

## NOTE

## A pressurising system for long-term study of marine or freshwater organisms enabling the simulation of cyclic vertical migrations\*

ALFREDO DAMASCENO-OLIVEIRA<sup>1,2</sup>, JOSÉ GONÇALVES<sup>1,2</sup>, JOÃO SILVA<sup>3</sup>,  
BEGOÑA FERNÁNDEZ-DURÁN<sup>1</sup> and JOÃO COIMBRA<sup>1,2</sup>

<sup>1</sup>Laboratório de Ecofisiologia, Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Universidade do Porto, R. dos Bragas 177, 4050-123 Porto, Portugal. E-mail: aoliveir@icbas.up.pt

<sup>2</sup>Instituto de Ciências Biomédicas de Abel Salazar (ICBAS), Universidade do Porto, Lg. do Prof. Abel Salazar 2, 4099-003 Porto, Portugal.

<sup>3</sup>Instituto Superior de Engenharia do Porto (ISEP), R. Dr. António Bernardino de Almeida 431, 4200 - 072 Porto, Portugal.

**SUMMARY:** A computer-controlled pressurising system enabling large aquatic organisms like marine fishes to be maintained for long periods is described. The paper presents a system that is capable not only of maintaining constant hydrostatic pressures but also of producing any desired cyclic variations of that parameter, allowing the close and reliable simulation of vertical migrations that many species of fishes and other aquatic animals perform during some stages of their life cycles.

*Key words:* hydrostatic pressure, vertical movements, semi-diurnal cycle, fish behaviour, flounder.

**RESUMEN:** UN SISTEMA DE PRESURIZACIÓN PARA REALIZAR ESTUDIOS DE LARGA DURACIÓN CON ORGANISMOS MARINOS O DE AGUA DULCE, CAPAZ DE SIMULAR MIGRACIONES CÍCLICAS VERTICALES. – Es descrito un sistema de presurización controlado por computador que permite realizar experiencias de larga duración con animales acuáticos de considerable tamaño, manteniéndolos siempre en óptimas condiciones. Este artículo presenta un sistema que es capaz, no solo de mantener presiones hidrostáticas constantes sino también de producir variaciones cíclicas de dicho parámetro permitiendo realizar una rigurosa simulación de las condiciones que se dan en las migraciones verticales que muchas especies de peces y otros animales acuáticos realizan durante algunas etapas de su ciclo vital.

*Palabras clave:* presión idroestática, movimientos verticales, ciclo semidiurno, comportamiento de los peces, platija.

The thermodynamic variable pressure has been studied for a long time by a broad spectrum of disciplines but the effects of pressure *per se* on living organisms have proven to be much more difficult to study. The main difficulty is to dissociate the effects of pressure from those of the partial pressure of the gases in air-breathing animals. However, in fish and

other aquatic organisms that difficulty does not exist (Fenn, 1967).

The main problem with long-term exposure to high hydrostatic pressures is the maintenance of aquatic organisms both under pressure and in an environment with stable water conditions. For more than a hundred years a number of different techniques and systems have been used to achieve this (Nishiyama, 1965; Theede, 1972; Avent and Men-

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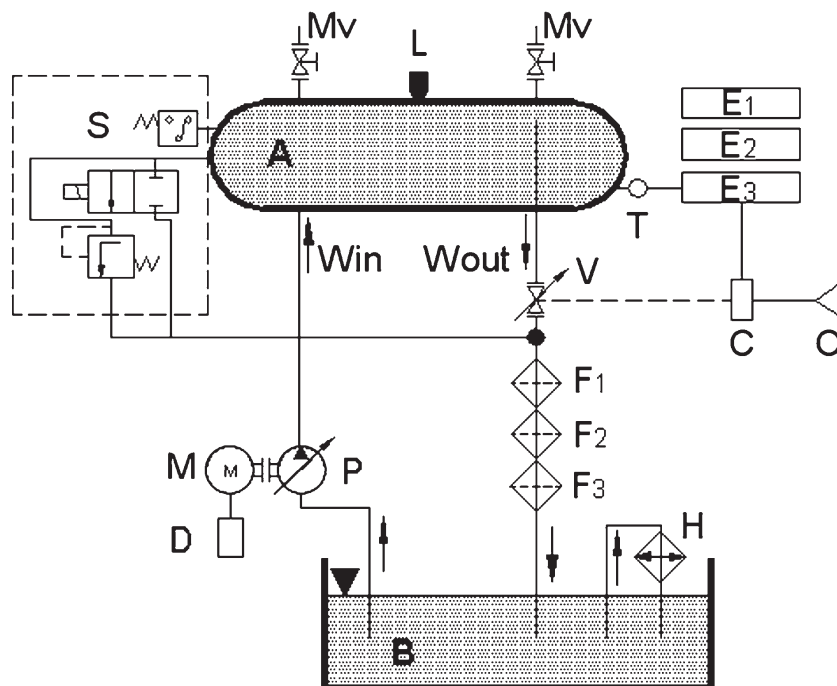


FIG. 1. – Pressure system. A: hyperbaric test chamber; B: reservoir tank; C: electropneumatic converter; D: variable speed drive; E1: computer; E2: serial interface; E3: programmer; F1: mechanical filter; F2: biological filter; F3: charcoal filter; H: cooler/heating unit; L: light source; M: electric motor; Mv: manual valve; T: pressure sensor; O: compressed air supply; P: positive displacement pump; S: security system; V: proportional control valve; Win: water input; Wout: water output.

zies, 1972; Lowenstam and Wastphal, 1972; Meek and Childress, 1973; Tytler and Blaxter, 1973; Avent, 1975; Fontaine *et al.*, 1985; Quetin and Childress, 1980; Sébert *et al.*, 1990; Smith and Baldwin, 1997; Sébert, 2002). However, most of the systems used were limited in their capacity to hold large or adult fishes. Furthermore, it has been suggested that cyclic variations in hydrostatic pressure rather than constant or abnormally high pressures could have much more dramatic effects (Gibson, 1982, 1984), but equipment suitable for studying cyclic hydrostatic pressure effects has not been available.

Numerous species of aquatic organisms experience (at least during some stages of their life cycles) those rhythmic pressure changes associated with vertical movements and there are usually two distinct patterns of vertical movements: a diurnal pattern and a semi-diurnal pattern related to tides and involved in the selective tidal stream transport (Greer-Walker *et al.*, 1978). Plaice, eel and cod are some of the fish species with the best-characterised patterns of cyclic vertical movements during spawning migrations, although they are quite different in terms of cycle duration and level of pressure exposure (Metcalf *et al.*, 1994; Tesch, 1979, 1989; McCleave and Arnold, 1999; Godş and Michalsen, 2000; Stensholt, 2001).

The present paper describes a pressurising system (Fig. 1) designed to study seawater fish, enabling for the first time the automatic simulation of those vertical migrations with the capacity to maintain them for long periods under stable water parameters. This will allow us to perform studies on the physiological effects of cyclic hydrostatic changes on, for example, reproductive development.

The system has two main units, the hyperbaric test chamber (A) and a reservoir (B). The test chamber was built by Iberco (Cartagena, Spain) and it is an AISI type 316L stainless steel cylinder with an internal diameter of 45 cm and a volume of 153 litres. This chamber has a working pressure capability of 8 MPa and was tested with hydrostatic pressures up to 12 MPa. It has two main ports ( $3.5 \times 10^{-2} \text{ m}^2$  each) through which the organisms are introduced. Each port is a flanged end closed by a 316 stainless steel plate 3 cm thick with eight screws 2 cm in diameter and sealed by a rubber o-ring. At each end of the cylinder there is an observation window ( $2.8 \times 10^{-3} \text{ m}^2$  each) made of polymethylmetacrylate (PMMA). There is a lighting port (L), also made of PMMA ( $2.8 \times 10^{-3} \text{ m}^2$ ), at the top of the chamber, where a 50 W halogen lamp is installed, delivering  $23.7 \mu\text{E m}^{-2} \text{ s}^{-1}$  at the bottom of the chamber. A Theben timer (Haigerloch, Germany) con-

trols the photoperiod regimes. There are also several small connections for pressure sensors and measuring equipment. Two manual valves (Mv) at the top allow the removal of all air trapped inside the chamber at the beginning of each experiment. A small chamber (10 cm<sup>3</sup> volume) can be connected to the top of the test chamber through a manually operated ball valve. The introduction of the food in this small chamber and, after water pressurisation, the opening of the ball valve allows the test organisms to be fed if necessary.

The rate of water exchange required to maintain good water quality in the hyperbaric test chamber is ensured through the reservoir coupled to a complete water treatment system with mechanical (F1), biological (F2) and charcoal filters (F3) and oyster-shell chips (buffer). The reservoir, in which the flowing water has a surface open to the atmosphere, has a capacity of 1000 l. Trials developed in this system may operate in a flow-through configuration or alternatively through water treatment and reuse. The filtration circuit is independent from the hyperbaric test chamber supply circuit, ensuring fully operational bio-filtration at all times, even during test chamber maintenance shutdown.

The water is pumped from the reservoir by a Hydracell positive displacement pump (Wanner International, Minneapolis, USA) (P), creating a pulse-free hydrostatic pressure inside the chamber that could reach 8.3 MPa (the maximum for this model). This pump has a variable speed control (LG, Barcelona, Spain) (D) coupled with the electric motor (M), allowing a variable flow that can be freely settled up to 8.3 l min<sup>-1</sup>. With this feature it is possible to change the flow through the pressurising chamber, which must be strong enough to allow the mixing of the water and the maintenance of a proper environment inside the chamber, i.e. with water of a predetermined composition and without the accumulation of metabolic by-products. The water flows from the chamber back to the reservoir through a pneumatic-operated proportional control valve (Conflow, Milano, Italy) which maintains the desired pressure inside the chamber.

The desired pressure (fixed or with variations with any amplitude or frequency) is set by controlling the opening of the control valve (V). A proportional/integral/derivative (PID) programmer (Eroelectronic, Novara, Italy) (E3) controls the opening/closing of this valve through an electropneumatic converter (Conflow, Milano, Italy) (C). This converter receives a constant supply of compressed air

of 172.5 kPa (O) and converts the 4-20 mA signal from the programmer into a proportional pneumatic control signal to the valve. A pressure sensor (Valcom, Milano, Italy) (T) inside the chamber with an output of 4-20 mA is connected to the programmer and provides an input for the real pressure inside the chamber. The programmer permanently receives these datasets and compares them to the value (set-point) that the pressure should have at that time, adjusting the flow through the valve.

A microprocessor-based serial interface converter (Eroelectronic, Novara, Italy) (E2) allows for communication between the programmer and a PC (E1) through a SDDE driver using the MODBUS protocol. The PC also enables easier programming and control of the PID programmer and is constantly recording several variables such as real pressure inside the chamber and theoretical pressure.

The safety of the unit is ensured by (S) a flow switch (Yamatake, Tokyo, Japan) that stops the system during water supply failure, a pressure relief valve and a pressure switch (Telemecanique, Lisbon, Portugal) adjusted to the desired pressure. In cases of power cuts the proportional valve closes the output of water from the test chamber and, since the water volume inside the chamber is large, the animals can survive in good condition for several hours.

An experiment was performed over a period of 15 days with three independent systems in order to determine whether with this design it is possible to provide optimal levels of environmental parameters (pH, dissolved oxygen, temperature) and at the same time to have hydrostatic pressure and photoperiod strictly controlled. One system was used as a reference (control) at 100 kPa (~ 1 atm) of absolute hydrostatic pressure (simulating a surface fish), another at a specified and constant hydrostatic pressure (800 kPa) and finally another one in which we simulated a tidally-associated vertical migration with a semi-diurnal pressure cycle of 600 kPa amplitude (between 200 and 800 kPa) and 6.20 h period. The compression rate used was 100 kPa min<sup>-1</sup> up to 800 kPa in the system for constant pressure and up to 200 kPa in the system with cycles of hydrostatic pressure. All the systems were assembled in a temperature-controlled room at 20 °C. In this trial adult flounder (*Platichthys flesus* Linnaeus, 1758) averaging 358.1 ± 50.2 g (mean ± SE) were used. Six fish were randomly placed in each test chamber and the flow through each system was set at 4 l min<sup>-1</sup>.

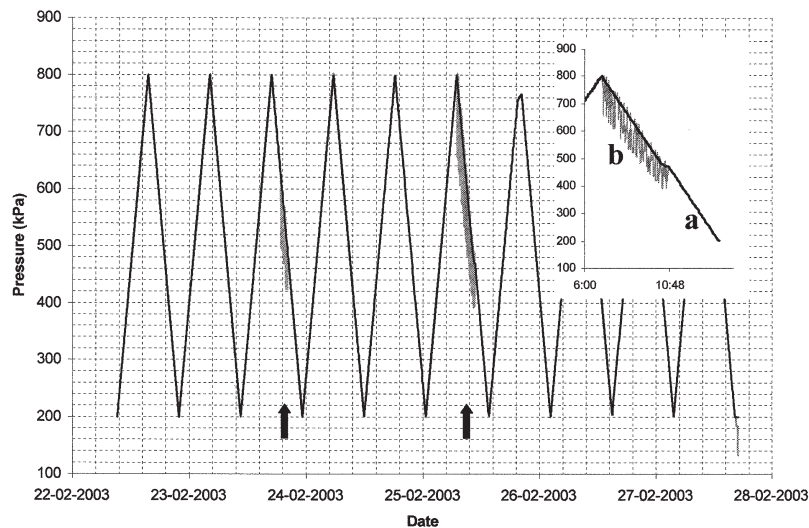


FIG. 2. – The theoretical and the real pressure cycles of 600 kPa amplitude and 6.20 h period (only six of the fifteen days of experiment are represented). The arrows indicate two periods when these cycles were not coincident. The insert shows more clearly the second of those periods (a, theoretical pressure cycle; b, real pressure cycle inside the experimental chamber).

The temperature, pH, salinity and oxygen, ammonia, nitrite and nitrate content of the effluent from each test chamber, and the water in each respective reservoir tank were measured daily. These parameters were measured with a WTW Multi 340i meter (WTW, Weilheim, Germany), a coupled oxygen/temperature probe (WTW CellOx 325), a pH-sensor (WTW Sentix 41) and a salinity sensor (WTW Tetracon 325). Ammonia, nitrite and nitrate were measured using a Palintest Photometer 7000 (Palintest Ltd., Tyne & Wear, UK).

At the end of the experiment all the systems proved to work in good condition without any interference from the operator. In the two systems with constant hydrostatic pressure this parameter remained stable at 100 kPa and 800 kPa with maximum fluctuations of 0.5%. In the system with cycles of hydrostatic pressure these cycles were almost always identical to those programmed (Fig. 2), except in power surges when some slight differences were detected between the theoretical and the real pressure cycle. This problem was corrected with the installation of an uninterruptible power supply with a surge protector and an electric generator.

In the effluents of the three test chambers the ranges of oxygen content were 7.55 to 7.65 ppm, pH 7.95 to 7.98, salinity 34.2 to 34.6‰ and temperature 14.3 to 14.7°C. The differences between the effluent and the water in the reservoir tanks never exceeded 4, 1, 0.3 and 3% respectively. The ammonia, nitrite, and nitrate concentrations did not exceed 0.22 mg total ammonia-N l<sup>-1</sup>, 0.17 mg NO<sub>2</sub>-N l<sup>-1</sup> and 4.40 mg NO<sub>3</sub>-N l<sup>-1</sup> respectively, which are considered within

acceptable limits. No mortality was observed during the duration of the experiment and the fish exhibited no abnormal behaviour.

These results show that by using this system it is possible to provide similar conditions for all the test organisms inside the three chambers during the study, making it possible to compare the effects of cyclic or constant hydrostatic pressure. The use of this novel apparatus, capable of producing a wide range of cycles in a feasible and non-operator-dependent way, will allow a large diversity of studies on the physiological effects of cyclic hydrostatic pressures in fishes performing vertical migrations.

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