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A Study of Some Effects of Water Chemistry on Growth of *Betta splendens*¹

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SYNOPSIS: Experimental (varying water chemistry) and Control (constant water chemistry) quart jars containing single specimens of the fish were tested over an eighteen-week period. Organisms were weighed before and after this period to determine net growth, possibly associated with changes in the ecosystems. Alkalinity, carbon dioxide, dissolved oxygen, total hardness, nitrates, nitrites, pH, and bacteriological parameters were tested. Statistical analyses

revealed that chemical parameters exist correlated with particular light levels. Changes in water chemistry could not be correlated with growth. Information obtained suggests light may affect growth of inhabiting Bettas but more information is needed to confirm this. Certain chemical parameters appeared to be required for visible algal blooms to appear. These blooms were found only in experimental containers receiving maximum light. They were preceded by increasing alkalinity and carbon dioxide levels.

INDEX DESCRIPTORS: Water chemistry, fish growth, Siamese Fighting Fish, *Betta splendens*.

Fish respond in various ways to pollutants in water. Bonnet (1932) found guppies to be flourishing in sewers of Guadalupe, showing extreme tolerances to various deleterious chemical parameters. Mackay (1970) found mild forms of pollution accompanying eutrophic conditions to be inhibitory to growth of trout and graylings. Cairns, Sparks and Waller (1970) found that by continuous monitoring of aquatic life (goldfish) in a stream, they could detect increases in levels of pollutants long before they reached danger levels.

Inhabiting organisms (fish, etc.) may contribute nitrogen in various forms. According to Prosser and Brown (1961) pathways of nitrogen metabolism in fish have not yet been totally and systematically explored, but information suggests parallels with the metabolic pathways of higher vertebrates. This is indicated by the occurrence of the usual main forms of non-protein nitrogen in the urine. There appears to be a relatively low urinary nitrogen excretion in teleosts due to the importance of branchial ammonia excretion. Six to ten times as much nitrogen is secreted by the gills as in all nitrogenous compounds of the kidneys. Branchial excretion is comprised of highly diffusible products, such as urea and ammonia, while the less diffusible products, creatine and uric acid are excreted by the kidneys.

Delaney (1931) showed that ammonia is the chief product of metabolism in all aquatic organisms, freshwater and marine, from simple protozoans to the most complex metazoa. This, in turn, may eventually be converted to nitrates. There are many advantages to ammonia excretion. Martz and Romeu (1964) showed that ammonia possessed the ability to exchange with sodium (Na⁺) absorption by the gills of freshwater fish, which is important in maintaining salt and water balance. In freshwater organisms, the exchange of NH₄⁺ for Na⁺ serves a dual purpose, in nitrogenous end product elimination and in the accumulation of Na⁺ for osmotic balance. Imbalances of either may have detrimental effects on the organisms.

In this study, an attempt was made to follow chemical

changes occurring in quart jars (after Linn, 1965) containing single specimens of sibling *Betta splendens*. These jars were located in various light concentrations. It was hypothesized that organisms housed in containers in which water was changed regularly would grow larger. By contrast, the growth of organisms kept continually in the same water would be inhibited, presumably due to pollutants, their own wastes.

Following preliminary experimentation, it was believed that chemical changes in the water would occur related to various light levels and time. By weighing Bettas before and after a specific testing period, net growth could be ascertained. These weight increases were then analyzed for significant differences. An attempt was made to correlate these differences with light concentration and/or water chemistry.

MATERIALS AND METHODS

A set-up similar to that used by Linn (1965) was used for this study, without aeration of test containers. Test containers were also quart-sized, rather than gallon as utilized in the original. Furthermore, since the organisms were in individual containers, the possibility of disease or serious competition affecting all of the fish (*Betta splendens*) was practically eliminated. In order to reduce particulate sources of carbon, no substrate was provided, thus ensuring greater dependence upon the alkalinity system for a source of carbon dioxide for plant life.

Test organisms and controls were treated alike, except that the water of the controls was changed every three days. This three day period was found to be optimum for maintaining chemical parameters constant. Organisms were given daily feedings of brine shrimp hatched in a saline solution. These were rinsed in fresh water before being introduced into the test containers. This food was suspended in a freshwater medium and distributed to the test containers with a plastic syringe. Water temperature was maintained relatively constant at 27°C ± 3°C.

Twenty experimental and twenty control Bettas were individually weighed on January 31, 1970. They were reweighed again on June 15, 1970 to check for any net growth and ultimately the causes of any variations.

After weighing, the fish were transferred to test containers filled with 800 ml of Des Moines tap water which had been

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aged for three days to normalize parameters which could cause severe shock to the introduced organisms. A supply of this aged water was kept available for use at all times during the experiment.

Compensation was made for the water removed from the experimentals during testing by refilling to the 800 ml mark with aged water. Containers were then tagged with adhesive labels (E₁-E₂₀ and C₁-C₂₀). The containers were placed upon a steel shelf, about 50 inches from the floor. Light concentration decreased progressively from the front to the back containers, producing three distinct groups.

Fluorescent ceiling lights connected to a modified Diehl (24 hour) timer produced a sixteen hour daily exposure. The test containers were approximately six feet from the light source. All containers were covered with a flat sheet of plastic to reduce excess dust and bacteria. Three different light energy levels resulted in approximation of various settings in stagnant pools.

Pilot tests (alkalinity, carbon dioxide, dissolved oxygen, total hardness, nitrate nitrogen, nitrite nitrogen and pH) were run on a bi-weekly basis from January 31 to February 10, 1970 with reagents and equipment from a portable water test kit, "Hach Direct Reading Engineer's Laboratory Model DR-EL". All pilot and regular tests were run at approximately the same time daily (between 10 A.M. and 1 P.M.). The Hach kit was judged sufficiently accurate for the tests in this study.

The actual testing was begun on February 13, 1970. Each group was followed throughout the 18 week study to check for progressive chemical changes, their eventual causes, growth effects and any other significant alterations. Containers were tested alternately to assure a more accurate picture. Initially, four *Betta* experimentals and four controls were sampled weekly. This pattern was continued until it was observed that there were no differences within the groups in the *Betta* control containers, thus the number tested weekly was decreased. The method of alternating test containers is indicated in Table 1.

TABLE 1. PATTERN OF TEST ROTATION

| WEEK | EXPERIMENTALS | CONTROLS |
|------|---------------|---------------|
| 1 | 1, 6, 11, 16 | 1, 6, 11, 16 |
| 2 | 2, 7, 12, 17 | 2, 7, 12, 17 |
| 3 | 3, 8, 13, 18 | 3, 8, 13, 18 |
| 4 | 4, 9, 14, 19 | 4, 9, 14, 19 |
| 5 | 5, 10, 15, 20 | 5, 10, 15, 20 |
| 6 | 1, 6, 11, 16 | 1, 6, 11, 16 |
| etc. | | |

All chemical data were analyzed for significant variations between experimentals and corresponding controls, utilizing computer analysis by an intercorrelation program modified to handle missing data. Analysis of weight increase of *Betas* was a comparison of experimentals and corresponding controls, and between groups in different light levels. Due to smaller quantities of data, it was possible to use the "Olivetti Underwood Programma 101" to calculate the Student's t distribution.

RESULTS AND DISCUSSION

Certain chemical parameters in experimental units varied significantly from their controls. These variations in water

chemistry exhibited extensive interaction. Figure 1 shows these parameters for Experimental Group 1 (bright light), Figure 2 for Group II (Low light), and Figure 3 for the Control Groups.

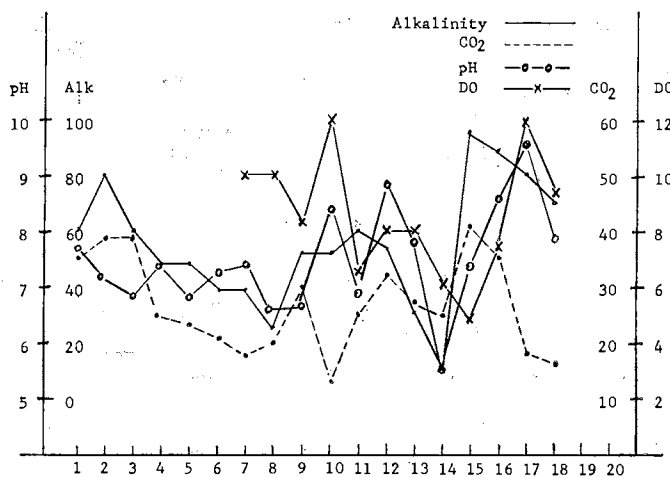


Figure 1. Interrelation of pH, DO, Alkalinity and CO₂ of experimental group 1

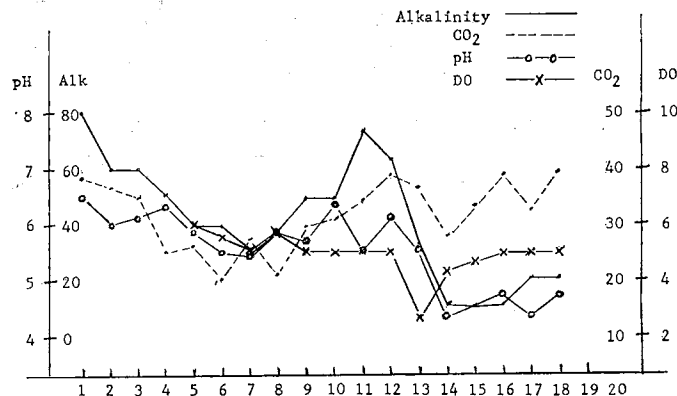


Figure 2. Interrelation of pH, DO, Alkalinity and CO₂ of experimental group 2

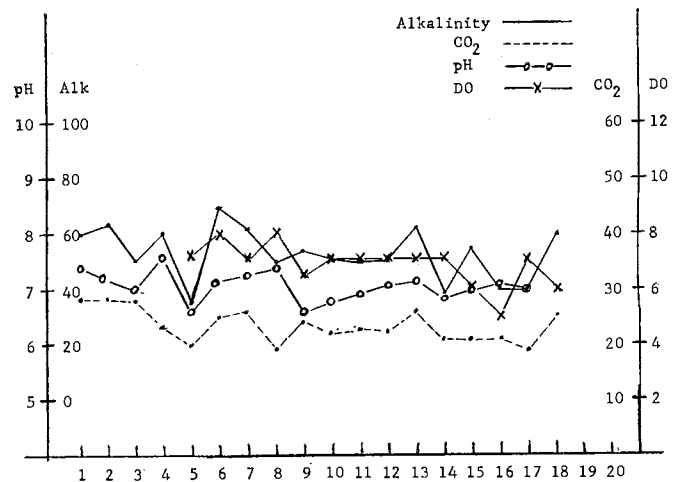


Figure 3. Interrelation of pH, DO, Alkalinity and CO₂ of control organisms

The information compiled indicated no differences in weight increase between experimentals and controls in parallel light levels. Low light levels, however, appear to be inhibitory to growth, but more information is needed to confirm this (Table 2).

It was also considered noteworthy that in group I there always seemed to be a significant rise in CO₂ and alkalinity just prior to each visual algal bloom. It may be of further importance that the cyclic peaks in water chemistry were being progressively amplified. This feature could not be checked further, since the experiment had ended.

TABLE 2. WEIGHT INCREASE ANALYSIS, EXPERIMENTALS AND CONTROLS

| Container | N | Range | Mean | S.D. | t | P |
|-----------|----|-------------|--------|------|-------|-------|
| E1 | 9 | 410-740 mgs | 537 mg | 113 | 0.721 | 0.500 |
| C1 | 7 | 220-1110 | 617 | 250 | | |
| E2 | 10 | 370-670 | 499 | 84 | 0.108 | 0.500 |
| C2 | 10 | 190-790 | 492 | 176 | | |

Chemical data in Experimental Group II also exhibited cyclic fluctuations. In this group, however, pH, alkalinity, and dissolved oxygen showed a general decline throughout the study. Alkalinity dropped from 80 mg/l to 15 mg/l, pH fell from 7.4 to 5.5 and DO descended from 5 mg/l to below 3 mg/l.

Data for the Control organisms showed moderate variations. The normal range was much more restricted with no long range cycling or significant shifts in chemistry detected.

It might be surmised from this study that organisms (*Betta splendens*) seem to be unaffected by natural water quality deterioration. Information suggests, however, inhibition in growth with lower light levels.

It may become possible to predict ensuing algal blooms in enriched closed systems, or its parallels, following analysis of cyclic chemical changes related to the alkalinity system. Such indicative changes as rises in carbon dioxide and alkalinity prior to blooms may prove useful. This might also be carried a step further in proposing ways of curtailing eutrophication, where harmful, through control of these chemical changes at critical periods. In contrast, where such lush algal growth is desired, conditions could be maintained for growth sustenance.

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