second year of operation will be expanded to house approximately 6,000 frogs. The plant has space for approximately 12,000 bullfrogs under maximum operation. Although the market potential looks promising, we must develop an active advertising program to create the demand before we can operate at full capacity.

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A RESPIROMETER FOR DETERMINING THE EFFECT OF PESTICIDES AND RELATED POLLUTANTS ON OXYGEN CONSUMPTION OF ESTUARINE FISHES[1]

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ABSTRACT

A continuous-flow respirometer was constructed to measure the effect of pesticides and related pollutants on oxygen consumption of estuarine fishes. The parts of the respirometer in contact with pollutants were constructed of glass and teflon for efficiency in cleaning. Filtered, irradiated sea water of constant temperature and salinity was gravity-fed through ten experimental and ten control respiration chambers in which individual fish were held. Flow rates through the chambers were controlled by stopcocks and measured by flowmeters; dissolved oxygen was determined by the Winkler method before and after water passed through each chamber. Pollutants were metered into the experimental chambers by syringe pump.

INTRODUCTION

This research was initiated because (1) little is known about the effects of pesticides and related pollutants on the oxygen consumption of fishes, and (2) the criterion for effect in almost all acute fish/pesticide bioassays in previous years has been death. Recently, investigators at this laboratory have begun to use behavioral and physiological responses of fishes to measure sublethal effects of pollutants (Hansen, 1969; Cop-

[1] Gulf Breeze Contribution No. 135.

page, 1971). I began respiration research to permit determination of another possible sublethal effect of exposure to pollutants in both acute and chronic laboratory bioassays, namely, increased or decreased oxygen consumption.

METHODS AND MATERIALS

Sea water was pumped from Santa Rosa Sound through a series of cellulose or polypropylene core-type filters (15, 5, and 1 micron porosities) into a 2.5 kiloliter reservoir constructed of plywood and fiberglass. The filtered water was then pumped over a heating or cooling unit and two ultraviolet lamps into a elevated constant-level tank. Water flowed by gravity from the tank into the respirometer and overflow from the tank returned to the reservoir (Figure 1). Tap water was added, when necessary, to obtain desired salinity.

The respirometer, mounted on a frame in the laboratory, consisted of an experimental and control manifold. I attached ten stopcocks to each manifold, and attached flowmeters by standard-taper ground glass joints to each stopcock. Disposable flexible tubing connected the flowmeters to each of twenty glass respiration chambers which were closed at each end with one-hole rubber stoppers. Each chamber was connected to a 300 milliliter BOD bottle by tubing (Figure 2). Water collected in the bottles was analyzed for dissolved oxygen by the Winkler method (Strickland and Parsons, 1968).

Water level was adjusted in two tanks which contained manifolds, respiration chambers, and BOD bottles so that all these parts were under water and more stable temperature was maintained.

Pollutants were dissolved in polyethylene glycol (molecular weight 200) or acetone and metered into the experimental manifold neck by syringe pump. The same amount of solvent was metered into the control manifold.

Fish were collected locally and acclimated to laboratory conditions. Small groups of fishes were anaesthetized with ethyl m-aminobenzoate methanesulfonate (MS-222). Each individual was weighed, placed in a respiration chamber, and a cover was placed over the chambers. The fish were left undisturbed for 24 hours before oxygen determinations were made. Fish were held for periods up to seven days in the respirometer.

RESULTS

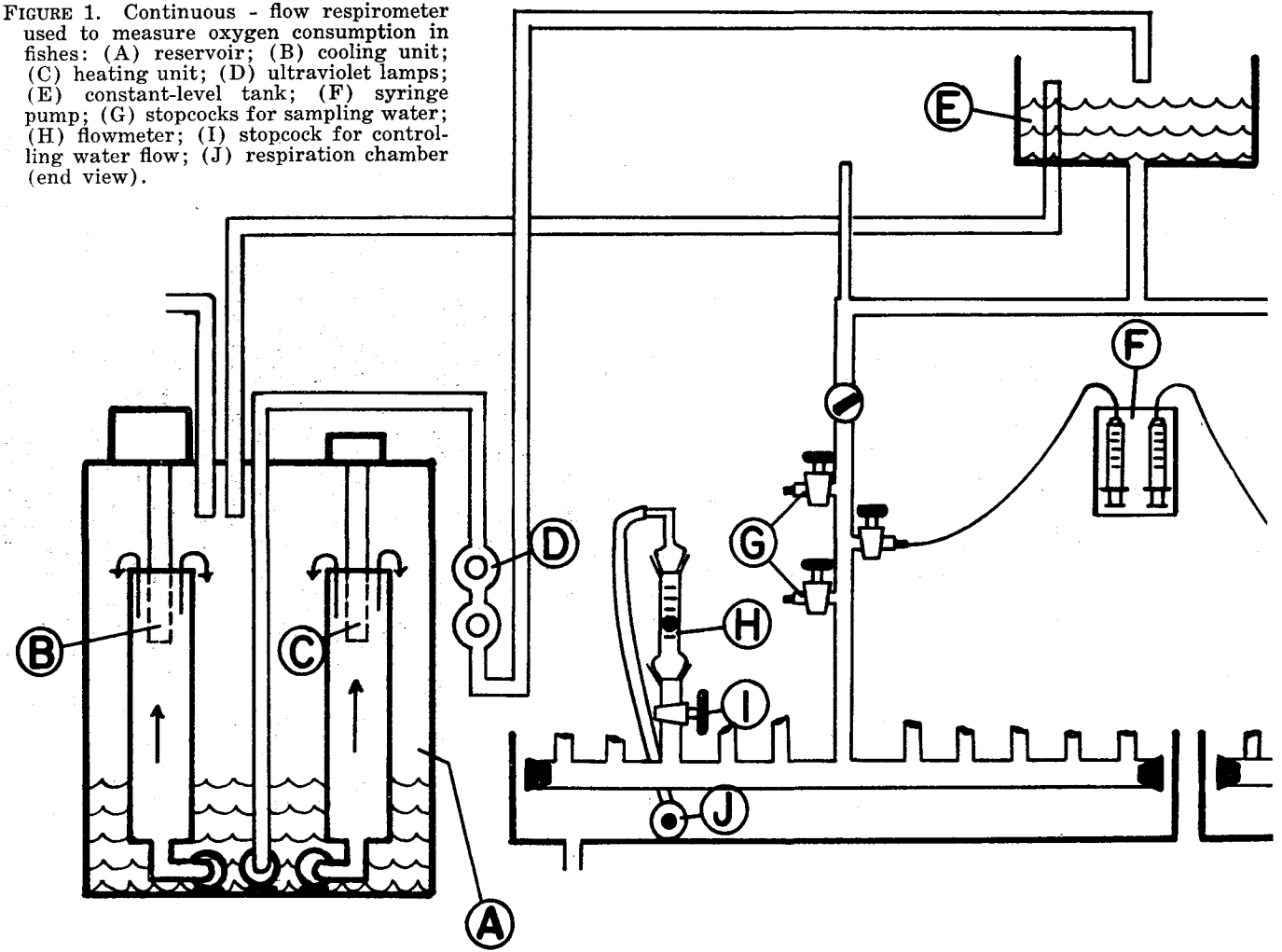
A series of tests to determine routine oxygen consumption rates (as defined by Beamish and Mookherjii, 1964) for juvenile pinfish, *Lagodon rhomboides*, was conducted before pollutants were introduced into the respirometer. Values obtained generally agreed with those of Hoss (1967) and Cameron (1969).

Water samples were analyzed by gas chromatography for pollutant content to insure that each of the ten respiration chambers was receiving the same amount of pollutant. At the calculated concentration of 100 parts per billion (ppb, micrograms per liter) of p,p'-DDT, a composite sample of water from all ten experimental chambers contained 62 ppb (not adjusted for recovery efficiency). Water samples collected simultaneously from each of the ten experimental chambers ranged from 50 to 64 ppb of DDT, and averaged 57 ppb. There was no apparent relationship between location of a chamber and DDT content in the water.

DISCUSSION

The value of my respirometer lies not in its uniqueness but in its application. Flowing water respirometers have long been used to determine oxygen consumption rates of fishes (Keys, 1930; Moss and Scott, 1961; Hoss, 1967; Hill and Potter, 1970) and also to measure the effect of pesticides on oxygen consumption of fresh water fishes (Mount, 1962; Cairns and Scheier, 1964; Huner, Dowden, and Bennett, 1967). However, to my knowledge, no worker has attempted to study the effect of pesticides and related pollutants (such as the polychlorinated biphenyls) on oxygen consumption of estuarine fishes. Indeed, little work has been done on routine respiration rates of estuarine or marine fishes.

FIGURE 1. Continuous - flow respirometer used to measure oxygen consumption in fishes: (A) reservoir; (B) cooling unit; (C) heating unit; (D) ultraviolet lamps; (E) constant-level tank; (F) syringe pump; (G) stopcocks for sampling water; (H) flowmeter; (I) stopcock for controlling water flow; (J) respiration chamber (end view).



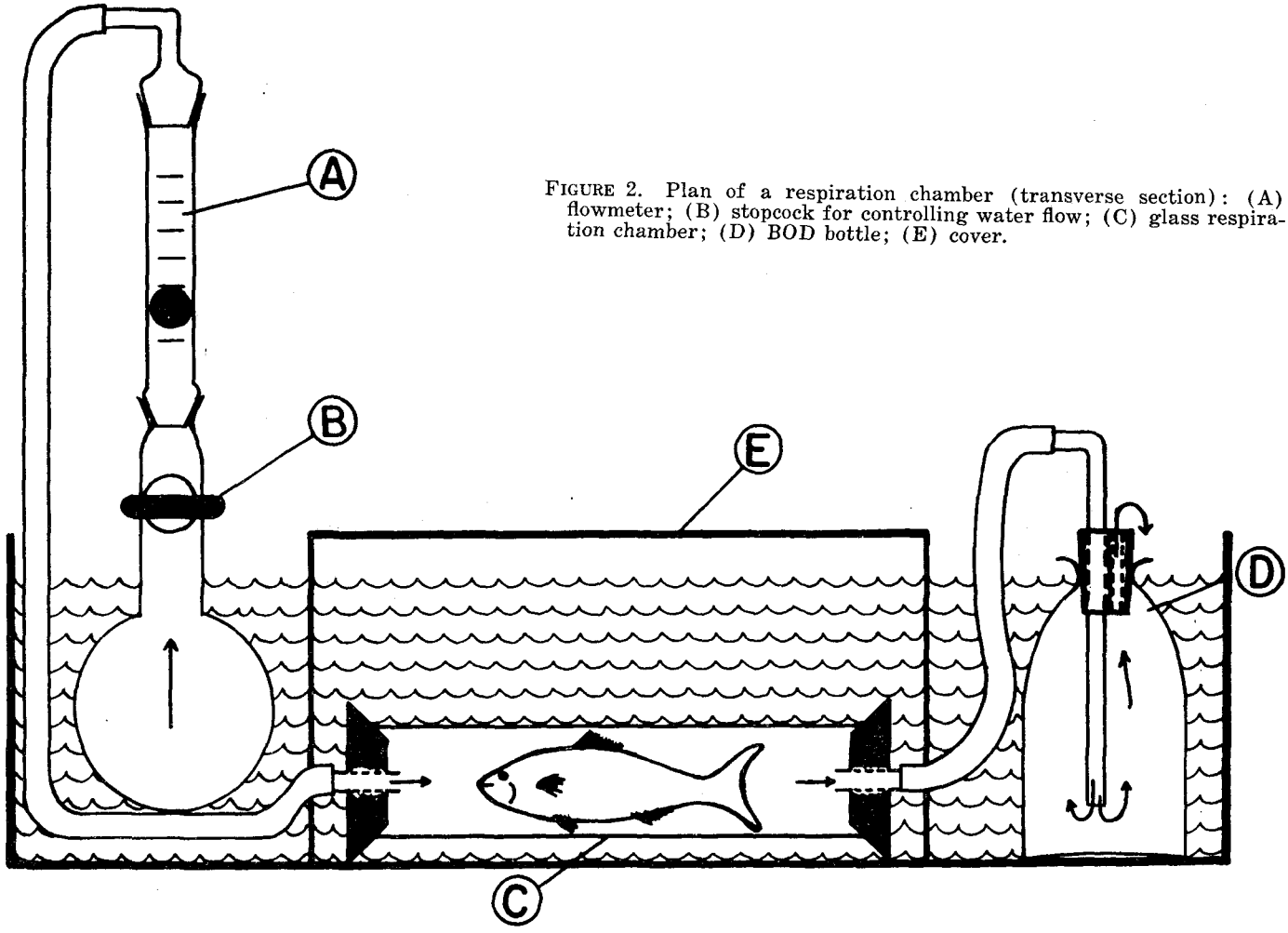


FIGURE 2. Plan of a respiration chamber (transverse section): (A) flowmeter; (B) stopcock for controlling water flow; (C) glass respiration chamber; (D) BOD bottle; (E) cover.

My respirometer is versatile. Fishes of various sizes can be exposed to pollutants at a desired temperature and salinity. Further, fishes can be exposed to pollutants within the respirometer or tests may be conducted on fishes that have been exposed in either field or laboratory. Since parts of the respirometer likely to contact pollutants are made of glass or teflon for efficiency in cleaning, routine oxygen consumption measurements can be made in the absence of pollutants.

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