A STUDY OF SOME EFFECTS OF WATER CHEMISTRY AND LIGHT ON GROWTH OF *Betta splendens*

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INTRODUCTION AND LITERATURE REVIEW

Fish respond in various ways to pollutants in water. Bonnet (1932) found guppies to be flourishing in sewers in Guadalupe, showing extreme tolerances to various deleterious chemical parameters. Mackay (1970) found mild forms of pollution accompanying eutrophic conditions to be inhibitory to the 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 reach 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 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 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 nitrogenous end 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 NH_4^+ 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 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 the 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 analysed for significant differences. An attempt was made to correlate these differences with light and/or water chemistry.

MATERIALS AND METHODS

Organism. Siamese Fighting Fish (Betta splendens) are a Southeast Asian air breathing labyrinth fish of the family Anabantidae (Brown, 1957). The adaptive respiratory structures are shown in Figure 1.

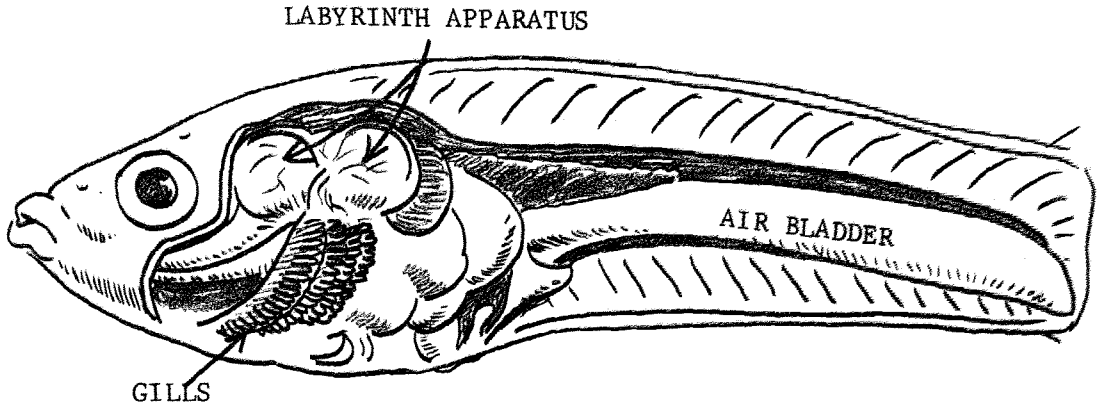

Figure 1. Respiratory apparatus, Betta splendens.

Bettas typically inhabit warm brackish waters and play a part as a food reserve in the lives of the native peoples. They are very tolerant of stagnant, mildly polluted waters. They produce large, consistent spawns, are easy to handle, economical to maintain, and small enough to permit extensive numerical studies for genetic or ecological studies in a limited space.

Teleost fishes are known to excrete nitrogenous waste products readily converted to nitrates by bacteria. Phosphates are also added to water as a by-product of respiratory metabolism. A pilot study was made for phosphates to get an idea if the quantity excreted by the Bettas would be sufficient to support an algal bloom. A single Betta was placed in a covered, acid washed container with 200 ml of three day aged tap

water. The fish was not fed over a five day testing period. The metapolyphosphate level was measured at the onset of the experiment (0.010 mg/1) and again after five days (0.015 mg/1) indicating a net increase of 0.005 mg/1. It was concluded that Bettas release small amounts of phosphate but that quantities were probably not sufficient to have a noticeable effect.

Experimental Conditions. A set-up similar to that used by Linn (1965) was used for this study, without aeration. Test containers were quart-sized, rather than gallon. Furthermore, since the organisms were in individual containers, the possibility of a disease or serious competition affecting all of the organisms 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.

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. 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 fresh water medium and distributed to the test containers with a plastic syringe. Water temperature was maintained relatively constant at $27^{o}C \pm 3^{o}C$.

Twenty experimental and twenty control Bettas were individually weighed on a balance scale on January 31, 1970. They were re-weighed on June 15, 1970 to check for growth. The organisms had to be inactivated for this purpose. This was accomplished by placing four to five

fish in a solution of MS-222, tricane methane sulfonate (Bell, 1964), until the fish became docile. They were then placed in a small plastic container (63 mm x 30 mm) filled with water and weighed. By subtracting the tare of the box and water, the weight of the organisms could be obtained.

The fish were then transferred to test containers which were filled with 800 ml of Des Moines tap water which had been aged for three days to normalize parameters which could cause severe shock to the introduced organisms. The condition of this water when first drawn and following aging can be seen in Table 1. A supply of this aged water was kept available for use at all times during the experiment.

VARIABLE	BEFORE AGING	AGED 3 DAYS
Dissolved Oxygen	7 mg/l	6 mg/l
Carbon Dioxide	0	10
Total Hardness	95	50
pH	8.5	7.2
Nitrate Nitrogen	8.5 mg/l	8.0 mg/l
Nitrite Nitrogen	0.01	0.06
Temperature	24.5°C	27.0°C

Table 1. Effects of aging tap water.

The method for changing water was to pour the contents of the container through a moist, fine mesh net, the test organism being retained in the net. The container was then cleaned with tap water and sponge, rinsed with tap water then rinsed with aged tap water. Finally, it was

refilled with aged tap water and the organism replaced.

Compensation was made for the water removed from the experimentals during testing by refilling to the 800 ml mark with aged water (150 ml). 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 in the arrangement indicated by Figure 2.

Figure 2. Arrangement of test containers.

Light concentration decreased progressively from the front to the back containers producing two distinct groups. Utilizing the Science and Mechanics Direct Current Microampere Light Meter, it was determined that the light energy levels encountered were as shown in Table 2.

GROUP	ENERGY LEVEL ENCOUNTERED
Group I	39.5 microamperes per cm^2 per min.
Group II	16.0

Table 2. Varying light levels encountered.

Fluorescent ceiling lights connected to a diehl (24 hr.) 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. Two different light energy levels resulted approximating various environmental settings in stagnant pools.

Pilot tests (alkalinity, carbon dioxide, 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 10:00 A.M. and 1:00 P.M.). The Hach kit was judged sufficiently accurate for the test run in this study (Hach Methods Manual).

> "Standard Methods for Examination of Water and Waste water, published jointly by the American Public Health Association (APHA), the American Water Works Association (AWWA) and the Water Pollution Control Federation (WPCF)... this book is the standard reference for water analysis. Most procedures contained in this manual are based on the standard methods." (Hach Methods Manual)

The actual testing was begun on February 13, 1970. Each container was followed throughout the eighteen 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 in the Betta control containers, thus the number tested weekly was decreased. The method of alternating

test containers is indicated in Table 3.

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.		

Table 3. Pattern of test rotation.

At the onset of the experimentation there were difficulties with dissolved oxygen tests, therefore, some results are missing. Chemical water parameters are compared in various ways to discern any notable differences in progressive changes in groups or individual containers.

Methods of Statistical Analysis.

1. 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.

2. Analysis of weight increase of Bettas was a comparison of Experimentals and corresponding Controls, in corresponding 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.

3. An Analysis of Variance test was then run on chemistry and light effects on weight changes of Experimentals and Controls.

The Null Hypothesis stated that there would be no difference in the weight gains of Experimentals and Controls. Should the null hypothesis be rejected, an attempt would be made to find other differences and factors responsible.

A "P" value of 0.05 or less for t-tests was taken as sufficient grounds for rejecting the null hypothesis and accepting the research hypothesis that a significant difference did exist between the means of the Experimentals and Controls, etc. If in fact, no difference existed between the means in the populations from which the Experimentals and Controls were drawn, application of the 0.05 criterion would result in rejecting the null hypothesis 5% of the time (Snedecor, 1959).

Because the research hypothesis generally states that a difference exists without signifying the direction of the difference, two-tailed tests for the significance of the differences were used. Generally, a t value greater than 2.0 indicates a significant variation between the two groups being compared, enough so to reject the null hypothesis.

RESULTS

Upon visual appearance of algal blooms in Experimental group I (in weeks 7, 10, 12 and 17) specific types were identified. It was observed that Chlorella vulgaris predominated, with an interspersion of Volvox platydorina (Prescott, 1964). Neither Experimental group II nor any of the Control groups showed algal growth at any time throughout the study.

The overall results of chemical testing are shown in Appendix Table 1 (p. 23-28) indicating progressive changes throughout the study. Statistical comparisons of chemical parameters were made between Experimental and Control groups in similar light intensities (Tables 4 and 5).

Results	Alk. mg/l	Bacteria no./ml	CO_2 mg/l	Total Hardness mg/l	DO mg/l	NO_3^--N mg/l	NO_2^--N mg/l	Test Group
N_1	10	10	10	10	9	10	10	E1
Mean	45.0	8.19×10^6	18.2	135.7	5.2	12.1	0.18	E1
S.D.	13.2	3.64	2.8	6.8	1.6	2.6	11.7	E1
Range	21-68	2.30×10^5 - 1.10×10^7	13-24	127-149	3-8	8-16	.07-.44	E1
N_2	8	4	7	8	8	8	8	C1
Mean	54.9	6.15×10^6	14.0	105.1	5.1	7.5	0.14	C1
S.D.	7.6	2.96	1.9	9.8	0.8	1.3	11.4	C1
Range	45-70	3.01×10^6 - 1.10×10^7	11-17	94-118	4-6	6-9	.04-.37	C1
t	1.878	0.971	3.508*	7.018*	0.151	4.565*	0.545	
P	0.100	0.400	0.005	0.001	0.500	0.001	0.500	

Table 4. Comparison of water chemistry of Group I Experimentals and Controls.

Results	Alk. mg/l	Bacteria no./ml	CO_2 mg/l	Total Hardness mg/l	DO mg/l	NO_3^--N mg/l	NO_2^--N mg/l	Test Group
N_1	10	10	10	10	10	10	10	E2
Mean	41.3	6.96×10^6	21.3	141.8	3.6	12.6	0.19	E2
S.D.	9.0	3.95	1.5	9.5	0.7	2.8	1.07	E2
Range	23-56	7.80×10^5 - 1.11×10^7	19-24	127-160	2-4	8-18	.08-.38	E2
N_2	10	4	10	10	8	10	10	C2
Mean	53.1	3.74×10^6	14.7	112.8	5.6	5.9	0.12	C2
S.D.	12.8	4.33	3.0	17.7	0.5	1.1	0.96	C2
Range	20-65	6.90×10^5 - 1.10×10^7	8-20	98-160	5-6	5-8	.07-.40	C2
t	2.257*	1.138	5.965*	4.329*	7.056*	6.580*	1.480	
P	0.025	0.200	0.001	0.001	0.001	0.001	0.200	

Table 5. Comparison of water chemistry of Group II Experimentals and Controls.

The results of weight changes in Experimental Bettas and Controls, throughout the study, can be seen in Tables 6 and 7 respectfully. The organisms are further designated as to sex and critical light groups.

Organism	1/31/70	6/22/70	Wt. Inc.	Sex	Lt. Conc.
1	100 mgs	510 mgs	410 mgs	F	1
2	50	790	740	M	1
3	60	610	550	M	2
4	120	570	450	F	2
5	80	630	550	F	1
6	30	470	440	F	1
7	90	540	450	F	2
8	120	530	410	F	2
9	90	770	690	F	1
10	110			M	1
11	90	670	580	F	2
12	120	660	540	F	2
13	60	570	510	F	1
14	60	680	620	F	1
15	90	590	500	F	2
16	120	590	470	M	2
17	100	530	430	F	1
18	130	580	450	F	1
19	100	770	670	M	2
20	110	480	370	F	2

Table 6. Weight increases of Experimental Bettas.

Organism	1/31/70	6/22/70	Wt. Inc.	Sex	Lt. Conc.
1	90 mgs	Died 6/16/70		F	1
2	80	Died 6/14/70		F	1
3	60	550	490 mgs	F	2
4	40	400	360	F	2
5	110	330	220	M	1
6	100	670	570	M	1
7	70	790	720	F	2
8	120	310	190	F	2
9	80	720	640	M	1
10	90	750	660	F	1
11	120	710	590	F	2
12	110	670	560	F	2
13	160	1270	1110	F	1
14	110	790	680	F	1
15	150	650	500	F	2
16	200	640	440	M	2
17	110	Died 6/14/70		F	1
18	120	560	440	M	1
19	110	900	790	F	2
20	320	600	280	F	2

Table 7. Weight increases of Control Bettas.

DISCUSSION AND CONCLUSIONS

The information summarized in Table 8 shows no significant weight changes between Experimentals and Controls in Group I (high light) or II (low light). Thus the null hypothesis was accepted. Graphic representation of changes in weights can be seen in Figure 3.

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		

Table 8. Weight increase analysis, Experimentals and Controls.

This suggests that Bettas are quite tolerant of self induced water quality deterioration, or this chemical situation has not reached a critical threshold inhibitory to normal growth.

A two way analysis of Variance test, using weight as the dependent variable and light and chemistry as independent variables, revealed no highly significant variations in growth rate (Table 9). The F-ratio of 2.019 is borderline significant, suggesting a trend toward this effect. More tests are obviously needed to confirm such a suggestion.

Further tests on the effects of light on weight increase were run on Experimental groups I and II as well as Control groups I and II. The results of these tests (Table 10, p. 17) indicate no significant

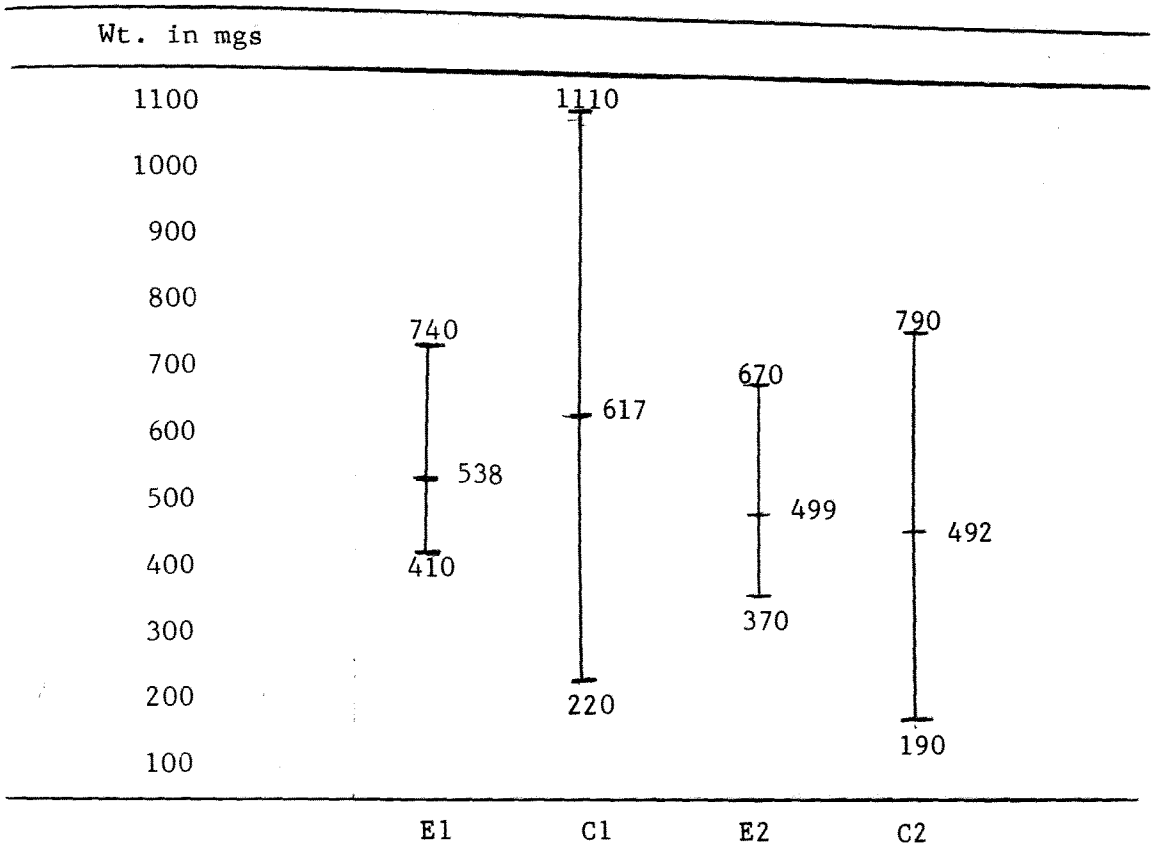

Figure 3. Range and mean values of weight increases for Bettas.

Group	Mean Wt. Inc.	F-ratio
Light (I)	577.5 mgs	2.019
Dark (II)	495.5	
Exp. (Variable Chem)	518.4	0.393
Cont. (Constant Chem)	554.6	

Table 9. Analysis of Variance test: effects of light and chemistry on weight increase of Bettas.

light influenced weight changes.

Group	N	Range	Mean	S.D.	t	P
E1	9	410-740 mgs	537.7 mgs	114	0.791	0.450
E2	10	370-670	499.0	84		
C1	7	220-1110	617.1	250	1.060	0.300
C2	10	190-790	492.0	176		

Table 10. Weight increase analysis by light levels.

Although chemical parameters revealed no significant effects on weight increase of Bettas, they were analyzed for interactions and patterns of differentiation. Significant variations did exist between CO_2, total hardness and nitrates in both light groups. Significant chemical variations between Experimentals and Controls in alkalinity and dissolved oxygen showed up only in low light groups (II).

In both Controls the oxygen levels were maintained relatively constant (5.3 mg/1 $\pm$ 0.3). In the high light Experimental group algal growth developed, thus presenting a source of oxygen renewal (Tables 4 and 5). In the reduced light levels of Experimental group II through the processes of decomposition and various oxidizing processes oxygen level was reduced with no source of renewal.

The higher alkalinity values appear to be correlated with high light concentrations. E1 mean alkalinity was 45 mg/1 (Range 21-68 mg/1), while group II Experimental was 41 mg/1 (Range 23-56 mg/1). Mean alkalinity for Control I was 55 mg/1 (Range 45-70 mg/1), while Control II was 53 mg/1 (Range 20-65 mg/1).

Further analysis of water chemistry revealed some interesting interrelations by light concentrations. In group I Experimentals (Figure 4), after a period of seven weeks, visual (as previously defined) algal blooms occurred in cyclic patterns. It also appears that certain chemical conditions had to be met in order for a bloom to occur and maintain itself. In general, alkalinity, pH and dissolved oxygen levels seemed to parallel these blooms very closely, while, as would be expected, CO_2 levels fell. King (1969) noted similar changes related to the alkalinity system.

Alkalinity and pH followed this cyclic pattern, but with each successive bloom both continued to rise significantly higher. Conversely, between each successive bloom, dissolved oxygen, alkalinity and pH fell even lower.

In Experimental group II (Figure 5) dissolved oxygen levels fell from 5 mg/1 to 2 mg/1 while pH showed a decline from 7.5 to 5.6. This is interpreted as a sign of progressive change due to the presence of the fish and without the benefit of photosynthesizing organisms.

Data for the Control organisms (Figure 6, p. 20) showed moderate variations, as might be expected. The normal range was much more restricted with no long range cycling or significant shifts in chemistry detected.

From these interactions it may become possible to predict ensuing algal blooms in enriched, closed systems following analysis of cyclic chemical changes. 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 algal blooms,

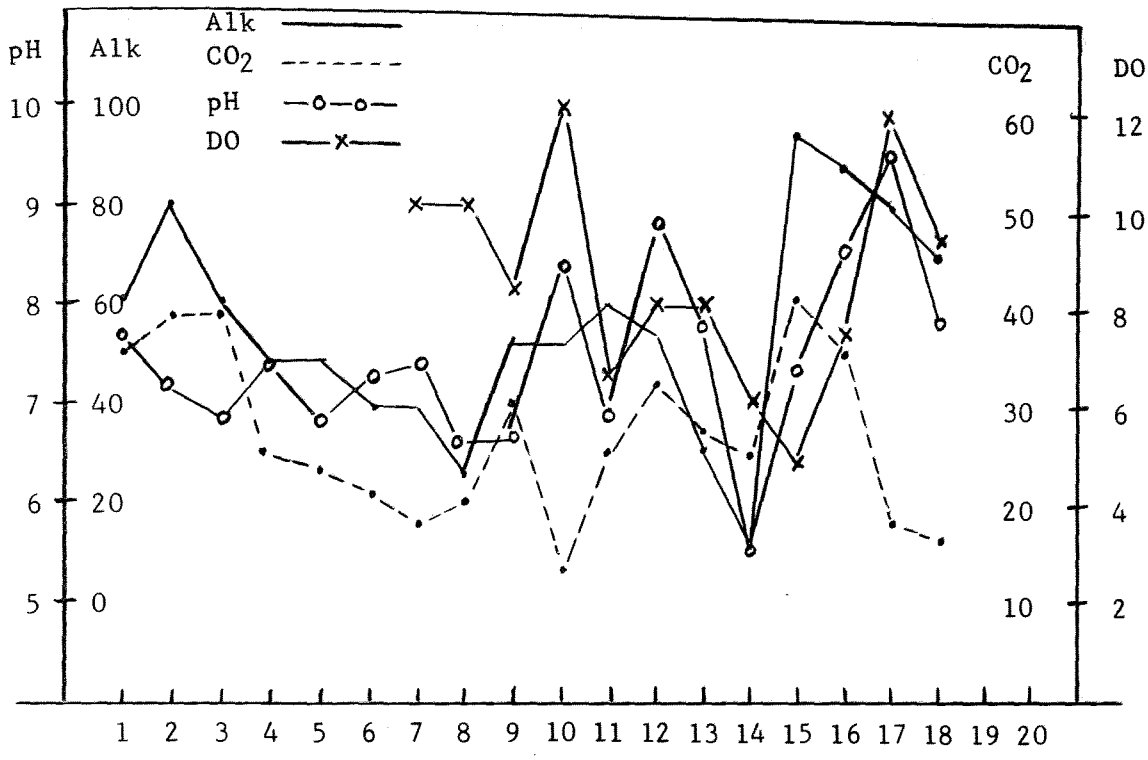

Figure 4. Interrelation of pH, DO, Alkalinity and CO_2 of Experimental Group I.

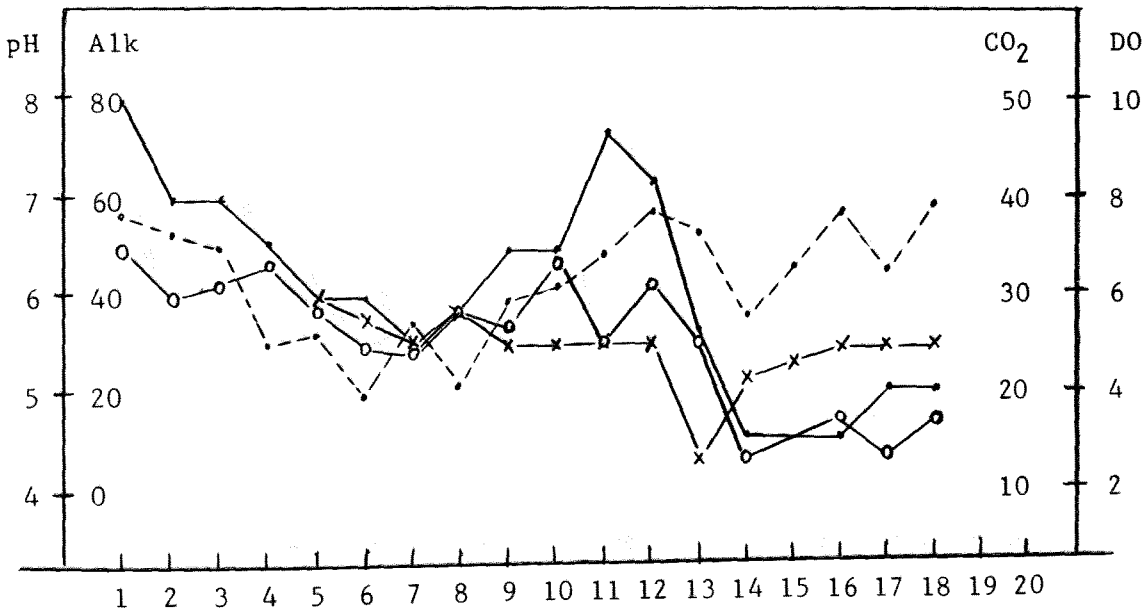

Figure 5. Interrelation of pH, DO, Alkalinity and CO_2 of Experimental Group II.

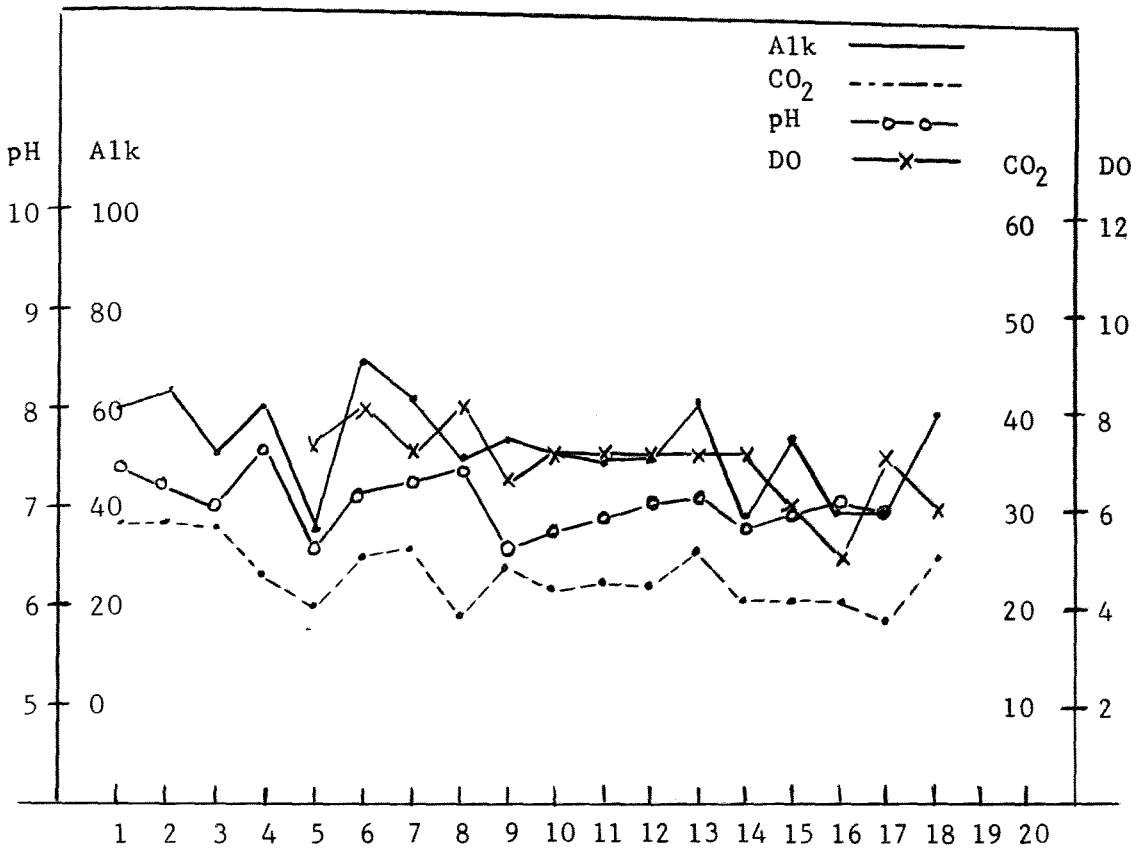

Figure 6. Interrelation of pH, DO, Alkalinity and CO_2 of Control organisms.

where undesirable, through control of these chemical changes at critical periods.

It becomes apparent that weight increases can not be correlated with the parameters measured (light and chemical variations). Either the wrong influential parameters were selected, or more extensive studies with the same parameters are needed. At any rate, it can be suggested that changing of aquarium water or other regular renewal of water is unnecessary for Betta splendens to exhibit normal growth rates. Further studies should elucidate the effects of borderline significant light influence.

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APPENDIX

Container	Week	Temp. °C	Lt. Conc.	Alk. mg/l	Bacteria no./ml	CO_2 mg/l	DO mg/l	Total Hardness mg/l	NO_3^--N mg/l	NO_2^--N mg/l	pH
E1	1	28.0	1	60		24		120	6	.16	7.5
E1	4	24.0	1	50	1.05×10^7	16		170	9	.02	7.3
E1	9	26.0	1	50	1.11×10^7	20	5	140	14	.14	6.8
E1	14		1	15		14	4	100	20	.20	5.2
C1	1	28.0	1	60		20		110	5	.03	7.4
C1	4	24.0	1	60	5.76×10^6	14		120	8	.07	7.6
C1	9	27.0	1	50		16	5	100	10	.11	6.7
C1	14	23.0	1	35		12	5	110	12	.01	7.4
E2	5	25.0	1	50	1.37×10^5	16	4	120	10	.14	6.9
E2	10	29.0	1	50	3.31×10^5	24	5	140	10	.06	7.3
E2	15	28.0	1	20		32	3	140	31	.03	5.6
E2	18	31.0	1	20		24	4	130	22	.09	5.4
C2	5	26.0	1	30		12	4	150	7	.22	6.6
C2	10	27.5	1	55	4.86×10^6	12	5	100	5	.03	7.4
C2	18	30.5	1	60		16	4	100	14	.35	6.9
E3	2	29.0	2	80		24		160	19	.60	7.0
E3	6	29.0	2	40		16	5	150	8	.22	7.5
E3	11	28.0	2	85	8.60×10^5	32	5	150	9	.08	7.2
E3	16	25.5	2	20		24	2	145	21	.15	6.6
C3	2	27.0	2	60		20		110	6	.08	7.2
C3	6	29.5	2	80		16	6	110	6	.30	7.0
C3	11	28.0	2	50	6.87×10^5	12	5	95	5	.02	6.5

Appendix Table 1. Results of chemical tests of Experimental and Control containers.

Container	Week	Temp. °C	Lt. Conc.	Alk. mg/l	Bacteria no./ml	CO_2 mg/l	DO mg/l	Total Hardness mg/l	NO_3^--N mg/l	NO_2^--N mg/l	pH
E4	7	29.5	2	30		16	4	130	23	.05	6.5
E4	12	26.0	2	60	1.10×10^7	28	4	150	2	.40	7.0
E4	17	28.0	2	20		22	4	160	13	.04	5.4
C4	2	27.0	2	60		20		110	6	.08	7.2
C4	7	29.0	2	60		16	5	105	9	.08	6.9
C4	12	26.5	2	50	1.10×10^7	12	5	110	2	.04	7.0
E5	3	28.0	1	60		28		130	5	.02	6.9
E5	8	27.0	1	25	7.03×10^6	10	8	120	14	.09	7.2
E5	13	27.5	1	30		16	6	130	8	.95	7.8
C5	3	28.0	1	70		20		100	4	.40	7.0
C5	8	27.0	1	50		8	6	100	6	.02	7.3
C5	13	28.0	1	60		16	5	100	6	.05	7.2
C5	16	26.0	1	40		12	3	75	9	.16	7.1
E6	1	28.0	1	60		24		120	6	.16	7.3
E6	4	24.0	1	50	1.02×10^7	16		170	9	.02	6.9
E6	9	26.0	1	50	1.10×10^7	20		140	14	.14	6.8
E6	14	23.0	1	15		14	3	130	20	.02	5.6
C6	1	28.0	1	60		18		110	6	.03	7.4
C6	4	24.0	1	60		12		100	6	.35	7.5
C6	15	28.0	1	60		12	4	90	15	.40	6.8

Appendix Table 1 (continued).

Container	Week	Temp. °C	Lt. Conc.	Alk. mg/l	Bacteria no./ml	CO_2 mg/l	DO mg/l	Total Hardness mg/l	NO_3^--N mg/l	NO_2^--N mg/l	pH
E7	5	25.0	2	50	7.35×10^5	16		120	6	.18	6.8
E7	10		2	60	1.04×10^7	24		130	15	.04	
E7	15	28.0	2	20		28	2	140	30	.08	5.7
E7	18	30.0	2	20		20	4	150	21	.03	5.4
C7	5	26.0	2	40		12	6	130	5	.08	6.5
E8	2	27.0	2	60		26		135	17	.40	7.0
E8	6	29.0	2	40		12		130	11	.22	6.5
E8	11	27.5	2	70	8.84×10^5	24		130	10	.48	5.9
E8	16	26.0	2	15		28	4	145	23	.08	5.6
C8	2	27.0	2	60		20		110	10	.05	7.2
C8	6	29.5	2	70		14	6	100	5	.14	7.0
E9	7	29.5	1	40		8	8	130	18	.03	7.3
E9	12	26.0	1	50	1.10×10^7	24	6	160	6	.40	8.9
E9	17	28.0	1	80		8	10	140	9	.22	9.5
E10	3	28.0	1	60		28		130	6	.06	7.1
E10	8	27.0	1	25	6.40×10^6	8		130	15	.07	6.7
C10	3	28.0	1	60		20		100	4	.40	7.3
C10	15	28.0	1	50		12	4	90	15	.40	6.8
C10	17	27.5	1	40		8	5	80	6	.32	7.0

Appendix Table 1 (continued).

Container	Week	Temp. °C	Lt. Conc.	Alk. mg/l	Bacteria no./ml	CO_2 mg/l	DO mg/l	Total Hardness mg/l	NO_3^--N mg/l	NO_2^--N mg/l	pH
E11	1	28.0	2	60		24		120	7	.18	7.4
E11	4	24.0	2	40		12		150	7	.01	7.2
E11	9	26.0	2	50	1.10×10^7	20		140	13	.14	6.9
E11	13	28.0	2	30		24	3	150	12	.02	5.8
C11	1	28.5	2	60		18		110	8	.03	7.4
C11	3	24.0	2	60		12		100	4	.14	7.2
E12	5	25.0	2	40	7.97×10^5	16	5	120	10	.15	6.8
E12	10	29.0	2	50	1.03×10^7	22		140	11	.06	7.3
E12	14	23.0	2	15		16	3	150	10	.07	5.3
E12	18	30.0	2	20		28	4	140	18	.03	5.8
C12	5	26.0	2	20		8	6	160	5	.07	6.6
E13	2	27.0	1	80		24		140	16	.30	7.1
E13	6	29.0	1	40		12		130	7	.07	7.2
E13	11	28.0	1	60	1.11×10^7	16	4	130	8	.50	6.9
E13	15	28.0	1	90		25	3	110	27	.90	7.7
C13	2	27.0	1	70		18		110	6	.07	7.2
C13	6	29.5	1	70		16	6	110	6	.03	7.2
E14	7	29.5	1	50		20		135	20	.34	6.6
E14	12	26.0	1	20	1.09×10^7	16		160	6	.05	6.3
E14	16	26.0	1	15		24	3	150	18	.08	5.5
E14	17	28.0	1	20		20	4	140	11	.03	5.4

Appendix Table 1 (continued).

Container	Week	Temp. °C	Lt. Conc.	Alk. mg/l	Bacteria no./ml	CO_2 mg/l	DO mg/l	Total Hardness mg/l	NO_3^--N mg/l	NO_2^--N mg/l	pH
E15	3	28.0	2	60		24		130	6	.01	7.1
E15	8	27.0	2	40	8.83×10^6	12		130	14	.04	7.0
E15	13	28.0	2	30		28	2	140	11	1.10	5.6
C15	3	28.0	2	55		20		105	5	.40	7.3
E16	1	29.0	2	80		28		130	7	.08	7.5
E16	4	24.0	2	50		14		150	8	.05	7.3
E16	9	27.0	2	50	1.10×10^7	20	4	130	14	.28	6.8
E16	14	23.0	2	15		20	3	130	15	.05	5.4
C16	1	28.0	2	50		18		110	8	.03	7.4
C16	4	24.0	2	60		12		120	2	.08	7.5
C16	9	27.5	2	60		12	4	100	10	.20	6.7
C16	14	23.0	2	40		12	5	80	11	.01	7.4
E17	5	25.0	1	50	1.39×10^6	14		120	11	.20	6.8
E17	10	29.0	1	50	4.38×10^6	4	10	140	4	.05	8.4
E17	15	28.0	1	99		32	4	120	10	.25	7.3
E17	18	31.0	1	50		8	7	150	6	.02	7.9
C17	5	26.0	1	40		10	6	140	6	.03	6.6
C17	10	27.5	1	50	3.01×10^6	12	5	95	8	.05	7.0

Appendix Table 1 (continued).

Container	Week	Temp. °C	Lt. Conc.	Alk. mg/l	Bacteria no./ml	CO_2 mg/l	DO mg/l	Total Hardness mg/l	NO_3^--N mg/l	NO_2^--N mg/l	pH
E18	2	28.0	1	80		24		140	9	.40	7.1
E18	6	29.0	1	30		12	6	140	6	.05	7.0
E18	11	28.0	1	30	1.05×10^7	12	5	135	13	.06	5.7
E18	16	28.0	1	10		20	4	150	16	.06	5.6
C18	2	27.0	1	70		20		115	8	.08	7.3
C18	6	29.5	1	60		14	6	110	5	.03	7.5
C18	11	27.5	1	50	1.10×10^7	14	5	90	6	.01	6.7
E19	7	29.5	2	30		20	4	140	20	.20	6.8
E19	12	26.5	2	20	1.10×10^7	16	5	150	10	.10	6.3
E19	17	28.0	2	20		20	4	160	16	.10	5.5
C19	7	29.0	2	70		16	5	105	9	.10	7.0
C19	12	26.5	2	50	1.67×10^5	12	5	115	0	.02	7.0
E20	3	28.0	2	60		24		130	2	.06	7.2
E20	8	27.0	2	35	4.05×10^6	12	5	120	14	.02	7.0
E20	13	28.0	2	30		24	2	130	9	.90	6.5
C20	3	28.0	2	60		20		110	3	.40	6.9
C20	8	27.5	2	50	3.12×10^6	10	6	85	6	.02	7.1
C20	13	28.0	2	65		16	5	100	6	.02	7.2

Appendix Table 1 (continued).