and Chevaldonné, 2005, 2006; Benzid et al., 2006; Lejeusne et al., 2006).

Its easy operation and small size are ideal for cave divers, who already have to carry cumbersome equipment and must be careful of their movements. Although not physically necessary, designing the device (pipe and bottle) with transparent material further improves its efficiency; the operator can check whether the animal is still in the pipe or has been efficiently sucked in the bottle. It is obvious that the device can be successfully used in a variety of aquatic environments commonly presenting a number of challenges for researchers, such as rubble filled bottoms, branching coral reefs, small holes, or cracks on rocky surfaces, where sampling small sized motile organisms is difficult. We hope that publishing for the first time the simple design of this improved "Sket bottle" will help aquatic researchers to recover bits of the largely unknown biodiversity of submerged cave systems.

Acknowledgements

The authors are indebted to Donat Petricioli for bringing the original "Sket bottle" design to the attention of one of us (PC). One anonymous reviewer of an earlier version of this manuscript, and F. Schram provided useful comments. We must acknowledge the support of the many divers having participated to our researches for the past 6 years. The last author would like to acknowledge Fundação para a Ciência e a Tecnologia (scholarship SFRH/BPD/18009/2004) from the Portuguese government for their financial support.

References

Bakran-Petricioli, T., J. Vacelet, H. Zibrowius, D. Petricioli, P. Chevaldonné, and T. Rada. 2007. New data on the distribution of the "deep-sea" sponges *Asbestopluma hypogea* and *Oopsacas minuta* in the Mediterranean Sea. Marine Ecology – An Evolutionary Perspective 28: (Suppl. 1):10-23.

Benzid, D., L. De Jong, C. Lejeusne, P. Chevaldonné, and X. Moreau. 2006. Serotonin expression in the optic lobes of cavernicolous crustaceans during the light-dark transition phases: role of the lamina ganglionaris. Journal of Experimental Marine Biology and Ecology 335: 74-81.

Bianchi, C. N., R. Cattaneo-Vietti, F. Cinelli, C. Morri, and M. Pansini. 1996. Lo studio biologico delle grotte sottomarine del Mediterraneo: conoscenze attuali e prospettive. Bolletino dei Musei e degli Istituti Biologici dell Universitá di Genova 60-61: 41-69.

Boxshall, G. A., and D. Jaume. 2000. Discoveries of cave misophrioids (Crustacea : Copepoda) shed new light on the origin of anchialine faunas. Zoologischer Anzeiger 239: 1-19.

Bozanic, J. E. 1985. Equipment and techniques developed for scientific exploration of oceanic caves. Oceans 17: 962-966.

Bussotti, S., A. Terlizzi, S. Fraschetti, G. Belmonte, and F. Boero. 2006. Spatial and temporal variability of sessile benthos in shallow Mediterranean marine caves. Marine Ecology Progress Series 325: 109-119.

Calado, R., P. Chevaldonné, and A. dos Santos. 2004. A new species of the deep-sea genus *Bresilia* (Crustacea: Decapoda: Bresiliidae) discovered from a shallow-water cave in Madeira. Journal of the Marine Biological Association of the United Kingdom 84: 191-199.

Chevaldonné, P., and C. Lejeusne. 2003. Regional warming-induced species shift in north-west Mediterranean marine caves. Ecology Letters 6: 371-379.

Denoël, M., P. Joly, and H. H. Whiteman. 2005. Evolutionary ecology of facultative paedomorphosis in newts and salamanders. Biological Reviews 80: 663-671.

Harmelin, J.-G. 1997. Diversity of bryozoans in a Mediterranean sublittoral cave with bathyal-like conditions: role of dispersal processes and local factors. Marine Ecology Progress Series 153: 139-152.

———, and J. Vacelet. 1997. Clues to deep-sea biodiversity in a nearshore cave. Vie et Milieu 47: 351-354.

———, ———, and P. Vasseur. 1985. Les grottes sous-marines obscures: un milieu extreme et un remarquable biotope refuge. Téthys 11: 214-229.

Hart, C. W., Jr., R. B. Manning, and T. M. Iliffe. 1985. The fauna of Atlantic marine caves: evidence of dispersal by sea floor spreading while maintaining ties with deep waters. Proceedings of the Biological Society of Washington 98: 288-292.

Iliffe, T. M., C. W. Hart, Jr., and R. B. Manning. 1985. Biogeography and the caves of Bermuda. Nature 302: 141-142.

Kensley, B., and D. Williams. 1986. New shrimps (families Procarididae and Atyidae) from a submerged lava tube on Hawaii. Journal of Crustacean Biology 6: 417-437.

Lejeusne, C., T. Pérez, V. Sarrazin, and P. Chevaldonné. 2006. Baseline expression of Heat Shock Proteins (HSP) of a 'thermotolerant' Mediterranean marine species largely influenced by natural temperature fluctuations. Canadian Journal of Aquatic and Fisheries Sciences 63: 2028-2037.

———, and P. Chevaldonné. 2005. Population structure and life history of *Hemimysis margalefi* (Crustacea: Mysidacea), a "thermophilic" cave-dwelling species benefiting from the warming of the NW Mediterranean. Marine Ecology Progress Series 287: 189-199.

———, and ———. 2006. Brooding crustaceans in a highly fragmented habitat: the genetic structure of Mediterranean marine cave-dwelling mysid populations. Molecular Ecology 15: 4123-4140.

Ott, J. A., and A. Svoboda. 1976. Sea caves as model systems for energy flow studies in primary hard bottom communities. Pubblicazione de la Stazione Zoologica di Napoli 40: 477-485.

Vacelet, J., N. Boury-Esnault, and J.-G. Harmelin. 1994. Hexactinellid cave, a unique deep-sea habitat in the SCUBA zone. Deep-Sea Research I 41: 965-973.

———, and ———. 1995. Carnivorous sponges. Nature 373: 333-335.

RECEIVED: 5 June 2007.
ACCEPTED: 30 August 2007.