

BLUE-WATER SCUBA COLLECTION OF PLANKTONIC FORAMINIFERA

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The best way to collect free-floating planktonic foraminifera for laboratory study and experimentation is to capture them in glass jars held by scuba divers at 3 to 5 m depth in open-ocean conditions. This blue water diving method is preferred over collection with a plankton net because it minimizes trauma and enhances the survival of taxa that are easily damaged or killed when they come into contact with the mesh of a plankton net. Planktonic foraminifera can be discriminated from other groups of marine plankton (e.g., radiolaria, acantharia) by focusing from 50 to 80 cm distance, whereas species-level identification is best accomplished at a range of 20 to 50 cm. One fine-mesh collection bag containing wide-mouthed collection jars (~130 ml) that are each filled with seawater is hung onto a 25 m length vertical down-line that is passed through a float and tethered to the dive boat. An empty collection bag is also attached to the line to receive jars with captured specimens. Divers are tethered to the down-line through a safety-diver monitored trapeze to ensure continuous contact with the diving platform. Foraminifera are collected by gently rotating the jar around a specimen as the lid is slowly lowered onto the jar top. After the lid is firmly replaced the jar is put into the receiving sack. Using this method, as many as 60 to 80 specimens/hr can be collected depending on planktonic foraminifera abundance. Upon return to the laboratory the collected specimens are transferred to culture vessels using a wide-mouthed pipette. At this point, the researcher is ready to initiate a desired experiment.

BACKGROUND

Planktonic foraminifera comprise a group of free-floating protozoa that inhabit surface waters throughout the world's major oceans. The microscopic (<1 mm) calcium carbonate shells of these organisms are preserved as fossils in deep sea sediments dating back to the middle Jurassic Period, over 150 million years ago. Changes in the species composition of deep sea sediments or microvariations in the chemistry of the foraminifera shells yield information about the oceanic environment when the foraminifera were alive. Because of their long geological history and sensitivity to environmental

conditions, planktonic foraminifera have long been used to interpret the geological age in which they were deposited and the ecological/climatic conditions that existed when they were alive. Thus, it is important to understand their modern vertical and horizontal distribution patterns, the ecological parameters that control shell growth, shell morphology, and changes in the chemical composition of their shell throughout their development.

Collection of living planktonic foraminifera for culture study is best accomplished in the open ocean, and preferably in blue-water, as specimens are rare in nearshore marine environments. Some species can be collected for laboratory study using a plankton net with a 75 to 202 μm mesh, but other species are traumatized by such collection methods and do not survive the entanglement of their spines and rhizopodia. These species require collection by scuba divers.

BLUE-WATER SCUBA DIVING

ARRANGEMENT FOR TETHERED DIVING

Several precautions must be taken when scuba diving in blue-water environments because of: 1) the lack of visual references for maintaining a constant depth position; 2) the risk of sinking quickly beyond the reach of rescuers in the case of a diving accident; and 3) the danger of shark attack. An array of tether lines is typically utilized whereby each diver is connected to a trapeze that is attached to a vertical down-line. In addition, a safety diver is present on each dive to disentangle the research diver tether lines and to look out for sharks and other potential hazards that might arise.

One setup that is used by divers when capturing plankton at 3-5 m water depth is illustrated in Figure 1. A line is tied from the dive boat to a float and from the float to a 2 kg weight that hangs about 25 m below the float. The down-line is continuously connected to the diving platform through the float. A light weight (circa 2-3 kg) and "flopper stopper" (e.g., the top of a 5 gal pickle bucket) are placed at the end of the down line to serve as a shock absorber to dampen vertical line movement due to surface sea conditions. To further dampen the vertical heave of the line from surface waves a 0.2 m rubber line is linked to a 0.5 m section of slack in the vertical line immediately below the float.

The safety diver is stationed by the trapeze about 2 m below the specimen collection sacks. This diver carries a shark billy, which is simply a 2 m length of PVC pipe, to ward off inquisitive sharks. Shark billies with an explosive head are avoided since there is substantial risk that these could be triggered accidentally. Each end of the trapeze has holes for 6 m safety lines that are attached to the collection scuba divers with quick release clips at their buoyancy compensators. An 85 g fishing weight is tied to the other end of the tether line to take up line slack and minimize tangling the line.

SCUBA COLLECTION OF PLANKTONIC FORAMINIFERA

Once the dive boat has reached the offshore collection site the engine is shut off. If sea conditions are rough or a breeze is blowing, bow and stern plankton nets are deployed to serve as sea anchors to slow down drift. Next, the tether and trapeze lines are deployed with the mesh collection sacks attached. The upper sack contains wide-mouthed collection jars (~130 ml) that are filled with seawater and the lower sack, which is empty, is used to receive the collection jars containing captured specimens. Once all the lines are deployed, the divers enter the water, with the safety diver taking position on the trapeze and the collection divers hooking up to the safety tethers.

Planktonic foraminifera are quite easy to distinguish from other groups of marine plankton (e.g., radiolaria, acantharia, and the colonial cyanobacterium *Trichodesmium*). The divers maintain a depth of 3-5 m, and look upwards towards the bottom of the dive boat. By focusing approximately 50 to 80 cm distant, planktonic organisms are readily apparent against the dark background of the hull of the boat because sunlight reflects off the organisms. This method creates an effect that is analogous to dark field lighting on a microscope. Foraminifera are particularly obvious, looking like a small ball of fine flexible

hairs (calcite spines) that radiate from a central point (the shell). Although a foraminifera shell is typically <1 mm in maximum diameter (Fig. 2), the long spines give the animal a cross-section that is generally between >3-4 mm to 6-8 mm (Fig. 3).

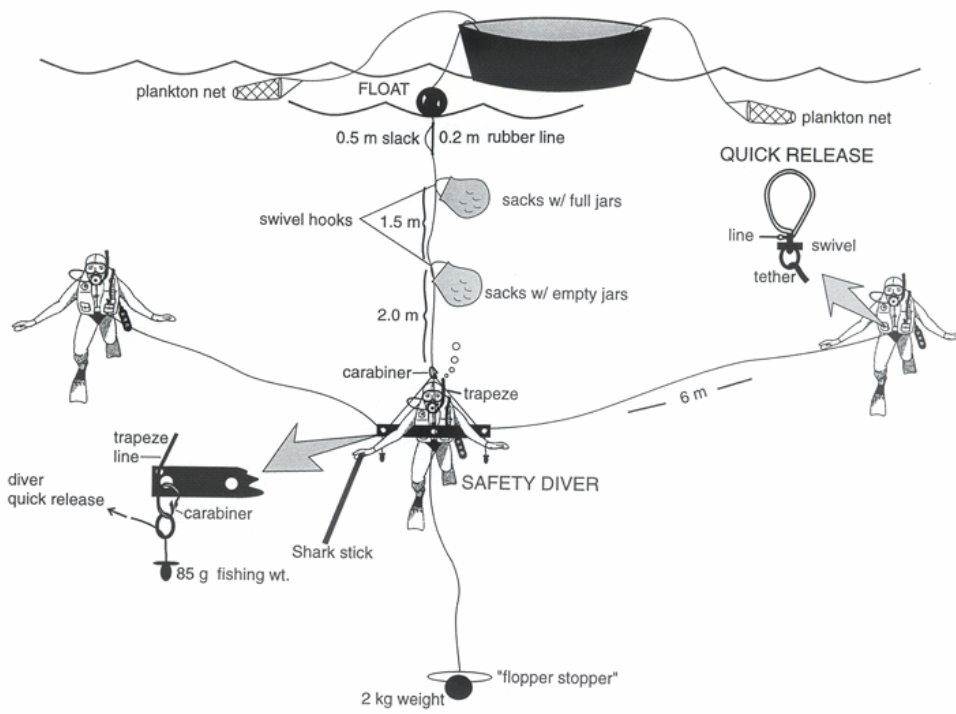


Figure 1. Arrangement of tether line assembly, equipment, and scuba divers for collecting surface plankton in blue-water environments.

Given sufficient experience, a diver can not only distinguish foraminifera from other planktonic organisms, but can accurately identify specimens to the species level. Such identification is possible because of variations in the density, arrangement and length of the spines and by the organization of the rhizopodial network. As many as eight different spinose taxa can be identified with some practice (Tables 1, 2). However, non-spinose species cannot be distinguished by scuba divers because the shell alone is too small to identify among a mixed planktonic community. Hence, species lacking spines must be collected using a plankton net.

Once a specimen is identified for collection, the diver slowly swims over to the floating foraminifera and positions the collection jar next to it. The collection jar is slowly rotated around the specimen as the lid is slowly lowered onto the jar top. After the lid is firmly reattached, the jar is put into the receiving sack. Using this method, as many as 60 to 80 specimens can be collected within an hour provided planktonic foraminifera are sufficiently abundant.

Because this particular type of blue-water diving takes place close to the surface, extreme care should be taken to control buoyancy and to breathe at a normal rate. Our divers generally adjust their weight belts so that a slightly negative buoyancy is attained at the surface. At a depth of 3-5 m the divers are then negatively buoyant and can easily maintain a desired depth with minor fin movement. Finally, we prefer steel tanks to aluminum cylinders because the latter become buoyant as they empty making buoyancy control difficult at these shallow depths.

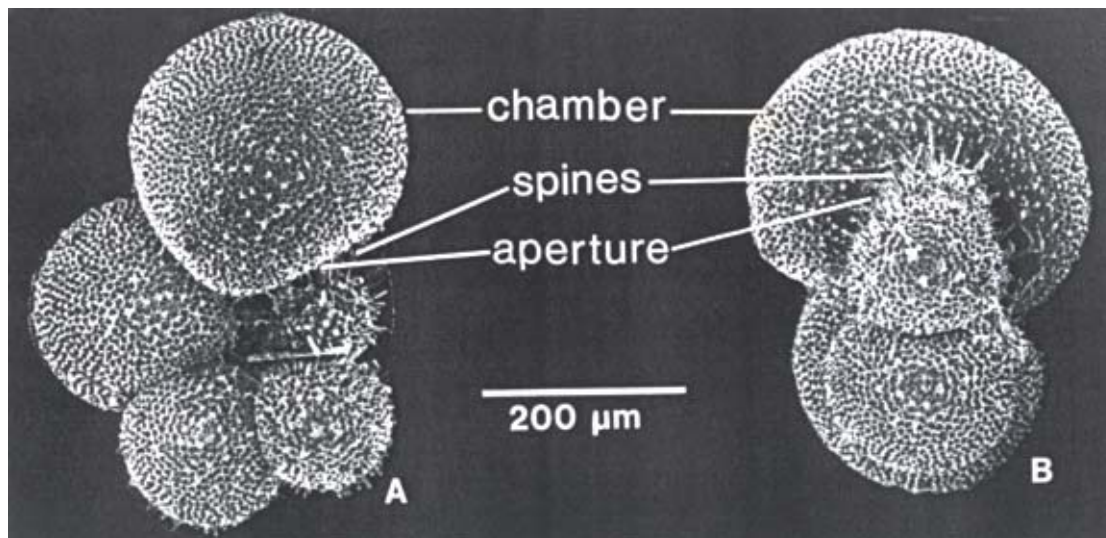


Figure 2. Scanning Electron Micrographs showing side (fig. 2A) and edge (fig. 2B) views of the spinose planktonic foraminifer shell of *Globigerinella siphonifera* Type I. Specimen was collected from blue-water by a scuba diver and underwent gametogenesis (reproduction) in the laboratory. Most of the spines were discarded during gametogenesis or broken during preparation for SEM observation.

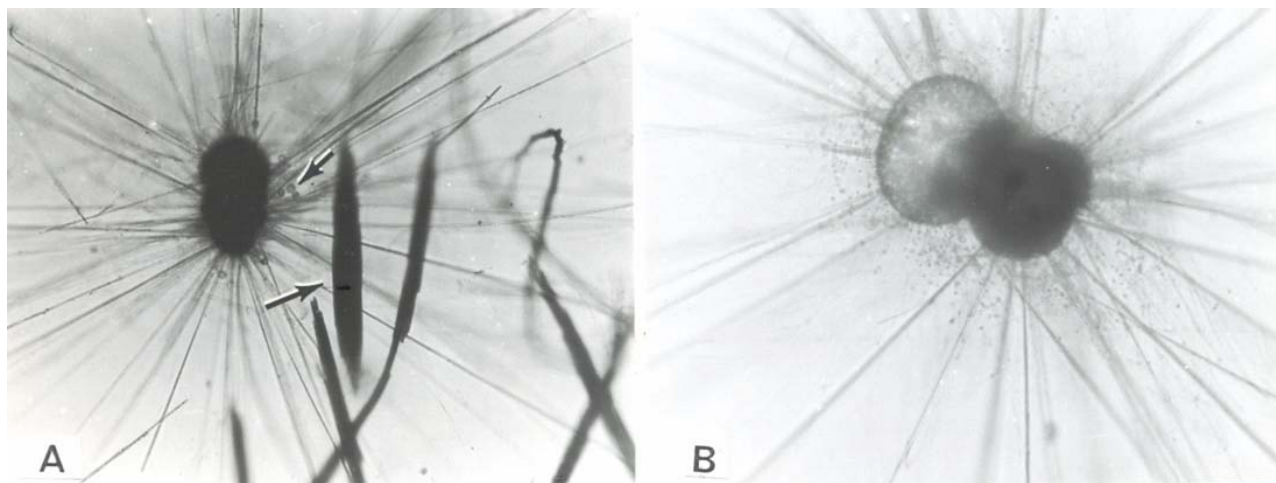


Figure 3. Inverted light microscope photographs of blue-water collected living specimens of *Globigerinella siphonifera* Type I. Figure 3A: specimen with cytoplasm withdrawn into shell (dark area at center of spines) and commensal dinoflagellates (arrow pointing to small dots) and the cyanobacterium *Trichodesmium*. Figure 3B: specimens with woolly rhizopodial network extruded out of the aperture of the final chamber and between the spines. Note the presence of endosymbionts (tiny dots surrounding shell). Shell size of specimen in Figure 3A ~600 μm; shell size of specimen in Figure 3B ~500 μm.

Following a dive, the collection jars are removed from the water and placed into a water-filled pail with a tight fitting cover. The water keeps the specimens from heating up, and the lid shuts out direct sunlight that would affect the symbiotic algae that are typically associated with these organisms.

TABLE 1. List of tropical planktonic foraminifer taxa that can be identified by SCUBA divers and their distinguishing features.

PLANKTONIC FORAMINIFERA	DISTINGUISHING FEATURES
Positive Identification	
<i>Globigerinella siphonifera</i> Type I	woolly, rhizopodial network; spine density relatively low; spines radiate in all directions; white color; frequent commensal association with <i>Trichodesmium</i> ; diam. up to 3 mm
<i>Globigerinella siphonifera</i> Type II	rhizopodial network cannot be distinguished; spines often concentrated in two tufts; brown color; no association with commensals; diam. up to 2 mm
<i>Hastigerina pelagica</i>	cytoplasmic 'bubbles' surrounding shell; spines are stiff and radiate in all directions; dark red color; most abundant around full moon; large size (up to 6-8 mm)
<i>Orbulina universa</i> (adult)	spherical chamber; spines radiate in all directions, high density, individual spines hard to see; spherical shell; light green to white color; symbionts may be arranged in halo; diam. up to 5 mm; most abundant around full moon
More Difficult Identification	
<i>Globigerina bulloides</i>	spines radiate in all directions, density low, less orderly arrangement than in <i>O. universa</i> ; diam. up to 1.5 mm
<i>Globigerinoides conglobatus</i>	spines radiate in all directions; diam. up to 1.5 mm
<i>Globigerinoides ruber</i>	spines radiate in all directions, density low; white to pink color; diam. up to 2 mm
<i>Globigerinoides sacculifer</i>	spines radiate in all directions, density high; shell white to green color; diam. up to 2 mm
<i>Orbulina universa</i> (pre-adult))	spines radiate in all directions, high density, individual spines hard to see; symbionts may be arranged in halo; diam. <5 mm

TABLE 2. Identification key for blue-water collection of planktonic foraminifera living in tropical surface waters.

1) relatively long spines, low density.....	3)
2) relatively short spines.....	5)
3a) bubble capsule.....	<i>H. pelagica</i>
3b) no bubble capsule.....	4)
4a) shell dark brown, no <i>Trichodesmium</i>	<i>G. siphonifera</i> II
4b) shell white, woolly rhiz. network, mostly assoc. w/ <i>Trichodesmium</i>	<i>G. siphonifera</i> I
5a) spines moderately high density.....	6)
5b) spines very high density.....	8)
6a) no symbiont halo.....	<i>G. bulloides</i>
6b) symbiont halo.....	7)
7a) white color.....	<i>G. ruber</i> white
7b) pink color.....	<i>G. ruber</i> pink
8a) individual spines hard to discriminate.....	<i>O. universa</i>
8b) individual spines can be discerned.....	9)
9a) symbionts present.....	<i>G. sacculifer</i>
9a) no symbionts present.....	<i>G. conglobatus</i>

LABORATORY ANALYSIS

Upon return to the laboratory the collected specimens are transferred to glass vessels using a wide-mouthed pipette and are placed in a temperature- and light-controlled environment for study and/or experiments. The specimens may be observed in glass vials with an optically clear and flat bottom using an inverted light microscope, and they can be kept alive by feeding them one *Artemia* shrimp nauplius per day. Growth of the shell by chamber addition, extension of the rhizopodia outside the shell, and observation of cytoplasmic streaming are clear indications that the cultured specimen is healthy. Spinose species will often be found floating in culture vessels some time after they have been emplaced. Gametogenesis will occur in healthy, mature individuals and is recognized by withdrawal of the rhizopodia, change from translucent to opaque appearance of the cytoplasm as gametes are produced, and expulsion of the gametes and emptying of the shell of cytoplasmic material. The time that it takes a scuba collected juvenile to grow to adult size and produce gametes may vary from several days to more than a week.

Gamete fusion leading to production of multiple generations of foraminifera has never been observed in the laboratory, so specimens must be constantly replenished for successful completion of various laboratory experiments. Specimens are studied in culture to learn more about their biology, ecology, and shell chemistry. The effects of changes in temperature, salinity, feeding rates, ambient water chemistry, and light intensity have been used to determine the effects on shell growth rates, longevity, and shell chemistry. Results from such studies provide a baseline for reconstructing environmental conditions and ecological relationships for fossil foraminifera in the geological past.

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