

## RESEARCH NOTE

# A DOSING SYSTEM TO VARY pH, SALINITY AND TEMPERATURE

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**Abstract**—Construction of a dosing system to expose small aquatic organisms to varying pH and salinity under different temperature regimes is discussed. The design allows for three controlled pH levels and two salinity levels across three temperature regimes. The system is flexible and may be adapted for other experimental paradigms including pH depression, elevation or maintenance as well as use with various dilution media or "toxicants" other than salt.

**Key words**—aquatic toxicology, bioassay, dilutor, fish larvae, pH, salinity, temperature

This paper outlines a simple continuous flow dosing system designed to expose small aquatic organisms to varying pH, salinity and temperature regimes. The design utilizes components of a pH controlling system, a delivery/dilutor system and test chambers in temperature controlled water baths (Fig. 1). This dosing system was intended for use with "soft reconstituted" bioassay water (Marking and Dawson, 1973) as a dilution medium (pH 7.2-7.6) where experimental pH ranges were to be lowered and maintained throughout an acute exposure period. Salinity levels were maintained via a salt stock metered into the delivery system.

The design that follows yields two salinity levels (i.e. one adjusted level and a control) in replicate for each pH exposure regime. However, should more than two salinity levels be required, features of a proportional dilutor system (Mount and Brungs, 1967; Riley, 1975) or use of a multichannel peristaltic pump with variable tubing sizes could be incorporated into the design.

The system design flexibility permits pH elevation or depression as well as pH maintenance over time. This system could be adapted for use with other dilution media or with "toxicants" other than salt.

### *pH controlling system*

Our initial experimental design required up to 400 l. of flow-through dilution medium for each pH regime over an acute exposure period. The pH controlling system (for each experimental pH regime) features the following components:

- (1) 600-l. polyethylene tapered cylindrical reservoir tank, Chem-tainer®;
- (2) recirculating pump, March AC-5C-MD;

- (3) pH/ORP controller, Jenco 2671;
- (4) in-line pH electrode, Phoenix 5993-70;
- (5) dosing pump, Hanna K-2;
- (6) stock carboy containing dilute (0.01 M) H<sub>2</sub>SO<sub>4</sub> acid.

Combination pH controller/acid pump units are available but are considerably more expensive.

The described recirculating pump has a maximum flow of 16 gpm which was sufficient to maintain adequate circulation in the reservoir tank when filled to capacity. An in-line globe valve was installed in the recirculating pump return line. This permitted control of recirculating flow rates in the 600-l. reservoir tank as necessary. For each pH regime operated, a separate reservoir tank and pH controlling system is required.

For convenience, the pH electrode and the acid infusion line were placed in-line to the reservoir tank recirculating system (Fig. 2). The high set-point of the pH controller is set at the desired experimental pH (the low set-point would be used if pH levels were to be elevated). The neutral plug wire of the acid pump is rerouted to the normally-open high set-point relay of the controller. This design activates the acid pump when the pH is above the desired set-point. Four parameters are responsible for maintaining the desired pH set-point; (1) sufficient sensitivity of the pH electrode, (2) adequate circulation in the holding tank, (3) flow rate from the acid pump when activated and (4) strength of the acid stock. The electrode, controller and acid pump (set at 10-20% flow) as previously described operated satisfactorily under our test conditions. In the reservoir tanks, pH was able to be maintained at  $\pm 0.1$  pH units of the desired set-point.

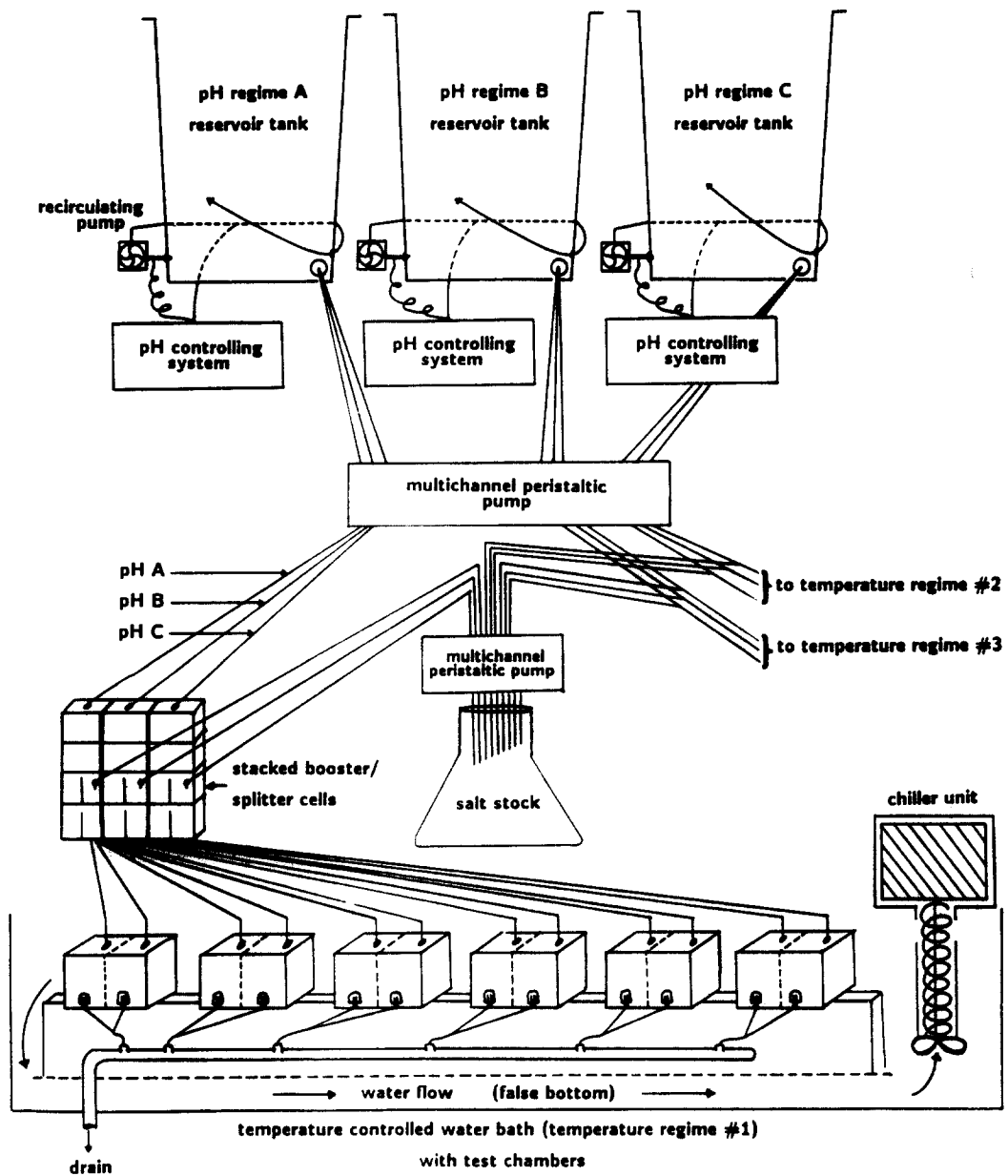


Fig. 1. Schematic overview of dosing system (not to scale).

When using "soft reconstituted" bioassay water the pH equilibrium of the control medium, after aeration and introduction of fish, is 7.2–7.6. The pH in the 600-l. reservoir tanks may be 0.0–0.2 pH units higher than in the exposure chambers. Water passing through the booster (accelerator) and splitter cells is aerated to some extent, however, a weighted airstone in the recirculating reservoir tank may be necessary depending on test parameters.

#### Delivery system

For each experimental pH used per temperature regime, a stacked series of two booster cells and two splitter cells are required (Fig. 3). The stacked series of cells rest atop a shelf either above or to the side

of the temperature-controlled exposure vessels (Fig. 4). The cells may be held in alignment on the shelf by means of a backing board. Our backing board consisted of a plexiglas sheet anchored to the shelf side. A second piece of plexiglas sandwiched the cells and was held in place with elastic straps.

Water first enters the primary booster cell which accelerates the flow by means of a cap siphon for the primary splitter. This splitter divides the flow into two approximately equal portions, half for pH regime-A with  $X$  salinity level and half for pH regime-A with  $X + N$  salinity level.  $X$  represents the control salinity in the holding tank water and  $N$  is the additional salinity added from the salt stock for the higher salinity conditions. The secondary booster cell

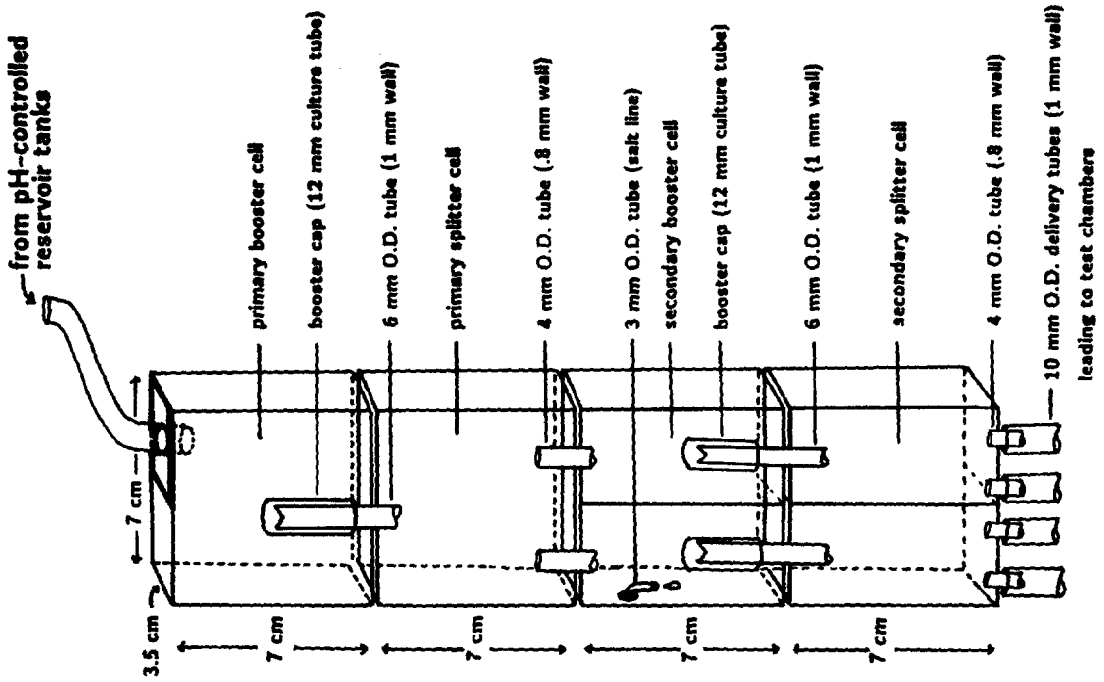


Fig. 3. Detail of a single booster/splitter cell series.

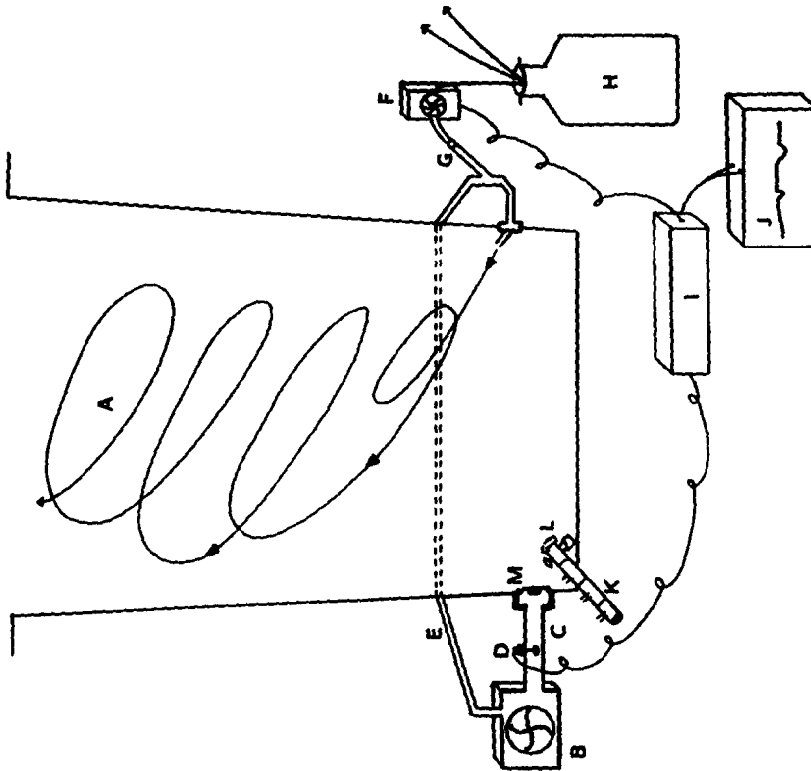


Fig. 2. Schematic drawing of single pH controlling system. (A) 600-l. reservoir tank, (B) recirculating pump, (C) 1 in. pump inlet line, (D) in-line pH electrode, (E) 1/2 in. pump return line, (F) acid pump, (G) acid infusion line, (H) acid stock carboy, (I) pH controller, (J) optional chart recorder (K) 1/2 in. outlet manifold with drain (polypropylene or celcon<sup>®</sup>) and three tubing inserts (1/4 in. insert by 1/2 in. male pipe thread adapter) for connection to peristaltic pump [an in-line ball valve (not shown) may be installed between the tubing inserts and the drain], (L) 1/2 in. threaded bulkhead fitting and (M) 1 in. threaded bulkhead fitting.

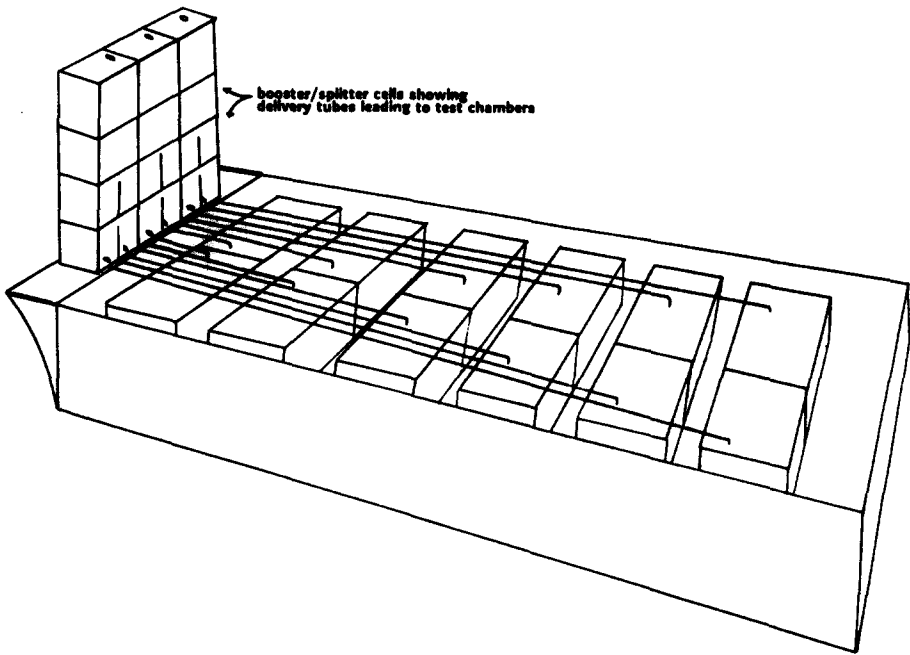


Fig. 4. Schematic diagram of booster/splitter cells on top of shelf at one end of a temperature controlled recirculating water bath (recirculating chiller unit not shown).

acts as a mixing chamber in the one cell which receives the salt stock. The mixed solution is accelerated to a secondary splitter which then delivers test solutions, through delivery tubes, to replicated test chambers. Actual water quality measurements showing pH depression and salinity dilution are illustrated in Fig. 5. The rate of change in these parameters is affected by media strength and flow rate.

The volume of water delivered to the exposure chambers per primary booster cell cycle is regulated by the height of the notched glass tube and/or the length of its siphon cap inside the primary booster cell. For cell calibration, silastic tubing or a one-hole tapered stopper may be used to make the seal between the glass tubing and the drilled holes in the bases of the booster and splitter cells. Careful splitter

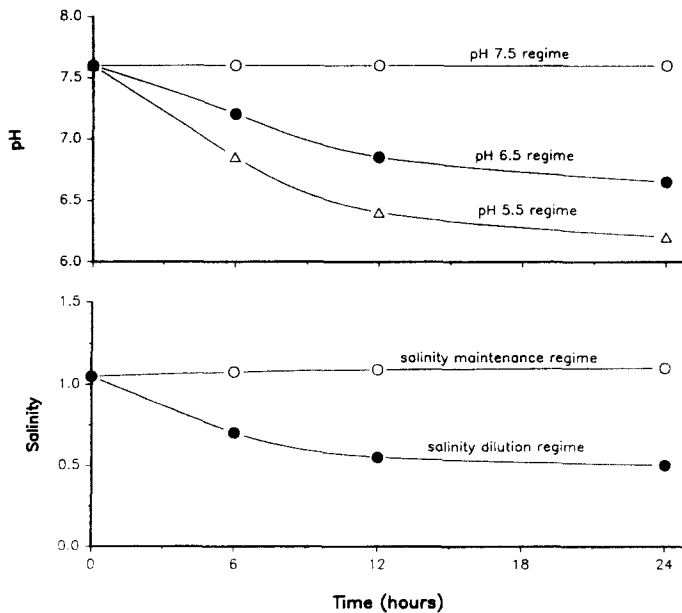


Fig. 5. Water quality measurements showing pH depression and salinity dilution from an acute 24-h exposure period.

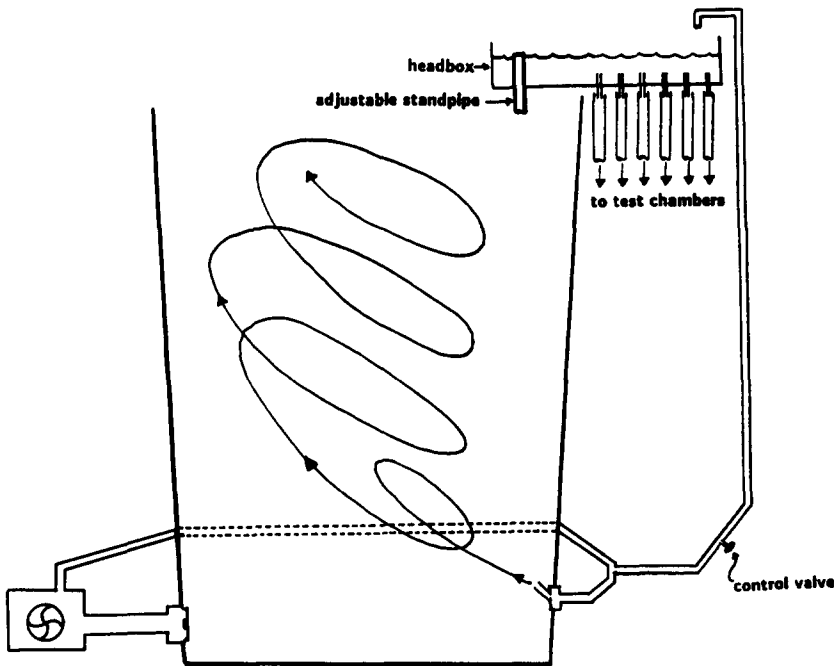


Fig. 6. Gravity fed alternative for pH controlled media distribution.

cell calibration will permit even distribution of test media ( $\pm 5\%$ ) to test chambers. For information regarding glass cutting, assembly and associated supplies, the reader may consult Mount and Warner (1965), Mount and Brungs (1967) or Benoit *et al.* (1981).

A multichannel Masterflex™ pump was used to transport the water from a manifold at the base of the 600-l. reservoir tank, near the recirculating pump inlet, to the primary booster cell (Fig. 1). The manifold should have as many tubing inserts for pump tubing as there are temperature regimes. For example, if three temperature and three pH regimes are being tested three tubing inserts are required in each manifold. For our protocol a total of nine inserts were needed since three pH-controlled recirculating tanks were necessary. A 10-channel Masterflex™ peristaltic pump with # 24 tubing (Cole-Parmer Company, Chicago, Ill.) was satisfactory for our requirements. System flow rates are regulated by increasing or decreasing flow from the peristaltic pump. However, too low a flow may not sufficiently prime the booster cell for complete cycling.

An inexpensive gravity-fed alternative for the multichannel pump can be instituted in situations where reservoir tanks and exposure chambers are in close proximity to one another ( $\leq 2$  m) when running only one pH or temperature regime. The pump drive is replaced by a headbox situated above the reservoir tank (Fig. 6). The headbox receives the pH-controlled media from a small-bore line fed by way of a 45° wye from the recirculating pump return line, upstream of the acid injection point. An in-line control valve regulates the flow to the headbox. A standpipe in the

headbox maintains appropriate constant head pressure for the adjustable delivery tubes leading to the primary booster cells. The headbox height above the primary booster cell(s) should be approximately half the distance between the headbox and the booster/splitter cells to insure unrestricted gravity flow.

A Masterflex™ low-drive pump with a multichannel microhead meters a flow of salt stock from a stock carboy to one side of each secondary booster cell. Tygon tubing (1/8 in.) was used to deliver the salt stock to the booster/splitter cells (Fig. 1).

#### Test chambers

Test chambers described were designed to hold a functional volume of 2 l. Adjacent chambers share a common wall for ease of construction (Fig. 7). An internal standpipe with a siphon cap was installed in a corner of each chamber. This permits circulation and periodic flushing within exposure vessels. Test chambers are submerged in the temperature-controlled water bath to maintain constant temperature. Our water baths consisted of model LSW-700 "Living Stream" systems (Frigid Units, Inc. Toledo, Ohio). Test chambers are submerged in the water bath to the internal volume of the chamber. Our chambers were supported on a frame constructed of 1/2 in. PVC pipe open at the corners and ends. This prevented the structure from floating. Test chamber standpipes were connected externally to the water bath drain via a series of 45° wyes and tees. Internal exposure vessels may be constructed of 16 or 32 ounce high density polyethylene containers with three or four staggered 1-in. holes cut in the side wall at least 3 cm from the bottom. This permits careful

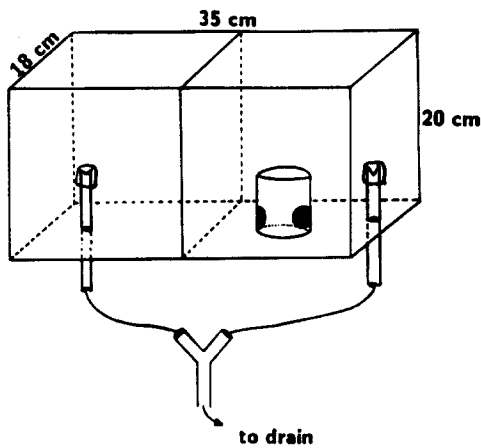


Fig. 7. Design of test chambers. Individual exposure vessels may be placed within. Water level is controlled by standpipe height. Drain level fluctuations are controlled by standpipe siphon cap length [standpipe made from 6 mm o.d. tube (1 mm wall); siphon cap made from 12 mm culture tube].

removal of the exposure vessels for counting or observation of test organisms without subjection to undue handling stress. The holes in the side walls are screened with 400 micron nitex® mesh (Tetko, Inc. Elmsford, N.Y.) to prevent larvae from escaping.

Mention of product names or manufacturer is for identification purposes only and does not necessarily constitute endorsement.

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