

## Chapter III.5 Deep-Sea Respirometry: In Situ Techniques

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### 1 Introduction

The deep-sea environment occupies over half the earth's surface. However, inaccessibility has greatly restricted the study of this unique environment characterized by high pressure and low temperature. Technological advances over the past 15 years permit us to "enter" the deep-sea environment either directly with submersibles or indirectly with sophisticated instrumentation to make observations, collections and measurements and to conduct experiments. With these advances, the biological dynamics of the deep-sea ecosystem are slowly being resolved. Our contributions to this resolution are related to energy flow and, more specifically, the metabolism of deep-sea organisms. The integral component of our instrumentation used in these studies is the polarographic oxygen sensor (POS).

There are two basic approaches to studying the metabolism of deep-sea organisms, (1) a laboratory approach and (2) an in situ approach. The first method involves capturing the organisms in their environment and returning them to the surface to conduct measurements and experiments in the laboratory [2, 11, 19, 24]. Many limitations of this approach must be resolved or acknowledged. (A) The capture process, no matter how gentle, stresses the organism. This is evident from initial oxygen consumption rate increases noted immediately following entrapment [15, 17, 18]. (B) Organisms captured at depth and brought to the surface unprotected will undergo decompression and temperature change. Decompression problems are being addressed with pressure-retaining mechanisms that have been employed in traps [25] and net cod ends [9]; temperature control problems are being addressed with insulated traps [25] and net cod ends [3]. (C) Solar or artificial light sources can adversely affect visual pigments of deep-sea organisms accustomed to low ambient light levels [4, 10]. (D) Organisms are generally returned to a surface ship where they experience varying degrees of abnormal motion (rolling, yawing, pitching). (E) Confinement in containers for both laboratory maintenance and metabolic measurements places physical and biological constraints on organisms. (F) Organisms are generally held in surface seawater, ignoring the water quality differences between surface and deep sea.

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The in situ methodology is based on capturing and measuring the metabolism of organisms in their environment. There are limitations to this approach which must be considered. (A) The capture process, with its associated stress to the organism, is a problem pertaining to in situ work as well as to the laboratory approach mentioned above. (B) When submersibles are used for in situ operations, the artificial lighting will have a temporary blinding effect on animals with visual pigments which normally experience attenuated light levels [4, 10]. (C) There is a severe limitation on the number of measurements and the complexity of the experiments which can be performed. (D) Containment of organisms in either flowthrough or closed chambers is an abnormal situation. These effects are believed to be minimal from comparisons of the observed behavior of entrapped versus free animals [15, 17, 18].

After analyzing the limitations of the two methodologies, we chose to use the in situ approach because it allows measurements of metabolism to be made under conditions more closely approximating the natural environment from which the organisms have been removed.

### 2 In Situ Methodology

We have developed equipment for measuring the metabolism (oxygen consumption and excretion) of both individual animals and communities of organisms in situ. These instruments were developed for deployment with manned submersibles and with free vehicles (systems autonomous from the surface ship). The emphasis of our research has been directed toward the deep-sea benthos and the water column immediately overlying the sediment-water interface which together constitute the benthic boundary layer. The biota of this layer can be divided into two basic components, the sediment community and the pelagic community. Specific equipment has been developed to examine the metabolism of various components of these two communities.

#### 2.1 General Equipment

The two parameters which we measure as indicators of the metabolic activity of deep sea organisms are rates of oxygen consumption and nutrient exchange within sealed enclosures. Changes in dissolved oxygen tension are measured with a POS [8]. Nutrient flux measurements are made by the chemical comparison of an initial and final water sample taken by a syringe withdrawal system from an enclosure incubated in situ. Organic enrichments, biological poisons, or radioactive tracers, useful in compartmentalizing O<sub>2</sub> uptake, may also be introduced into the respiration chambers by syringe injection systems. The electronic and mechanical systems designed for oxygen measurement and the syringe injection/withdrawal system are, with a few variations, the same for all experimental enclosures used. The dimensions and configuration of the enclosure vary depending on the organism (fish, amphipod, crustacean) or community (benthic or pelagic) being studied and the type of vehicle used for equipment deployment and recovery (deep-sea submersible or surface ship).



### 2.1.1 Polarographic Oxygen Sensor/Amplifier System

Each respirometer unit is capable of monitoring changes in dissolved oxygen tension by means of a POS of the type described by Kanwisher [8]. This sensor can easily be made in the laboratory at minimal expense. The basic unit consists of a Ag-Ag<sub>2</sub>O cylinder (1 cm diam., 1 cm length), which is concentric around a platinum disk (5 mm diam., 0.02 mm thick) and a thermistor (provides automatic temperature compensation). The whole assembly with attached electrical cables is cast in epoxy (Hysol resin R-2039, hardener HD 3561). Prior to use, the sensors are filled with electrolyte (1 mol dm<sup>-3</sup> KOH), covered with a Teflon membrane (0.03 mm), and calibrated using air saturated and nitrogen purged chilled seawater. The effects of hydrostatic pressure on the sensors are corrected for, using laboratory generated pressure/output curves for each sensor (see also Chap. I.8).

The amplifier and recorder, as well as the other associated electronic circuitry, are contained in an aluminum pressure-resistant housing. A POS is inserted into each respirometer enclosure to record the relative oxygen tension continuously. A power regulator provides a 1 V excitation voltage to each of two sensor anodes (Fig. 1). The resulting sensor current, which is proportional to dissolved oxygen tension, passes through a preamplifier before being recorded. These preamplified signals from both sensors are multiplexed so that the two outputs can be recorded on one chart recorder (Rustrak model 288/F137). This multiplexing is done by selecting one of two transmission gates with a switching signal.

For greater resolution, the preamplified signal is processed by an autoscaling circuit which splits the full scale of the recorder (zero to saturated dissolved oxygen solution) into five divisions, so that each chart width represents a 20% change in oxygen concentration. This circuit consists of a comparator, oscillator, counter, resistor ladder, step amplifier, and a reference voltage. Its function is to generate a step voltage which increases or decreases (by counting up or down in steps of 20% of full scale) until the sum of the step voltage and the preamplified sensor voltage is within the range of the meter movement. This sum is then amplified by a current follower to drive the recorder meter movement.

### 2.1.2 Stirring Motor

A stirring motor is inserted into each respiration chamber to circulate the water over the POS and prevent stratification within the enclosure. This stirring motor consists of three electromagnetic coils, symmetrically spaced about the axis of a ceramic magnetic rotor with attached Teflon impeller (Fig. 2). These coils, embedded in epoxy (Hysol resin R-2039, hardener HD 3561), are consecutively pulsed to attract the rotor.

The electronic circuitry which drives the coils is a simple waveform generator (Fig. 2c). An oscillator coupled with a monostable multivibrator creates the drive pulse and an oscillator (clock) signal. A sequencing circuit, built around a three-stage counter and driven by the oscillator, generates a holding pulse and sequentially routes it to each one of three outputs (A, B, and C, Fig. 2d). These holding pulses (with individually adjusted amplitudes) are summed with the power drive pulse at the bases of three

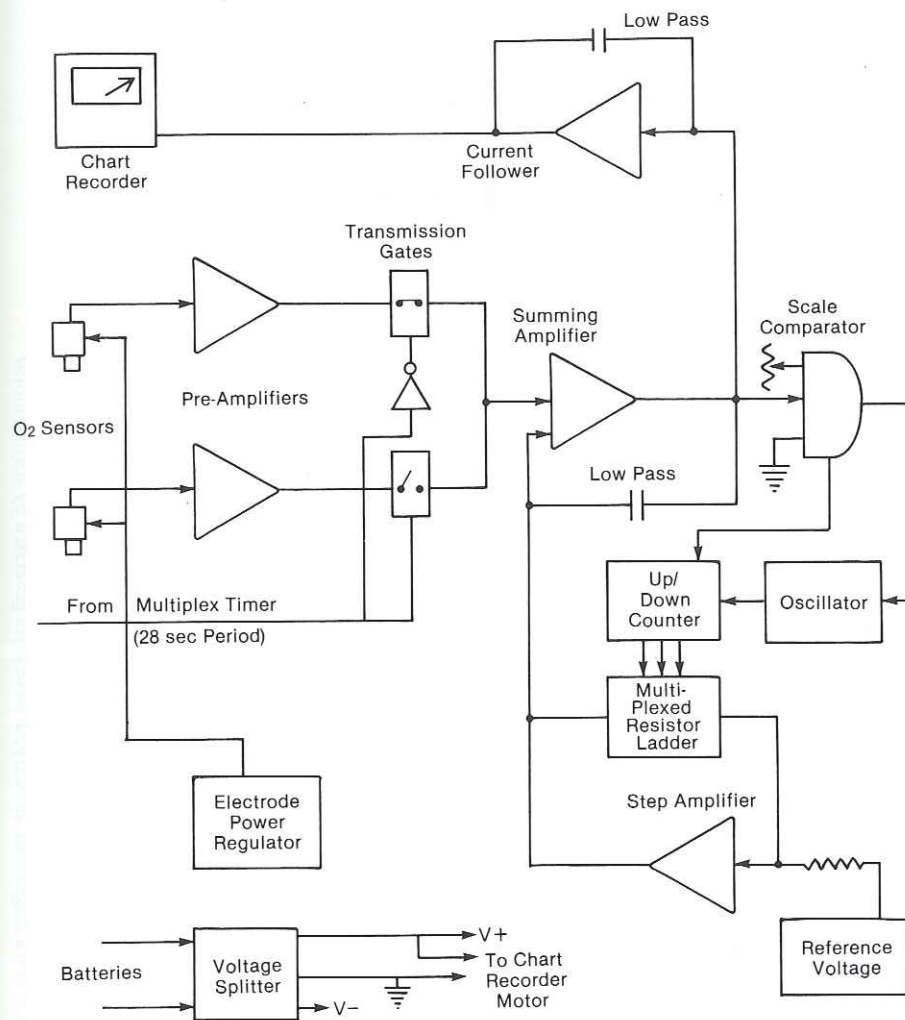


Fig. 1. Block diagram of autoscaling POS amplifier

transistor coil drivers, to activate the three coils. As each driver is sequentially selected, the corresponding coil is activated to attract and turn the rotor. A variable resistor adjustment permits water flow rates to be set between 0.28 to 5.65 cm<sup>3</sup>/s.

### 2.1.3 Syringe Injection/Withdrawal System

Nutrient flux determinations (e.g., excretion and nitrification) are another useful measure of metabolic activity. For these measurements, we have developed a system capable of withdrawing 50 cm<sup>3</sup> of seawater from closed chambers for nutrient determination. An optional and interchangeable injection unit may be added for

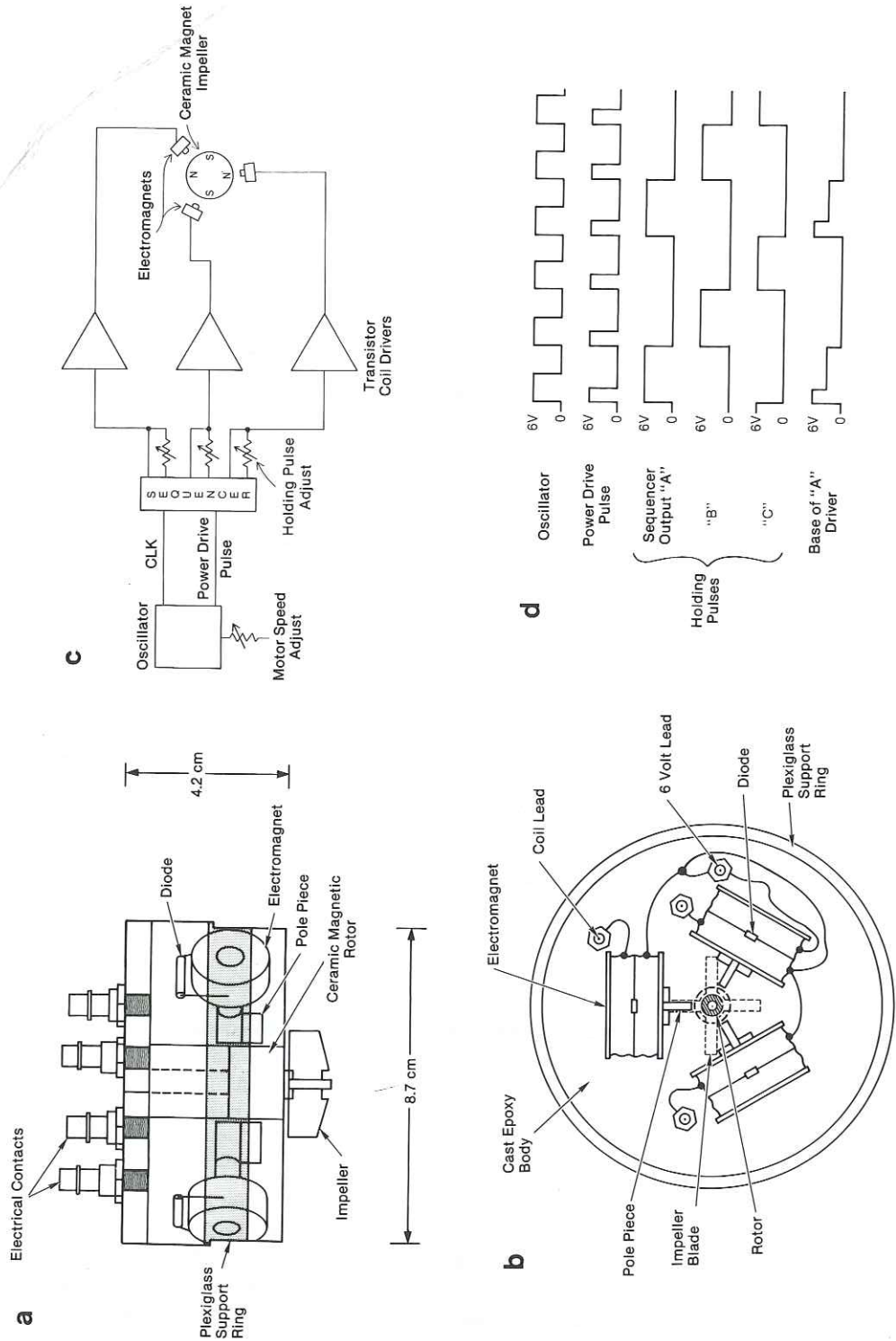


Fig. 2a-d. Stirling motor: a side view, b top view, c block diagram of circuitry, d wave form generated by stirring motor

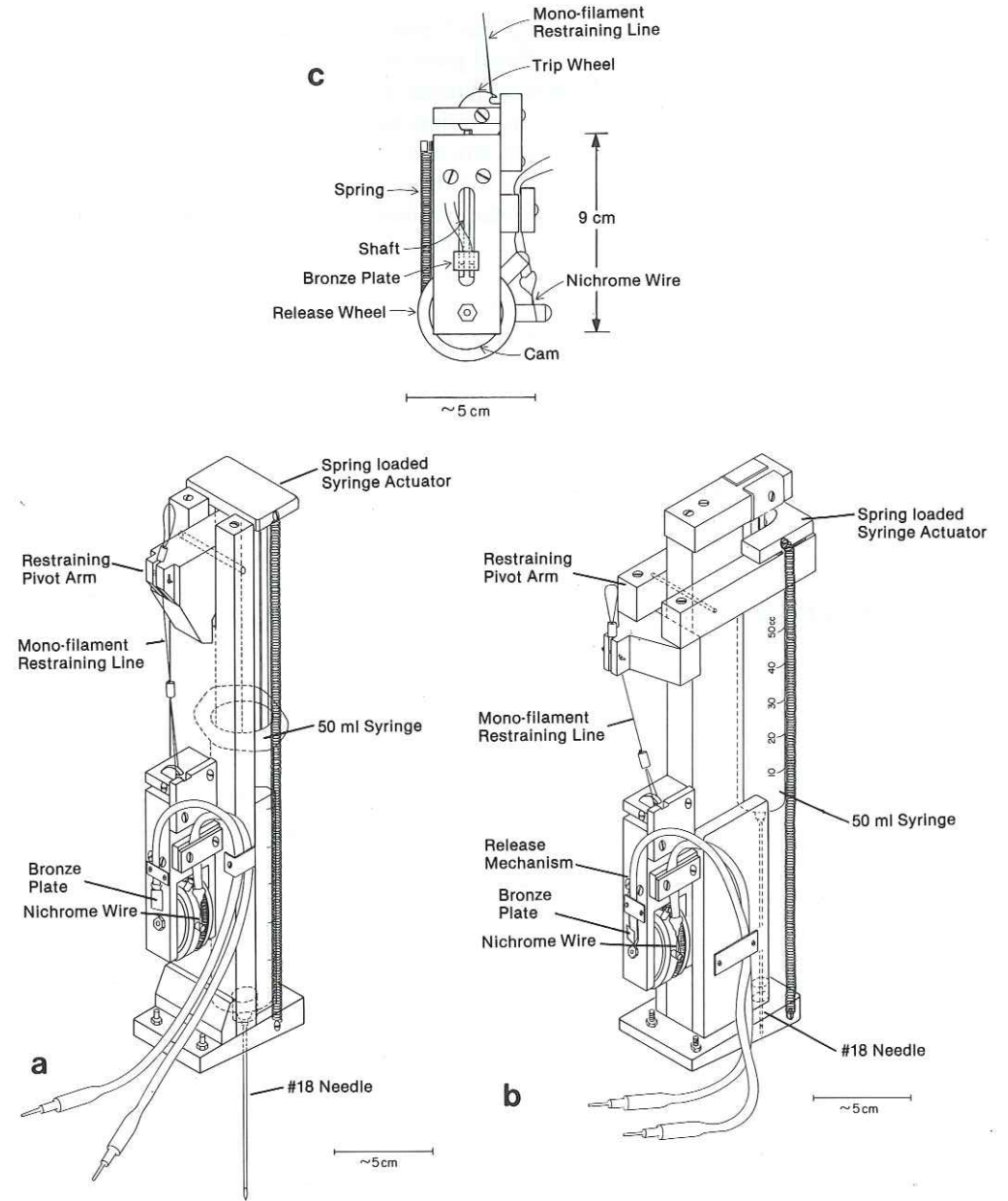


Fig. 3a-c. Syringe injection/withdrawal system: a injection syringe, b withdrawal syringe, c nichrome release

inoculating the chamber with organic enrichments or poisons. The nichrome releases associated with these syringes are controlled by a 12-event electronic timer with crystal oscillator. Selected activation times of the syringes may be preset in increments of 7.5 min for up to a maximum of 1250 h.



The injection unit consists of a 50 cm<sup>3</sup> plastic syringe (B-D Plastipak) supported by a Plexiglas stand (Fig. 3a). In a filled position, the syringe plunger is held by a restraining pivot arm. Atop the syringe plunger is a polyvinyl chloride plate (spring-loaded syringe actuator) with extension springs fastened on both sides and attached to the base of the stand. When the pivot arm is released, these springs drive the plunger down thus forcing the contents of the syringe into the grab chamber below through a self-sealing neoprene septum. The release mechanism for the pivot arm is mounted on the back of the stand and represents a modification of one designed by T.R. Folsom (Scripps Institution of Oceanography, personal communication) (Fig. 3c). It is constructed of Delrin and consists of a spring-loaded release wheel with an offset cam and fused shaft which engages a stainless steel trip wheel. A notch in the trip wheel holds a monofilament restraining line which prevents the pivot arm from releasing the syringe plunger while in the cocked position. The release wheel is held in the loaded position by a loop of nichrome wire (0.127 mm diam.) fastened around a pin on the periphery of the wheel. Located on the side of the release is a bronze plate (7 × 10 × 0.3 mm). When current is applied to the nichrome wire (cathode) an electrolytic cell is formed with the bronze (anode). A current of 300–400 mA applied for 10 to 20 s causes the oxidation of the nichrome wire. Upon breaking, the release wheel and cam rotate, disengaging the shaft and trip wheel and freeing the monofilament restraining line. The pivot arm then rotates and the syringe plunger is driven down by the resulting force on the spring-loaded syringe actuator.

The withdrawal system employs essentially the same principle as that of the injection system, however the plunger of the 50 cm<sup>3</sup> syringe is maintained in a fixed position by a bracket on the top of the stand (Fig. 3b). A pivot arm, much like that on the injection system, prevents the barrel of the syringe from being forced down by a spring-loaded actuator while in the cocked position. When released, the barrel of the syringe is driven down, but the plunger is held stationary, thus withdrawing a water sample from the respiration chamber. All samples are filtered in situ through a 25-mm filter holder containing a Whatman GF/C filter.

## 2.2 Specific Equipment

### 2.2.1 Sediment Biota Respirometers

**Sediment Community Respirometers.** The development of instruments to measure in situ oxygen consumption by the deep-sea sediment community has undergone a gradual progression toward more complex and auto-controlled systems. Our first instruments, bell jar respirometers, were placed with submersibles [20]. These units were subsequently designed into a free vehicle bell jar respirometer which could be deployed untethered from a surface ship in depths of water up to 7000 m [16, 21]. A similar respirometer was developed to measure the oxygen consumption of deep sea sediments using O<sub>2</sub> analysis of initial and final water samples [6].

One critical limitation of the bell jar respirometer was the inability to recover the sediments upon which the respiration measurements were made. Hence we developed a grab respirometer for use with a submersible [22]. The prohibitive cost, depth restrictions, and limited availability of the submersible prompted us to develop this system

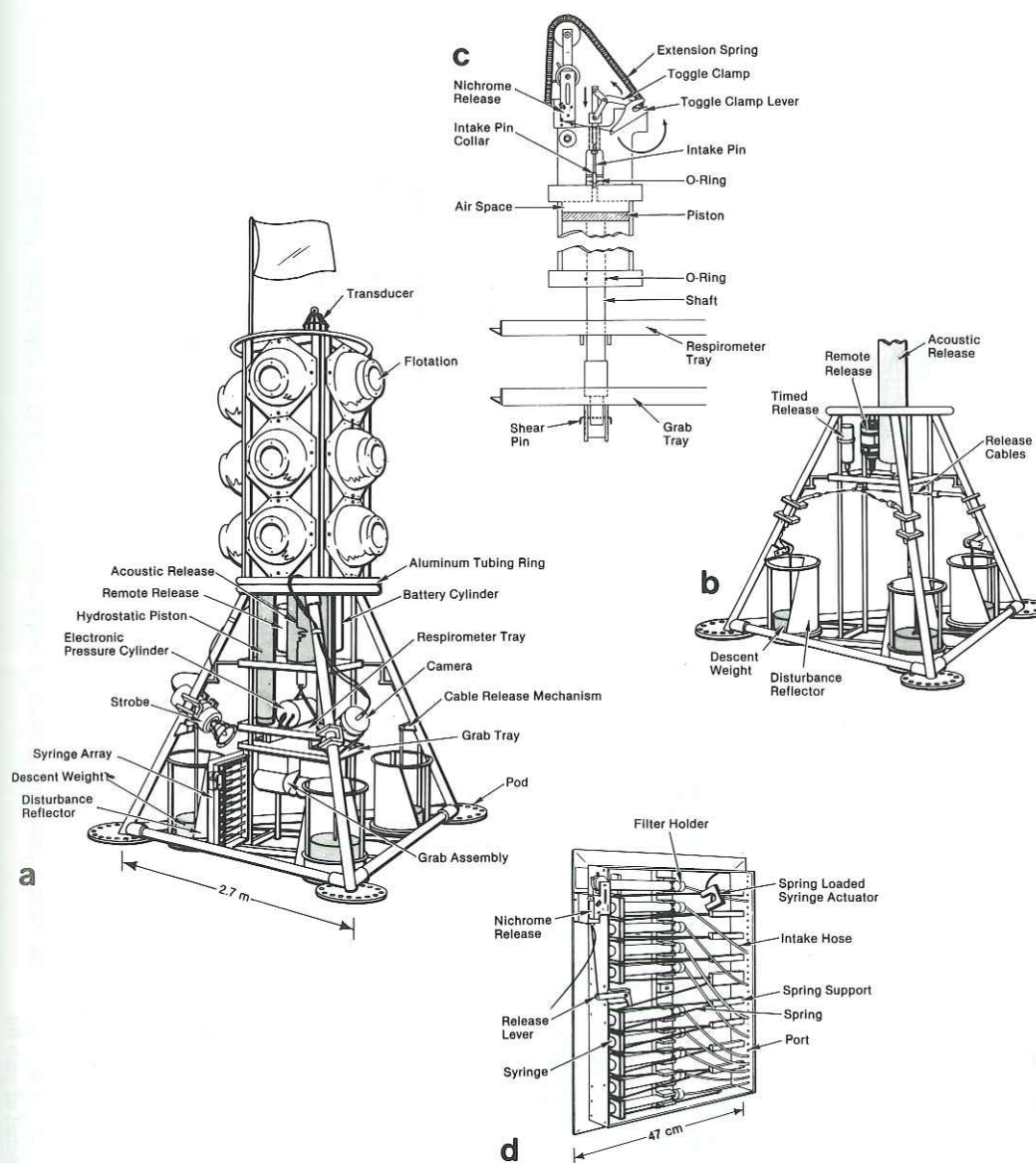


Fig. 4a–d. Free vehicle grab respirometer: a entire assembly, b descent weight release system, c hydrostatic piston assembly, d syringe array

further into a free vehicle [23]. This most recent stage of development in sediment community respirometers is described below.

The free vehicle grab respirometer (FVGR) consists of a tripodal frame which supports the acoustic control and grab respirometer instrumentation, and a flotation package (Fig. 4a). The tripod frame (2.4 m high × 2.7 m wide) is constructed of alu-



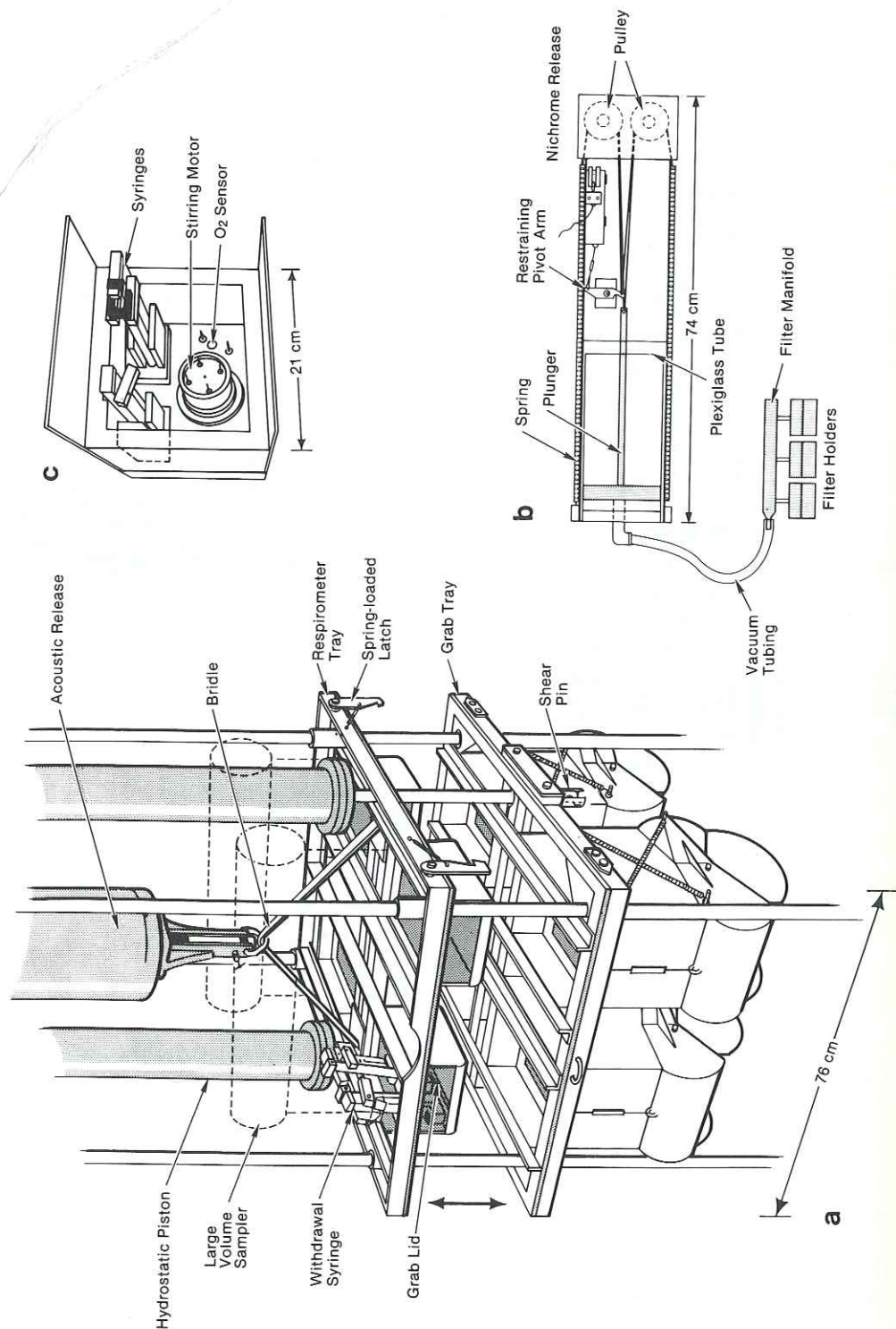


Fig. 5a-c. Detail of respirometer and grab tray of free vehicle grab respirometer: a respirometer and grab tray in deployment position, b large volume sampler, c respirometer tray with detail of grab lid

minimum tubing (6.5 cm OD, 5 cm ID) which has been sleeved and bolted at all joints for easy disassembly. Each leg has a perforated disk pod at the base and is bolted to an aluminum tubing ring at the top (diam. 1.1 m). The upper ring also provides a base for the flotation array.

Central to the frame are the square grab and respirometer trays which are supported at each of four corners by a vertical guide rod (aluminum tubing, 2.5 cm ID). The grab tray consists of four stainless steel grabs (20.3 × 20.3 × 30 cm × 2.7 mm gauge) opened at the top and mounted on a rigid square stainless steel frame (Fig. 5a). Each grab has two spring-loaded jaws which can penetrate the sediment up to 20 cm and enclose a surface area of 413 cm<sup>2</sup>. Two nichrome releases located on opposite sides of the frame control the closure of two grabs each through a mechanical linkage. Two hydrostatic pistons pass freely through the above positioned respirometer tray and attach to the perimeter of the grab tray on opposing sides. Each unit consists of an aluminum anodized cylinder (76.2 cm length, 10.2 diam.) with an internal stainless steel piston (76.2 cm throw).

The respirometer tray consists of a square stainless steel frame with cross bars supporting lids for each grab. Within each lid is a POS, a stirring motor and a pair of interchangeable injection/withdrawal syringes (Fig. 5c). This tray also supports the pressure cylinder with associated electronics for the respirometry system. Power for the respirometry electronics and releases is provided from a separate battery cylinder (Fig. 4a). The respirometer tray is held in place by a centrally positioned acoustic release (AMF, Model 324).

The cylindrical flotation rack is fastened to the top ring of the tripodal frame and consists of twenty spherical glass floats (eighteen 43.2 cm OD, two 25.4 cm OD Benthos floats) (Fig. 4a). These floats are enclosed in protective plastic covers and arranged in six vertical racks. On the top of the flotation rack is an acoustic transducer which relays coded messages between the shipboard transducer and the centrally located acoustic release.

For deployment to the deep-sea floor, the free vehicle grab respirometer is in a loaded configuration (Figs. 4a and 5a) i.e., both trays are in the raised position with the respirometer tray held by the acoustic release and the grab tray, with grab jaws open, held by the hydrostatic pistons.

Once on the bottom, a period of up to 1 h is allowed for the associated disturbance of the initial landing to dissipate. The disturbance reflectors on each tripod leg help prevent resuspended sediment from entering the central measurements area. After this initial period, the two hydrostatic pistons are actuated simultaneously by a pre-set nichrome release attached to the top of the piston (Fig. 4c). When the release fires, the toggle clamp lever swivels, thus freeing the toggle clamp which is pulled to a vertical position by the extension spring. The rotation of this clamp pushes the collar of the intake pin down, thereby allowing a fine stream of ambient seawater to enter the "air" space which can be backpressured between the top of the cylinder and the piston. The ambient pressure at depth is sufficient to slowly drive (1–2 cm/min) the pistons and the attached grab assembly into the sediment to a depth of approximately 15 cm. A high pressure line between the two pistons assures equal force on both plungers as the grabs are being driven down. In the event of a malfunction, shear pins at the point of attachment on the grab assembly prevent excessive force being directed to a single sides of the frame.



Once the hydrostatic pistons have had ample time to position the grab tray assembly in the sediment, a command from the surface ship activates the release of the upper respirometer tray which free falls down the four guide rods to engage the grab assembly. Four spring-loaded latches secure the two assemblies together and silicone gaskets assure a watertight seal between chamber units (Fig. 5a). A 35-mm camera and strobe system mounted on the tripod legs (Fig. 4a) are activated by the release of the respirometer tray through a magnetic reed switch. The self-winding camera with timer and solenoid activated shutter takes pictures at 0.6-s intervals before, during, and after release of the respirometer tray [1]. The strobe is synchronously triggered by the camera.

Once the grab respirometer chambers are sealed, the oxygen tension in the water overlying the enclosed sediment is monitored continuously by the POS system. At this time initial water samples may be withdrawn from the chambers for nutrient and dissolved oxygen analyses or injection syringes may inoculate the chambers with enrichments or poisons.

In addition to the syringe systems associated with the grabs, there are two other water samplers mounted on the FVGR and controlled by electronic timers: two large volume syringe samplers (LVS) and a vertical syringe array. The LVS consists of a 1.7 dm<sup>3</sup> syringe constructed of 10 cm ID Plexiglas cylinder with a spring-loaded PVC plunger (Fig. 5b). When released, the plunger inside the barrel is withdrawn by two sets of springs and the water is pulled into the LVS through three 4.7 cm filter holders, containing pre-combusted Whatman GF/C filters. The filter intake is located on the outside of the respirometer tray, 5 cm above the level of the intake for the grab syringes.

The syringe array consists of ten 50 cm<sup>3</sup> syringes (B-D Plastipak) mounted horizontally on a vertical frame, the bottom of which rests on the sediment surface (Fig. 4d). Each syringe is equipped with a filter (Whatman GF/C) and intake hose which can be adjusted from heights of 0 to 72 cm above the sediment surface at 2-cm intervals. The operating principle is similar to that of the individual withdrawal systems, except that all ten syringes are released at once by a single restraining pivot arm which is held by a nichrome release. The array is designed to collect water samples for the detection of chemical gradients (i.e., nutrients) immediately above the sediment-water interface. This information, coupled with the analysis of pore water extracted from the grab sediment provides a vertical profile from 13 cm below to 72 cm above the sediment-water interface for comparison with nutrient fluxes determined from the withdrawal syringe samples taken inside each grab.

At the termination of the grab respirometer incubation, which usually lasts from 2 to 6 days, final syringe withdrawal samples are taken from each grab and the grab jaws are closed by a pre-set electronic timer and nichrome wire-release system. Final water samples are also taken with one LVS and the vertical syringe array systems.

The final event is the acoustic command given from the surface ship to release the descent weights via the remote release. Suspended from each tripod leg is a cable release mechanism which holds a descent weight (99 kg). This cable release is controlled by the remote release through a series of three wire cables (Fig. 4b). The remote release, part of the main acoustic release system, is controlled by one of the two command modes via the shipboard transducer. This descent weight release system has a

backup unit consisting of a pre-set timed release [13] connected in tandem with the remote release. On command, the descent weights are dropped and the free vehicle grab respirometer ascends to the surface at a rate of 50 to 60 m/min. The FVGR weighs 259 kg in water; the descent weights add another 297 kg (3 × 99 kg), yielding a net negative buoyancy (negative buoyancy minus positive flotation buoyancy) of 94 kg on deployment. Upon release of the descent weight, the FVGR achieves a positive buoyancy of 203 kg minus the additional weight of the sediment in the grabs. Surface recovery time has been minimized with the addition of a transmitter (OAR ST206-100), flasher (OAR SF500) and a meter square fluorescent flag mounted on a two meter mast above the float array. The time from surfacing to shipboard recovery is usually within 30 min, thus preventing significant surface warming of the water samples and sediment.

**Megafauna Respirometer.** The animals which occupy the sediment surface and are considered an integral part of the sediment community are the epibenthic megafauna. These animals include such taxa as ophiuroids, asteroids, holothurians, coelenterates, crustaceans, and gastropods [5] which are generally not enclosed in the grabs of the free vehicle grab respirometer because of their large size and spatial distribution.

The problems associated with locating and measuring the in situ respiration of these large animals have been solved by developing a respirometer for use with a submersible. The visual and manipulative capabilities of a submersible are essential for selecting and sampling specimens for placement in individual chambers of the megafauna respirometer.

The megafauna respirometer consists of dual Plexiglas cylinders (29 cm ID × 9.5 cm high) mounted on a 35 cm × 72 cm plate which has been reinforced with aluminum angle (3 × 3 cm) (Fig. 6). This plate is hinged at the back side and mounted on a 29 cm × 35 cm × 72 cm frame of fiberglass angle (5 cm × 5 cm). The top plate pivots so that the respiration chambers seal on two neoprene gaskets which are glued to the respirometer base plate. An aluminum pressure resistant cylinder containing the POS electronics, stirring motor circuitry, timers, and battery power is mounted horizontally beneath the respirometer base. Each chamber has a stirring motor, POS, and two injection/withdrawal syringe systems mounted on the top plate.

The manipulator arm of the submersible uses a 0.5 cm mesh basket (15.5 cm ID × 5.6 cm high) to scoop up animals from the sediment surface, thus sieving out the associated sediment. The basket with the captured animal is then placed on the respirometer base and the top portion of the animal chamber is manipulated to the closed position. A T-handle locking mechanism assures the integrity of the chamber seals. Usually the megafauna respirometer is rigged so that the syringes take initial and final samples from each chamber for excretion product analysis. Measurements are made for periods up to 3 days, depending on the size of the animal and suspected respiration rates.

### 2.2.2 Pelagic Biota Respirometers

The large numbers of animals that inhabit the water column of the deep-sea benthic boundary layer can be behaviorally divided into two groups: the mobile scavengers and



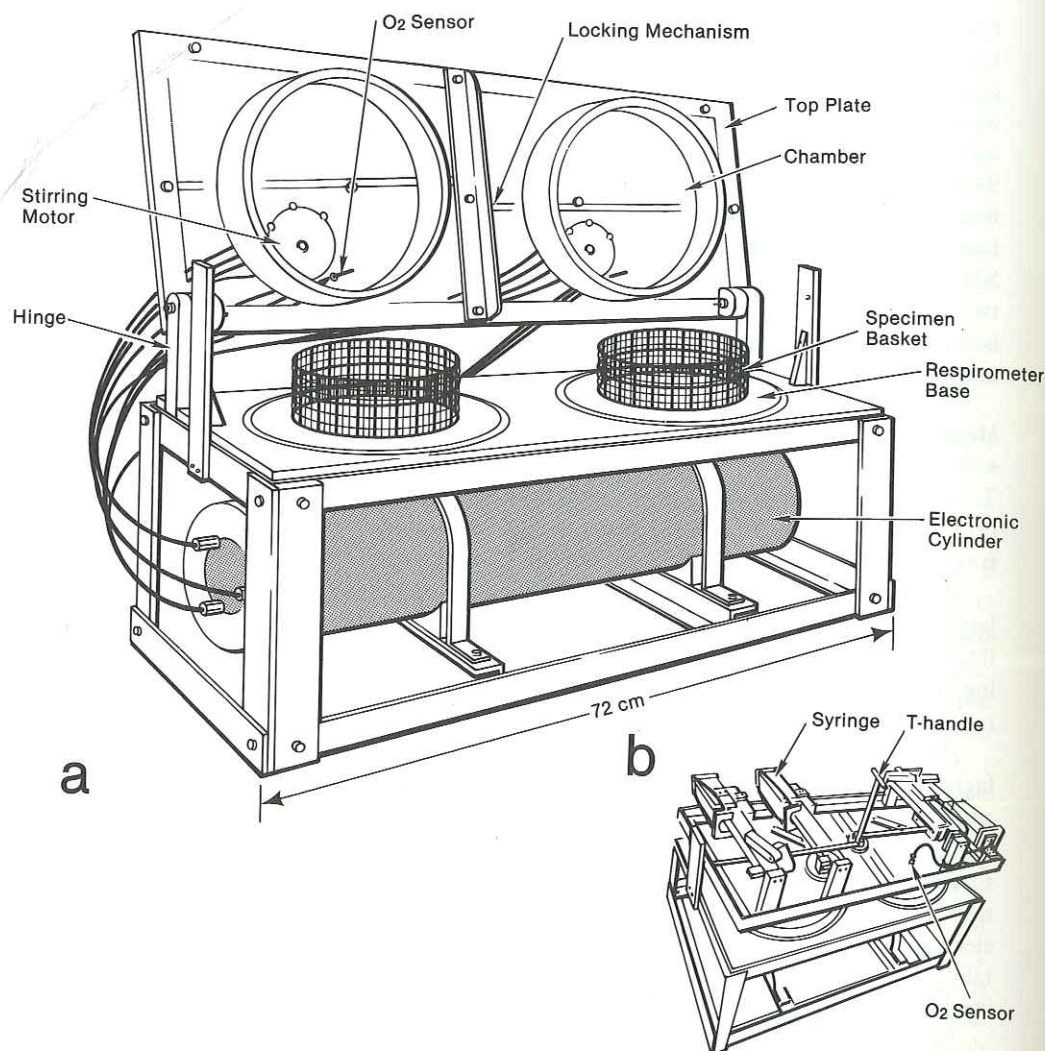


Fig. 6a,b. Megafauna respirometer: a open position, b closed position

the mobile nonscavengers. The mobile scavengers are those animals which are readily attracted to and captured in baited trap respirometers and include such organisms as rattail fishes, amphipods, and decapods. Mobile nonscavengers are those pelagic animals which are not attracted to such baited traps and include organisms such as myctophid and gonostomatid fishes, siponophores, chaetognaths, and copepods.

We have developed both free-vehicle and submersible-operated equipment to measure the respiration and excretion rates of both groups. The scavenging fishes and amphipods are lured into free-vehicle fish and amphipod trap respirometers. Non-scavenging organisms are actively captured using a submersible-operated slurp gun respirometer. These three respirometers and associated instrumentation are described below.

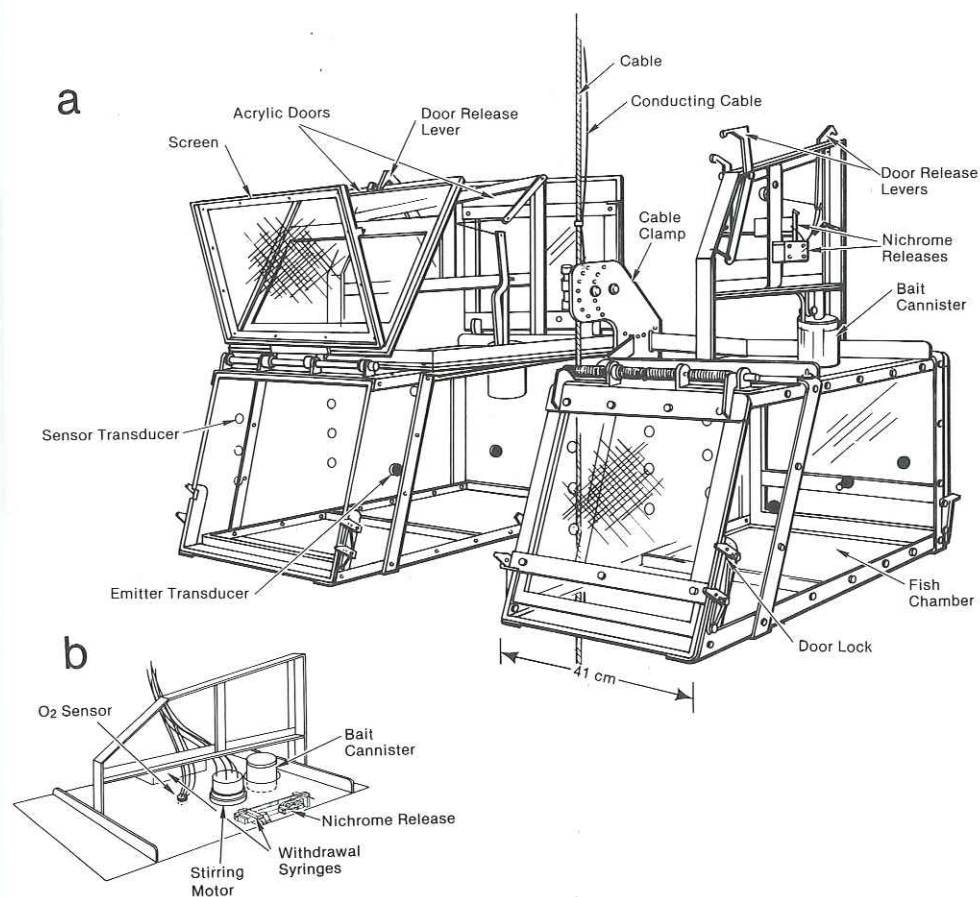


Fig. 7a,b. Fishtrap respirometer: a open and closed positions, b detail of top of trap

**Fish Trap Respirometer.** The fish trap respirometer (FTR) is a free-vehicle system designed for capturing pelagic scavengers attracted to bait and measuring their metabolic rates (Fig. 7a) [21]. Dual acrylic chambers of trapezoidal shape ( $91 \times 74 \times 41 \times 41$  cm) are joined together with a stainless steel frame. A shielded conducting cable connects the FTR with its electronic control center which is housed in a pressure cylinder 1 m above the trap. Each chamber has a total volume of  $53.7 \text{ dm}^3$  and is equipped with independent POS, stirring motor and syringe withdrawal system (Fig. 7b). A bait canister is located in the top of each trap to attract a scavenger but is automatically withdrawn from the chamber once the animal is enclosed to prevent contamination from bait oxygen consumption and nutrient flux.

An acoustic sensing system is used to detect the presence of pelagic organisms in the trap and to record their movement during the metabolic measurement (Fig. 7a). A series of three emitter transducers is aligned on one wall of the trap and emits a pulse train of 200 kHz. Nine sensor transducers are positioned in an evenly spaced  $3 \times 3$  array on the opposite wall and function as on/off switches. The sensors are sequentially



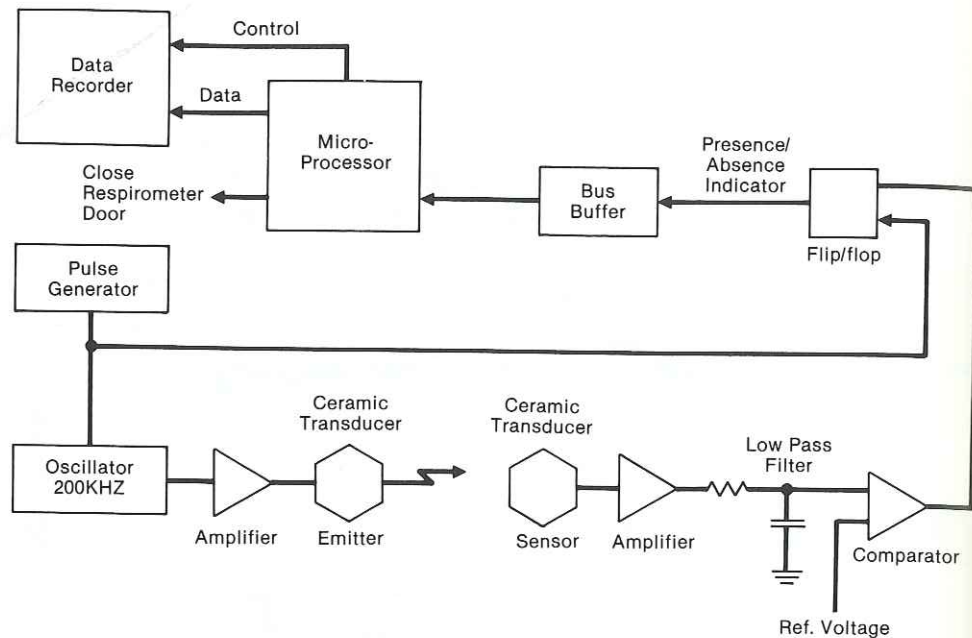


Fig. 8. Block diagram of acoustic sensor system for fish and amphipod trap respirometer

monitored by a microprocessor to check for a received signal (Fig. 8). The first continuous disruption of received acoustic pulses indicates an animal within the trap and a signal is given to the closing mechanism on the door. Once an animal is trapped the sensors are monitored sequentially and their on/off states are recorded to provide a record of the activity of the organism during the respiration measurements.

Dual chamber construction provides for a control to be run simultaneously with the measurement. Each chamber is equipped with a spring-loaded double-door system separate locking latches at the sloped end. During free vehicle deployment, the FTR descends with the control screen door closed and the experimental chamber screen door open; both bait canisters are filled and in the lowered position within the trap (Fig. 7a, left trap). When the acoustic sensor responds to the presence of a fish, the experimental chamber screen doors close via a battery-powered nichrome wire release system. After a selected period of time, all acrylic doors close and the spring-loaded bait canisters are withdrawn by electronic control. The delayed final closing of the acrylic doors allows the fish to become accustomed to the trap environment before initiation of respiration measurements and excretion sample withdrawal.

The combination acrylic/screen doors with separate timer-controlled release levers allow starvation studies to be performed in situ. Fish can be held for extended periods of time within the screened chamber, removed from their normal food supply. Free exchange of seawater through the screen eliminates metabolic waste accumulation and oxygen depletion within the chamber. The fine mesh covered bait canister prevents the animal from feeding on the bait.

A free vehicle mooring line system is used to deploy and recover the fish trap respirometer. The basic components of the mooring system are (1) a mast assembly,

(2) a main flotation package, (3) a nylon mooring line, and (4) a release system with disposable descent weights. The mast assembly is topmost, consisting of two 33 cm Benthos glass floats secured to a counter weighted 3.1 m fiberglass mast, a radio transmitter, submersible strobe light, and flag [7, 12, 14]. The mast system is connected to two 43 cm Benthos floats (25.5 kg total positive buoyancy) by a 7.6 m nylon line (Samson braid) of 0.95 cm diameter. The 43 cm spheres are connected to the mooring line (0.64 cm Samson nylon line) which supports the electronics pressure cylinder, the respirometer, the tandem timed-release system [2, 13] and the anchor weights. The mooring line is of variable length so that the FTR units can be positioned at any distance above the sediment-water interface.

The array is deployed by streaming the mast system off the stern of the ship followed by the 43 cm floats, electronics cylinder, respirometer and the mooring line while the ship is underway at 0.5 to 1 knot. The tandem timed-release system with anchor weight is deployed last.

On completion of the experiment, the preset releases are actuated, the anchor weight is dropped and the array ascends to the surface at approximately 50 m/min. Once on the surface, the free vehicle is located via the radio transmitter, strobe light and flag. The mast system and floats are recovered first, followed by the electronics cylinder, respirometer, mooring line and dual releases.

**Amphipod Respirometer.** A free vehicle amphipod respirometer (FVAR) was developed to capture amphipods or other small scavengers attracted to bait in four independent acrylic chambers. These chambers are mounted horizontally between two acrylic disks, perforated to decrease water resistance during descent and recovery operations (Fig. 9a). The disks are strengthened by two aluminum tubing rings (61 cm in diameter) and are held parallel to each other by four stainless steel support rods (22.5 cm long). The bottom disk supports four acrylic cylindrical chambers (16 × 7 cm ID – total volume of 615 cm<sup>3</sup>) each attached to the center acrylic bait container and each having an independent acoustic sensing system, POS, stirring motor and closure device (Fig. 9b). The top disk supports five withdrawal syringes (Fig. 9c). The extra withdrawal provides the initial water sample for the four chambers, while the other four are taken as final samples in each of the chambers. The POS system, electronic control of nichrome releases and acoustic detection system have been described above. The aluminum pressure cylinder housing the control and sensing electronics and power supply is clamped onto the free vehicle mooring line 1 m above the respirometer and communicates via shielded conducting cables.

At the time of deployment, bait is placed in the center cage with nylon mesh (300 μm mesh opening) permitting water exchange between each chamber and the bait but preventing feeding. As an amphipod or other scavenger enters a chamber, the acoustic sensor system within that cylinder triggers the nichrome release system; a spring-loaded clamp constricts the flexible polyethylene tube opening to the chamber, thus sealing the animal in the respiration cylinder. This acoustic sensing system is identical in design to that described for the fish trap respirometer. When all four traps have been individually triggered and closed by the presence of an animal, the center bait container rotates 45° within the central housing which then excludes the bait from all four chambers.



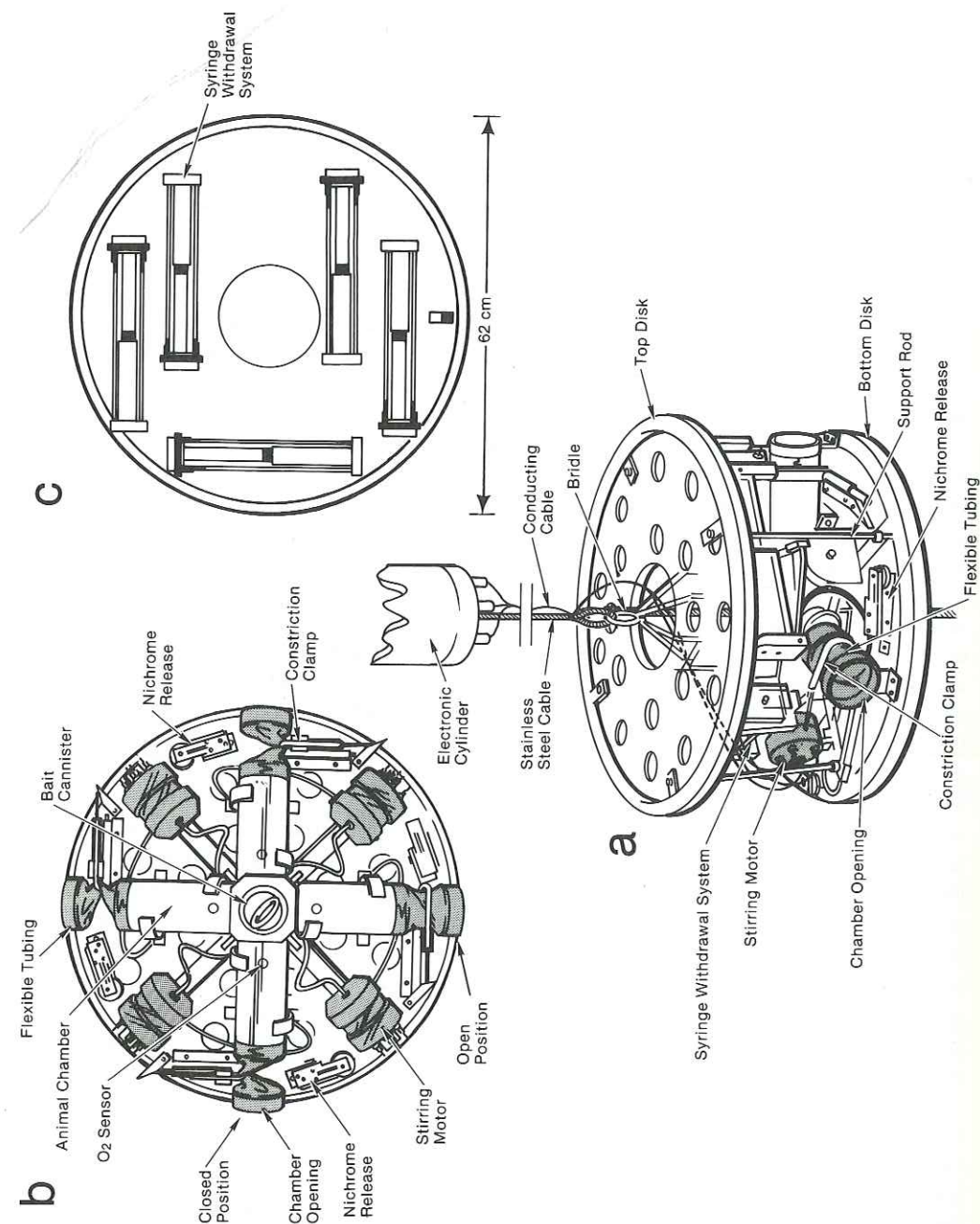


Fig. 9a-c. Amphipod respirometer: a complete assembly, b bottom disk, c top disk

The amphipod respirometers are deployed and recovered on free vehicle mooring lines as described above. These units with the accompanying electronics cylinder can be positioned at any desirable height above the bottom on this mooring line.

**Plankton/Nekton Respirometer.** The slurp gun respirometer was developed to capture pelagic animals (plankton/nekton) not attracted to the baited trap respirometers [21]. This instrument requires the visual and manipulative qualities of a manned submersible to collect such organisms in the water column of the deep-sea benthic boundary layer. The primary criteria considered in developing the slurp gun respirometer were: (1) collection of multiple discrete samples with continuous oxygen consumption measurement and withdrawal of water samples for excretion rate analyses, (2) gentle capture of organisms ranging from fast-swimming fishes to gelatinous zooplankton with minimum stress to the organism, (3) ability to change the volume of the animal chamber as warranted by the size or suspected respiration rate of the organism, and (4) ability to conduct these in situ metabolic measurements over periods in excess of a normal manned submersible dive (10 h). These four criteria were all considered in the final design.

The slurp gun respirometer consists of three to four modules, each of which includes a horizontal acrylic animal chamber (40.5 cm × 8.8 cm ID) with a right-angle valve at either end (Fig. 10). One valve is connected through a sliding manifold to a flexible reinforced intake hose (10.5 cm ID × 2 m) and collecting funnel 0.5 m diam. opening). This valve is closed by an acrylic spring-loaded plunger which has a volume-adjusting piston. A worm-gear driven piston can be turned into the animal chamber to adjust the volume to between 2460 and 200 cm<sup>3</sup>. The other valve is connected through the sliding manifold to a variable speed pump (30 V dc; 0.3–3 dm<sup>3</sup>/s) via a flexible reinforced vinyl hose (4 cm ID). The power for the pump is supplied through quick-disconnect cables from the submersible. This pump valve is closed by a spring-loaded acrylic plunger which has the stirring motor inserted into the end facing the animal chamber. An interchangeable nylon screen (50–300 μm mesh) is positioned between the animal chamber and the pump hose valve aperture to prevent animals from being sucked beyond the chamber or coming in direct contact with the stirring motor impeller.

Each animal chamber has a POS and two withdrawal syringes inserted through the bottom wall near the pump valve and stirring motor (Fig. 10b). The associated amplifying, recording, and timing electronics required by these systems are housed in a control electronics cylinder mounted on the respirometer frame. Battery power for these electronics is supplied from a contiguous pressure cylinder with all electronic communication via shielded cables.

A series of three to four of these modules are assembled in parallel on an acrylic platform reinforced with structural aluminum angle (the number of modules is dependent on the weight restrictions of the submersible) (Fig. 10a). Each module communicates with the intake hose and pump hose via a common sliding manifold which is driven by spring motors that sequentially engage and disengage each module.

The slurp gun respirometer with electronic cylinder, battery unit, and pump is mounted on a rigid aluminum tubing support frame which is then fastened to the front of the submersible with a solenoid release and slip-sleeves.



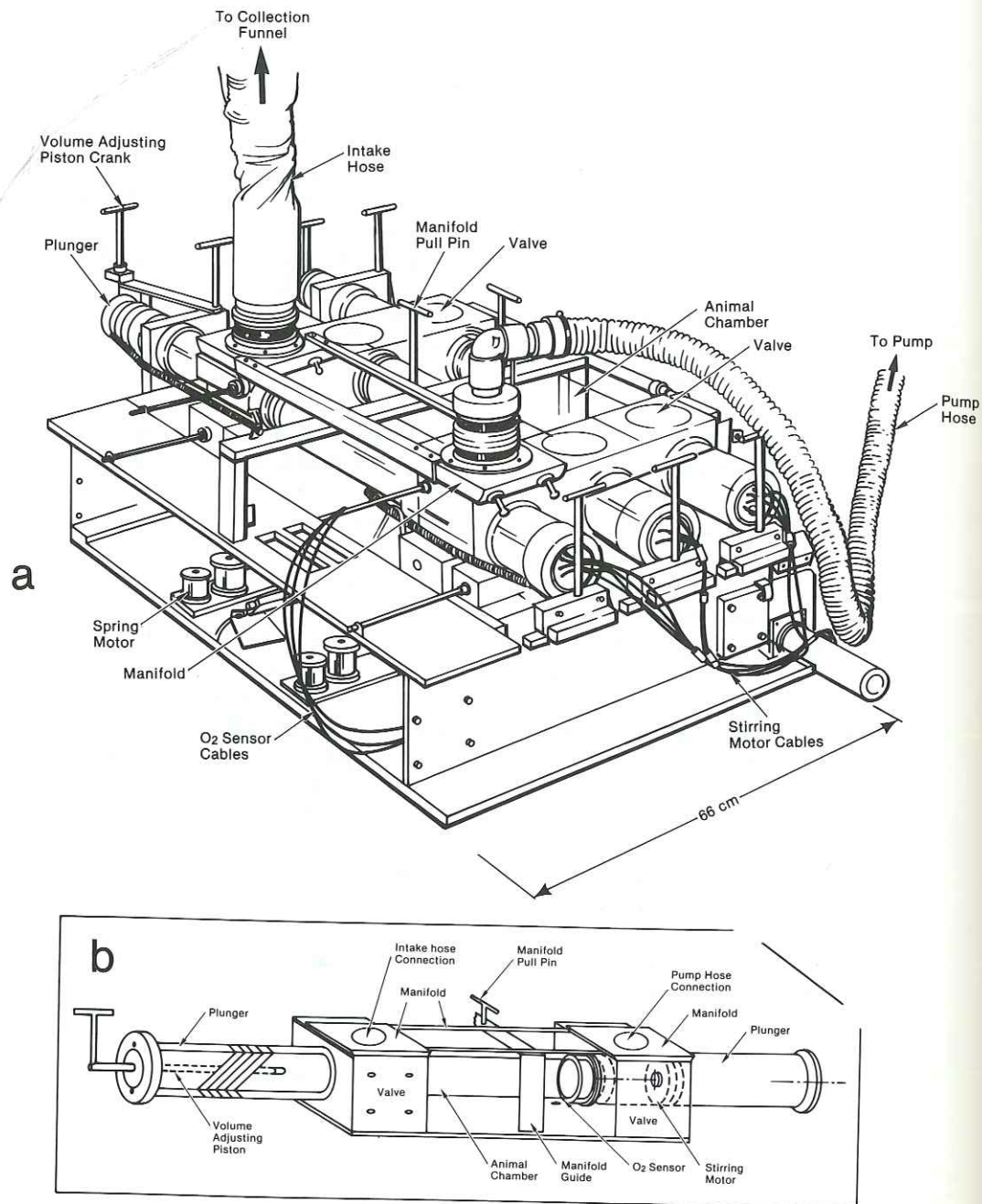


Fig. 10a,b. Slurp gun respirometer: a complete assembly, b single module

On deployment, the valve of each module is held open by pull pins, the manifold is positioned over the first module, and the volume-adjusting pistons are fully retracted into the valve plunger. Planktonic and nektonic organisms are visually located from the submersible and the intake hose and funnel are positioned for collection. The pump is activated and the speed adjusted so that suction is just sufficient to move the animal into the transparent animal chamber. At this point, the pump is turned off and the two right-angle valves on either end of the animal chamber are closed by releasing pull pins with the submersible manipulator. The volume of the animal chamber is then adjusted (200–2460 cm<sup>3</sup>) for the size and the suspected metabolic activity of the enclosed organism by cranking the volume-adjusting piston. The common manifold is then released by another pull pin and it advances to engage the next respirometer module. The same procedure is followed in filling each animal chamber. In some cases, instead of collecting single organisms, the funnel is placed in a forward-looking position, the submersible driven forward at a known speed, and the pump activated for a specific time to supply a mixed plankton sample at a particular depth above the bottom. One module or an attached Niskin water sampler (5 dm<sup>3</sup>) serves as a control.

When all the modules are filled, the slurp gun respirometer is detached from the submersible by the quick-release solenoid and tethered to a free-vehicle mooring line (similar to that described above) at the depth where the animals were collected. This allows the slurp gun respirometer to incubate for a period in excess of the normal dive time of the submersible (> 10 h). These incubations are generally for periods up to 3 days during which time the dissolved oxygen tension within each animal chamber is monitored continuously. The withdrawal syringe system is pre-set to take a chamber water sample (50 cm<sup>3</sup>) at the initiation and termination of the incubation. A pre-timed release or acoustic release on the mooring line is then activated, thus permitting the free vehicle with attached slurp gun respirometer to ascend to the surface for recovery.

### 3 Conclusions

We have presented our approach, with the associated instrumentation, to measuring the metabolism of organisms and communities in the deep sea. Inaccessibility of the deep-sea environment coupled with submersible and surface ship expense and availability serve to limit the number of measurements and the complexity of experiments which can be performed in situ. We feel the best direction for further research in deep-sea energetics is a combination of both in situ and laboratory studies, establishing meaningful comparisons between the two methodologies. However, in conducting such deep-sea metabolic studies, full cognizance of the artificial constraints being imposed on the organisms must be maintained.

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