

A fluvium with controlled water quality for preference—avoidance experiments with fish and invertebrates

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Abstract

The effect of environmental variables on movement of aquatic organisms can be examined using preference-avoidance behaviors. Fluviums have been used to examine such preference-avoidance behaviors in fish, including choices associated with various water quality variables. The sharp gradient in fluviums provides aquatic organisms with a choice of (usually) two water qualities, sharply demarcated at a relatively narrow boundary zone. A detailed description of a recently constructed and up-dated fluvium, however, is missing from the literature. The fluvium described in this paper integrates hydrodynamic design and extensive control of water quality to provide consistent experimental conditions, while at the same time being affordable. The system described was designed and used to examine acid avoidance behavior in juvenile fish and prawns, as part of a larger study on migration barriers in aquatic environments. An assessment of the fluvium with juvenile snapper, *Pagrus auratus*, showed that the system worked as desired. The design described here, however, can be used or modified to study behavioral responses of aquatic biota to a wide variety of environmental variables, including habitat quality or combinations of two or more environmental variables.

Sustainable management of aquatic ecosystems includes maintaining adequate water quality for aquatic fauna. Inadequate water quality may elicit a response in aquatic fauna, such as fish and mobile invertebrates, including preference, physiological adjustment, or avoidance. For example, discharges of inadequate water quality may create barriers to movement, potentially affecting migration of fish and invertebrate species. If adults avoid such discharges, spawning migrations may be affected, whereas migrations to nursery habitats may be affected if juveniles avoid such discharges. As a result, the capacity of habitats beyond the discharge point to act as spawning or nursery areas may be reduced, with potential effects on population genetics as well as stock size.

Behavioral research into the role of chemoreception in fish has expanded to include potential environmental stressors, such as temperature (Richardson et al. 1994), acidification (Jones et al. 1985a, 1985b; Peterson et al. 1988), turbidity (Cyrus and Blaber

1987; Boubée et al. 1997), and aquatic pollutants (Brown et al. 1981). This work has built on the knowledge and expertise gained from behavioral research on individual recognition (Olsén 1989; Olsén and Winberg 1996), schooling (Keenleyside 1955), homing in salmonids (Cooper and Hirsch 1981; Erkinaro et al. 1999), reproductive behavior (Liley and Stacey 1983; Olsén et al. 1998), and alarm substance (Pfeiffer 1981).

Fluviums, in particular, have been used to examine preference-avoidance behavior in fish. The sharp gradient in fluviums provides aquatic organisms with a choice of (usually) two water qualities, sharply demarcated at a relatively narrow boundary zone. Behavioral responses examined in fluvium studies have included choices associated with pH (Davies 1991; Åtland and Barlaup 1996; Åtland 1998; Kroon 2003), waste discharge (Smith and Bailey 1990), pheromones (Bjerselius et al. 1995), and chemical cues released by siblings (Olsén and Höglund 1985; Olsén 1986, 1989; Olsén and Winberg 1996).

The fluvium described in this paper, as most fluviums used to date, was modified from the design of Höglund (1961). A detailed description of a more recently constructed and updated fluvium, however, is missing from the literature. The fluvium described in this paper integrates hydrodynamic design and extensive control of water quality to provide consistent experimental conditions, while at the same time being affordable. The system described was designed and used to examine acid avoidance behavior in juvenile fish and prawns (Kroon 2003), as part of a larger study on migration

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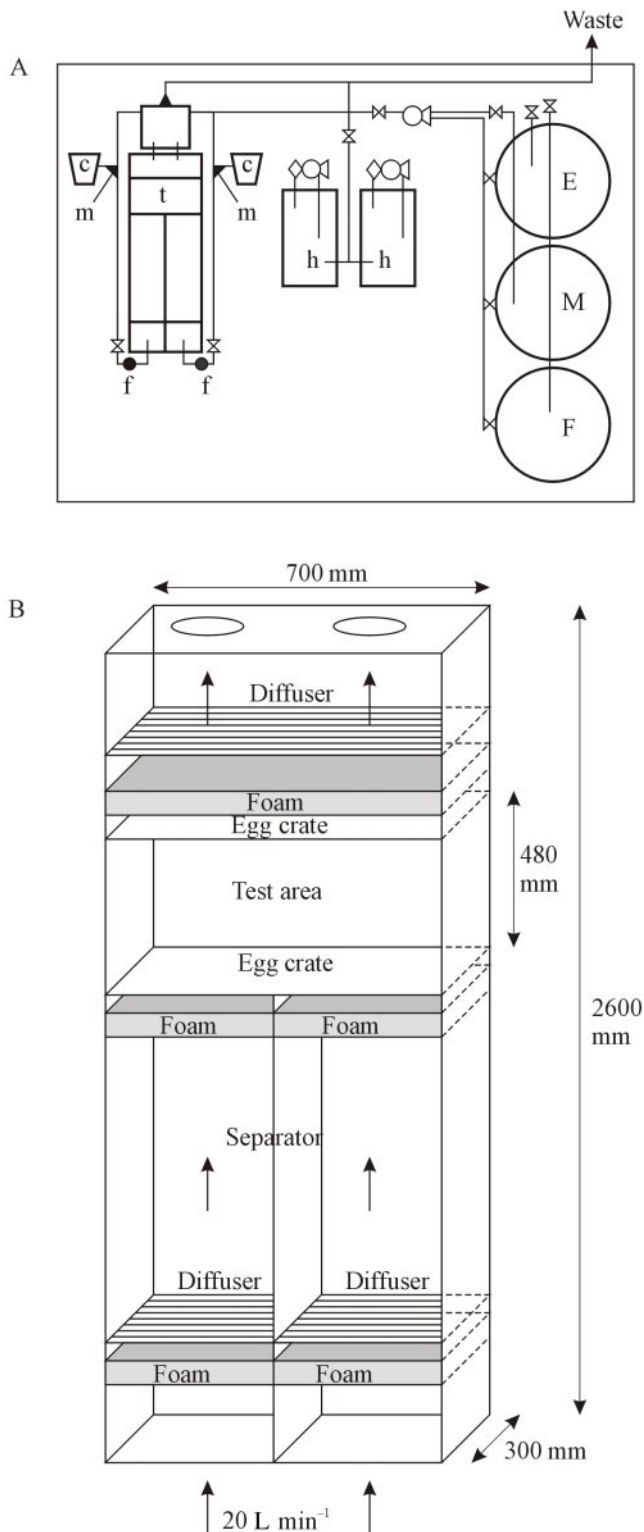


Fig. 1. Fluviarium. (A) General layout of the facility and (B) detailed outline of the fluvium. Abbreviations: c = container with acid solution or water; f = flowmeters; h = holding tanks; m = Mazzei injectors; t = test area; E = estuarine water input; F = freshwater input; M = mixing tank. Dimensions are in mm, and arrows in B indicate direction of flow. See text for detailed description.

barriers in aquatic environments. However, the design can be used or modified to study a wide variety of preference-avoidance responses in fish and invertebrates, including choices associated with substances other than sulphuric acid.

Materials and Procedures

Design considerations of fluvium—Design of the system was based upon the constraint that limited amounts of fresh and salt water (i.e., maximum of 4000 L day⁻¹) would be available for this study. Furthermore, the combination of materials and equipment used to construct the system was selected on the basis of minimum cost.

The fluvium was modified from the design of Höglund (1961) and Olsén and Höglund (1985) and is detailed in Fig. 1A and 1B. The entire fluvium was constructed of fiberglass (inner dimensions: 2600 mm length × 700 mm width × 300 mm height), and consisted of two channels with a test area (inner dimensions: 480 mm length × 700 mm width × 300 mm height) (Fig. 1B). To provide the strongest possible contrast with the experimental animals, the inside of the fluvium was coated in white gelcoat. In addition, two baffles consisting of 800 μm nitrile mesh on white plastic egg crate (a type of diffuser for suspended ceiling lighting) with a cell configuration of 10 × 10 mm were placed upstream and downstream from the test area (Fig. 1B).

To establish and maintain a laminar flow in the system, we included three diffusers, one separator, and five baffles (Fig. 1B). The diffusers and separator were constructed of fiberglass and coated with white gelcoat. Two diffusers were placed at the upstream end, and one diffuser was placed at the downstream end of the fluvium (Fig. 1B). The separator started at the upstream end and was placed exactly in the middle of the fluvium (Fig. 1B). Baffles consisting of foam blocks (low density) were placed upstream from the two diffusers (two blocks, 350 mm length × 200 mm height × approximately 100 mm width), as well as upstream (two blocks, 350 mm length × 200 mm height × approximately 100 mm width) and downstream (one block, 710 mm length × 200 mm height × approximately 100 mm width) from the test area (Fig. 1B). All baffles were held in place by compression against the sidewalls and by a bar from above. This was sufficient to allow foam of this porosity to resist a flow of 20 L min⁻¹.

The fluvium was fed from either one of three, 4000-L polypropylene round feeder tanks (Fig. 1A); one for estuarine water from Port Stephens, one for fresh water from a local bore, and one for mixing to achieve a desired salinity. Each feeder tank had air stones for continuous aeration to ensure the test water was well mixed and to minimize CO₂ concentration in the test water. The aims of the latter were to obtain stable pH levels during experiments and prevent the formation of free CO₂ during acidification of test water, as free CO₂ itself can elicit a preference-avoidance response in fish (Jones et al. 1985b). Water was pumped at approximately 20 L min⁻¹ through 260 μm filters into each of the two independent

constant-head boxes of the fluvium channels (Fig. 1B), using a centrifugal pump (Onga model 413 400 watt, 145 L min⁻¹ at 145 kPa). From here, the water flowed through the test area into the outflow pipes (Fig. 1B). The two outflow pipes established water height at 110 mm (Fig. 1B).

During experiments, animals were kept in either one of two rectangular holding tanks (400 L poly-ethylene) (Fig. 1A). Water in these tanks, whether fresh, marine, or brackish, was recycled and filtered through a vortex XL Diatomaceous earth filter (1 µm capacity). Both tanks had air stones for continuous aeration and were kept indoors in artificial illumination at natural daylight hours at ambient temperature.

The entire system was established on reinforced concrete and enclosed by a tunnel house (9000 by 6200 cm) covered in panda plastic (black inside, white outside), providing blackout conditions. The tunnel house had a second clear layer inside providing an insulating air gap. The test area was illuminated from above by two pairs of 1200-mm red color fluorescent tubes (GE 36 watt) fitted with translucent acrylic plastic, one pair at each side of the fluvium. Such diffuse illumination minimized reflections or shadows in the test area, and thus on the videotapes. Finally, to minimize potential observer effects on the animals (Martin and Bateson 1993), panda plastic was placed around the fluvium during experiments.

Precise control of water quality—The fluvium was constructed to examine acid avoidance behavior in juvenile fish and prawns (Kroon 2003). To this effect, sulfuric acid (98% tech grade; Allied Pacific Specialty Chemicals, Parramatta) was added to the water to regulate levels of acidity. Approximately 2 L of H₂SO₄ were metered using a pump (Acemite Lubrequip) from a 200-L drum into Winchester bottles. Using a bottle-top dispenser, mixed predetermined volumes of H₂SO₄ were mixed with 20 L water to obtain precise levels of acidity. Apparatus for constant pH control in the fluvium consisted of two Mazzei injectors (Model 584 from PPS), one attached to either side of the continuous-flow dilution system (Fig. 1A), with an uptake of 20 L 70 min⁻¹ of pH-adjusted water. To confirm a drop in acidity, acidity profiles of water flowing through the test area were obtained by measuring pH in the experimental lateral half of the intake area every 30 s. The acidity profile was measured until it stabilized, generally 5 min after the Mazzei injectors were turned on. Subsequently, the pH of the untreated channel was also checked.

Behavioral observations using video—The behavioral response of juveniles in the system described here was noted as location (Olsén and Höglund 1985; Peterson et al. 1988; Bjerselius et al. 1995; Newman and Dolloff 1995; Olsén and Winberg 1996) but could also include duration and activity (Olsén and Höglund 1985; Smith and Bailey 1990; Winberg et al. 1993; Åtland and Barlaup 1996; Åtland 1998). The positions of the individual juveniles were recorded continuously with a digital video camera (Sony Digital Handycam, DCR-TRV 520E PAL) on digital videocassette (Sony Digital Recording 8-mm Videocassette) using Infra-Red Images mode. The video camera was

positioned approximately 150 cm above the test area, ensuring that it covered the whole area. Subsequently, the image analyses program "Scion 4.02 Beta release" was used to enhance the infrared images.

Standard operating procedures—In control runs, untreated water was added to both lateral halves of the fluvium. In experimental runs, untreated water was added to one lateral half, whereas a test solution (e.g., acidified water) was added to the supply line of the other half. The experimental and control channel were alternated randomly to negate any bias fish may have for either side.

Before starting a run, a container with acid solution (experimental run) or water (control run) was prepared and placed under one of the Mazzei injectors (Fig. 1A). The fluvium was then filled with water (i.e., not acidified), using the recycling mode, ensuring that the two flow meters (Dwyer, VFC-152; 0–40 L min⁻¹) (Fig. 1A) were set correctly for laminar flow. A flow of 20 L min⁻¹ resulted in a laminar flow. The video camera was positioned correctly and the pH probe was set up in the experimental channel (i.e., channel that was going to receive acidified water).

Ten naïve juvenile fish or prawns were gently transferred from one of the holding tanks into the test area. They were left to settle for at least 15 min to establish themselves in the test area. Panda plastic around the test area was closed to prevent visual disturbance from affecting fish behavior. Time of introduction of juveniles was noted, and video recording was started remotely.

Fifteen minutes after introduction, a 70-min run was started by changing from recycling mode to flow-through mode and by turning on the Mazzei injectors. This resulted in acid solution (experimental run) or water (control run) being added to the water entering the test area via the experimental channel. The drop in acidity was confirmed as described above. Subsequently, the run continued without anyone present in the tunnel house to minimize potential observer effects on the animals (Martin and Bateson 1993).

At the end of each run, all 10 animals were collected from the test area and returned to the holding facilities. To ensure that individuals were tested only once, used animals were kept in a separate holding tank. The fluvium and foam blocks were rinsed and cleaned thoroughly between experiments.

Behavioral response of juveniles—The behavioral response of juveniles to two different water qualities (i.e., pH levels) was assessed as follows. One image was grabbed every minute using the frame grabber Scion LG3, totaling 60 images or frames per run. To ensure juveniles had sufficient time to show a preference, and thus prevent a mean biased over time, the first frame was grabbed 10 min after a run had started. For each frame, the position of each juvenile (i.e., position of the snout) was determined visually, and the number of individuals in each lateral half of the test area was counted. This procedure was repeated for all 60 images, and the total number of individuals in each lateral half of the test area over all 60

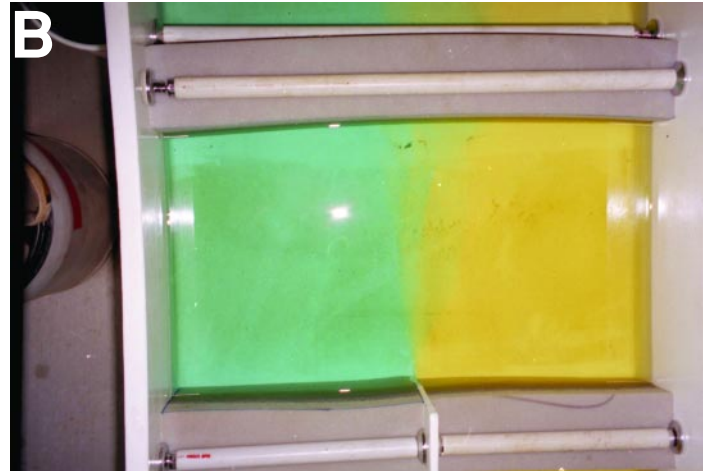
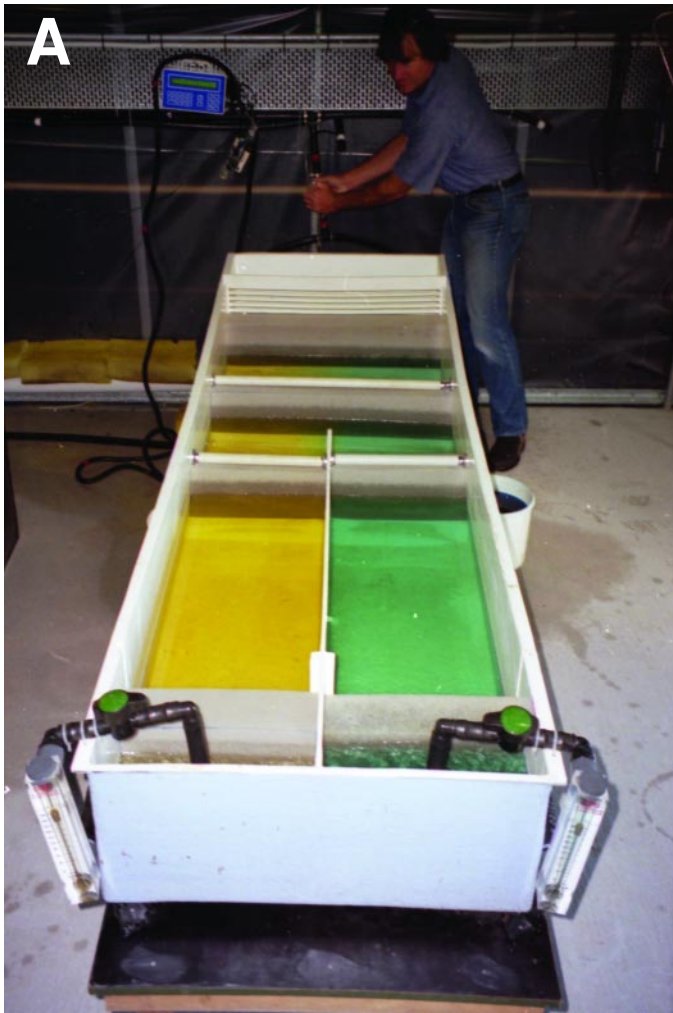


Fig. 2. Fluviarium. (A) Testing the stability and sharpness of demarcation of the two water masses in the fluviarium using dye tracers (food coloring) and (B) detail showing the demarcation within the test area.

images was calculated. Subsequently, the mean percentage of juveniles in each lateral half of the test area was determined for all 60 frames combined. This provided us with the mean proportion of juveniles in the experimental half of the test area over a 60-min run. This mean proportion was considered the behavioral response of the juveniles in that particular run and was entered as a single replicate in the data analysis. In control runs, a flip of a coin determined the “experimental half” of the test area.

Assessment of the fluviarium—To determine the stability and sharpness of demarcation of the two water masses within the test area, dye tracers (food coloring) were used (Fig. 2A). Water intake was adjusted to achieve proper demarcation. A flow of 20 L min^{-1} , measured with the two flow meters (Fig. 1A), resulted in a laminar flow with stable and sharp demarcation (Fig. 2B). Dye tracer tests were repeated on a regular basis to ensure proper demarcation of the two water masses across time.

To examine whether animals would distribute themselves evenly in the test area under control conditions, the system

was tested as follows. Juvenile snapper (*Pagrus auratus*, 20 to 40 mm SL) were obtained from the Aquaculture Marine Hatchery facilities at the Port Stephens Fisheries Centre in July and August 2001. Four control runs were conducted according to the standard operating procedures described above. The results revealed that the behavioral response of juvenile snapper, i.e., the mean proportion of juveniles in the experimental half of the test area over a 60-min run (mean $49.95\% \pm 4.90 \text{ SE}$, $n = 4$), was not significantly different from the expected distribution (mean 50.00%). These results showed that the system worked as desired and could be used to study preference-avoidance responses in fish and mobile invertebrates.

Discussion

The fluviarium described in this paper offers an affordable and working system to study preference-avoidance behaviors of aquatic biota. The system provides consistent experimental conditions through the ability to control in detail the acidity levels

in the experimental channel. Using this system, such extensive control can and has been achieved with other water quality variables, such as levels of salinity (James Knight pers. comm. 2002).

An important feature of this design includes fish being continuously exposed to both water qualities without getting trapped in either of the two channels. This ensured that fish were able to continuously choose between the two water qualities. Consequently, the behaviors observed were true reflections of preference-avoidance responses. In some studies on preference-avoidance behaviors, fish were given a one-off choice between two different water qualities after which they became trapped in either one of the channels (Rehnberg and Schreck 1987; Newman and Dolloff 1995; Boubée et al. 1997). Changes in behavior are not possible in such a set up, and results may not necessarily reflect true preference-avoidance behavior. This is of particular concern when schools of individuals are tested (Rehnberg and Schreck 1987; Newman and Dolloff 1995; Boubée et al. 1997), and the behavior of individuals is not independent of behavior of other individuals in the school.

Proper experimental design and data analyses are critical aspects of measuring behavior (Martin and Bateson 1993), including examining behavioral responses in two-choice maze studies. Experimental design requires careful consideration and should take into account the use of controls, as well as order effects and interactions (Martin and Bateson 1993). Our experiments were designed to examine acid avoidance behavior in juvenile fish and prawns (Kroon 2003). To this effect, we compared the behavioral response of juveniles in experimental runs to that in control runs, and we used individuals only once to avoid potential order effects. Interaction effects were not an issue in our experiments as the effect of only one water quality component (i.e., pH) was examined. However, if the effect of more than one variable is to be examined simultaneously (e.g., effect of temperature, salinity, and pH combined), each combination of variables must be represented in the experimental design. This may provide constraints on the feasibility of such a study as the number of runs that need to be conducted to obtain sufficient statistical power would increase exponentially.

Correct data analyses assume that individual data points are statistically independent of one another. This assumption of independence is often violated in behavioral research, including two-choice maze studies. A common error is to take multiple records of an individual animal and treat them as if they were independent (so-called "pooling fallacy"; Machlis et al. 1985). Rather, multiple records on the same subject should be averaged to obtain an individual data point for that subject. In our experiments, we averaged repeated measurements (i.e., 60 video frames) on the same subjects within a run to obtain a single replicate for that run for data analysis. Thus, our sample size was equal to the number of runs conducted and not the number of video frames grabbed.

In fluviariums, the behavioral response of aquatic organisms is generally examined in individuals (Olsén and Höglund

1985; Olsén 1986, 1989; Bjerselius et al. 1995; Olsén and Winberg 1996), but has included examination of schools of up to 22 individuals (Richardson et al. 1994). Using individuals may not necessarily be a good reflection of the situation in the field, but provides data on completely independent individuals. On the other hand, using schools means that the behavior of one individual is not independent of that of other individuals. In our experiments, the use of groups of 10 juveniles as opposed to individual juveniles was thought to be more appropriate, because juveniles generally move in schools. The assumption made was that if individual runs were conducted and analyzed over an appreciable time (i.e., 60 min), the average equilibrium behavior of the 10 individuals in the school would reflect the properties of the run and would not be affected by the initial state of the run. Our results from the four control runs conducted with juvenile snapper show that this was a reasonable assumption.

Comments and recommendations

The system described here can be used or modified to study behavioral responses of aquatic biota to a wide variety of environmental variables, including habitat quality or combinations of two or more environmental variables. For example, the presence of suitable habitat may alleviate strong avoidance behavior to low acid levels as has been shown for zinc avoidance by fathead minnows (*Pimephales promelas*) (Korver and Sprague 1989). The choice of (combination of) variables is limitless but is dictated by the nature of the problem under investigation. In any choice experiment, however, it is vital to know the values of the studied variable in the field to ensure that the experiment reflects choices that may be encountered in the field.

Behavioral studies conducted with the system described can complement LD₅₀ studies. LD₅₀ trials provide valuable information regarding the physiological tolerances of species to certain substances, however, they do not provide information on a species' behavior in response to these substances. A combination of laboratory studies, including preference-avoidance experiments and LD₅₀ trials, as well as field observations on natural populations and environmental variables, will provide a solid combination of scientifically sound data for sustainable management of aquatic ecosystems.

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