A Versatile Nematode Water Bath Apparatus

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The activity of nematodes in unstirred water frequently decreases due to the depletion of oxygen from their surrounding microenvironment (1,2,3,). In in vitro studies on the activity of *Nothanguina phyllobia* Thorne we found it essential to prevent this by constantly agitating nematode suspensions. Various physiological processes are undoubtedly affected at oxygen tensions which inhibit activity, and the need for agitation arises in studies of nematode survival, lipid utilization, and toxicology. Nematode activity and metabolism are also sensitive to temperature; the rate of movement of *N. phyllobia* juveniles, for example, increases 25% over the range 19–24 C (Robinson, unpublished data). To solve these problems, we designed and built a space-efficient, multi-temperature water bath apparatus specially adapted for nematodes. It provides shaker capability as well as precise microscope stage temperature control (± 0.2 C) (Fig. 1). It is constructed from readily obtained parts and requires < 10 A (115 V) under full load. The basic design may be modified to provide any number of temperatures simultaneously. With our particular arrangement, we are able on a 1-m length of laboratory bench to store, shake, and microscopically observe twenty 25-ml samples at five temperatures in the range 0–60 C (100 total samples).

The apparatus consists of four assemblies (Fig. 2), each of which can be built and installed separately: a water bath assembly (B), an electrical power/water pressure manifold (M), an observation well assembly (W), and five water bath reservoirs (RES). The machine requires external pressurized hot and cold water sources. We use an ice-cooled Styrofoam (polystyrene) container with a 0.03 kW output magnetic drive pump for cold water and a commercially manufactured external circulating thermostatic water bath for hot water.

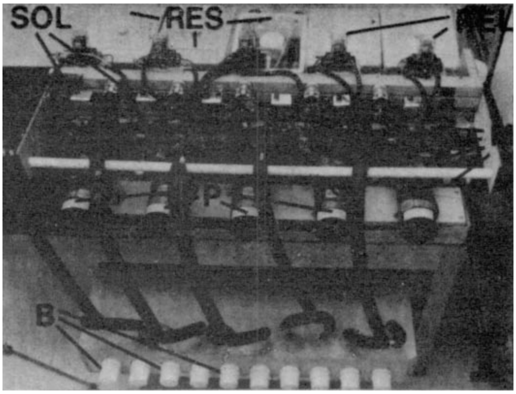

Fig. 1. Temperature control apparatus with observation well assembly removed. Note bath units (B) of the water bath assembly; relays (REL), solenoid valves (SOL), circulator pumps (P), and pressurized hot and cold water manifolds (M) of the manifold assembly; and water bath reservoirs (RES).

The water bath assembly consists of ten 42-mm-o.d. × 117-cm-long tubes mounted in a glass-wool-packed plywood and sheet metal box. The box is bolted to a platform shaker (Fig. 2). The tubes are constructed entirely from polyvinyl chloride (PVC) pipe and pipe fittings. Threaded plastic caps give access to both ends of each tube for the loading of experimental vials.

The manifold assembly consists of a simple plywood frame (Fig. 3) through which holes are bored to permit the passage of water lines and to which pressurized hot and cold water manifolds (12.5-mm PVC pipe and pipe fittings), two electrical plug molds, five relays, six solenoid valves, and five water pumps are secured. As such, the manifold assembly is the heat, cold, pressure, and power source for the entire apparatus. Vinyl tubing (6.5-mm i.d.) is used to interconnect manifold, water bath, and reservoirs. The universal use of hose barbs permits the quick rearrangement of water lines for maximum functional flexibility. The glass wool packing within the bath assembly and the use of tubular refrigeration insulation on all vinyl lines limit temperature variation within the system to < 0.2 C.

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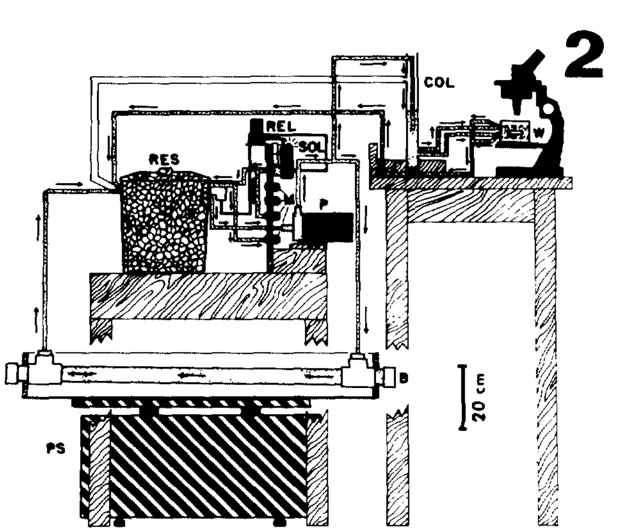

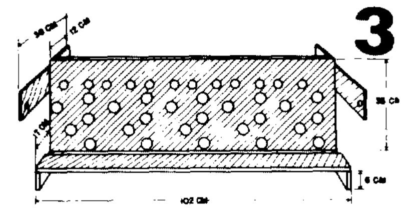

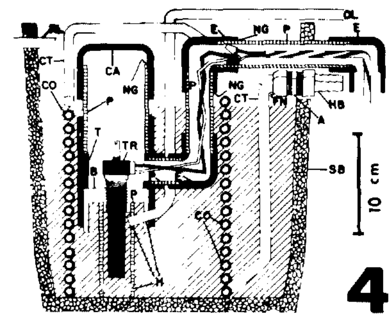

Figs. 2-4. 2) Cross-sectional view of temperature control apparatus. Note bath unit (B) of the water bath assembly; relay (REL), solenoid valve (SOL), circulation pump (P), and pressurized hot and cold water manifolds (M) of the manifold assembly; water column (COL) and well (W) of the observation well assembly; and a water bath reservoir (RES). Bath assembly is mounted to platform shaker (PS). Arrows indicate water flow direction. 3) Plywood frame of manifold assembly. 4) Cross section of water bath reservoir. The PVC parts shown are elbows (E), pipe (P), slip cap (CA), tee (T), and bushing (B). Other parts are Styrofoam box (SB), thermoregulator (TR), water bath output line (OL), return line (RL), input and output copper tubing (CT) to the coil (CO), flare nut (FN), tubing to pipe thread adapter (A), and hose barb (HB). Holes (H) are drilled into PVC at three points. Three PVC connections are not glued (NG).

Each water bath reservoir (Fig. 4) consists of a Styrofoam container into which are fitted a heat transfer coil (7.6 m of 9.5-mm o.d. copper tubing), a thermoregulator housing (constructed entirely from PVC pipe and pipe fittings), and a thermoregulator (Fenwall Thermoswitch 17100-0, Fenwall, Inc., Ashland, MA). Working in conjunction with each reservoir are a relay, a solenoid valve, and a water pump mounted to the manifold frame. When in operation, the thermoregulator of each reservoir controls the relay which in turn opens or closes the solenoid valve, thereby regulating hot or cold water flow through the heat transfer coil. Temperature is adjusted by turning the adjusting stem of the thermoregulator while watching an indicator lamp wired in parallel with the solenoid.

The observation well assembly consists of a series of observation wells and vertical cylinders that deliver temperature-controlled water to the wells by gravity flow. Vertical cylinders are made from 40-cm lengths of 32-mm-d acrylic tubing. The bottom of each cylinder is rubber-stoppered and inserted into a hole in a plywood holder. Three-centimeter lengths of 6.5-mm-o.d. acrylic tubing are glued into holes drilled in the side of the cylinder to provide connections for latex lines going to observation wells. A vinyl line from the circulator pump supplies the cylinder with temperature-controlled water. Water level within the cylinder and, consequently, water flow through the wells is adjusted by differentially squeeze-clamping the water line running from the pump to the water bath assembly. An overflow line returning to the bath reservoir ensures that the cylinder never overfills.

Each observation well and its cover are made in such a way that the experimental suspension is completely surrounded by circulating water > 13 mm thick (Fig. 5). Trapped air pockets above and below the water minimize heat transfer. Wells are easily constructed by chemically welding (using methylene chloride as a solvent) three sizes of polystyrene petri dishes. A well bottom requires three 88- × 15-mm petri dish bottoms and one 57- × 10-mm petri dish cover; a well cover requires two 88- × 15-mm bottoms and one 90- × 9-mm cover.

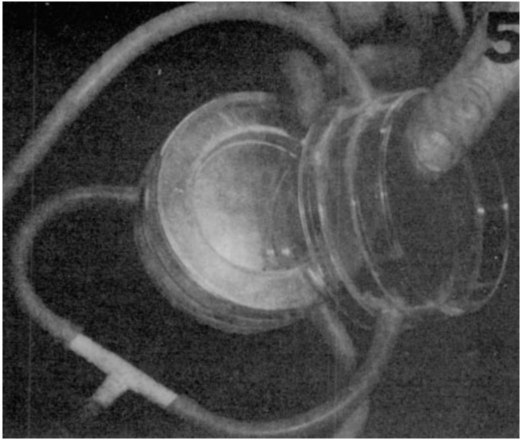

Fig. 5. Observation well bottom and cover with temperature-controlled water lines attached.

A filler putty made by dissolving acrylic shavings in methylene chloride firmly secures tubing connectors (four 2-cm lengths of 6.5-mm-o.d. acrylic tubing) within holes melted in the sides of wells. Highly flexible latex tubing (6.5-mm i.d.), interconnecting vertical cylinders, wells, and return lines to the reservoirs, enables the easy maneuvering of wells on the microscope stage and bench. The wells yield good optical resolution and are easily cleaned.

A complete list of parts is available from the authors.

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