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Techniques for live capture of deepwater fishes with special emphasis on the design and application of a low-cost hyperbaric chamber

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A cost effective, simple, portable hyperbaric chamber was constructed from polyvinyl chloride to aid in the collection of adult rockfishes *Sebastes* sp. to hold as broodstock. This system was designed to recompress fishes quickly once brought to the surface on hook and line, and to allow for decompression over a period of days. The hyperbaric chamber is capable of continuous stable operation at $<1\,033\,515$ N m^{-2} and can accommodate fishes up to 91·4 cm in length and 26·8 cm in diameter. Pressure in the chamber is maintained by a Goulds Booster pump that delivers continuous pressure and supplies sea water at a rate of 3·8 to 7·6 l min^{-1} to as many as four chambers. The hyperbaric chamber operated very effectively and allowed successful decompression of 12 cowcod *Sebastes levis* captured at depths of 90·2 to 146·3 m.

Key words: chamber; decompression; hyperbaric; portable; pressure; *Sebastes*.

INTRODUCTION

In 2002 Hubbs-SeaWorld Research Institute (HSWRI) initiated a project to evaluate the feasibility of restoring depleted rockfishes of the genus *Sebastes* by first breeding them in captivity and then releasing the offspring into the wild. The primary target species were bocaccio *Sebastes paucispinis* Ayres, cowcod *Sebastes levis* (Eigenmann & Eigenmann) and vermilion *Sebastes miniatus* (Jordan & Gilbert). These species were selected because of their commercial and ecological importance, depleted status and recognition that population rebuilding times would be long (Butler *et al.*, 2003; PFMC, 2002). As adults, these three species generally inhabit rock embankments with the greatest overlap in occurrence between 90 and 300 m (Love *et al.*, 2002; Williams & Ralston, 2002; Butler *et al.*, 2003). In order to establish captive breeding stocks, adults must be collected from depth and acclimated to surface pressures. Although *S. paucispinis* and *S. miniatus* have been successfully collected and displayed

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in public aquariums, *S. levis* are apparently more sensitive and have never before been kept alive. For this reason, *S. levis* was the focus of this study.

When fishes are captured at depth, rapid depressurization can cause serious injuries, including gas bubbles in the blood vessels, gills, skin and brain, exophthalmia, internal and external haemorrhage, everted stomachs, disoriented swimming, altered behaviour, and death (Gotshall, 1964; Beyer *et al.*, 1976; Feathers & Knable, 1983; Rogers *et al.*, 1986; Gitschlag & Renaud, 1994). Specialized collection techniques are needed to minimize barotrauma among deepwater fishes that are collected live for public display or breeding programmes. Collection techniques will vary based on the depth of capture and sensitivity of the target species.

Techniques used to collect fishes from depth typically include cages, trawls, and hook and line fishing. Without special handling, fishes collected using these methods are typically brought to the surface either moribund or dead. Special handling techniques include gradual decompression and swimbladder deflation (Gotshall, 1964; Lee, 1992; Keniry *et al.*, 1996). Swimbladder deflation has enabled collection of some specimens from the sea, but mortality is highly variable and increases with depth of capture. To increase survival, more time must be taken to decompress fishes. In order to accomplish this, specialized pressure-controlled chambers have been developed. These hyperbaric chambers are typically designed to trap fishes at depth and return them to the surface under pressure (Jannasch *et al.*, 1973; Yayanos, 1978; Macdonald & Gilchrist, 1978; Wilson & Smith, 1985; Koyama *et al.*, 2002).

Hyperbaric fish traps have made it possible to collect fishes from the deep sea (600–5700 m). These systems, however, are very costly to construct and do not allow visual monitoring during collection. Problems with these systems have been reported to include loss of pressure upon ascent due to door mechanism malfunction at depth and inadvertent collection of non-target species (Wilson & Smith, 1985). Recognizing these limitations, there is a need to develop a low-cost solution that allows human interaction and monitoring, especially for fishes collected at shallower depths (90–150 m). This paper describes the development of a portable hyperbaric chamber that is capable of recompressing fishes quickly to pressures up to 1 033 515 N m^{-2} (10·2 atmospheres) and then decompressing them at staged intervals lasting days if necessary, all of this being accomplished while observing the fishes, maintaining stable water temperatures and ensuring high water quality standards.

MATERIALS AND METHODS

There were three trial phases for development of the hyperbaric chamber. Phase I included basic assessment of various gear types and ascent rates to determine species-specific catch per unit effort and relative sensitivities to barotrauma. Gear types included hook and line, and traps, although traps were not successful. Phase II was designed to test the use of a hyperbaric chamber for onboard recompression and to define all protocols associated with fish handling. Phase III included practice of the refined protocols and full implementation of a four chamber hyperbaric system (only two chambers were available in Phase II) specifically for the collection of *S. levis*.

In all three phases of this study some or all of the *S. levis* caught were placed in the 4000 l bait wells on board the vessel after being caught on hook and line. This

technique was the standard for phase I trials, which yielded survival of species other than *S. levis*. Thus, although this technique cannot be considered as a 'control' treatment, it does represent the most basic method of collection for comparison with the hyperbaric chamber methods used in phases II and III.

HYPERBARIC CHAMBER DESIGN AND CONSTRUCTION

The hyperbaric chamber described in this paper was designed based on the following criteria: 1) portability for transport of live fishes from the field to the laboratory; 2) visual monitoring of fish health and behaviour; 3) water quality monitoring, especially temperature and dissolved oxygen; 4) pressure stabilization at a maximum of 1 033 515 N m^{-2}; 5) manual control of decompression; 6) temperature chilling and control; 7) use of standardized parts that are readily available to reduce costs.

Designing the complete apparatus to meet portability goals required a multi-part decompression system that could be disassembled into manageable sections. These sections included primary and secondary manifolds, and the pressure chambers. The primary manifold was used to maintain stable pressures for the secondary manifolds. The secondary manifolds were designed to maintain stable pressure and water flow in the chambers. The chambers were used to hold the fishes during the recompression and decompression sequences, and also to maintain a constant pressure during 'lock down' when the fish was being transported from the boat back to the laboratory without water circulation.

PRINCIPAL COMPONENTS

Primary manifold

Pressure in the primary manifold was created by a 3.0 hp Goulds' booster pump (25GBS3014P4; Goulds manufacturing, Seneca Falls, NY, U.S.A.) that delivered 94·6 l min^{-1} of sea water to the apparatus. Pressure in the system was controlled by a 2·5 cm pressure relief valve that vented excess sea water back to the sump and created stable back pressure in the system. A portion of the back-pressured sea water was diverted before the pressure relief valve and served as the source for the secondary manifolds. Four 1·9 cm ball valves operating in parallel from the primary manifold controlled the flow of sea water to four secondary manifolds. Pressure in the primary manifold was monitored on a 10·1 cm face gauge up to 1 378 020 N m^{-2}. Two 5·1 cm ball valves were also installed on the primary manifold and opened during startup procedures to reduce initial water-hammering. For portability, the primary manifold and pump were bolted to a hand truck (Fig. 1).

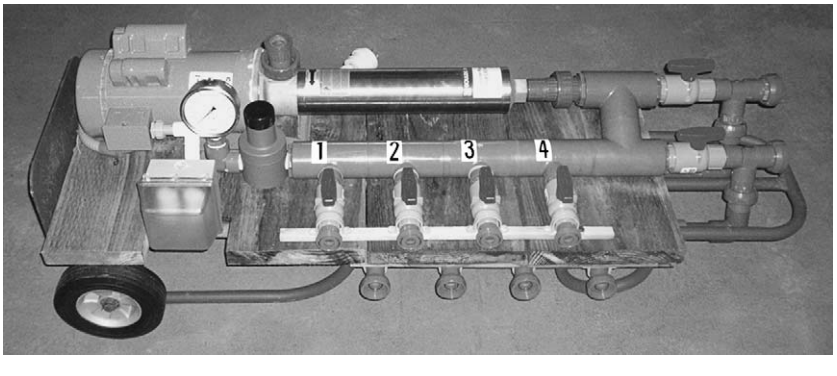

FIG. 1. Portable primary manifold and booster pump attached to a hand cart for ease of transport.

Secondary manifold

A secondary manifold was constructed for each of the four chambers that allowed individual pressure and flow control to the chambers, as well as safety and bypass loops. Water from the primary manifold was diverted through a check valve and a needle valve designed to control the amount of water delivered to the chamber. Water could enter or bypass the chamber through a 1·9 cm ball valve. This bypass allowed operation of the secondary manifold without sea water flowing through the chamber. After sea water was routed through the chamber or bypass it was pumped through a t-strainer before being routed through a 0·6 cm pressure relief valve. Downstream of the pressure relief valve, a 1·0 cm normally closed solenoid valve was installed that operated as a safety valve when power to the pump was interrupted. Beyond the solenoid valve, sea water was routed through a rotary flow indicator and two 0·7 cm labcock valves used to divert outgoing flow to a cylindrical reservoir where water quality measurements could be taken, either by collecting a water sample or immersing a probe. Two other 1·3 cm ball valves were also used to bypass the solenoid and pressure relief valves in the event of a component failure or to operate the system at ambient pressure (Fig. 2).

Chamber

The hyperbaric chamber was constructed from 30·5 cm schedule 80 polyvinyl chloride (PVC) pipe with an internal diameter of 28·6 cm. It was 91·4 cm in length and had two 30·5 cm flanges glued to each end. On one end a 5·1 cm thick acrylic viewing window and gasket assembly was bolted to the flange. An opaque black fabric cover was used to enclose the window and to eliminate light from entering the chamber. Two 1·3 cm bulkhead fittings were installed in the side of the cylinder. A 1·3 cm ball valve was connected to each of the bulkhead fittings with a pressure gauge located on opposite sides of the valves directing water in and out of the chamber. A cradle

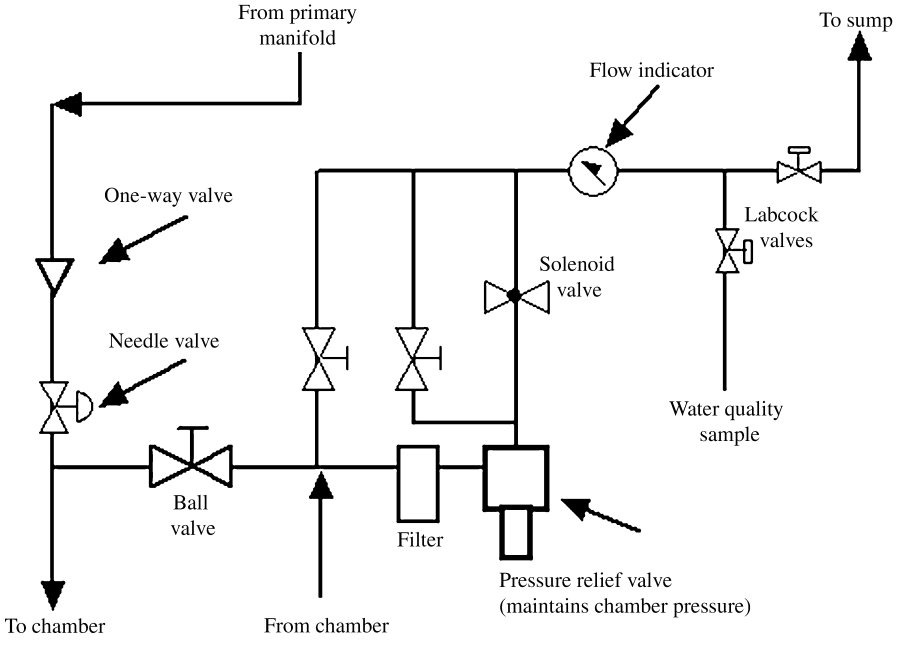

FIG. 2. Schematic drawing of the secondary manifold (located under each chamber) that is supplied with pressurized sea water from the primary manifold.

was designed to support the chamber when strapped to a 273 kg capacity hand truck and to protect the secondary manifold located beneath the chamber. Connections from the chamber to the secondary manifold were made with a high pressure hose. A PVC blind flange and gasket assembly was attached to the open end of the cylinder to complete the chamber. Pressure in the secondary manifold and chambers reached a maximum of 962 588 N m^{-2}.

SEAWATER SUPPLY

Seawater supply to the apparatus depended on its location, either in the field or at the laboratory. During field operation, the primary manifold was supplied with chilled water from the fish hold onboard the vessel using a 0.5 hp Flotec® submersible sump pump (Delavan, WI, U.S.A.). At the laboratory, sea water was pumped from Mission Bay (San Diego, CA, U.S.A.; 32°46′38″ N; 117°14′01″ W), filtered using rapid sand filters, and chilled in a re-circulating sump. The temperature regulation systems at both locations were computer controlled, resulting in consistent supply temperatures (±0·7° C). Because laboratory holding tanks ranged from 10 to 12° C, 11° C was used as a target standard for all trials.

PRESSURIZATION SEQUENCE

Prior to receiving a fish, each chamber was oriented vertically (open end up) and filled with chilled sea water (Fig. 3). Water pressure was regulated and stabilized in

FIG. 3. Hyperbaric chamber set on end prior to receiving a fish. Secondary manifold is connected and visible on the left side of the chamber as viewed in the photograph.

the primary manifold at 1 104 443 N m^{-2} and in the chamber bypass line of the secondary manifold at 891 660 N m^{-2}. Once a fish was caught and brought to the surface, it was placed inside the chamber in a head down position. A gasket and blind flange assembly was then attached to the top of the chamber with 12 stainless steel bolts. The bolts were torqued in a star pattern to 13·1 m kg^{-1}. Once sealed, the valves isolating the chamber were opened, which equalized pressure from the secondary manifold. This began the recompression sequence. Once at the maximum pressure, the valve bypassing the chamber on the secondary manifold was closed forcing sea water to flow-through the chamber.

MONITORING ENVIRONMENTAL CONDITIONS AND CHAMBER WATER QUALITY

Subsurface sea conditions were monitored using a Hydrolab DataSonde (Loveland, CO, U.S.A.). The data were used to identify target water temperatures to maintain in the chamber, as well as to improve understanding of the target species' natural environment. The Hydrolab DataSonde was factory fitted with probes for depth (±1 m), dissolved oxygen (±0·1%, ±0·01 mg l^{-1}), temperature (±0·1° C), pH (±0·01), salinity (±0·2), and photosynthetically active radiation (PAR) (±1·0). Measurements were recorded every 30 s as the probe was lowered to the depth of the targeted fishing areas. Other variables monitored included simulated depth inside each chamber, depth of fish capture and rate of fish ascent. These data were collected with a Reefnet Sensus Pro® depth recorder (Mississauga, Ontario, Canada) programmed to log depth measurements every 10 s when attached to the fisherman's line, and every 30 s when placed in the chamber. It measured depth to the nearest 0·3 m.

Water quality was monitored inside and outside the chamber. Externally, water quality was monitored downstream of each chamber using an Oxyguard Handy Gamma probe (Birkerød, Denmark). The probe was factory fitted with temperature (±0·1° C) and dissolved oxygen (±0·1%). Measurements were recorded at 1 h intervals while on the boat and every few hours in the laboratory during the daytime. Measurements and observations were also recorded at night between staged decompression plateaus. For continuous in-chamber measurements an Onset Tidbit® temperature probe (Bourne, MA, U.S.A.) was deployed to record temperature every 30 s to the nearest 0·01° C.

DECOMPRESSION SEQUENCE

Fishes were stabilized at pressures equivalent to their capture depth for 20·0 to 25·5 h before beginning their decompression sequence. The first 4 to 6 h of the stabilization period took place on the research vessel. After the initial stabilization period the chambers were isolated (valves closed and disconnected from the water source on the boat) and transported back to the laboratory. Transport took no longer than 25 min at which point water flow was re-established to the chambers from the laboratory supply. After the stabilization period, the fishes were decompressed at a rate that was consistent with recommendations reported in the United States Navy diving manual for saturated human divers (United States Navy Diving Manual, 1999). This rate varied from 1·8 to 0·9 m h^{-1} depending on the depth. The ascent rates were: 1·8 m h^{-1} below 61·0 m, 1·5 m h^{-1} between 30·5 and 61·0 m, 1·2 m h^{-1} between 15·2 and 30·5 m and 0·9 m h^{-1} between 0 and 15·2 m. Because the chamber pressure was controlled manually, fishes were decompressed in stages typical of those performed by scuba divers practicing a safety stop. A relatively rapid decrease in pressure was followed by an extended stabilization period of 5–11 h at the new pressure. This extended stabilization period allowed fishes to equalize internal gas concentrations with those of the environment, reducing the possibility of bubble formation in the circulatory system. No attempt was made to feed the fishes in the chamber because it was unlikely they would feed immediately after capture and because of the difficulties with feeding and cleaning the chambers while under pressure.

MONITORING FISH BEHAVIOUR AND HEALTH

Fishes were caught on hook and line and brought to the surface at a target rate of 11 m min^{-1} from depths between 94·2 and 146·3 m. Once on board each fish was examined externally before being placed into the chamber or bait well. The severity of pop-eye and the extent of stomach extrusion were scored based on predetermined criteria described below. The eyes were scored either as normal, protruding or 'crystallized' (severe protrusion, causing the corneal layers of the eye to separate and crack), and the stomach was rated either as normal, partially extruded or fully extruded (Fig. 4). The orientation, respiration rate (this was measured by counting the number of completed fish ventilations in a 60 s period; timing began at the termination of the previous ventilation and three counts were performed to verify the correct number of ventilations) and buoyancy of each fish were assessed by looking through the viewing window of the chamber after pressurization. Observations were made every hour during transport on the vessel and then every few hours while in the laboratory. Observations of fish condition were made immediately before and after each decompression sequence between staged plateaus. A veterinarian was readily available for consultation during all phases of decompression. At any stage during the decompression cycle, if fishes appeared distressed (*e.g.* erratic swimming, increased ventilation rate, change of colour or loss of buoyancy), the decompression sequence was halted immediately and the system was re-pressurized until the fishes appeared 'comfortable'. The decompression sequence was then re-started 3 h later.

TERMINATION OF DECOMPRESSION

At the end of each decompression sequence, the chamber was again oriented vertically (blind flange up) and the blind flange was removed. Fishes were then sluiced into a custom-built, vinyl sling and inspected for damage around the gills and inside the mouth prior to being placed into a recovery pool. Once they were in the pool, the swimming orientation and general behaviour were monitored daily. After 1 week they were offered food daily until they began to feed. Fishes were rated as 'survivors' once they began feeding.

At the end of each trial the chambers were cleaned with fresh water and scrubbed as needed. Each apparatus was also inspected and maintenance performed as needed.

FIG. 4. Initial presentation of trauma in an adult *Sebastes levis* caused by rapid decompression from 96·0 m. This is one of 12 *S. levis* successfully recompressed, transported, decompressed and actively feeding at the Hubbs-SeaWorld Research Institute.

RESULTS

In phase I, *c*. 8% of *S. miniatus* and *S. paucispinis* survived while none of the three *S. levis* that were caught survived (Fig. 5). Unlike the other species, *S. levis* died within 1 h after capture suggesting that they were much more sensitive to capture than the other targeted species. Because of this sensitivity, *S. levis* were designated the target species for phases II and III trials. A total of 41 *S. levis* weighing between 2·2 and 10·0 kg (52·0 to 74·5 cm fork length, L_F) were caught throughout all phases.

Of the 14 *S. levis* caught during phase II, eight were recompressed and decompressed in the chamber but only one of those survived to feed actively. In phase III, 16 *S. levis* were recompressed and decompressed in the hyperbaric chamber, and 11 survived to feed, yielding a survival rate of 69% (Fig. 5). As observed in phase I, all *S. levis* caught in phases II and III that were not recompressed in a chamber expired on the boat within 1 h after reaching the surface. Survival of *S. levis* was dependent on re-pressurization (two-by-two contingency table with no fixed margin, $n = 41$, $P < 0{\cdot}01$) and there was no significant difference in L_F between the fish that died or survived when treated in the chamber (Mann–Whitney *U*-test, $n = 24$, $P > 0{\cdot}05$).

CHAMBER OPERATION

The apparatus was used to recompress and decompress a total of 24 *S. levis* in phases II and III of this study. Once a fish was brought on board, it took mean ± S.D. of 8·0 ± 1·4 min to recompress it to a pressure equivalent to 84·7 ± 2·2 m in depth. Mean recompression time was 1·8 ± 0·9 min at a mean ± S.D. rate of 63·6 ± 37·6 m min^{-1}.

Following recompression, *S. levis* were allowed to stabilize for 20 to 25·5 h. After this period fish were decompressed at a rate of 1·4 m min^{-1} to sequential target stabilization depths (plateaus). These stabilization depths typically were

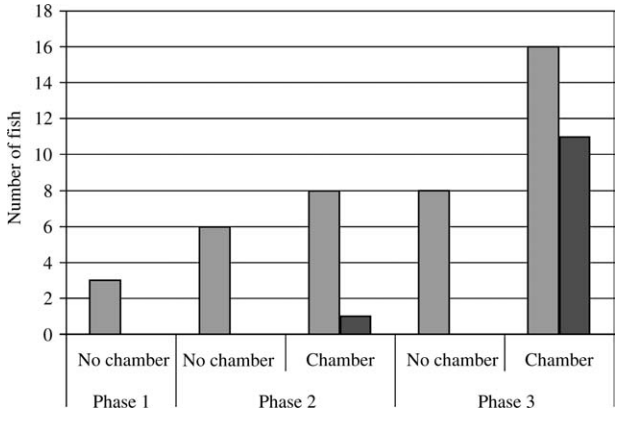

FIG. 5. Number and placement of *Sebastes levis* caught on hook and line in each phase of the trial (▒, the number of *S. levis* treated; ■, the number that survived. Survival was based on a fish actively feeding once decompression was completed).

75·8 ± 0·6 m, 60·4 ± 0·5 m, 47·0 ± 0·2 m, 31·5 ± 0·5 m, 18·0 ± 0·2 m, 11·1 ± 0·4 m, 4·1 ± 0·3 m and 3·6 ± 1·0 m (mean ± S.D.). Stabilization times at these depths ranged from 5·4 to 11·0 h depending on scheduling, initial stabilization depth, appearance and behaviour of individual fish. Total recompression–decompression treatments lasted from 73·4 to 150·4 h and resulted in complete recovery and acclimation of 12 *S. levis* (Fig. 6).

FISH HEALTH AND BEHAVIOUR

At the surface *S. levis* exhibited a range of symptoms associated with barotrauma. While some fish exhibited no external damage, others had fully extruded stomachs with protruding or 'crystallized' eyes. All *S. levis* brought to the surface were lethargic with their mouths agape. Once placed in the chamber and re-pressurized their mouths closed and they began to respire more noticeably. Typically, fish would remain lying on their side throughout the recompression and decompression sequence.

In the chamber, the respiratory rates of *S. levis* increased slowly from a low of two ventilations min^{-1} up to 22 while on the boat. Typical respirations measured back in the laboratory ranged from 20 to 32 ventilations min^{-1} regardless of pressure change during decompression. Any deaths while in the chamber usually occurred in the first 24 h after recompression.

Fish behaviours during decompression included lying on one side, maintaining an upright position, swimming aggressively against the window and attempting to turn around inside the chamber. Aggressive swimming occurred immediately after being recompressed on the boat and was minimized by covering the window with a dark cover. Out of 24 attempted decompressions only two fish are known to have turned themselves around in the chamber, and only three fish were observed swimming aggressively against the glass. During scheduled decompression periods, fish showed no change in buoyancy, respirations or behaviour.

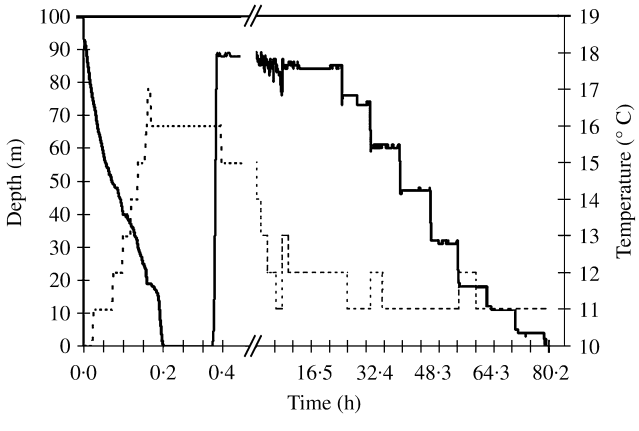

FIG. 6. Decompression profile for one of 12 *S. levis* successfully collected by the Hubbs-SeaWorld Research Institute. Pressure readings converted to actual or simulated depths (——) with water temperature (- - -) also shown.

SEAWATER TEMPERATURE

Ocean surface temperatures during collection trips ranged from 14·5 to 19·9° C. Seawater temperature ranged from 9·7 to 10·4° C at collection depths of 93·7–106·7 m. During capture fishes ascended through a 4·3–10·3° C temperature differential before being recompressed in a chamber or placed in the bait well. Initial chamber water temperature was warmer than desired because chilled sea water was not circulated through the chambers while waiting for fishes to be captured. During the recompression sequence when chilled sea water was re-circulated through the chamber, the water temperature decreased quickly from a maximum of 18° C to the target temperature of *c.* 11° C. Temperature stabilization usually occurred in the first 60 min of operation and thereafter the temperature stayed relatively constant. Large temperature differentials of 9–10° C did not appear to affect survival when the hyperbaric chamber was used as evidenced by the fact that 11 of 12 surviving *S. levis* were caught during the warmest collection months of June, July and August.

DISSOLVED OXYGEN

Dissolved oxygen at the collection sites ranged from 52·3 to 59·5% saturation at depths of 93·7–106·7 m. These levels increased to 112% in surface waters <6·1 m. During operations on the boat, dissolved oxygen levels in the chambers ranged from 91 to 120%. When the fishes were transported back to the laboratory dissolved oxygen concentrations decreased to a minimum of 81% before circulation was re-established. This stabilized oxygen concentrations between 84 and 98% for the remainder of the treatment. After complete decompression fishes were placed in sea water with dissolved oxygen concentrations >90%.

DISCUSSION

Survival time for deep ocean species brought up from depth and maintained under pressure without water exchange have been documented to range from 2 to 9 days (Jannasch *et al.*, 1973; Macdonald & Gilchrist, 1978; Yayanos, 1978; Wilson & Smith, 1985), and up to 64 days with exchange (Koyama *et al.*, 2002). Systems used in these studies were designed to capture fishes, amphipods and bacteria from 1200 to 5700 m and to maintain them under pressure for observation and measurement. The hyperbaric apparatus described in this paper is different from these systems because it was built to not only maintain fishes caught from depths <146·3 m, but to decompress them as well.

The primary limitation of decompression-traps is that the traps are indiscriminate and capture both target and non-target organisms. This limitation becomes unacceptable if the target species is never caught, while work crews are left to deploy, catch and retrieve the heavy cumbersome trap systems without result. The ability to manually catch fishes on hook and line and place them in a relatively small manageable hyperbaric chamber will increase the chances of being successful and reduce time spent at sea.

Another benefit of using a portable hyperbaric chamber system is that it allows for rapid decision making and problem solving. This is not the case for many of the deep-sea collection devices. Some systems have been reported to lose internal pressure during ascent (Yayanos, 1978; Wilson & Smith, 1985), and not be large enough to collect organisms >2·5 cm in diameter (Macdonald & Gilchrist, 1978; Yayanos, 1978). Manual techniques used in the present work allow real-time adjustment of the apparatus according to fish behaviour and correction of minor equipment malfunctions, thereby preventing loss of pressure or other system failures. The chamber and opening hatch is also large enough to accommodate most fishes pulled up from target depths.

The versatility of the portable hyperbaric system allowed re-pressurization of fishes caught from depth, and then transport in a controlled environment back to the laboratory where customized decompression schedules could be implemented. This was possible because temperature controlled sea water was circulated through the chamber under pressure. Simulating depth is not a new concept. Previous work has been completed in the laboratory where depth was simulated with air pressure (McCutcheon, 1966; Tsvetkov *et al.*, 1972; Hoss & Blaxter, 1979), or regulated with a single pressure relief valve (Hill & Caulton, 1974). Resulting depth profiles in the air pressure studies were limited due to build up of metabolites, decreases in oxygen concentration and increases in gas partial pressures when pressure was reduced. Use of a pressure relief valve to control pressure did allow simulated depths of 60 m for extended periods of time, but the system was restricted to the laboratory because it was not portable.

The portable hyperbaric chamber described in this paper was used successfully to recompress, transport and decompress a species never before maintained in captivity. The survival rate of *S. levis* in the final phase of this research was 69%. The apparatus is portable and capable of stable operation under a variety of field and laboratory conditions. The system is also relatively inexpensive to build, operate and maintain.

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