Effects of depressed pH on reproduction, growth and survival of flagfish, *Jordanella floridae* (Goode and Bean).

by

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ABSTRACT

Breeding communities of flagfish (*Jordanella floridae*) were exposed to adjusted pH levels of 6.0, 5.5, 5.0, 4.5 and 4.0 for 21 days. Control water (pH 7.5) received no acid treatment. Egg production was significantly reduced ($p<.05$) at pH 6.0 relative to control, and a termination of spawning occurred below this test level. Control eggs were incubated at test levels where spawning had not occurred. Hatching success was impaired at pH 4.5 with a significant decrease ($p<.01$) at pH 4.0. Hatching time was variable but no significant relationship was found between hatching time and hydrogen ion activity.

Fry mortality was significantly increased (p .01) with decreasing pH between treatment groups pH 6.0, 5.5 and 5.0. All fry died within 72 hours at pH 4.5 and no fry survived the first 24 hours at pH 4.0. The final mean weights and lengths of the fry were significantly reduced ($p<.01$) at pH 5.5 relative to control.

Spawning resumed within three days following the return of the pH 4.5 test levels to control conditions. A histological examination of the ovaries from the control and pH 5.5 females revealed no obvious differences.

Results of the present study concur with other reproductive investigations of flagfish, brook trout and fathead minnows indicating a pH decline below 6.5 may result in reproductive impairment.

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INTRODUCTION

Bodies of water within the pH range 6.5 to 8.5 harbor the most successful fish populations (Welch, 1952). Field studies of certain waterways however, have revealed an accelerated decline in pH accompanied by a decrease in the populations of various aquatic organisms (Sprules, 1975; Beamish, 1974a; Hagen and Langeland, 1973; etc.). These observations have prompted a full scale investigation into the possible acid sources and their long term effects.

It has been demonstrated that a wide variety of industrial activities are responsible for the accelerated pH decline found in many bodies of water around the world.

Decreased recruitment of young fish into a population has been cited as the primary factor leading to the gradual extinction of fish populations in acidified waters. Failing recruitment has been attributed to the inability of females to attain spawning conditions (Beamish and Harvey, 1972) and to a selective lethal effect on the egg and fry stages (Jensen and Snekvik, 1972).

The present study was conducted to determine the site of impairment in the reproductive cycle of flagfish (*Jordanella floridae*) and establish the relationship between decreasing pH and the concurrent decline in fish populations.

LITERATURE REVIEW

Acidification of natural waters

Natural acidification

Natural waters obtain vast quantities of chemicals from their contact with the various types of soils; some are acquired through runoff and others from the substrate. Owing to the great variety of substances which makes contact with water, numerous compounds may be present which contribute ionized hydrogen.

Some inorganic contributors are: the hydrolysis of multivalent metal ions (Welch, 1952); bacterial reduction of sulfate to H_2S (Dugan, 1972); bacterial oxidation of H_2S to H_2SO_4 (Reid, 1961); and, oxidation of pyrites found in both clay and coal-bearing strata (Ruttner, 1963). Another important source is sphagnum, which is known to accumulate sulfur from the atmosphere and release it as sulfuric acid (Reid, 1961). Volcanoes and certain hot springs may yield acidic waters by adding gases such as HCl and SO_2 (Ruttner, 1963).

There are also numerous organic sources which contribute to the acidification of water. Anaerobic decomposition of organic material yields methane gas which can undergo oxidation and contribute ionized hydrogen. The ability of carbon dioxide (CO_2) to combine with water (H_2O) to form carbonic acid (H_2CO_3) (Reid, 1961), makes it one of the most important acidic constituents in natural waters (Stumm, 1970). Bio-

logical activities such as photosynthesis, respiration and bacterial decomposition, as well as the physical occurrences of turbulence and ground seepage, influence pH through their respective abilities to decrease or increase the concentration of dissolved CO_2. Extremely acidic conditions are not generated by organic matter however, as carbonic acid is predominantly unionized below a pH of about 6.4 (Barnes and Romberger, 1968).

Acidification by industrial wastes

A variety of acids are constituents of many industrial effluents (Doudoroff and Katz, 1950) and in many instances are discharged directly into adjacent waterways.

Coal mining activities exposing pyrite and marcasite (FeS_2) have also resulted in the acidification of many of the natural waterways. Iron pyrite present in the coal-bearing rock, oxidizes in the presence of oxygen and water to produce ferrous sulfate ($FeSO_4$) and sulfuric acid. Further oxidation produces ferric hydroxide [$Fe(OH)_3$] and liberated hydrogen (Barnes and Romberger, 1968). Other reaction mechanisms resulting in the production of ionized hydrogen have been described by Hanna (1963) and Barnes and Clark (1964). Consequently, rainfall and the resultant ground seepage that comes in contact with the exposed coal, becomes highly acidified and eventually incorporated into the neighboring waterways. This rapid acidification has resulted in a significant reduction in aquatic flora and fauna in many bodies of water

and a complete obliteration in others (Jewell, 1922; Lackey, 1939; Parsons, 1952; Carrithers and Bulow, 1973; Nichols and Bulow, 1973).

Inasmuch as atmospheric contaminants have been shown to be transported in substantial amounts over distances of 1000 to 2000 km (Overrein, 1976), the focus of attention has been directed to the sources and potential environmental hazards they represent. Precipitation in southern Norway, Sweden and Finland contains large amounts of H^+, $SO_4^=$ and NO_3^- that originate as air pollutants in the heavily industrialized areas of Great Britan and central Europe (Holt-Jensen, 1973; Hagen and Langeland, 1973; Wright *et al.*, 1976). As a result of the increase in atmospheric H_2SO_4, many lakes in southeastern Norway have a pH below 5.0 and sulfate has replaced bicarbonate (HCO_3^-) as the major anion (Wright and Gjessing, 1976). Sudbury, the worlds largest single source of atmospheric SO_2 (Summers and Whelpdale, 1976) emits to the atmosphere one million tons of sulfur per annum, chiefly as SO_2 (Gorham and Gordon, 1960). This magnitude of SO_2 emission is reflected in the surrounding watershed where many lakes have a pH of less than 4.5 (Beamish and Harvey, 1972).

Many shield lakes and those found in the southern Scandinavian countries are largely oligotrophic or dystrophic lakes with low buffering capacity (Hagen and Langeland, 1973). With the accumulation of acidic air pollutants in snow (Beamish and Harvey, 1972; Elgmork *et al.*, 1973; Hagen and Langeland, 1973; Holt-Jensen, 1973), these poorly buffered lakes undergo rapid pH declines in the surface layers during the critical spring runoff period (Hagen and Langeland, 1973; Hultburg, 1977).

Atmospheric sulfur oxides are derived principally from the combustion of fossil fuels and the smelting of minerals containing sulfur impurities. During combustion, sulfur compounds are oxidized to sulfur dioxide and very small amounts of sulfur trioxide (Revelle, 1974). The SO_2 produced in the combustion process is emitted from the stack. There is no evidence to suggest that SO_2 in the atmosphere could cause adverse affects in man at the concentrations now present in urban air (Cheng *et al.*, 1971). However, studies of atmospheric chemistry have shown that SO_2 does not remain unaltered in the atmosphere, but is converted to sulfuric acid (Cheng *et al.*, 1971).

The atmospheric oxidation of SO_2 to H_2SO_4 involves both water and dissolved oxygen (Foster, 1969). As the rate and subsequent yield of this reaction is extremely slow, it has been suggested that the atmospheric oxidation of SO_2 involves other reaction processes. The most important are: photochemical oxidation (Cox and Penkett, 1970; Gerhard and Johnstone, 1955); catalytic oxidation (Cheng *et al.*, 1971); and catalyic oxidation in the presence of ammonia (NH_3) (Junge and Ryan, 1958; Scott and Hobbs, 1967).

Although the exact mechanism of SO_2 conversion remains somewhat in question, the resultant product (sulfuric acid) and its biological implications have been fully realized. The fact that sulfur fallout can occur up to several thousand kilometers from the emission source, the biological impact not only exists near industrialized areas, but in many remote areas around the world.

Effects of depressed pH on fish

The loss of fish populations from unexploited lakes, accompanied by a corresponding decline in pH, has been demonstrated in many areas around the world (Harrison, 1958; Jensen and Snekvik, 1972; Beamish, 1974a; Nichols and Bulow, 1973; Almer *et al.*, 1974; Schofield, 1976). Attempts to determine the toxic nature of increased hydrogen ion activity in the environment reveal that several factors need be taken into consideration. The degree of toxicity at a given pH level varies from species to species (Beamish and Harvey, 1972; Almer *et al.*, 1974), within individuals of a given species (Dunson and Martin, 1973; Robinson *et al.*, 1976; Falk and Dunson, 1977), with size and maturity (Packer and Dunson, 1972; Robinson *et al.*, 1976), with water hardness, alkalinity and dissolved CO_2 (Lloyd and Jordan, 1964; Mount, 1973; Nichols and Bulow, 1973) and with water temperature (Kwain, 1975; Robinson *et al.*, 1976). There is, however, sufficient information available to allow some general conclusions to be made concerning the range of acidity that is acceptable to fishes. Based on intensive surveys (Ellis, 1936; Doudoroff and Katz, 1950; etc.), The European Inland Fisheries Advisory Commission (1969) have established that most freshwater fishes can live in waters above pH 5.0. The suitability of water close to pH 5.0 however, is questionable for the successful reproduction of the more sensitive species.

The loss of fish species from a given body of water may be attributed to one of two factors: a direct lethal effect on the existing

fish; or, the failure of young fish to be recruited into the population (Beamish, 1974a). Consequently, the effects of depressed pH on fish will be reviewed under two subtitles: 1) The effect of sublethal exposure levels, 2) The toxicity of lethal exposure levels.

1) The effect of sublethal exposure levels

Recruitment failure generally reduces the population of a given body of water before the pH reaches levels low enough to kill the larger fish. This decline is made apparent by the fewer, but larger fishes caught (Jensen and Snekvik, 1972; Hagen and Langeland, 1973). Recruitment failure has been attributed to the inability of the females to attain spawning conditions (Beamish and Harvey, 1972) and a selective lethal effect on the egg and fry stages (Jensen and Snekvik, 1972). Beamish and Harvey (1972) point out however, that at a given pH level, all species are not equally affected. The decline in numbers of the various fish species occurred at different times during their study as the water became increasingly acidic. Studies in fish hatcheries in southern Norway revealed that salmon species fail to spawn when the pH falls below 5.0. In the pH range 4.7 to 4.8, trout species also stop spawning (Holt-Jensen, 1973). Johansson and Kihlstrom (1975) suggested that it might be possible for pike species to spawn within the pH range 4.0 to 4.5.

Tolerance to acidic conditions does in fact vary from species to species (Beamish and Harvey, 1972; Almer *et al.*, 1974), but the level

of acidity within their sublethal range appears to determine the effect. At pH 5.2 the fathead minnow (*Pimephales promelas*) failed to spawn, but a gross examination revealed the females were heavy with eggs and sexually mature (Mount, 1973). Mount (1973) reported good egg production at the pH 5.9 test level, but the eggs were extremely fragile and there was a marked decrease in per cent hatch. Chronic tests conducted with brook trout (*Salvelinus fontinalis*) in acid waters are also in agreement (Menendez, 1976; Smith, 1978). Down to pH 5.0, brook trout egg production remained relatively high. The lethal effect at pH 5.0 and above was on the hatchability of eggs and on fry survival. Similar observations were made by Craig and Baksi (1977) when mature flagfish (*Jordanella floridae*) were exposed to a depressed pH gradient.

A histological examination revealed a reduction of mature ova in breeding communities of flagfish held for three weeks in depressed pH conditions (Ruby *et al.*, 1977).

Field observations revealing a reduction of fish growth in acidified waters (Campbell, 1961; Beamish, 1974b) are consistent with laboratory findings (Menendez, 1976; Smith, 1978). It was earlier thought that the reduction in fish growth was the result of a reduction in the available food supply. Menendez (1976) and Smith (1978) report that an abundant food supply was readily available to the fish at all test levels and that the reduction in growth was a result of a decrease in feeding activity. White suckers (*Catostomus commersoni*) held for several months in sublethal concentrations of acid also exhibited a reduction in feeding intensity (Beamish, 1972).

High serum calcium levels normally associated with maturing female fish (Oguri and Takada, 1967), were absent in female white suckers exposed to acid waters (Beamish, 1974a; Lockhart and Lutz, 1977). Beamish (1974a) suggested a change in the normal serum calcium of female white suckers contributed to the reproductive failure of fishes in his study lakes. Beamish (1974a) also found the occurrence of spinal deformities in the white sucker and fathead minnow populations as the pH of one test lake approached 5.0. These spinal deformities could be the result of low serum calcium levels.

2) The toxicity of lethal exposure levels

Fish mortalities may be expected below a pH of 5.0. Some species, however, have been found to live in waters with a pH below 4.0. (Nichols and Bulow, 1973).

As demonstrated in the previous section, in an increasingly acidic environment there is a corresponding decrease in the feeding activity of fish. Smith (1978) reported that the feeding activity of brook trout terminated after a short exposure to pH 4.0 and at this test level, he attributes actual death in part to starvation.

Packer and Dunson (1970) suggested that the inability of brook trout to live in waters with a pH less than 5.0 is related to a drop in blood pH (acidemia). Brook trout exposed to an environmental pH of 3.0 to 3.3, exhibited a drop in mean blood pH from 7.39 to 6.97 (Packer and Dunson, 1970). Smith (1978) however, found that following long term

exposure to pH levels as low as 4.0, brook trout were able to maintain normal blood pH levels.

Excessive sodium (Na) loss in brook trout exposed to pH 2.0 to 3.5 has also been reported (Packer and Dunson, 1970). Brook trout, placed in a saline solution (150mM NaCl), lived longer at pH 3.25, but not at pH 2.0. As a result, it was suggested that the loss of body Na is of secondary importance as the cause of death when fish are exposed to extremely acidic conditions.

Packer and Dunson (1972) suggested that the lethal effect of extremely acidic waters is an inhibition of oxygen (O_2) uptake. Potassium cyanide (KCN) treatment which results in anoxia, occurred in about the same time as death caused by exposure to very low pH. A common explanation of fish death at low pH has been suffocation due to coagulation of mucus on the gills (Westfall, 1945). There are however, other explanations which could account for anoxia. Acidemia, which has been demonstrated in brook trout exposed to very acidic waters (Packer and Dunson, 1970), would decrease the O_2 carrying capacity of the blood and consequently the O_2 uptake. As anoxia and sodium loss have been linked together (two primary exchange elements at the gill epithelium), simple destruction of gill epithelium at these extremely corrosive pH levels may possibly be an alternative explanation.

It has been suggested by Doudoroff and Katz (1950) that the increased CO_2 tension and not the increased hydrogen ion activity is the lethal factor involved when fish are exposed to these extremely low pH levels.

MATERIALS AND METHODS

Methods developed at the Environmental Research Laboratory - Duluth (Duluth, Minnesota), were applied for chronic testing with the flagfish.

Physical and chemical systems

The test facility comprised a header tank, a diluter, a toxicant delivery system and 24 holding tanks.

De-chlorinated water was delivered under pressure to a 340 liter polypropylene header tank (Fig. 1), which was located directly above the diluter. This arrangement provided a gravity flow of water from the header tank to level I of the diluter. Diluter filling and the subsequent refilling of the header tank were controlled by a one half inch diameter solenoid valve and a one inch diameter solenoid valve respectively. These solenoids were in turn controlled by a five minute, electrical timer.

Located within the header tank was a thermostatically controlled, 4.5kw stainless steel immersion heater. With only one twentieth of the header tank volume released each cycle, the heater was capable of providing a 10°C boost in water temperature. Two, 30 cm airstones were located in the header tank to maintain a high level of dissolved oxygen.

The "equal volume" diluter employed in this study has been de-

scribed by DeFoe (1975). The diluter consisted of three levels (Fig. 2). Level I metered out two liters of water into each of six (I. D. 10cm X 10cm X 25cm high) cells with the excess water spilling into a seventh cell. The seventh and only cell of level I to "fire" at this time, flooded the siphon initiator cell of level II. The float switch in the siphon initiator cell which was tripped by the initial flooding of the cell and the subsequent "firing" of the cell, triggered the pneumatic injector system. The "firing" of the siphon initiator cell also generated a vacuum in the vacuum manifold (venturi vacuum Fig. 2), resulting in the simultaneous delivery of water from the remaining six cells of level I to corresponding cells of level II.

The water delivered from level I to level II and the water acid mix delivered from level II to level III was accomplished via standpipe siphons. The siphons consisted of an inner 10mm O. D. downpipe and an outer 18mm O. D. glass tube. Level three provided a secondary mix of the acid and water and delivered an equal volume (500ml) to each of the duplicate adult and progeny test tanks via four, 6mm O. D. gooseneck siphons located in each of the six cells. This system provided a 90% replacement in each test tank in approximately 12 hours.

The pneumatic injector system employed to deliver the sulfuric acid has been described by Smith *et al.*, (1977). Each syringe was calibrated to deliver a given volume of sulfuric acid in each cycle, maintaining the prescribed pH levels in the test tanks. No acid was delivered to the control test tanks. The exhaust phase of each acid delivery permitted each syringe to refill from a 60 liter polypropylene reser-

voir containing 0.1N sulfuric acid. The reservoir was filled every third day.

The test tanks consisted of a duplicate set of six spawning tanks and a corresponding set of progeny tanks. Figure 3 illustrates the random assignment of test tanks, with duplicates arranged on either side of the diluter. Spawning and progeny tanks were the same size, approximately 55cm X 30cm X 25cm high, with a water volume of 30 liters.

Each spawning tank could be divided in half by a removable glass partition, with the inflow and outflow pipes located on opposite sides. A centrally located, 7.5cm opening covered by No. 20 mesh stainless steel screen allowed water circulation from compartment to compartment when the partitions were in place. The progeny tanks, located beneath the corresponding spawning tanks, were divided by a similar glass partition.

In order to maintain test tank temperatures at 26°C, each test tank was equipped with a 100w immersible heater. In addition, a small airstone was placed in each test tank.

The laboratory had ceiling fixtures, each containing an equal combination of Sylvania Gro-lux and General Electric Chroma 50 fluorescent tubes. Two, 1.5m long fixtures were placed above each bank of spawning tanks and fitted with General Electric Chroma 50 fluorescent tubes. All lights were controlled by a Tork timer. In addition, a light circuit with three incandescent bulbs was connected to a seperate Tork timer used to simulate dawn and dusk. The regulated length of the photoperiod was 16 hours of "daylight" and eight hours of darkness and was

maintained throughout the experiment.

Temperature, dissolved oxygen, and pH were recorded daily, Dissolved oxygen readings were taken with a Y. S. I. #54A dissolved oxygen meter.

A radiometer pH electrode (#GK2301C) was located in each of the spawning test tanks. The six electrodes (alternating from bank to bank weekly) were connected to a Bach-Simpson SAS1 switching unit. A Radiometer pH M63 pH meter, connected to the switching unit provided a scan of all test levels every three minutes. This allowed for a compensating adjustment on the injector as soon as a deviation occurred at any test level.

Monthly water samples were collected from each test tank and analysed for hardness, alkalinity, chloride, pH, conductivity, sodium, potassium, calcium, magnesium and sulfate by the Thunder Bay Regional Laboratory of the Onatrio Ministry of the Environment.

The spawning substrates were constructed of green orlon yarn which was pre-boiled to remove excess dye. The yarn was tightly wrapped in parallel strands on a 10cm X 15cm "V" shaped stainless steel frame.

Egg incubation cups were constructed from 120ml, 5cm O. D., round glass jars with the bottoms removed. Stainless steel screen (No. 40 mesh) was fastened to the bottom with silicone seal. The centers of the bakelite caps were removed with similar screening attached over the opening. A stainless steel hook was fastened to each cap, providing a point of attachment for the S-shaped stainless steel rod which was in turn attached to a "swivel snap" on the rocker arm assembly.

The rocker arm assembly (Fig. 4) consisted of 12.7mm aluminum rod which served as the main shaft and was suspended above each bank of progeny test tanks. Collars were machined from 25.4mm aluminum rod with the inside diameter slightly larger than 12.7mm. This allowed the collars to rotate freely on the shaft. Each collar contained a set screw allowing it to be fixed in desired lateral and vertical positions. Two, 6.4mm aluminum rods, each with two points of attachment for egg cups, were press fitted into each collar. The egg cups were attached to the rods by swivel snaps. This arrangement allowed a maximum of 16 cups to be suspended in each progeny tank. This unit was powered by a five rpm electric motor with cam, connected to the main shaft by a two piece linkage system.

Fry retainers were constructed from winchester acid bottles. The bottoms and tops were removed leaving a 13cm high center section. No. 40 mesh stainless steel screen was attached to the bottom. The same screen was wrapped around the top of the fry retainer to extend the sidewall height an additional 3cm. Three glass vials, 6cm in height, were fastened to the bottom of each fry retainer which extended the top of the screen above the surface of the water. All parts were attached by means of silicone seal.

Biological system

Fifty males and 130 female flagfish were obtained from the Environ-

mental Research Laboratory - Duluth, Duluth, Minnesota. The males were initially kept seperate from the females. Following a one week acclimation period, the males and females were randomly united and distributed among the duplicate test tanks.

Throughout the experiment, the fish were fed on a diet of newly hatched brine shrimp (*Artemia salina*) and frozen adult brine shrimp, five times daily. All tanks received equal rations. Excess food and debris were siphoned from the tanks each morning.

Following a one month growth and maturity period, the fish were selectively culled (ie. similar size fish of both sexes were retained) to a ratio of five females to two males per test tank. In the event of any problem with the selected breeding stock, excess fish were distributed and maintained in two, 75 liter static tanks. At this time the spawning substrates were placed in each tank.

The substrates were removed and examined for eggs each afternoon. Clean substrates were returned to each tank immediately following removal. Following an initial period of erratic spawning, the substrates were removed from all test tanks, then replaced one week later to achieve synchronous spawning in all test tanks.

To facilitate egg removal, substrates were transferred to a shallow glass aquarium (12cm X 38cm X 13cm high), containing water from their respective test tanks. Eggs were seperated from the substrate by a scraping motion of the hand. The eggs were pipetted into a petri dish, examined under a microscope and unfertile or abnormal eggs were removed. A maximum of 50 fertile eggs from each spawn were placed in individual

egg cups and immersed in a solution of malachite green (4mg/l) for five minutes. The egg cups were then attached to the rocker arm assembly in their respective progeny tanks. Until hatching, all egg cups were treated as above on a daily basis. To obtain additional incubation data, excess eggs from control and pH 6.0 test levels were transferred and allowed to hatch at lower pH levels where spawning had terminated.

Until synchronous spawning was attained in all test tanks, and for an additional eight days, untreated "control" (pH 7.5 to 8.0) water was delivered to all test levels. Following this eight day (*predepression period*) spawning period, sulfuric acid stock (0.1N) was dispensed by the pneumatic injector system to each test level. The pH was depressed linearly over the following five days (*depression period*) to the prescribed pH levels 6.0, 5.5, 5.0, 4.5 and 4.0. These pH values were maintained for the remainder of the experimental period (*depressed period*). Spawning was allowed to continue for 21 days at the prescribed pH levels. Spawning was then terminated by removal of the substrates.

Following the 21-day acid exposure period, the females from control and pH 5.5 test levels were sacrificed. Ovaries were removed and prepared for histological examination.

To determine spawning recovery at a test level where spawning had terminated, the pH 4.5 test levels were returned to control conditions over a 24 hour period. Following the return to control conditions, the spawning substrates were replaced. Egg collection, determinations of viability and hatching success were also resumed at this test level for an additional 21-day period.

Three egg cups were retained at each test level (in duplicate) to determine growth rate and fry survival over a 31-day period. In lower treatment levels where spawning had ceased, fry were acquired from the control eggs set to hatch at these levels. The fry were maintained in the egg cups until the yolk sac was absorbed (approximately three days), at which time all fry were transferred directly to the fry retainers.

A feeding programme employing newly hatched brine shrimp and Tetramin fish starter was implemented at this time. The same diet was maintained throughout the 31-day period.

To minimize handling at this early stage, all fry were kept for 11 days, at which time the number of fry in each retainer (one per test level in duplicate) was reduced to 25. At this time, the first photographs for use in growth determinations were taken of each group of 25 fry.

The fry, with a minimum of water from their respective test level, were transferred with a pipet from their retainers to a glass container ($12cm^3$). The container was in turn placed on a grid ($5cm^2$) and the photographs taken. Immediately following the photographs, the fry were returned to their respective retainers. A second photograph was taken at each test level on day 24 and a final on day 31. Measurements of the fry were obtained from the photographs following the procedure outlined by Martin (1967). Following the final photograph, the fry were sacrificed and weighed on a Mettler Gram-Atic balance.

Differences in egg production, viability, hatching success and hatching time at different pH levels, were tested for significance by

a oneway analysis of variance. If a significant f-ratio was found, a Duncan multiple range test (Duncan, 1955) was employed to determine which pH levels yielded significantly different results at the 0.01 and 0.05 test levels. A post-hoc decision was made to include only data where the total spawn exceeded 19 for the analysis of viability, hatching success and hatch time. Fry growth rate was also subjected to analysis of variance. Control and pH 6.0 egg transfers, as well as the data obtained when the pH 4.5 test levels were returned to control conditions, were tested for significance by independent t-distribution. Fry survival was subjected to Chi-square analysis.

Figure 1. Cross sectional view of header tank.

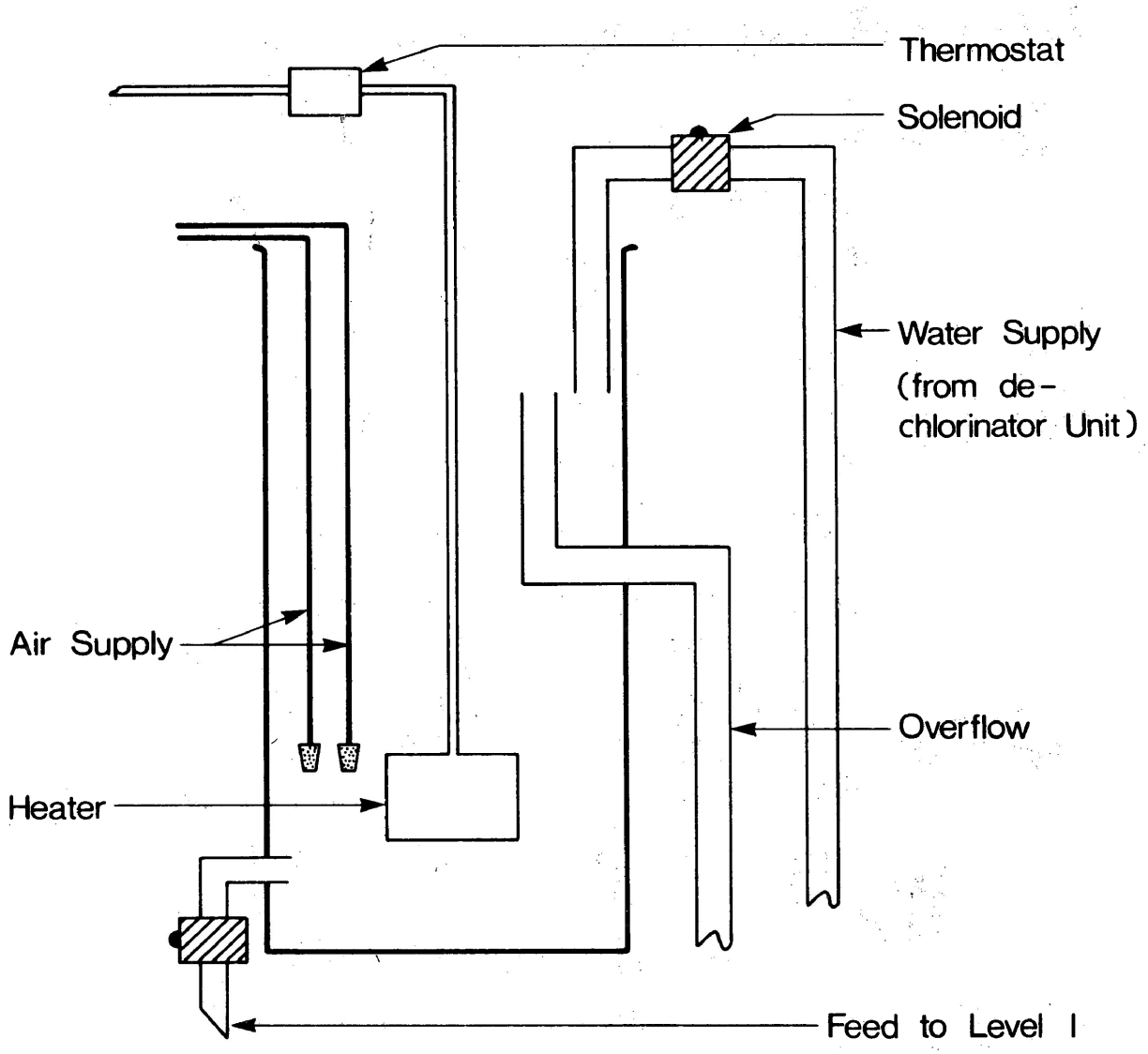

Thermostat
Solenoid
Water Supply
(from de-
chlorinator Unit)
Air Supply
Overflow
Heater
Feed to Level I

Figure 2. Front view of equal volume diluter.

From Water Res. 11: 347. A pneumatic dosing apparatus for flow-through bioassays. Smith *et al.*, 1977.

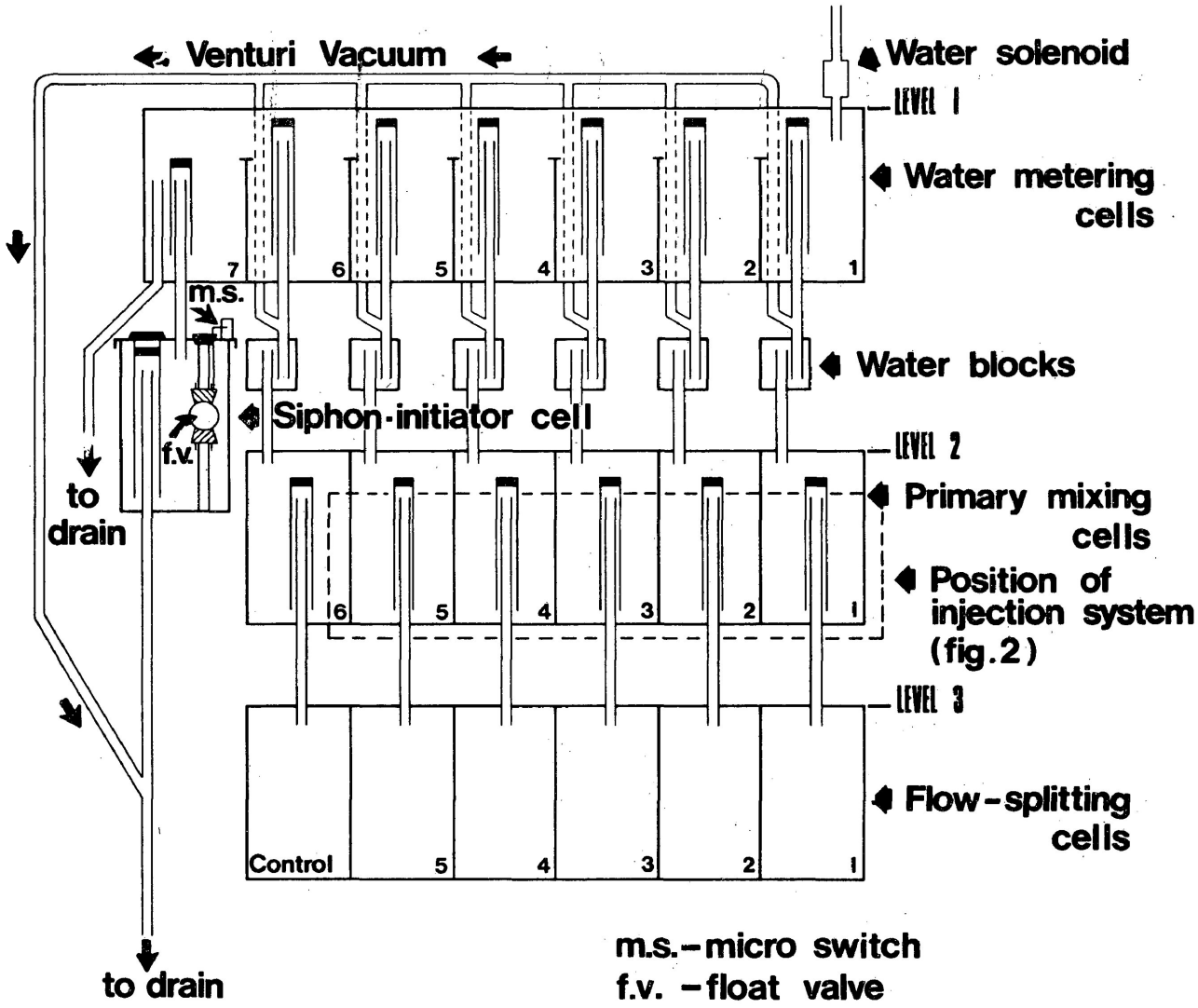

Venturi Vacuum
Water solenoid
LEVEL 1
Water metering cells
7
6
5
4
3
2
1
m.s.
Water blocks
Siphon-initiator cell
f.v.
to drain
LEVEL 2
Primary mixing cells
Position of injection system (fig.2)
6
5
4
3
2
1
LEVEL 3
Flow-splitting cells
Control
5
4
3
2
1
to drain
m.s.-micro switch
f.v. -float valve

Figure 3. Schematic illustrating test tank arrangement.

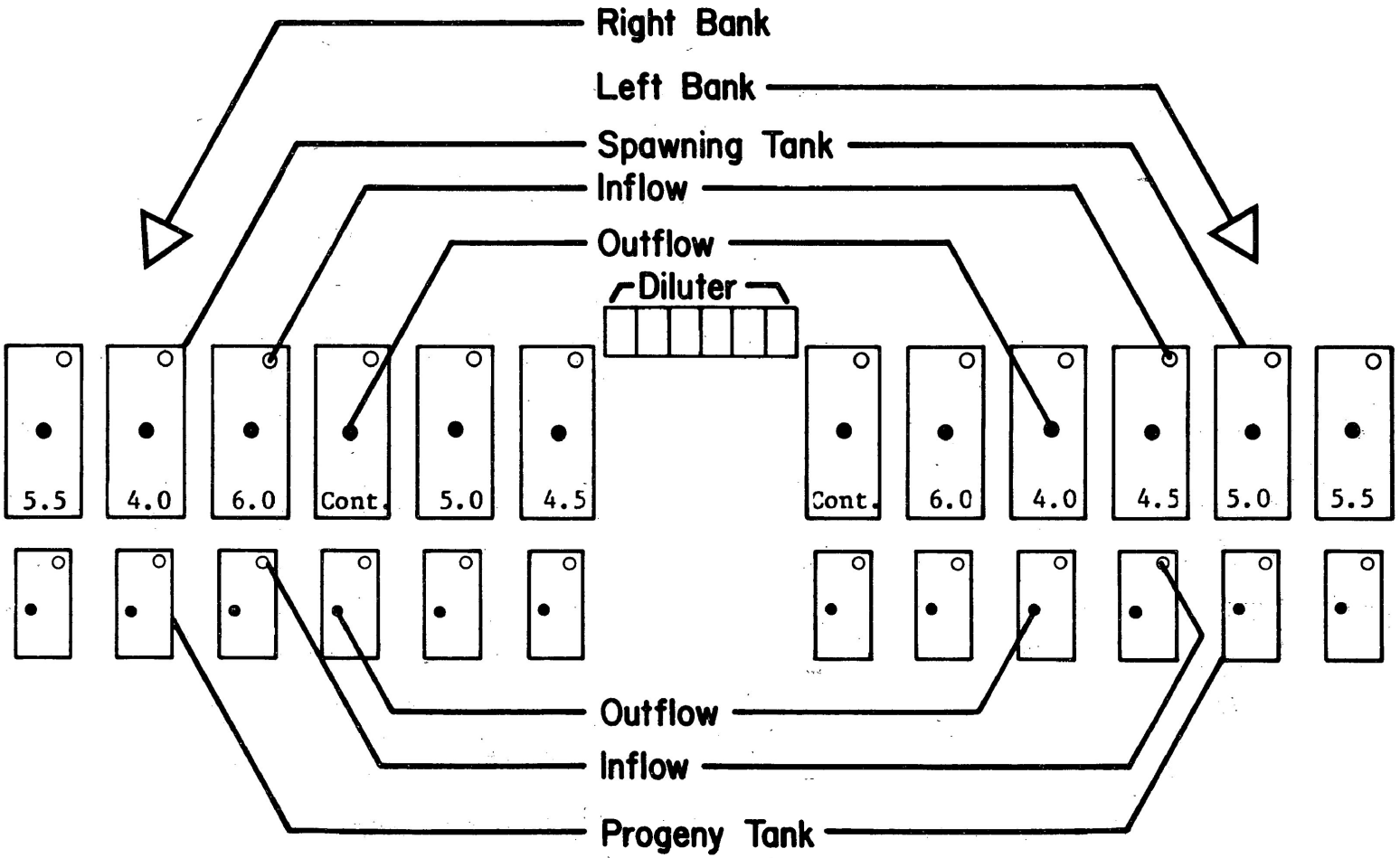
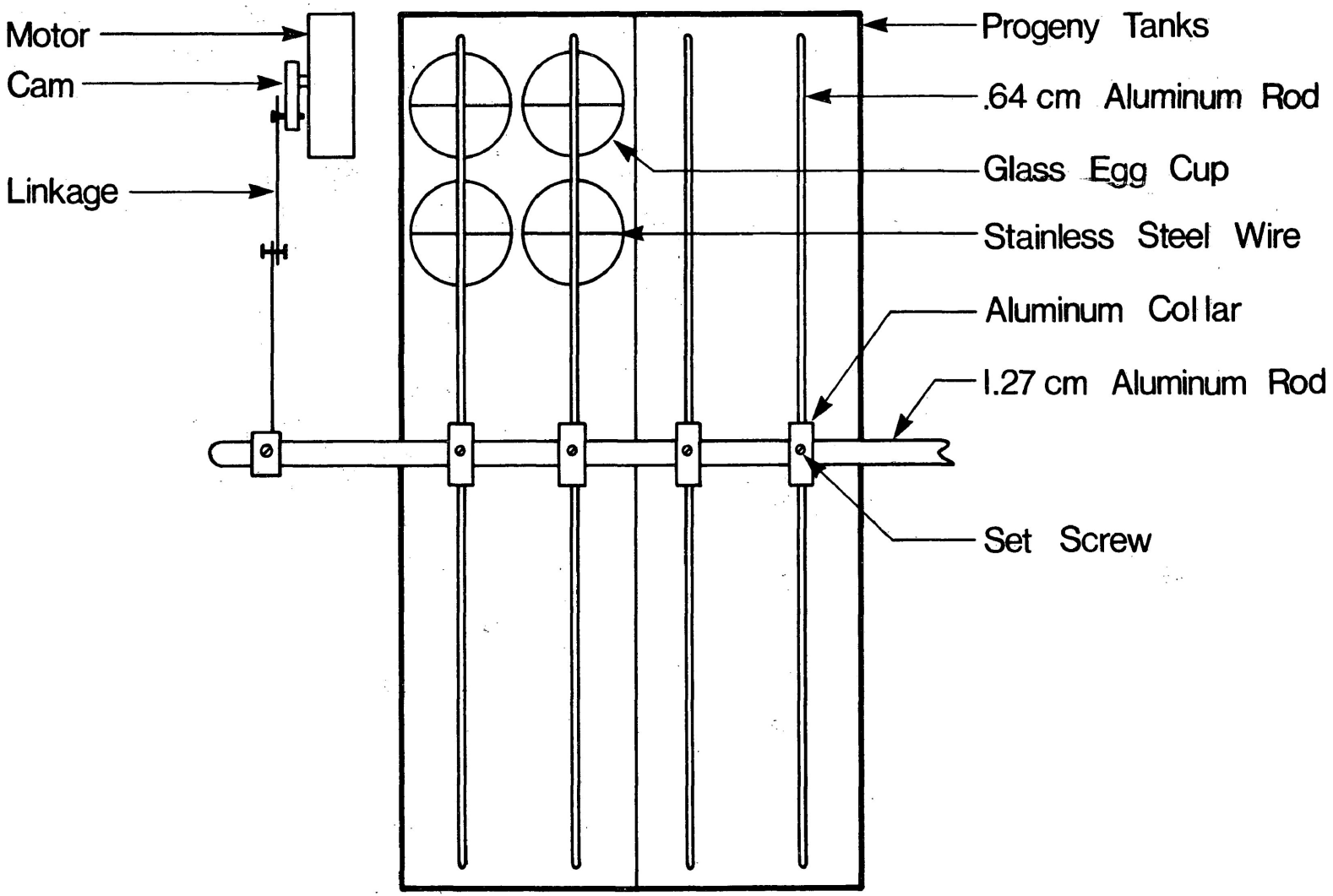

Right Bank
Left Bank
Spawning Tank
Inflow
Outflow
Diluter
5.5
4.0
6.0
Cont.
5.0
4.5
Cont.
6.0
4.0
4.5
5.0
5.5
Outflow
Inflow
Progeny Tank

Figure 4. Superior view of rocker arm assembly.


Motor
Cam
Linkage
Progeny Tanks
.64 cm Aluminum Rod
Glass Egg Cup
Stainless Steel Wire
Aluminum Collar
1.27 cm Aluminum Rod
Set Screw

RESULTS

Chemical

The results of the monthly water samples, January through March, are presented in Appendix 1. Tests indicated hardness, alkalinity and conductivity were consistently low. A change to an alternate water source during the month of April resulted in a slight elevation in pH and an approximate doubling of hardness, alkalinity and conductivity (Appendix 1). Even with the elevation in the above parameters, the test water remained moderately soft. With the exception of calcium, other ion concentrations remained low (Appendix 1). The sulfuric acid was introduced to the test water on June 1st. Alterations to the water chemistry as a result of the acid, are reflected in the test tank chemistry results for the months of May and June (Table 1). There was a direct relationship between decreased pH (increased H_2SO_4) and alkalinity and an inverse relationship with sulfate and conductivity.

Dissolved oxygen concentrations in all test tanks were maintained above 6.0Mg/ℓ (70% saturation) throughout the experimental period (Table 2). The temperature in all test tanks was maintained at 26°C with deviations of less than 0.75°C (Table 3). Following the establishment of the prescribed pH values, the tolerance of ±0.2 pH units was exceeded on only two occasions (Table 4).

Biological

No pH related adult mortality occurred at any test level during the two month exposure to acidic conditions. The one adult mortality recorded during the experimental period (left pH 6.0), resulted from a possible congenital disorder. There was however, a decrease in adult feeding intensity in the lower pH regimes during the depressed pH period.

Spawning began approximately one and one half months following the placement of the spawning substrates in the test tanks. The removal and subsequent replacement of the spawning substrates resulted in spawning at all test levels. All treatment groups were actively spawning before and during pH depression (Table 5 & 6), with no significant difference in egg production between the prescribed test levels in either of the duplicate banks. During chronic acid exposure however, spawning ceased below the pH 6.0 test level (Table 6). The reductions in total egg production are clearly evident when expressed as mean daily output for each test level (Table 7). During chronic acid exposure, egg production decreased significantly ($p<.01$) at all treatment levels compared with pH 6.0 and control. A significant reduction ($p<.05$) in egg production at pH 6.0 relative to control was also recorded in both banks during the same period. An analysis of egg production between periods, revealed that the high daily egg production demonstrated at all test levels during the predepression period continued throughout the experimental period in the control tanks. There was however, a significant decrease ($p<.01$) in all remaining test levels between the predepression and de-

pressed periods with the exception of left pH 6.0. Low initial egg production during the predepression period continued throughout the depression and depressed periods in this test tank.

Viable egg production was extremely high at all test levels during the predepression period, ranging from 98% to 99% (Table 8). During the depression period however, viable egg production was significantly reduced ($p<.01$) at the pH 4.5 treatment level compared to the remaining test levels. High viable egg production continued at the control and pH 6.0 treatment levels during the depressed pH period. As a result of extremely low productivity at the lower pH levels during the depressed pH period, these values were not included in the analysis.

Hatching success of the fertile eggs was extremely high in all test tanks during the predepression period, with no significant differences found between treatment levels. Depression and chronic acid exposure resulted in a significant decrease ($p<.01$) in hatching success at the pH 4.0 treatment level relative to the remaining test levels (Table 9). An extremely poor hatch in one of the egg cups at the pH 6.0 test level and in two egg cups at the pH 4.5 test level, resulted in high standard deviations at both levels (Table 9). Due to these large internal variances, no significance can be attributed to value differences in the remaining test levels during this period.

Egg incubation times ranged from four to six days, but neither depression nor chronic acid exposure appeared to alter this time. An analysis of egg incubation times revealed significant differences, but no apparent pattern was established (Table 10).

Tables 11 and 12 present the data obtained when excess control and pH 6.0 eggs were set to hatch at lower treatment levels. No significant differences were found between the two transfer groups with respect to hatching success and hatching time.

Spawning resumed within three days at the pH 4.5 test level following the return to control conditions (Table 13). Although the same pre-exposure, egg production levels were not reached ($p<.05$), an extremely good recovery was made. Viable egg production and hatching success did however reach the same pre-exposure levels (Table 13).

A histological examination of the ovaries from the control and pH 5.5 test level females revealed no obvious differences between the two groups (Figs. 5 & 6)

No fry survived the initial 24 hour period at the pH 4.0 test levels and all fry died within 24-72 hours when control eggs hatched in pH 4.5. The majority of fry mortality in the remaining test levels occurred in the first three weeks following hatching (Table 14; Fig. 7). The mortality of fry was significantly higher ($p<.01$) in the control test levels compared to the pH 6.0 treatment levels. Fry mortality was not significantly different between the control and pH 5.5 test levels. However, mortalities were significantly greater ($p<.01$) than control at pH 5.0. In addition, mortalities were significantly increased ($p<.01$) with decreasing pH, between treatment groups pH 6.0, pH 5.5 and pH 5.0.

The final mean weights of the 31 day old fry were reduced significantly ($p<.01$) at pH 5.5 relative to control (Table 15). There was no significant reduction in weight between the control and pH 6.0 test

levels. The same relationship was found to hold true for fry length (Table 16). The single observation made at pH 5.0 was excluded from analysis.

TABLE 1 Mean flagfish test tank chemistry results for May and June, 1976.

Parameter	Cont.	6.0	5.5	5.0	4.5	4.0
Hardness as $CaCO_3$	48.0	50.0	51.0	48.0	43.0	48.0
Alkalinity as $CaCO_3$	48.0	9.0	6.0	7.0	1.0	2.0
Chloride	3.5	2.5	2.5	2.5	3.0	3.0
pH at lab	7.3	5.7	5.3	4.9	4.4	4.1
Conductivity	105.0	122.5	123.5	125.5	132.0	132.0
Sodium	2.2	1.8	1.8	1.7	1.9	2.0
Potassium	0.7	0.6	0.6	0.6	0.7	0.7
Calcium	13.0	14.0	13.5	14.0	14.0	14.0
Magnesium	6.5	4.0	4.5	3.0	2.0	3.0
Sulfate	4.5	37.0	40.5	42.0	44.5	46.5

Note: Samples taken within the first few days of the month following that which is recorded.

Note: All analyses are reported in mg/l with the exception of conductivity, in micro-ohms/cm and pH in pH units.

TABLE 2 Flagfish test tank dissolved oxygen values in Mg/ℓ. Means and standard deviations for January through July, 1976.

pH level	Jan.	Feb.	Mar.	Apr.
Control	6.38 ± 0.28	6.41 ± 0.22	6.33 ± 0.22	5.78 ± 0.38
pH 6.0	6.35 ± 0.30	6.37 ± 0.20	6.32 ± 0.20	5.74 ± 0.39
pH 5.5	6.35 ± 0.30	6.35 ± 0.27	6.34 ± 0.27	5.81 ± 0.34
pH 5.0	6.44 ± 0.35	6.50 ± 0.21	6.41 ± 0.21	6.00 ± 0.33
pH 4.5	6.38 ± 0.36	6.51 ± 0.20	6.43 ± 0.20	5.96 ± 0.42
pH 4.0	6.29 ± 0.28	6.42 ± 0.16	6.26 ± 0.16	5.92 ± 0.31

pH level	May	June	July
Control	5.81 ± 0.32	5.74 ± 0.22	5.95 ± 0.31
pH 6.0	5.87 ± 0.43	5.69 ± 0.44	6.13 ± 0.43
pH 5.5	5.90 ± 0.29	5.73 ± 0.33	5.94 ± 0.43
pH 5.0	5.91 ± 0.34	6.01 ± 0.22	6.16 ± 0.31
pH 4.5	5.99 ± 0.38	5.66 ± 0.34	5.70 ± 0.35
pH 4.0	5.97 ± 0.23	5.90 ± 0.25	6.05 ± 0.26

TABLE 3 Flagfish test tank water temperature in °C. Means and standard deviations for January through July, 1976.

pH level	Jan.	Feb.	Mar.	Apr.
Control	26.5 ± 0.63	26.2 ± 0.53	25.8 ± 0.36	25.7 ± 0.36
pH 6.0	26.9 ± 0.63	26.0 ± 0.57	25.8 ± 0.63	25.7 ± 0.42
pH 5.5	26.6 ± 0.55	26.4 ± 0.64	25.8 ± 0.42	25.7 ± 0.46
pH 5.0	26.6 ± 0.72	26.4 ± 0.50	26.0 ± 0.41	25.8 ± 0.40
pH 4.5	26.8 ± 0.48	26.3 ± 0.46	25.8 ± 0.36	25.6 ± 0.49
pH 4.0	26.5 ± 0.50	25.9 ± 0.42	25.8 ± 0.34	25.9 ± 0.30

pH level	May	June	July
Control	25.6 ± 0.41	25.7 ± 0.28	25.9 ± 0.20
pH 6.0	25.4 ± 0.33	25.7 ± 0.31	25.8 ± 0.25
pH 5.5	25.5 ± 0.41	25.7 ± 0.36	25.9 ± 0.23
pH 5.0	25.7 ± 0.35	25.8 ± 0.37	25.8 ± 0.28
pH 4.5	25.8 ± 0.45	25.7 ± 0.34	25.9 ± 0.23
pH 4.0	25.6 ± 0.35	25.8 ± 0.32	25.8 ± 0.24

TABLE 4 Flagfish test tank pH values in pH units. Means and standard deviations for January through July, 1976.

pH level	Jan.	Feb.	Mar.	Apr.
Control	7.55 ± 0.037	7.65 ± 0.044	7.79 ± 0.033	7.55 ± 0.105
pH 6.0	7.56 ± 0.043	7.64 ± 0.055	7.78 ± 0.084	7.74 ± 0.053
pH 5.5	7.56 ± 0.042	7.56 ± 0.068	7.56 ± 0.112	7.61 ± 0.057
pH 5.0	7.56 ± 0.043	7.65 ± 0.093	7.68 ± 0.076	7.58 ± 0.093
pH 4.5	7.56 ± 0.046	7.61 ± 0.042	7.68 ± 0.067	7.66 ± 0.072
pH 4.0	7.56 ± 0.044	7.62 ± 0.035	7.62 ± 0.106	7.63 ± 0.053

pH level	May	June	July*
Control	7.95 ± 0.138	8.05 ± 0.116	8.17 ± 0.086
pH 6.0	7.80 ± 0.275	6.11 ± 0.097	6.09 ± 0.134
pH 5.5	7.74 ± 0.320	5.52 ± 0.201	5.55 ± 0.185
pH 5.0	7.72 ± 0.399	4.92 ± 0.194	5.11 ± 0.252
pH 4.5	7.81 ± 0.311	4.51 ± 0.195	4.43 ± 0.063
pH 4.0	7.68 ± 0.384	4.06 ± 0.080	4.05 ± 0.152

* July pH for a period of seven days following which it was returned to control conditions.

TABLE 5 Total number of flagfish eggs produced in each of the duplicate test tanks during three pH exposure periods

	pH level											
	Cont.		6.0		5.5		5.0		4.5		4.0	
Exposure period	*Rt.	Lt.	Rt.	Lt.	Rt.	Lt.	Rt.	Lt.	Rt.	Lt.	Rt.	Lt.
Pre-depression (8 days)	997	502	1057	81	527	612	833	253	692	658	753	695
Depression (5 days)	633	64	366	81	537	118	174	72	207	7	153	87
Depressed pH (21 days)	1865	1015	1414	213	1	0	20	0	2	6	0	0

* Rt. and Lt. = right and left bank respectively.

TABLE 6 Total number of flagfish spawnings† in each of the duplicate test tanks during three pH exposure periods

Exposure period	pH level											
	Cont.		6.0		5.5		5.0		4.5		4.0	
	*Rt.	Lt.	Rt.	Lt.	Rt.	Lt.	Rt.	Lt.	Rt.	Lt.	Rt.	Lt.
Pre-depression (8 days)	8	7	8	5	6	8	8	8	8	7	8	8
Depression (5 days)	5	3	5	4	5	3	4	2	4	5	3	3
Depressed pH (21 days)	21	20	18	15	1	0	3	0	1	1	0	0

* Rt. and Lt. = right bank and left bank respectively.

† A spawning is defined as one day of egg production.

TABLE 7 Mean number of flagfish eggs per spawning in each of the duplicate test tanks during three pH exposure periods.

Exposure period	pH level											
	Cont.		6.0		5.5		5.0		4.5		4.0	
	*Rt.	Lt.	Rt.	Lt.	Rt.	Lt.	Rt.	Lt.	Rt.	Lt.	Rt.	Lt.
Pre-depression[I]	124.6	62.8	132.1	11.6	87.8	76.5	104.1	31.6	86.5	82.3	94.1	86.9
Depression [II]	126.6	12.8	73.2	16.2	107.4	23.6	34.8	14.4	41.4	1.4	30.6	17.4
Depressed [III]	88.8	48.3	67.3	10.1	0.1	0.0	1.0	0.0	0.1	0.3	0.0	0.0

* Rt. and Lt. = right bank and left bank respectively.

Cont'd

TABLE 7 Cont'd

ANOVA - Between test levels

I	Rt. bank	F=00.86	Range test	5 3 6 4 1 2	
II	"	F=02.83	"	6 4 5 2 3 1	
III	"	F=40.88	"	6 3 5 4 2 1	
				6 3 5 4 2 1	($p<.05$)
I	Lt. bank	F=02.14	Range test	2 4 1 3 5 6	
II	"	F=00.56	"	5 1 4 2 6 3	
III	"	F=35.19	"	3 4 6 5 2 1	
				3 4 6 5 2 1	($p<.05$)

ANOVA - Between pH periods

1	Rt. bank	F=02.05	Range test	III I II
2	"	F=04.81	"	III II I
3	"	F=23.98	"	III I II
4	"	F=31.64	"	III II I
5	"	F=17.70	"	III II I
6	"	F=23.03	"	III II I
1	Lt. bank	F=02.39	Range test	II III I
2	"	F=00.27	"	III I II
3	"	F=20.77	"	III II I
4	"	F=10.21	"	III II I
5	"	F=23.62	"	III II I
6	"	F=13.26	"	III II I

Note: Numbered means (arranged in ascending order) not underscored by the same line are significantly different at the 1% level unless otherwise indicated.

TABLE 8 Per cent viable* egg production of flagfish in each of the test tanks during three pH exposure periods. Means and standard deviations.

	pH level					
Exposure period	Cont.[1]	6.0 [2]	5.5 [3]	5.0 [4]	4.5 [5]	4.0 [6]
Pre-depression [I]	98.2 ±2.2	98.3 ±1.7	98.9 ±1.3	98.8 ±1.9	98.5 ±1.4	99.4 ±0.9
Depression [II]	98.1 ±2.1	96.6 ±2.1	99.5 ±0.9	96.0 ±3.2	91.9 ±3.1	98.0 ±2.0
Depressed pH [III]	98.2 ±2.3	99.0 ±1.4				

I	Combined banks	F=0.99	Range test	1 2 5 4 3 6
II	"	F=5.79	"	5 4 2 6 1 3
III	"	F=0.35	"	1 2

Note: Numbered means (arranged in ascending order) not underscored by the same line are significantly different at the 1% level.

* A viable egg is defined as one devoid of abnormalities and undergoing active embryological development.

TABLE 9 Per cent hatching success of flagfish eggs in each of the test tanks during three pH exposure periods. Means and standard deviations.

	pH level					
Exposure period	Cont.[1]	6.0 [2]	5.5 [3]	5.0 [4]	4.5 [5]	4.0 [6]
Pre-depression [I]	93.8 ±6.2	91.5 ±6.3	95.0 ±3.4	88.7 ±5.5	88.8 ±9.3	87.3 ±13.2
Depression [II]	89.6 ±7.4	78.3 ±12.7	83.1 ±11.0	85.0 ±13.1	79.9 ±7.2	19.9 ±26.9
Depressed pH [III]	92.5 ±7.4	72.8 ±34.3	84.1* ±9.6	89.3* ±7.0	68.3* ±19.8	0.6* ±1.3

I	Combined banks	F=00.98	Range test	6 4 5 2 1 3
II	"	F=15.62	"	6 2 5 3 4 1
III	"	F=50.56	"	6 5 2 3 4 1

Note: Numbered means (arranged in ascending order) not underscored by the same line are significantly different at the 1% level.

* Eggs spawned under control conditions and transferred for incubation.

TABLE 10 Hatching time (in days) of flagfish eggs in each of the test tanks during three pH exposure periods. Means and standard deviations.

	pH level					
Exposure period	Cont.[1]	6.0 [2]	5.5 [3]	5.0 [4]	4.5 [5]	4.0 [6]
Pre-depression [I]	6.0 ±0.0	5.4 ±0.5	5.8 ±0.4	5.3 ±0.8	5.1 ±0.4	4.7 ±0.8
Depression [II]	6.0 ±0.0	5.0 ±0.0	5.7 ±0.5	5.3 ±0.6	6.0 ±0.0	6.2 ±0.5
Depressed pH [III]	5.4 ±0.5	5.2 ±0.4	6.0 ±0.0	5.9 ±0.3	6.1 ±0.3	6.0 ±0.0

I	Combined banks	F=5.16	Range test	6 5 4 2 3 1
II	"	F=8.57	"	2 4 3 1 5 6
III	"	F=5.67	"	2 1 4 3 6 5

Note; Numbered means (arranged in ascending order) not underscored by the same line are significantly different at the 1% level.

TABLE 11 Hatching success of flagfish eggs spawned in control test waters and incubated at various depressed pH levels.

	pH level			
	5.5	5.0	4.5	4.0
No. of spawns set to hatch	5	9	9	10
Total No. of eggs set to hatch	181	403	278	400
Total No. of eggs hatched	154	356	212	2
Mean % hatch	84.1 ±9.6	89.3 ±7.0	68.3 ±19.7	0.6 ±1.3
Mean hatching time (days)	6.0 ±0.0	5.9 ±0.3	6.1 ±0.3	6.0 ±0.0

TABLE 12 Hatching success of flagfish eggs spawned in pH 6.0 test waters and incubated at various lower pH levels.

	pH level		
	5.0	4.5	4.0
No. of spawns set to hatch	5	4	4
Total No. of eggs set to hatch	139	153	138
Total No. of eggs hatched	94	86	0
Mean % hatch	76.4 ±19.2	53.7 ±24.4	0.0 ±0.0
Mean hatching time (days)	5.8 ±0.5	6.0 ±0.0	- -

TABLE 13 *R*eproductive response of a breeding flagfish community subjected to pH 4.5 and returned to control conditions.

Exposure period	Reproductive response: Exposure time	Total No. of eggs	Total No. of spawnings
Pre-depression (Cont.)	8 (days) X 2	1350	15
*D*epressed (pH 4.5)	21 "	8	2
Control resumed	17 "	1494	26

Exposure period	Reproductive response: No. of eggs per spawn	Mean viable egg prod.	Mean % hatch
Pre-depression (Cont.)	90.0	98.2 ±2.2	93.8 ±6.2
*D*epressed (pH 4.5)	4.0	-	-
Control resumed	57.5 *	94.2 ±11.7	92.6 ±7.2

* $p<.05$ compared to pre-depression (Cont.)

TABLE 14 Survival of flagfish fry exposed to depressed pH for 31 days

	pH level			
Day	Cont.	6.0	5.5	5.0
11	50	50	50	50
14	45	48	40	26
17	43	43	35	8
20	40	43	32	2
23	35	43	25	1
26	34	43	25	1
29	34	41	25	1
31	34	41	25	1

Chi-square analysis of 31 day mortality levels 41 34 25 1

Note: Values not underscored by the same line are significantly different at the 1% level.

TABLE 15 *Final* weight of flagfish fry in gms following a 31 day growth period in depressed pH. Means and standard deviations.

	pH level			
	Cont. [1]	6.0 [2]	5.5 [3]	5.0
Total No. surviving	34	41	25	1
Mean weight	0.0123	0.0107	0.0060	0.0022
Standard Deviation	0.0060	0.0048	0.0031	0.00

Combined banks $F = 12.42$ Range test 3 2 1

Note : Numbered means (arranged in ascending order) not underscored by the same line are significantly different at the 1% level.

TABLE 16 Length of flagfish fry in mm at select intervals during a a 31 day growth period in depressed pH. Means and standard deviations.

Day		pH level Cont.	6.0	5.5	5.0
11	I	1.77 ±0.137	1.85 ±0.153	1.69 ±0.178	1.67 ±0.143
24	II	3.00 ±0.542	3.03 ±0.380	2.75 ±0.312	2.60 ±0.000
31	III	4.00 ±0.705	3.98 ±0.577	3.28 ±0.537	3.50 ±0.000

I	Combined banks	F=10.59	Range test	4 3 1 2
II	"	F=02.90	"	3 1 2
III	"	F=10.69	"	3 2 1

Note : Numbered means (arranged in ascending order) not underscored by the same line are significantly different at the 1% level.

Figure 5. Cross sectional view of ovary removed from control female flagfish.

*Stage three oocyte: 3 - deeply basophilic cytoplasm
- several nucleoli located around the periphery of the nuclear membrane.

*Stage four oocyte: 4a - deposition of primary yolk
- nucleoli peripherally located around the still highly visible nucleus

4b - late stage showing secondary yolk granules within the cytoplasm.

*Stage five oocyte: 5 - nuclear membrane has disintegrated
- increasing nos. of eosinophilic yolk globules indicating secondary yolk deposition in cytoplasm.

*Stage six oocyte: 6 - secondary yolk droplets have coalesced to form a solid mass.

*Developmental classification as in Ruby et al., (1977).

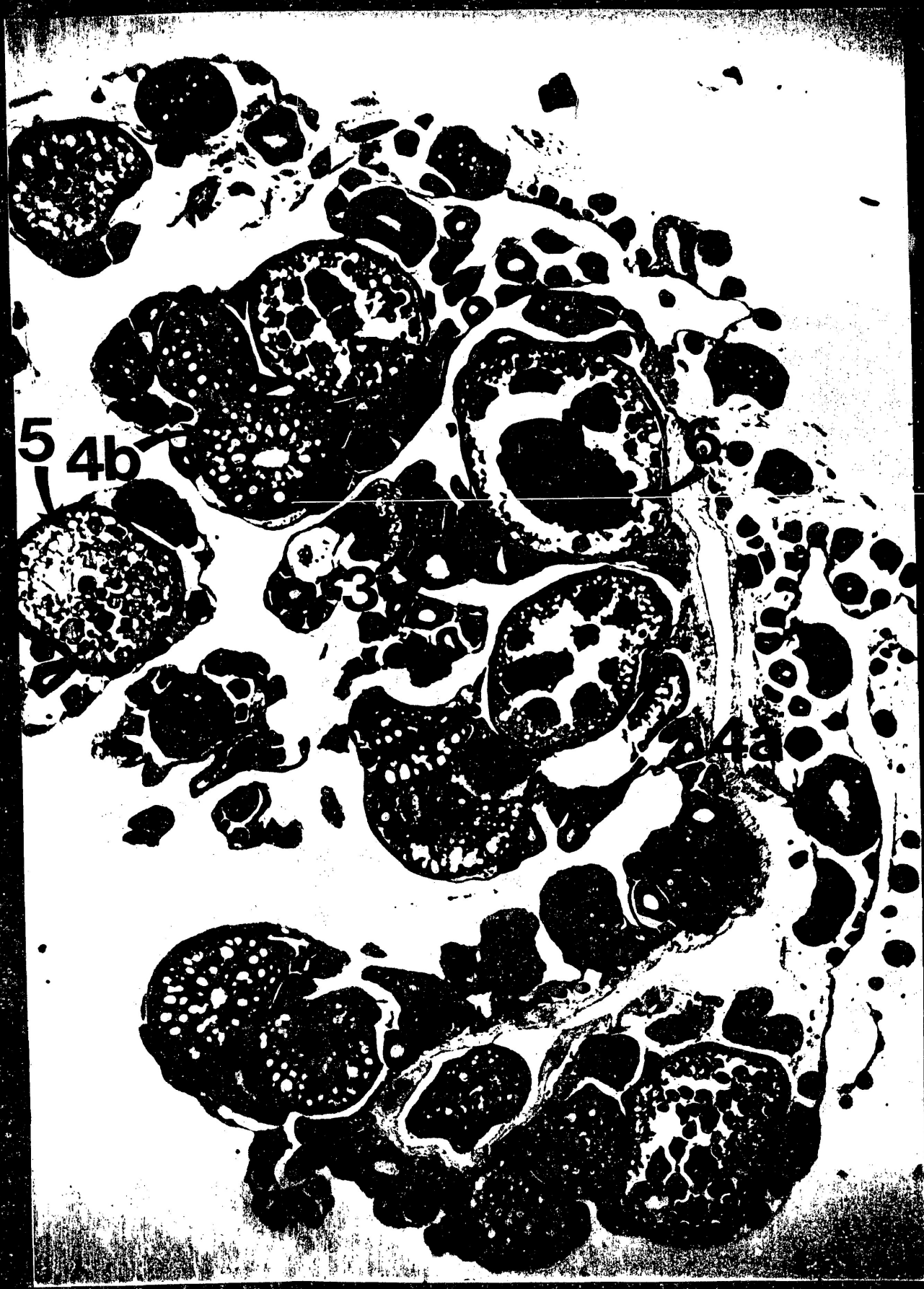

5
4b
6
3
4a

Figure 6. Cross sectional view of ovary removed from female flagfish exposed to pH 5.5 for 21 days.

*Stage three oocyte: 3 - deeply basophilic cytoplasm
- several nucleoli located around the periphery of the nuclear membrane.

*Stage four oocyte: 4a - deposition of primary yolk
- nucleoli peripherally located around the still highly visible nucleus

4b - late stage showing secondary yolk granules within the cytoplasm.

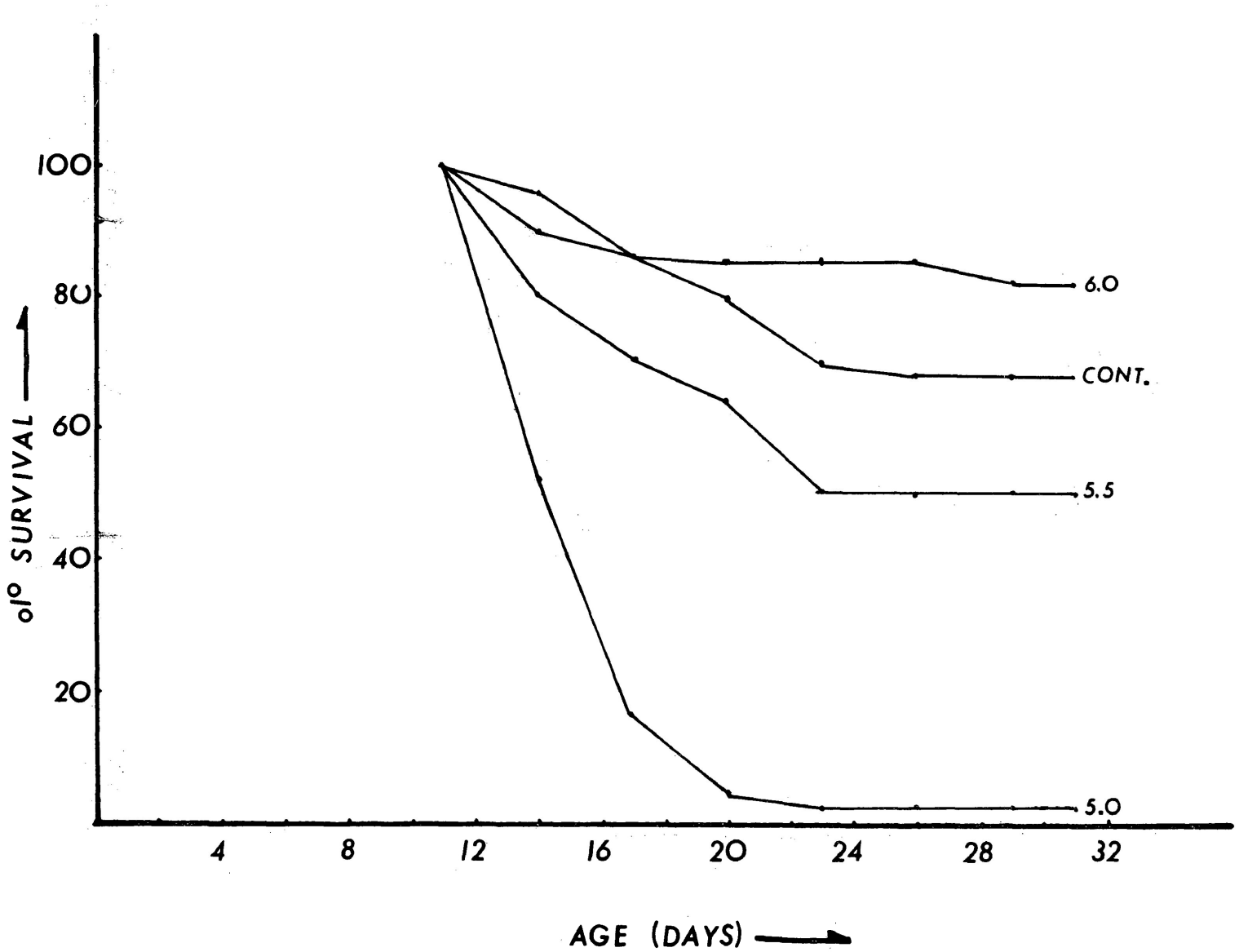
*Stage five oocyte: 5 - nuclear membrane has disintegrated
- increasing nos. of eosinophilic yolk globules indicating secondary yolk deposition in cytoplasm.

*Stage six oocyte: 6 - secondary yolk droplets have coalesced to form a solid mass.

*Developmental classification as in Ruby et al., (1977).

6
4b
4a
3
5

Figure 7. Survival of flagfish fry in depressed pH over a 31-day period.


100
80
60
40
20
% SURVIVAL
4
8
12
16
20
24
28
32
AGE (DAYS)
6.0
CONT.
5.5
5.0

DISCUSSION

In relatively soft water, adult flagfish survive a short term exposure to pH 4.0. One adult died during the experiment, an " egg bound " female exposed to pH 6.0. Acidic conditions were probably not the cause of death, as other females were not affected.

Craig and Baksi (1977) recorded a mortality of 71% in adult flagfish during a three week exposure to pH 4.5 in soft water (30ppm as Ca CO_3). Mount (1973) found 80% of his adult fathead minnows survived 13 months in hard water (200ppm as $CaCO_3$) at pH 4.5. This suggests that fathead minnows are either more tolerant of low environmental pH, or increased water hardness reduces the lethal effect of low pH. Lloyd and Jordan (1964) reported increased water hardness provided rainbow trout (*Salmo gairdneri*) an increased tolerance to acidic conditions. In waters 320, 40 and 12 ppm $CaCO_3$, 50% of the fish died in four days at pH values 4.18, 4.22 and 4.25 respectively. Menendez (1976) found no brook trout survived a five month exposure period to pH 4.5 at 61 ppm $CaCO_3$, Smith (1978) however, observed all brook trout survived when exposed for the same length of time to softer water (30 ppm $CaCO_3$) at pH 4.5. These variations in mortality suggest that more than water hardness need be considered when assessing the effects of acidified water on fish.

To the author's knowledge, there are no published data reporting an LC_{50} for sulfate ($SO_4^=$) or the effect of sublethal concentrations of this ion on discrete life stages of fish. Data compiled for the United States

Environmental Protection Agency (Water Quality Criteria Data Book, 1971) indicate the drinking water of 163 metropolitan areas in the United States have an average $SO_4^=$ content of 43.9 mg/ℓ. One would assume that each of these bodies of water support successful fish populations. Forty five per cent of the waters in the United States which support good game fish have a $SO_4^=$ content in the range of 32 to 90 mg/ℓ (McKee and Wolf, 1963). Based on this information, one can assume that either directly or indirectly, the H^+ ion is the toxic component of sulfuric acid and the elevated sulfate levels (Table 1) had little or no effect on the various stages of the flagfish life cycle.

Within sub-lethal temperature ranges, temperature does not appear to alter the resistance of brook trout to low pH (Daye and Garside, 1975), but free CO_2 is a factor that need be considered (Lloyd and Jordan, 1964). It has been suggested by Doudoroff and Katz (1950) that the increased CO_2 tension and not the increased hydrogen ion activity is the lethal factor involved when fish are exposed to extremely low pH levels. Although this parameter was not measured, a shift in the bicarbonate (HCO_3^-) to free CO_2 as indicated by the decline in alkalinity (Table 1) may have contributed to the toxicity of the lower pH levels.

Low plasma chloride (Leivestad and Muniz, 1976) in addition to low levels of sodium (Packer and Dunson, 1970) have been reported in fish exposed to low environmental pH. It has been demonstrated that acid tolerance is increased for eggs and fry of white suckers by increasing the salt concentration of the water. Similarly, brook trout exposed to low environmental pH lived longer in a slightly saline medium. (Packer and

Dunson, 1970). Low salt content as that recorded in the test waters used for the present study (Table 1), may also have contributed to the toxicity of the lower pH levels.

Following exposure to pH 4.5 and pH 4.0 it was observed that general activity and feeding behaviour was depressed. Similar observations were made with flagfish at pH 4.5 (Craig and Baksi, 1977), with brook trout at pH 4.5 and pH 4.0 (Smith, 1978) and in white suckers below pH 5.0 (Beamish, 1972). Based on the observation of declining feeding activity and the projection of a cessation in feeding at this treatment level, I feel that pH 4.0 is, or approaches the lethal threshold for mature flagfish.

Active spawning at all treatment levels prior to acid exposure continued at the control and pH 6.0 test levels during the depression and depressed periods (Table 6). A termination of spawning at the pH 5.5 test levels during chronic acid exposure suggests that a pH level between 6.0 and pH 5.5 inhibits flagfish reproduction. A significant decline in egg production at the pH 6.0 test level, indicates that even this level is marginal for the maintenance of a substantial population. Similarly, the egg production of fathead minnows terminated below pH 5.9 (Mount, 1973) and in smallmouth bass at a pH above 6.0 (Beamish, 1976). Craig and Baksi (1977) found that flagfish continued to spawn at a reduced rate below pH 6.0, with a termination of spawning below pH 5.0.

An examination of the flagfish ovaries from the control and pH 5.5 test levels revealed no detectable differences. The ability of the oocytes to deposit secondary yolk within the cytoplasm, as well as the

ability of the secondary yolk deposits to coalesce in mature eggs did not appear to be impaired. Ruby *et al.*, (1977) found mature female flagfish exposed for the same length of time to pH 6.0, 5.5, 5.0 and 4.5, exhibited a reduction in mature oocytes of 79.3, 84.2, 97.7 and 91.8% respectively. It should be pointed out here however, serial sections were not made, nor were the numbers of mature oocytes subjected to a reproductive index as were done by Ruby *et al.*, (1977). I think however, a sample section of each ovary within specified confidence limits, is an acceptable representation of the entire ovary. Based on my histological sections, as well as the sedentary behaviour displayed by the flagfish, I think that at pH 5.5, reproductive impairment may be a behavioural rather than a physiological phenomenon. Below a pH of 5.5, reproductive impairment may indeed be the result of a reduction in mature eggs. Fathead minnows subjected to pH 5.2 for 13 months failed to spawn. A gross examination of these females however, revealed they were sexually mature and heavy with eggs (Mount, 1973). Immature brook trout subjected to pH 4.0 failed to develop mature gonads, while the same fish held at pH 4.5 and higher did (Smith, 1978). Field observations of lake herring (*Coregonus artedii*) and rock bass (*Ambloplites rupestris*) at pH 4.5 showed abnormal development of ovaries (Beamish, 1974a).

Upon reaching sexual maturity, the female flagfish continues to produce eggs regardless of the time of year. The number of eggs produced and the relatively small physical size of the ovary, indicate rapid oogenesis. As a result, the ability of the flagfish to resume successful reproduction within a three day period following the return of the pH

4.5 test levels to control conditions, does not indicate the presence or the absence of mature eggs in the ovaries prior to the return to control conditions.

The rapid inhibition of spawning in a breeding flagfish community immediately following the onset of acidic conditions below pH 6.0 (Appendix 2), indicates the rapidity with which a change in the environment can affect reproduction. The accumulation of airborne SO_2 in snow has been shown to result in the decline of surface water pH to 4.2 at the onset of thawing, and return to pH 6.0 within a one month period (Hagen and Langeland, 1973). Should a pH drop such as this coincide with the spring spawning activity, the ecological ramifications of this phenomenon can be fully realized. If a pH recovery should occur within the spawning period, data in Table 13 suggest that spawning would resume but at a decreased rate. It is difficult to speculate on the long term effects of such a pH decline, but the possibility that reduced egg production would continue in subsequent spawns cannot be ruled out.

The extremely high viable egg production found during the predepression period continued through the depression period (Table 8). It is impossible to assess the effects of chronic acid exposure to viable egg production however, as so few eggs were produced at the lower treatment levels. A breeding flagfish community does in fact suffer a significant reduction in viable egg production at all treatment levels relative to control (Craig and Baksi, 1977). A similar reduction in viable egg production was found when brook trout were subjected to depressed pH conditions (Menendez, 1976: Smith, 1978).

The continual movement of water over the eggs provided by the rocker arm assembly, as well as the daily treatment in malachite green, resulted in extremely good hatching success as illustrated in Table 9. This treatment minimized fungal infection to which flagfish eggs are reported to be very susceptable (Craig and Baksi, 1977). During the depressed pH period, the first reduction in hatching success was observed at the pH 4.5 treatment level, with the strongest effect exerted at the pH 4.0 test level (Table 9). Comparable hatching success was found when brook trout eggs (Trojnar, 1977a) and white sucker eggs (Trojnar, 1977b) were subjected to similar conditions. The hatchability of fathead minnow eggs was significantly reduced at the pH 5.9 test level compared with both control and pH 6.6 treatments, with virtually no hatch at the pH 4.5 test level (Mount, 1973). Similarly, rainbow trout eggs (Kwain, 1976) and brook trout eggs (Menendez, 1976) failed to hatch at pH 4.5 and lower. Substantially greater acid tolerance has been recorded for embryos of Atlantic salmon (*Salmo salar*) (Daye and Garside, 1977), however, the data suggest that the pH should not drop below pH 5.5 during the time at which the eggs are hatching to ensure a relatively successful hatch.

Egg incubation time is temperature dependent (Kwain, 1975) and slight variations in the 25°C progeny tank temperatures could perhaps account for the four to six day range in incubation time (Table 10). Neither depression nor chronic acid exposure appeared to alter this time. With a mean incubation time of five days however, the validity of any significant differences could be questioned. Brook trout eggs, which have a 50 to 60 day incubation period, showed a reduction in hatching

time directly proportional to decreasing pH (Trojnar, 1977a). Menendez (1976), however, found no differences in incubation time when brook trout eggs were exposed to low environmental pH. Mount's (1973) observation that lowered pH increased the hatching time for fathead minnow eggs, leaves one questioning all these observations. No mention was made with regard to water temperature fluctuations and this could conceivably account for the differences in observations.

Within a 72 hour period, 100% fry mortality was recorded at the pH 4.5 and 4.0 test levels. Only one fry survived the 31 day exposure period to pH 5.0 (Table 14). The lethal limits for flagfish fry in the present study are similar to those of white suckers (Trojnar, 1977b) and fathead minnows (Mount, 1973) but considerably higher than those observed for Atlantic salmon (Daye and Garside, 1977) and northern pike (*Esox lucius*) (Johanson and Kihlstrom, 1975). Acid tolerance, however, is species specific. Despite this difference in fry survival, all results agree that the newly hatched fry are more susceptible to low pH than the developing egg. The suggestion that the chorion affords a form of environmental protection is a credible explanation (Craig and Baksi, 1977).

Unlike the flagfish, the eggs of most coldwater freshwater fish species require a great deal longer to hatch. Should eggs be laid under acceptable conditions, drops in environmental pH such as those recorded by Haltburg (1977) may not have such marked effects, provided of course the pH returned to original levels prior to hatching. Should the depressed pH conditions continue during the hatching period, fry survival would be greatly reduced.

The final mean weight and length of the fry were significantly reduced at the pH 5.5 treatment level compared with control. Craig and Baksi (1977), found flagfish fry growth was significantly reduced at both the pH 5.5 and pH 6.0 test levels relative to control. Brook trout fry exposed to the same pH gradient exhibited a similar reduction in growth (Menendez, 1976; Smith, 1978). Such a reduction in fry growth could possibly result in delayed sexual maturation. Should normal spawning behaviour not coincide with the optimum seasonal spawning period, this could conceivably result in the loss of a complete year class.

SUMMARY AND CONCLUSIONS

It can be seen that every stage of the flagfish life cycle can be altered by a depression in environmental pH. Although no adult mortality occurred at the lower pH levels, the dramatic reduction in food utilization would probably have resulted in their eventual death had these conditions continued.

There was a significant reduction ($p<.05$) in egg production at the pH 6.0 test level compared with control and a termination of spawning below this test level. Data collected by Craig and Baksi (1977), Mount (1973) and Smith (1978) all indicate a reduction in viable egg production with decreasing pH. One could speculate the same reduction would have occurred in the present study provided sufficient egg production had continued in the lower pH treatment levels.

Compared to the flagfish fry, the eggs exhibited a diminutive response to low environmental pH. Hatching success remained high to the pH 4.5 test level, but only one fry survived below the pH 5.5 treatment level. A significant reduction in fry growth at the pH 5.5 test level relative to control was also recorded.

When determining the hydrogen ion concentration that is detrimental to a given population, one must realize that the production of viable eggs is the primary key to the maintence of that population. Any environmental change that would inhibit successful egg production would ultimately destroy that population. The fact that flagfish fry will tolerate further pH depression is of little consequence if no eggs are

produced. My results indicate that recruitment failure in a flagfish population subjected to depressed pH conditions is the result of spawning impairment rather than high mortality of the egg and fry stages as reported by Jensen and Snekvik (1972).

As egg production terminated below the pH 6.0 test level, any depression below this level would definitely be detrimental to a breeding flagfish community. With a significant reduction in egg production at the pH 6.0 test level, this study, in agreement with all other researchers in the field, supports the need for environmental pH to be maintained above 6.5 to ensure unimpaired fish reproduction.

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APPENDIX 1 Flagfish test tank chemistry results for January through March (1) and April (2), 1976. Means and standard deviations

Parameter	(1)	(2)
Hardness as $CaCO_3$	25.6 ± 2.2	42.6 ± 1.0
Alkalinity as $CaCO_3$	19.2 ± 2.7	43.0 ± 0.0
Chloride	1.9 ± 0.2	3.0 ± 0.0
pH at lab	6.7 ± 0.2	7.2 ± 0.2
Conductivity	58.6 ± 0.6	97.3 ± 0.8
Sodium	1.1 ± 0.3	0.8 ± 0.2
Potassium	0.7 ± 0.1	0.6 ± 0.3
Calcium	6.2 ± 0.4	13.6 ± 0.4
Magnesium	2.6 ± 0.5	2.2 ± 0.4
Sulfate	5.8 ± 1.1	8.0 ± 0.0

Note: All analysis are reported in mg/ℓ with the exception of conductivity, in micro-ohms/cm and pH in pH units.

APPENDIX 2. Daily flagfish egg production during initial exposure to undosed water, linear pH depression and chronic exposure at a nominal pH.

	RIGHT BANK						LEFT BANK					
Predepression												
Day	7.5	6.0	5.5	5.0	4.5	4.0	7.5	6.0	5.5	5.0	4.5	4.0
1	43	113	0	7	6	3	112	0	6	2	39	2
2	54	204	0	93	85	29	183	3	4	22	34	227
3	165	219	31	127	69	180	52	4	52	7	129	207
4	155	88	31	139	145	126	95	14	135	2	189	57
5	151	126	102	149	213	159	30	0	132	46	76	69
6	229	96	155	121	91	135	4	0	117	87	65	15
7	122	135	123	100	71	64	0	12	103	17	126	79
8	78	76	85	97	12	57	26	48	63	70	0	39
	997	1057	527	833	692	753	502	81	612	253	658	695
Depression												
9	169	81	229	149	106	86	42	13	85	40	3	43
10	50	104	146	7	46	50	8	12	32	32	1	21
11	106	75	97	0	46	17	14	54	1	0	1	23
12	165	62	59	14	9	0	0	2	0	0	1	0
13	143	44	6	4	0	0	0	0	0	0	1	0
	633	366	537	174	207	153	64	81	118	72	7	87

Cont'd

APPENDIX 2 (Cont'd)

RIGHT BANK							LEFT BANK					
Depressed												
Day	7.5	6.0	5.5	5.0	4.5	4.0	7.5	6.0	5.5	5.0	4.5	4.0
14	116	166	1	1	0	0	97	26	0	0	0	0
15	79	80	0	14	0	0	60	0	0	0	0	0
16	36	151	0	5	0	0	93	9	0	0	0	0
17	120	138	0	0	0	0	77	3	0	0	0	0
18	75	82	0	0	0	0	40	2	0	0	0	0
19	102	25	0	0	0	0	38	9	0	0	0	0
20	61	109	0	0	0	0	44	0	0	0	0	0
21	47	35	0	0	0	0	101	7	0	0	0	0
22	18	24	0	0	0	0	11	18	0	0	0	0
23	139	8	0	0	0	0	100	8	0	0	0	0
24	97	3	0	0	0	0	13	0	0	0	0	0
25	5	0	0	0	0	0	61	0	0	0	6	0
26	45	0	0	0	2	0	0	0	0	0	0	0
27	82	0	0	0	0	0	51	0	0	0	0	0
28	102	55	0	0	0	0	73	1	0	0	0	0
29	97	52	0	0	0	0	16	3	0	0	0	0
30	179	70	0	0	0	0	20	3	0	0	0	0
31	134	117	0	0	0	0	63	2	0	0	0	0
32	76	79	0	0	0	0	12	33	0	0	0	0
33	181	144	0	0	0	0	38	28	0	0	0	0
34	74	76	0	0	0	0	7	61	0	0	0	0
	1865	1414	1	20	2	0	1015	213	0	0	6	0