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Development of a hyperbaric trap-respirometer for the capture and maintenance of live deep-sea organisms

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Abstract

A major obstacle to the investigation of deep-sea biology is the lack of instrumentation to retrieve deep-sea organisms from their habitat alive, particularly fishes with physoclistous swimbladders. To perform physiological experiments on deep-sea fishes under in situ but controlled conditions, we constructed a high-pressure fish trap-respirometer to capture deep-water fishes at depth and return them to the surface alive at in situ pressure and temperature. Pumps and instrumentation connected aboard ship or in the laboratory are used for maintenance of the animal and experimentation. The trap was designed so that respiration rates, pressure tolerance, and metabolic responses to various gas concentrations (CO_2 and O_2) could be examined in a controlled environment. The trap is deployed as an autonomous lander or free vehicle to depths of 4000 m. Once on the seafloor, a fish is captured on a baited hook that triggers the reeling of the fish into the pressure vessel and closure of its sealing door. Two fish, *Coryphaenoides acrolepis*, have been recovered live from 1450 m and maintained in the laboratory. Both fish were retrieved at ~95% of their in situ pressure and at temperatures of ~6°C. Oxygen consumption rates of these fish were 54.99 μmol O_2 kg^{-1} h^{-1} (1.158 kg, 65.0 cm total length, 23.5 cm pre-anal fin length) and 79.43 μmol O_2 kg^{-1} h^{-1} (1.305 kg, 66.5 cm total length, 24.5 cm pre-anal fin length). The latter fish was maintained for 3.5 d, and it survived gradual decompression to 76% of the pressure at its capture depth.

Since the *Challenger* expedition, investigations of deep-sea benthic and benthopelagic animals have been carried out using trawls, traps, and other fishing gears that retrieve dead or moribund animals. These studies have greatly advanced our knowledge of deep-sea fish ecology, biology, and, through the tissues of recovered animals, physiology (Somero and Hochachka 1984). Nevertheless, the sampling techniques used are inherently limited for studies of the biological activities and responses of live animals. Considerable information could be gained from experiments on animals in a controlled setting as is typical for studies of shallow water and terrestrial animals. Large fish are of particular interest because their abundance (Haedrich and Rowe 1977; Lauth 2000; Merrett 1992; Pearcy et al. 1982; Wakefield 1990) and dominant trophic role in deep-sea ecosystems (Drazen et al. 2001; Mauchline and Gordon 1984; Pearcy and Ambler 1974). Yet our inability to perform experiments to measure physiological rates and processes has hampered our ability to understand their impact in deep-sea communities and the mechanisms governing their distributions.

Experimental studies using live deep-sea animals fall within 2 groups. First, short-term studies have been performed on species which survive, at least briefly, recovery to the surface. These studies have focused on measurements of oxygen consumption, but for a few species tolerant of prolonged captivity, data on other energetic and physiological parameters have been obtained (Childress 1995 and references therein). This large body of work has led to the "light-limitation" hypothesis, suggesting that the depth-related declines in metabolic rates of midwater fishes (Childress 1971; Torres et al. 1979), crustaceans (Childress 1975), and cephalopods (Seibel et al. 1997) are due to a reduction in locomotory capacity in response to reduced reactive distances between predators and

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prey. Only fish species lacking gas bladders fall in this category of studies. Consequently, many of the dominant organisms in the deep sea remain unstudied. A second, smaller but growing, group of studies has measured rate processes in deep-sea animals in situ (reviewed in Smith and Baldwin 1997). These studies revealed very low metabolic rates as did the midwater studies, and the authors suggested that the low rates represented adaptation to a food limited environment (Smith and Brown 1983; Smith 1978; Smith and Hessler 1974). These and similar measurements subsequently were used to construct quantitative food webs and estimate trophic impacts (Smith 1982, 1992; Smith et al. 2001). Thus in situ studies are a very attractive option but logistic constraints have prevented manipulation of environmental variables (i.e., pressure, temperature, and dissolved gas concentrations) in all but the most recent investigations (Barry unpub. data unref.).

A variety of high pressure instruments have been developed to capture animals at depth and retrieve them alive to the surface under pressure. Due to engineering and operational constraints, most of them are small but very effective at retrieving small crustaceans and bacteria (Macdonald and Gilchrist 1978; Menzies et al. 1974; Yayanos 1978). A few traps have been developed to capture larger organisms. Phleger et al. (1979) constructed a large aluminum fish trap capable of retrieving fish at up to 1400 psi, which they used to collect fish for studies of lipid chemistry. This trap used a bait-actuated mechanism and a door that closed from the inside outward. Although their door seal worked well, the aluminum was not insulated, and it expanded as it warmed during recovery resulting in partial loss of pressure. A small window allowed for viewing but the trap was not designed for long-term maintenance of the animal. Later Wilson and Smith (1985) built a large (41.7 L) trap for the capture and decompression of macrourid fishes for energetic studies at depths of up to 1400 m. Their system used a large wedge-type door and a retracting baited hook to pull the fish inside the trap, similar to Phleger et al.'s design. Wilson and Smith's trap used an electronics housing to provide power to the ballast and door release mechanisms. Their system was insulated and included a gas charged accumulator (Yayanos 1978) to prevent pressure loss during recovery. The trap was designed so that the fish could be maintained inside the trap in a refrigerated room. A small window and internal light were used for viewing. They captured and kept two fish alive for 30 to 41 h in the laboratory and were able to partially decompress one animal. Unfortunately their trap was lost at sea shortly after their initial experiments. Most recently, Koyama et al. (2002) developed a pressurized sphere (20 L volume) with which they have captured small zoarcid fishes from 1170 m and maintained them under pressure. This instrument can be mated to a high pressure pumping system to maintain water flow through the trap and to instruments to measure metabolic rates. Thus the systems for the capture and maintenance of deep-sea fishes have evolved considerably over the last thirty years.

There is still a need for a device with which to capture and study large deep-sea fishes and other organisms under controlled conditions. We have built a large (89 L) hyperbaric trap-respirometer that is capable of capturing large fishes and other organisms at depths to 4000 m and maintaining these animals with flowing seawater in the laboratory. The goals of our study are to (1) measure the metabolic rates of deep-sea benthic and benthopelagic species, (2) examine the pressure tolerances and effects of pressure on metabolic rates, (3) examine the effects of elevated CO_2 on metabolic rates, and (4) use this technology to expand other fields of research such as behavior, chemosensory abilities, protein expression, membrane biochemistry, etc. Here we describe the design and construction of the instrument and present some preliminary data from its initial deployments.

Materials and procedures

The design of the hyperbaric trap centers around the cylinder used to enclose the fish (Fig. 1). A working pressure rating of 41.4 Mpa (6,000 psi) and a minimum size of 30.5 cm (12 inches) inside diameter and 122 cm (48 inches) in length were defined as requirements. A vessel of this size yields a volume of 89.1 L, an ample envelope for most deep-sea fishes that reach lengths to 90 cm even at 4000 m depth in the North Pacific (Drazen 2002). The trap was designed to the ASME B31.3 Power Piping Code using a safety factor of 3 to 1 to achieve a high level of confidence in the specifications and trap durability. The ends of the cylinder were flanged to simplify end cap attachment and ease machining requirements for end-cap tolerances.

Material selection for oceanographic systems is always a critical decision. The materials considered were titanium 6al4v, stainless steel 316, aluminum 6061 T-6, carbon fiber composite, and stainless steel 17-4 pH (1150). A titanium cylinder met all design criteria but was prohibitively expensive. Aluminum and even stainless steel 316 have relatively low yield strengths (276 Mpa or 40 kpsi for stainless 316) requiring unreasonably thick cylinder walls. A cylinder of carbon fiber could meet strength requirements with a reasonable wall thickness and would be affordable. However, with current technology flanges could not be formed or machined onto the carbon fiber cylinder. Therefore the end caps would have to encase the cylinder and be fastened by tie rods running the length of the cylinder. Considering the tensile properties of such long tie rods, it was determined that this design could not meet the required strength with a reasonable number of tie rods (< 20). Also, due to the noble nature of carbon fiber, the end caps would need to be manufactured of titanium. Thermal and strain disparity between these materials could also be problematic. We chose stainless steel 17-4 pH (1150) because of its extremely high yield strength, acceptable corrosion resistance, and its relative affordability. Furthermore, this material can be centrifugally cast with flanges (Delta Centrifugal Corporation). This is a process of forming cylinders by

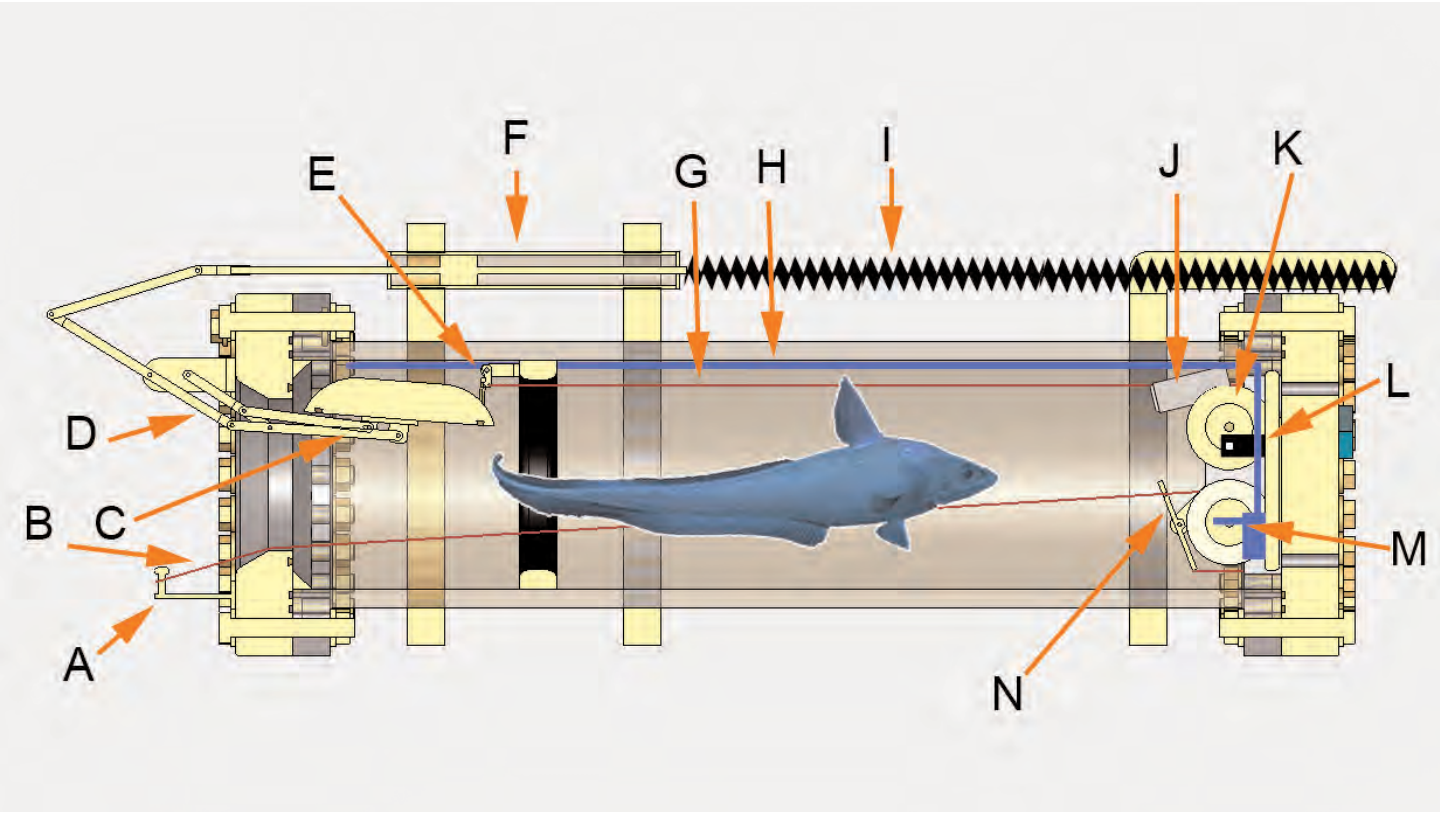

Fig. 1. Cross-sectional view of the trap with fish inside showing hooking and retraction mechanism. Lines are illustrated in red, and water circulation plumbing is in blue. (A) Pin to hold the release shackle/hook assembly, (B) retraction line connected to hook, (C) door, (D) door linkage system, (E) door latch, (F) hydraulic dampening piston, (G) line to door release plate, (H) tubing to draw water to pump, (I) conventional extension springs to close the door, (J) camera with integrated LED lighting, (K) wheels for coiled constant force springs, (L) oxygen optode, (M) water circulation pump and water outflow from circulation pump, and (N) release trigger for door.

pouring molten metal into a cylindrical mold spinning on its axis. The molten alloy is retained against the inside diameter of the mold by centrifugal force exerted by rotation. This rotation is maintained throughout the cooling process. The result is a cast cylinder with a grain density and a yield strength that exceeds wrought material. Post cast machining creates the appropriate finishes, tolerances, and hole patterns. This design represented the best compromise between the performance criteria and cost.

The end caps were designed as simple flat discs for attachment to the cylinder flanges (Figs. 2 and 3). Two o-ring grooves form the seal with the cylinder flanges. The front end cap is identical in size to the rear, but annular in design, and in addition to the two face seal o-rings, it has one internal o-ring that mates to the outward closing door.

A parametric modeling program (SolidWorks 3D, SolidWorks Corporation) was employed to derive the required wall thicknesses, bolt pattern, and preload torque. Finite element analysis (FEA) and an axisymmetric model were used to validate the design by assessing joint stiffness between the housing and end caps as well as the prying effects of the end cap flanges against fasteners. The result was a cylinder with 2.54 cm (1 inch) thick walls and flanges 5.08 cm (2 inches) thick and 48.25 cm (19 inches) in diameter. The end caps are 48.25 cm (19 inches) in diameter and 7.62 cm (3 inches) thick. The rear end cap has 6 penetrations and a 24-bolt hole pattern for 2.54 cm (1 inch) bolts. The front end cap has the same 24-bolt pattern and a 20.32 cm (8 inch) diameter opening functioning as the trap door. The tremendous forces generated by the internal pressure on end caps this size, required the torque on each bolt to be a staggering 746 Nm (550-foot pounds) to prevent prying effects and resulting o-ring extrusion.

Many deep-sea fishes will not enter confined traps for bait (Phleger et al. 1979; Wilson and Smith 1985, Drazen pers. observations unref.). Thus, it becomes necessary to capture the specimen outside, draw the animal into, and close a pressure-sealing door behind the animal. Our approach was to attract the specimen to a baited hook on the outside of the trap and pull it into the trap using a spring-loaded reel. A short leader attached to the hook is also the lanyard of a quick-release shackle attached to a pin at the trap entrance (Fig. 1 and 3). The shackle is connected by a longer lanyard to a spring loaded reel that generates a pull of 5.4 kg (12 pounds). When the shackle is released by the fish pulling on the hook the reel

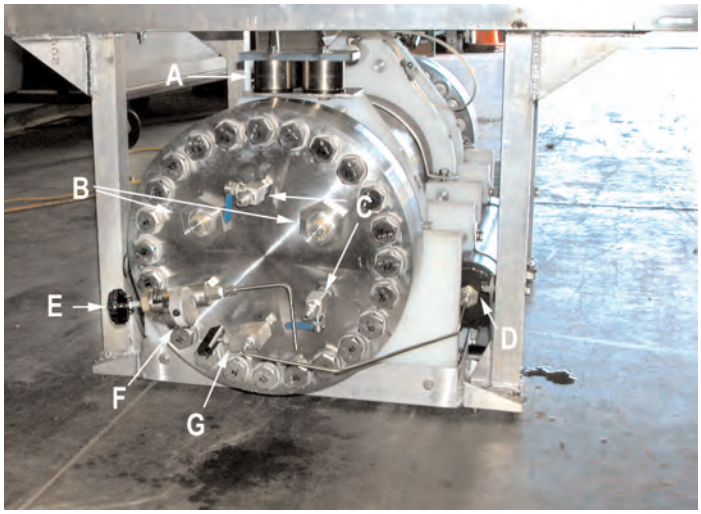

Fig. 2. View of the rear endcap of the hyperbaric fish trap-respirometer. In this image, the trap is attached to the float rack and the original constant force springs (A) are in place (*see* text). (A) Rotational door closing springs, (B) electrical penetrations for oxygen optode, internal water pump, and camera, (C) hydraulic penetrations and ball valves for water inflow and outflow ports, (D) pressure accumulator, (E) safety relief valve, (F) pressure gauge, (G) needle valve to hydraulic penetration for pressure accumulator.

is activated and the animal is drawn into the trap. The line on the reel passes through a secondary trigger located adjacent to the reel at the rear of the trap. When tripped, the secondary trigger releases the door, which closes outward from the cylinder interior. This outwardly closing door travels on a four-bar linkage allowing the door face to seal against the inner side of the front end cap. Two face seal o-rings, one on the door and the other on the inner side of the end cap are employed to make this seal. The four-bar linkage is driven by two linkage elements to a double-ended hydraulic cylinder, which acts as a dampener. The dampener is attached to two extension springs that generate 73 kg (160 pounds) of closing force that seat the o-rings. Complete door closure occurs in just a few seconds. The entire mechanism functions autonomously using only the stored energy of four springs.

The trap is deployed as a free vehicle with a descent weight and acoustic release. The trap and associated instrumentation weighs approximately 680 kg (1500 lbs) and requires a large flotation package for recovery. A frame 183 cm (72 inches) square (Fig. 4) was used to house 27 43.2 cm (17 inch) Benthos glass spheres, 85 L (3 cubic feet) of 4,000 meter syntactic foam, an acoustic release, a signal float, and the anchor-weight release mechanism. The float pack bolts directly to a frame containing the high-pressure cylinder forming a rigid package that can be deployed from a single pick point. The ballast may be released from the surface via the acoustic release or manually by ROV intervention at the seafloor.

The pressure gradient from inside to outside a pressure cylinder increases as it is brought to the surface from depth, causing the cylinder to expand. There is also potential thermal expansion of the material as it ascends through the thermocline. Insulation is provided by two layers of 2.5 cm thick open-celled foam that floods but does not compress under pressure, forming an effective water jacket around the trap. Due to the incompressibility of seawater, even small increases in the volume of the vessel can dramatically reduce the pressure of the contained seawater. To control this effect, a hydraulic accumulator (Parker-Hannafin) with a volume of 3.8 L (231 cubic inches), is plumbed into the system (Figs. 2 and 5). This accumulator is a large piston and cylinder. One side of the piston is open to the hyperbaric trap, the other side is charged with nitrogen. Assuming a maximum pressure differential of 41.4 MPa and a maximum temperature increase of 10°C (worst case scenario), the resulting increase in the trap's volume would be 312.6 mL. This increase in volume would result in a 20.6% loss in pressure. To compensate for this change the accumulator is charged to 90% of the pressure at the deployment depth. Upon descent, via the ambient pressure change, the accumulator is charged passively to the in situ pressure at the deployment depth. During its ascent, the hyperbaric cylinder expands and the accumulator forces seawater into the system. This volume of seawater, stored on descent, reduces pressure loss to the initial gas charge pressure (90% of in situ pressure). A needle valve between the accumulator and the cylinder controls the discharge potential of the accumulator should any leak develop while onboard ship. A pressure relief valve is integral to the system and provides an additional safety measure should the system be deployed beyond its 4,000 m working depth or should it be pressurized beyond 41.4 MPa in the lab.

The hyperbaric system contains several features to enable the maintenance and experimental manipulation and observation of the captured specimen. Inside the hyperbaric cylinder are an oxygen optode (Aanderaa 3830), a circulation pump, and a small CCD camera with integral LED lighting (Deep-Sea Power and Light LED Multi Sea Cam, Fig. 1). The optode uses the effect of dynamic luminescence quenching by molecular oxygen for measuring dissolved oxygen in salt water. Optodes have the advantage that their pressure response is predictable (Glud et al. 2001; Stokes and Somero 1999). The oxygen optode was set to sample oxygen concentration and temperature every 30 s and record the data to the laboratory computer. The pump maintains circulation within the hyperbaric cylinder. The camera allows the specimens behavior to be monitored simultaneously with oxygen consumption and pressure changes. These instruments are connected to power and dataloggers through two penetrations in the rear endcap, which are connected once the trap is on deck or in the laboratory.

Pressure control is accomplished with a high pressure hydraulic backpressure regulator (Tescom 54-2162Z24A) that controls the flow of seawater out of the hyperbaric cylinder (Fig. 5). This regulator is connected through a hydraulic port

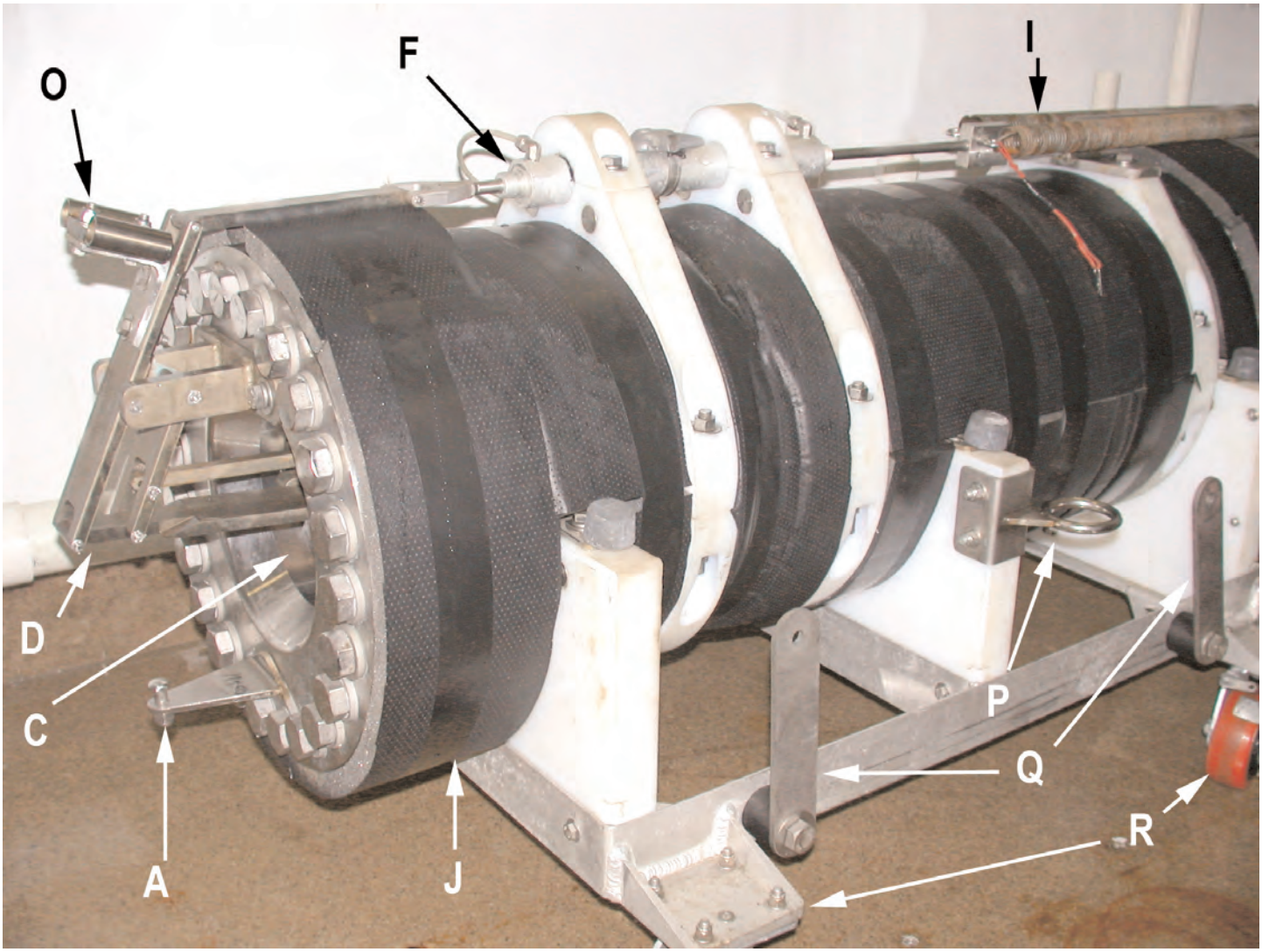

Fig. 3. View of the side and front endcap of the hyperbaric fish trap-respirometer. In this image, the float rack is not attached, and the trap is fitted with its wheels for placement in the temperature-controlled laboratory. Note also that the insulation is in place and that the rotational springs, seen in Fig. 2, have been replaced with conventional coiled springs. A-N are as in Fig. 1. (O) Guides for attaching lever arm to open door, (P) guide for line to ballast, (Q) support bars to limit lateral movement when float rack is attached, and (R) wheels.

on one of the rear end cap penetrations. The regulator is coupled to a computerized controller (Tescom ER3000FI-1) to achieve steady manual or automated pressure control sequences. Flow into the hyperbaric cylinder is achieved with a high pressure metering pump (LEWA*modular*® ELM5). This pump has a maximum flow of 14.8 l (3.9 gallons) per hour at 41.4 Mpa and allows for recompression, removal of wastes, and control of the internal environment.

Assessment

Laboratory testing—The pressure cylinder was tested by cycling its pressure from 0 to 44.8 Mpa (6,500 psi) for 10 cycles. Maximum pressure was held steady for 48 h during the final pressure cycle. The cylinder was then disassembled for o-ring inspection. Although the cylinder pressure was stable during the high pressure test and no leaks were detected, evidence of o-ring extrusion was observed on the annular end cap at the inner o-ring where the end cap meets the cylinder flange. This is the location predicted by the FEA to experience the greatest deflection. These o-rings were replaced with o-rings of a more extrusion resistant material (Parker nitrile NO552). The test was repeated and further inspection revealed no o-ring extrusion. Testing of the capture/door closure mechanism and flotation took place in the MBARI test tank. Several control respirometry experiments were conducted prior to field testing using surface seawater and a piece of bait (squid). Oxygen consumption was negligible over periods of 4 h.

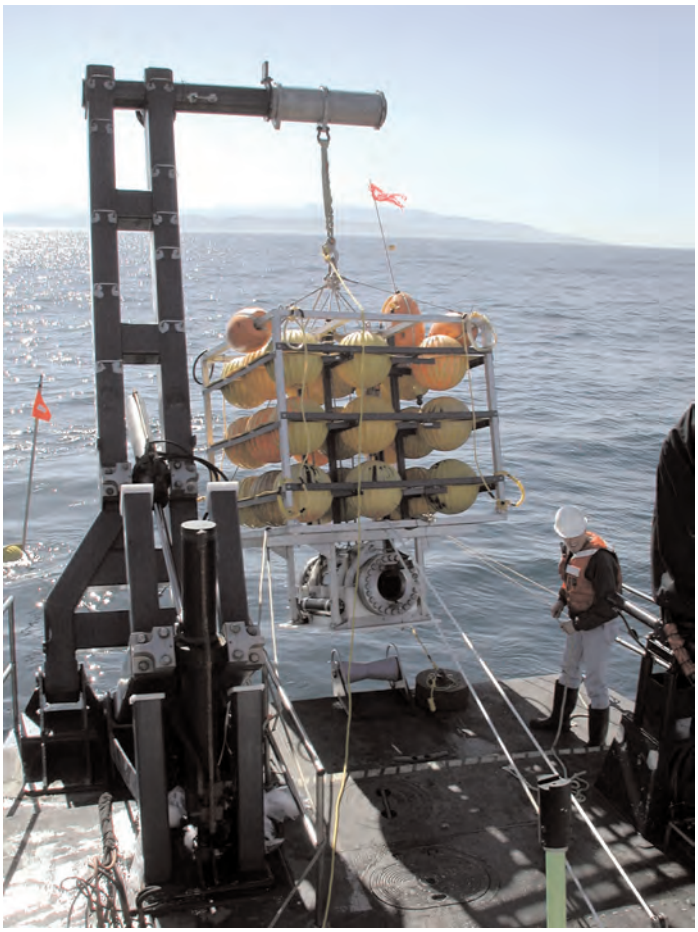

Fig. 4. The hyperbaric fish trap-respirometer during deployment in Monterey Bay from the RV *Zephyr*. The float rack contains 27 Benthos spheres and 3 cubic feet of syntactic foam.

Field testing—Seven deployments made in Monterey Bay, Calif., have been used for testing and initial operations (Figs. 4 and 6) and early results have been promising with conditions in the trap at recovery very near those in situ (Table 1). Deployments 3 and 5-7 were made with MBARI's ROV Ventana for trap recovery or observation of fish behavior. The ROV's CTDO allowed for in situ oxygen and temperature measurements to be made concurrent with trap deployments.

The trap retains pressure remarkably well. During the last four deployments, the trap maintained roughly 95% of the pressure at deployment depth. The door to the trap was closed after the first deployment but curiously the trap only contained 38% of the in situ pressure. This suggests that the trap was tripped during ascent. If the trap had been tripped during deployment or during descent, the door would have acted like a flapper valve allowing water to pass into the trap pressurizing it to bottom conditions.

The trap's insulation worked reasonably well. No insulation was present during the first deployment of the trap and the resulting recovery temperature (11°C) was considerably higher than that at the bottom (2.8°C) or below the thermocline (4-8°C) despite a rapid retrieval at the surface (~45 min). Future deployments of the trap used the open cell insulation as described above resulting in recoveries at ~6°C compared to average bottom temperatures at 1450 m depth of 2.84°C.

Oxygen concentrations within the pressure vessel (84-116 µmol O_2 L^{-1}) were always higher than those measured in situ by the oxygen electrode on the ROV (39.3-46.9 µmol O_2 L^{-1}). The trap floods with highly oxygenated surface water upon descent, which apparently does not flush completely during trap descent due do the restricted size of the vessel door. Oxygen toxicity upon exposure to high oxygen tensions has been observed in at least one invertebrate which inhabits the oxygen minimum zone (Van Dykhuizen and Seidel 1998) but *C. acrolepis* primarily inhabits depths below the oxygen minimum (Pearcy et al. 1982; Stein and Pearcy 1982) where oxygen tensions are similar to those measured in the pressure vessel. Oxygen concentrations where *C. acrolepis* have been observed in Monterey Bay were as high as 99 µmol O_2 L^{-1} (unpublished data) so it is unlikely that the oxygen concentrations in the trap were deleterious for the fish.

The capture rate of the trap, 3 of 7 deployments, is encouraging, particularly considering that these deployments were the testing phase of the project. All of the fish captured were the macrourid *Coryphaenoides acrolepis*, which was expected at the depth of deployment. Many macrourids are active scavengers rapidly attracted to bait (Priede and Bagley 2000) and *C. acrolepis* regularly consumes carrion as part of its diet (Drazen et al. 2001). Squid mantle muscle was used as bait and threaded onto a barbed (deployments 1-3) or barbless hook (all later deployments). To create a large odor plume, several small mackerel or sardines were split in half and placed inside wire mesh just below the door to the trap and the baited hook. Only one of the four deployments, which did not capture a fish, seemed related to a scarcity of animals. In this case the bait remained on the hook and the trap was retrieved untripped.

Our first fish capture occurred with deployment 3 (Table 1). The trap remained on the seafloor for 1 week during due to a failure of the release mechanism. The trap was recovered using MBARI's ROV *Ventana* to release the anchor weight. When observed by the ROV, the trap was tripped and its door was closed. However, when it was recovered at the surface the door was ajar and the trap was draining. The fish inside was dead but had unhooked itself. Failure of the door seal resulted from stress corrosion cracking and failure of the 302 stainless steel rotary constant force springs (Fig. 2). These close the door and provide the initial o-ring seat against the flange. These springs were replaced with regular extension springs that are available in more corrosion-resistant 316 stainless steel (Fig. 3).

Two *C. acrolepis* were captured and returned to the laboratory alive. They were kept in a refrigerated room at 4°C. The temperature of the vessel was allowed to equilibrate over several hours before data were collected to measure respiratory

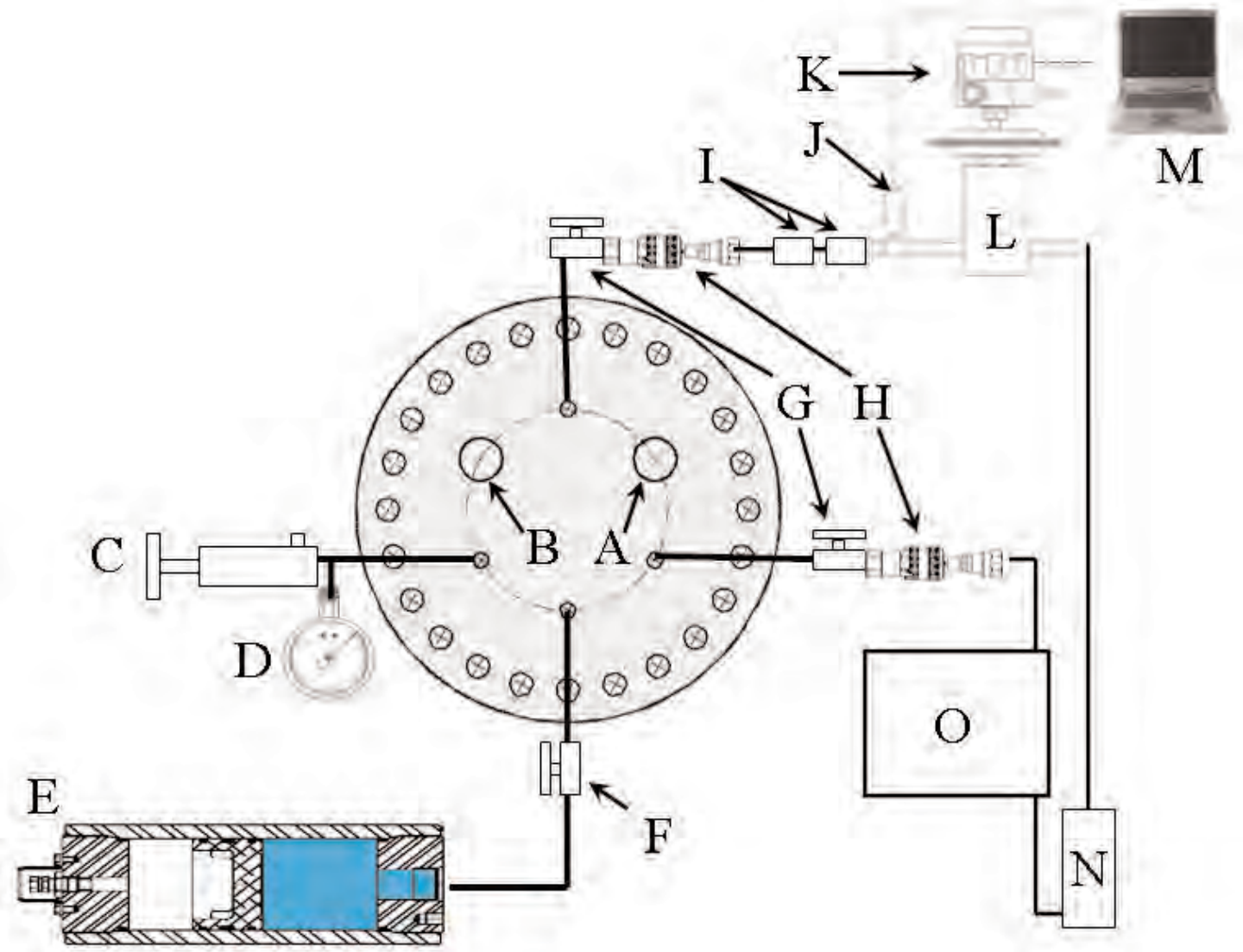

Fig. 5. Diagram showing the hydraulic and electrical connections between the trap and laboratory components. (A) Electrical penetrator to water pump and camera, (B) electrical penetrator to oxygen optode, (C) safety relieve valve, (D) pressure gauge, (E) pressure accumulator, (F) needle valve, (G) ball valves, (H) quick-connect couplings, (I) inline filters, (J) pressure transducer, (K) electronic pressure controller, (L) dome-loaded back pressure regulator, (M) laboratory computer, (N) inflow filter and gas exchange reservoir, (O) high-pressure metering pump.

rates. Following the first capture, water in the trap was initially very cloudy probably due to sloughing of mucus as described by Wilson and Smith (1985). Therefore we used the high pressure pump to flush the trap water for 5 h before closing the system and measuring the fish's respiration rate. During this flushing the water pumped into the trap was bubbled with nitrogen to reduce its oxygen content to near in situ levels. The final concentration was 88.33 μmol O_2 L^{-1}. The respiratory rate of the fish (1.158 kg, 65.0 cm total length, 23.5 cm preanal fin length) was 54.99 μmol O_2 kg^{-1} h^{-1} (Fig. 7). The pressure in the vessel was held between 13.1 Mpa (1900 psi) and 14.5 Mpa (2100 psi) during the entire experiment including water flushing (capture pressure at 1350m was 13.7 [1985 psi]). The fish did not free itself from the barbed hook used in this experiment. Although it generally swam weakly by undulations of its tail its condition appeared to deteriorate through the experiment. We found later that the fish had swallowed the hook, which lodged in its lower gill arches. Barbless hooks were used in subsequent deployments.

A second fish captured successfully during deployment 7 was maintained for 3.5 d. Water in the hyperbaric trap was not cloudy on retrieval, and the fish was observed swimming actively with slow undulations of its tail. During the course of the experiment, the fish would occasionally make sudden burst swimming behaviors and thrash its head to one side. Although a barbless hook was used and camera recordings

Table 1. Hyperbaric fish trap-respirometer deployment information*

Date	Depth (m)	Duration (h)	Pressure (%)	Temperature (°C)	O_2 (μmol L^{-1})	Species	Comments
12 Mar 04	1430	2.5	38%	11	95	None	Fish attacked bait but not captured
16 Mar 04	1430	3.75	0%	n/a	n/a	None	Bait untouched
26 Mar 04	1450	1 week	0%	n/a	n/a	*C. acrolepis*	Door springs broke
13 May 04	1350	25.5	95%	7.1	116	*C. acrolepis*	Fish maintained for 48 h
27 May 04	1450	3.75	93%	6.0	84	None	Trap sprung
5 Oct 04	1500	2.0	95%	6.0	90	None	Trap sprung and bait gone
6 Oct 04	1430	1.0	94%	5.9	110	*C. acrolepis*	Fish maintained for 84 h

*Duration of a deployment (to the nearest quarter hour) is the total time from beginning of descent to arrival at the surface, or for the last two deployments, to the time ROV observations indicated that the trap-door was closed. Acoustic monitoring of the trap showed that descent and ascent each required approximately 40 min for deployment depths of 1450 m. Pressure as a percent of deployment depth and temperature upon recovery are provided.

indicated that the fish was hooked superficially, the fish was unable to free itself. The fish's respiratory rate was 79.43 μmol O_2 kg^{-1} h^{-1} (1.305 kg, 66.5 cm total length, 24.5 cm pre-anal fin length), measured at a temperature of 7°C.

Decompression of the fish was attempted after the respiration measurements were completed. The fish was decompressed successfully to 11.7 Mpa (1700 psi) over 24 h. After this time, however, an unexpected pressure drop of 1.4 Mpa (200 psi) resulted in the fish quickly listing over to its side and vomiting its stomach contents. This clouded the water. Pressure was brought back up to 11.7 Mpa and the fish regained its attitude control. We then began gradual decompression to 11.0 Mpa (1600 psi). Another brief pressure drop to 8.6 Mpa (1250 psi) occurred the following day which resulted in thrashing and the loss of buoyancy control by the fish to the point of being ventral side up in the trap. When pressure was returned to 11.2 Mpa (1620 psi or 76% of pressure at capture depth), the fish regained some buoyancy control but it continued to list to the side. The fish's activity declined slowly and it died after a total of 84 h. Subsequent inspection of the back-pressure regulator revealed wear on the piston from small debris particles which prevented rapid closure of the regulator valve. Multiple filters have been placed in the line leading to the regulator to prevent this problem in the future. Subsequent laboratory pressure tests and regulator tuning proved that the system controlled pressure reliably and smoothly. Sudden uncontrolled pressure losses of even 10% resulted in loss of buoyancy by the fish and most likely led to its death.

Deployment 5 served as a field control experiment. The trap was tripped on the seafloor, bait was present but no fish had been captured. Oxygen consumption was measured as the trap cooled from 6.0°C to 4.0°C and was negligible.

Discussion

Considering success of the trap from the limited number of deployments during its testing phase, we are encouraged with the results. The hyperbaric trap-respirometer has captured animals, sealed at depth, returned fish to the laboratory under pressures and temperatures very similar to those in situ, and the trap has held pressure for several days. To our knowledge, this is the largest pressure vessel of its type and unlike other vessels currently in use it will permit experimentation on large deep-sea fishes and other animals.

The associated laboratory equipment has functioned properly and the difficulty with the pressure regulator has been resolved. Preliminary respiration data have been acquired to estimate metabolic rates and examine pressure tolerances. Our preliminary results are comparable with in situ measurements of macrourid metabolic rates. The metabolic rate of a single *C. acrolepis* was measured in the San Diego Trough at 1200 m and at 3.5°C (Smith and Hessler 1974). Their rate of 107 μmol O_2 kg^{-1} h^{-1} is slightly higher than measured here. *Coryphaenoides armatus*, an abyssal species, is the only other deep-sea macrourid fish for which we have metabolic rate information. The metabolic rates of three *C. armatus* measured by Smith (1978) ranged from 120-165 μmol O_2 kg^{-1} h^{-1}. A sim-

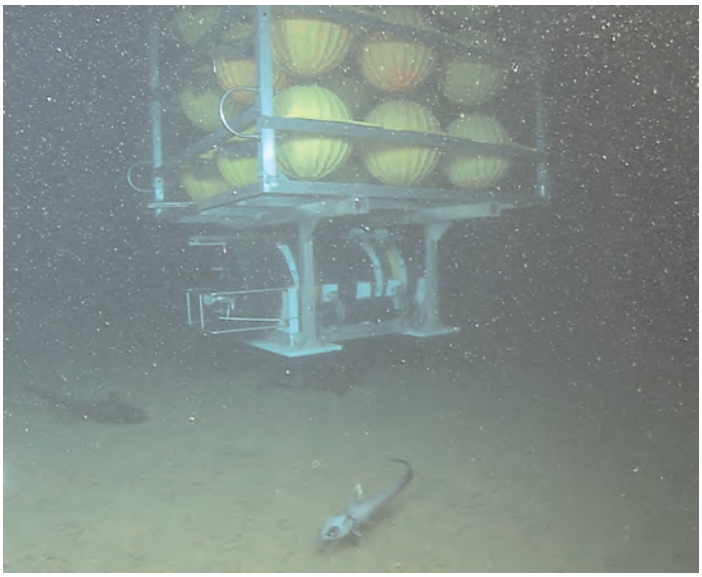

Fig. 6. The hyperbaric trap-respirometer on the seafloor at 1500 m. Two *C. acrolepis* can be seen, one is to the left of the trap and another is in the foreground.

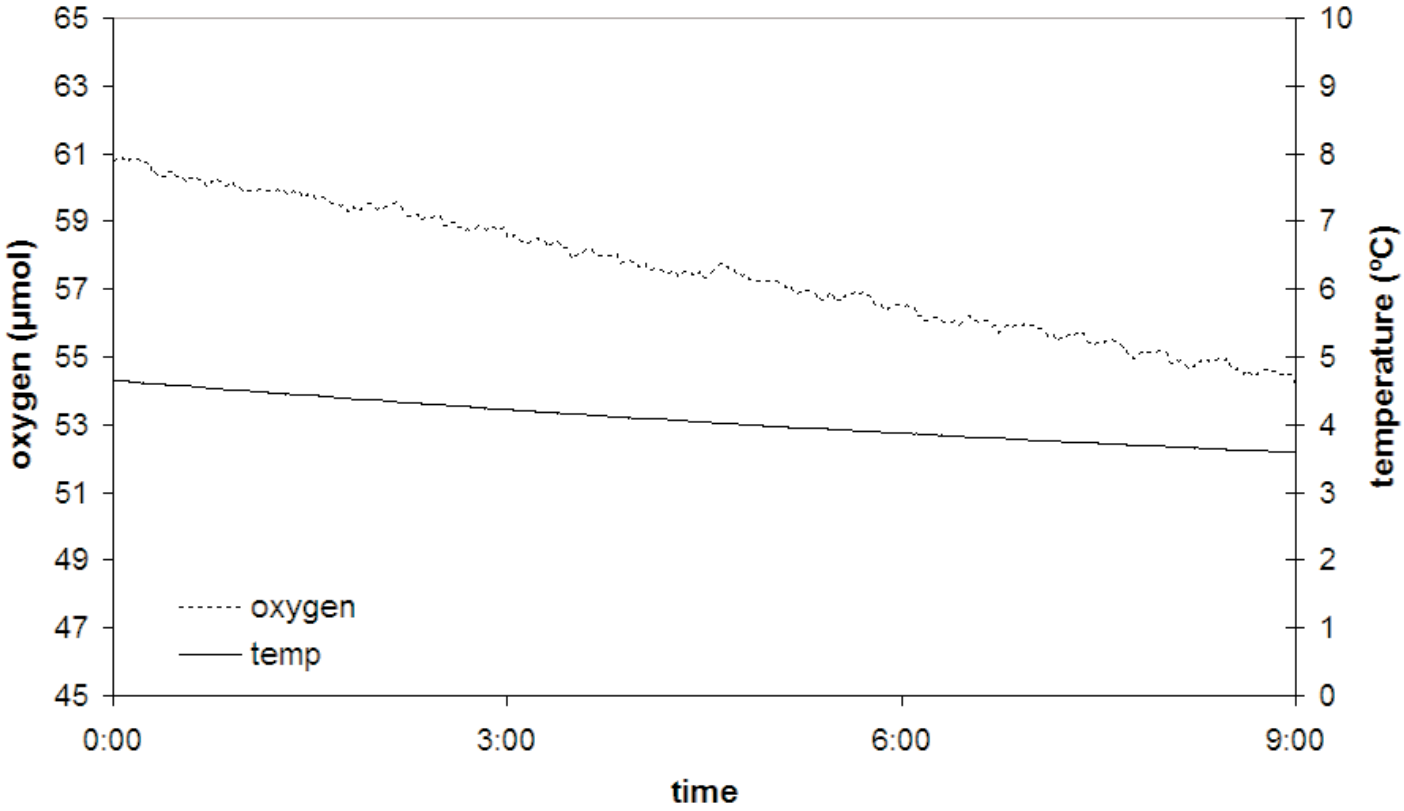

Fig. 7. Example of data used to calculate respiratory rate of the first fish. Changes in oxygen concentration and temperature are shown.

ilar rate was found for an individual in the North Atlantic (Bailey et al. 2002). These data and those for midwater fishes (Torres et al. 1979) have shown that their metabolism is much lower than would be expected by temperature effects alone. For midwater organisms, these declines are suggested to be the result of a decline in locomotory performance as a result of decreasing light levels and reactive distances between predators and prey (Childress 1995). Similar declines in benthic and benthopelagic fishes are likely as evidenced by studies on their enzyme activities (Siebenaller et al. 1982; Sullivan and Somero 1980) and by the limited studies directly measuring metabolic rate described above.

Unlike previous studies (Wilson and Smith 1985), our results suggest that macrourids do not readily unhook themselves and hook-related trauma almost certainly reduced the metabolic rate of the first fish examined. A mechanism that severs the hook leader immediately after the fish is trapped is under development. The second fish swam actively for days, and we believe that our estimate of oxygen consumption is reliable. At this point, it is premature to compare comprehensively our data to other small data sets.

From our second experiment, it was evident the *C. acrolepis* was very sensitive to pressure changes. Wilson and Smith (1985) made similar observations. In their experiment, a *C. acrolepis* listed after a 15% drop in pressure and only regained its normal attitude after 6 h. The animal was not able to regain attitude after a subsequent drop to 70% of its capture pressure. They suggested that full decompression would take approximately 8 d with 34 decompression steps of 13% reductions with 6 h between each. We were only able to decompress fish nr 2 about 25% (equivalent to 1090 m depth) over 3.5 d, suggesting that full decompression may take significantly longer. However, the barotrauma experienced by fish nr 2 could have damaged its gas bladder or internal organs or both, and hampered its ability to resorb gasbladder oxygen. It is interesting to note that macrourids, including *C. acrolepis,* are often found far off the bottom (Haedrich 1974; Pearcy 1976; Smith et al. 1979, 1992). Given their apparent slow resorption of gasbladder oxygen, these fish probably undertake slow vertical migrations or move horizontally off the continental slope, without changing depth greatly, where they are captured in deeper water.

The hyperbaric trap-respirometer has the potential to open many new avenues of research on deep-sea animals. Future deployments are planned to examine the effects of elevated CO_2 concentrations on metabolic rates as part of a study to examine the effects of carbon sequestration strategies on the deep-sea biota. The trap could also be used to determine the effects of pressure acclimation on protein expression, metabolite production, and membrane biochemistry. If we determine

that some species can acclimate to atmospheric pressure, this technology could be used to expand other fields such as energetics, behavior, and chemosensory studies of deep-sea fishes.

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