Volume per fish ratio effect on growth in <u>Lebistes reticulatus</u> (guppy) and pH as a control mechanism.

## A Research Paper

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# Table of Contents

Introduction		
Materials and Methods		
Results	Page	5
Discussion		
Summary	Page	15
Bibliography	Page	16

### Introduction

When considering factors limiting the growth of fish, it becomes obvious that no single factor is ever operating alone, but rather a combination of factors delicately interwined. Since fish are pokiliotherms, temperature obviously affects metabolism and hence growth rates. Diet, genetic factors, light, water movements, and growth hormones are all environmental factors affecting growth. Two others, the subject of this experiment, are space factors and pH.

This experiment was designed to test what effects, varying space factors had upon the growth of <u>Lebistes reticulatus</u> (guppy). Anyone raising tropical fish discovers that his fish grow more slowly when crowded and grow to a larger size in a larger tank. Comfort (1964), working with <u>Salmo trutta</u> (brown trout), stated that there are specific sizes characteristic of each size container and each level of nutrition, or alternately, of each population-density in a tank. He discovered that when a fish is moved from one such container to a larger, or when fish are removed from a tank population, a new size plateau is rapidly reached.

Brown (1960) grew groups of two year old <u>Salmo trutta</u> in tanks of different sizes, with different numbers of individuals. She found that very crowded fish (3 liters per fish) and uncrowded fish (50 liters per fish) grew more slowly than fish with 12, 23, or 35 liters per fish. Apparently there can be too much as well as too little space for

optimim growth.

No known research has been done with <u>Lebistes reticulatus</u> in this regard, and since growth rates and physical requirements vary widely among species of fish, research seemed appropriate.

Several mechanisms suggest themselves as possible explanations as to exactly how space affects growth. A subtle neurosensory control of the endocrine system might be operating, so that a fish "seeing" itself or "feeling" itself crowed might produce less growth hormone or might have a modified metabolism. Simpler to test, the pH as affected by number and size of fish per unit space, might identify a specific factor.

As concerns pH requirements, Brown (1957, p. 395) stated, "no one has yet demonstrated unequivocably that fish growth rates are directly affected by the ionic composition of the water, although evidence points in that direction." Suggested pH limitations, up to the present have been based on best breeding conditions and on mortality, rather than on growth rates.

### Materials and Methods

<u>Lebistes</u> <u>reticulatus</u> (guppy) was chosen for this experiment because it is both hardy and available in large numbers in the tanks at St. Meinrad College.

The 400 fry selected were between 7 mm. and 9 mm. in length. For measuring the fish, a piece of graph paper calibrated in millimeters, was mounted on a small board, covered with a piece of clear plastic, and the edges sealed with masking tape. A drop of water was placed on the plastic and a single fish placed in the drop of water. When all gross movement had stopped, the fish was maneuvered into place with a wet camels-hair artist's brush. A hand lens was used for greater accuracy. The measurement, known as the standard length, was taken from the tip of the snout to the end of the spinal column, the tail excluded since it varies widely.

The fish were measured on four occasions: at the start of the experiment, January 28, 1973; at the time of the first pH correction, February 9, 1973; at approximately the half-way point, April 2, 1973; and at the conclusion, May 20, 1973.

The fish were fed twice daily with a food prepared in the biology laboratory, consisting of powdered blood, Pablum, and ground CO-OP Thermo-Studed Dog Food. The mixture was combined in a blender.

Metal framed, glass aquaria were used for the experiment, one each of 2 gallon, 5 gallon, 10 gallon, and 20 gallon capacities in a control series and in an experimental

series. One half inch of clean, white sand was placed on the bottom of each tank.

In order to simplify controlled conditions, plants were omitted, but the tanks were artificially aerated. Glass plates were used to cover each tank to minimize evaporation, but distilled water had to be added periodically. Artificial light was provided from 8:00 am to 5:00 pm by 15 w florescents regulated by a timer. Since the temperature of the biology laboratory remained relatively constant, no heaters were needed.

At the end of each week the pH was measured with a Coleman Metrion (model 28AC) pH meter. This model reads on a scale of 0.0 pH to 14.0 pH. The control tanks were regulated weekly to a pH of 7.0. Sodium Biphosphate (Na<sub>2</sub>HPO<sub>4</sub>) was used as an acidifying agent. The experimental tanks were not regulated, but were measured and recorded.

# Results

The pH was measured and recorded, in general, at weekly intervals. The control tanks (series A) were adjusted to a neutral 7.0 pH. The experimental tanks (series B) were allowed to fluctuate. Table 1 indicates the pH measurements.

Table 1. pH values.

Week #	2 gallon tanks		5 gallon tanks		10 gallon tanks		20 gallon tanks	
	A	В	A.	В	$\mathbf{A}_{\cdot,\cdot}$	В	<b>A</b>	В
3	8.5	8.6	8.4	8.5	. 8.4	8.5	8.6	8.5
4	7.6	8.2	7.2	7.5	7.2	8.1	7.5	7.5
5	7.4	8.8	7.1	8.2	7.3	8.4	7.2	7.9
7	7.3	8.8	7.4	8.4	7.5	8.3	7.3	8.4
8	7.2	8.8	7:2	8.3	7.1	8.6	7.1	8.6
9 .	7.4	8.9.	8.4	8.6	7.2	8.5	7.4	8.5
10	7.3	8.8	7.1	8.5	7.2	8.5	7.2	8.4
11	7.3	8.9	7.3	.8.6	7.3	8.6	7.1	8.5
13	7.6	9.0	7.1	8.4	7.5	8.8	7.4	8.7
14	7.2	8.8	7.2	8.4	7.1	8.7	7.3	8.6
15	7.2	8.9	7.2	8.5	7.3	8.6	7.4	8.5
17	7.3	8.8	7.4	8.6	7.4	8.8	7.5	8.7

During the experiment each fish was measured four times and the lengths recorded. The average lengths for each tank appear in Table 2, and accumulative growth during the experiment is shown in Figures 1 and 2.

Table 2. Average lengths and amount of growth (mm.).

Tank #	0_	Wee	k # 10	17	Amount of Growth
2A	7.6	7.8	8.7	10.1	2.5
2B	7.5	7.6	8.1	9.5	2.0
5A	7.3	7.9	9.9	11.6	4.3
<b>5</b> B	7.5	8.0	8.6	11.4	3.9
10A.	7.6	8.0	13.5	16.2	8.6
10B	7.5	8.0	11.8	13.6	6.1
20A	7.4	7.9	10.4	14.9	7.5
20B	7.8	8.1	9.3	13.2	5.4

Figure 1. Accumulative growth, control vs. experimental, for the three growth periods.

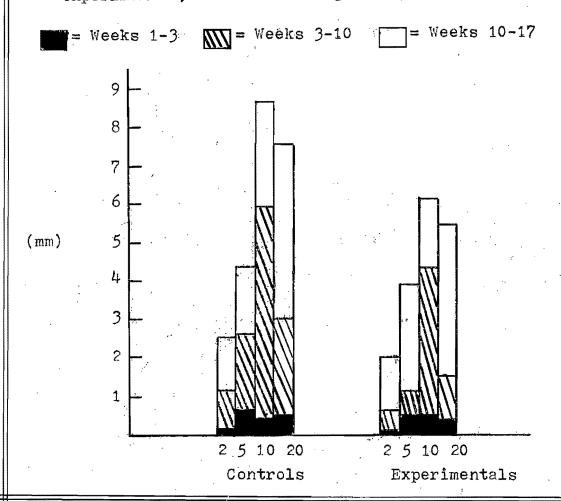
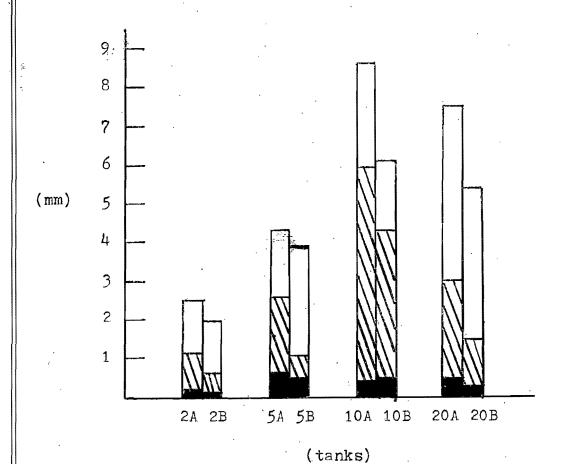


Figure 7. A comparison of accumulative growth of all controls and all experimentals for the three growth periods.

= Weeks 1-3 | | | = Weeks 3-10 | | = Weeks 10-17



The percentage growth per growth period as affected by mortality is presented in Table 3.

Table 3. Number of fish and percent of total growth per growth period.

	We	eks 1-3	Weeks 3-10_		Week	s 10-17
Tank #	# of fish	% total growth	# of fish	% total growth	# of fish	% total growth
2A	50	8	47	36	43	56
2B	50	5	46	25	40	70
5A	50	14	49	46	48	40
5B	50	13	50	15	42	72
10A	50	5	50	64	50	31
10B	50	8	50	62	50	30
20A	50	7	50	33	46	60
20B	50	6	47	22.	44	72

As a fish grows, the space available to it decreases.

Volume per unit length of fish (Table 4) rather than volume

per number of fish or surface area per fish appeared to yield

useful data.

Table 4. The number of cubic centimeters of water per millimeter of fish at the end of each growth period.

Tank #	3_	Week #	<u>-17</u>
2A	19	19	17.
2B	20	20	20
<b>5</b> A	48	39	34
5B	47	44	40
10A	95	58	47
10B	95	64	56
20A	192	146	110
20B	187	173	130

Growth rates are compared in Figures 3 and 4. The actual measurements for each growth period are plotted.

Figure 3. Growth rates for control tanks. (series A).

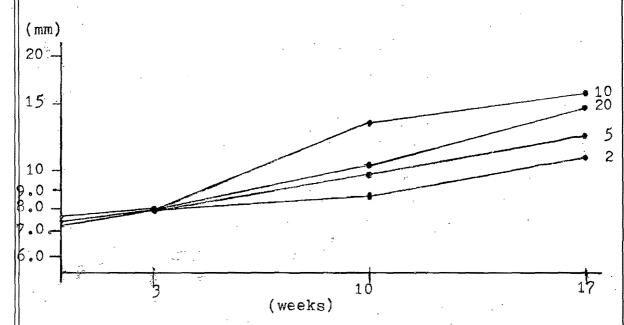
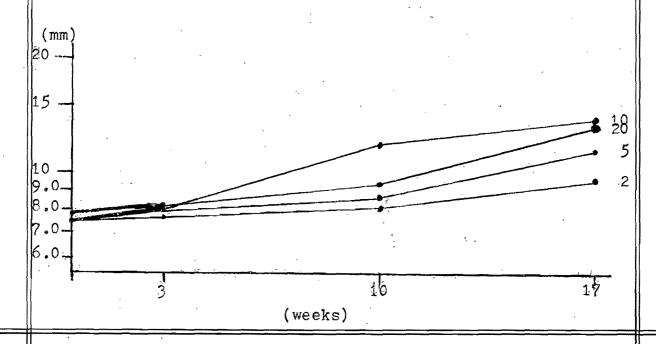


Figure 4. Growth rates for experimental tanks. (series B).



#### Discussion

The pH in the control tanks invariably increased after weekly adjustment to neutral, to an alkaline condition (Table 1). The experimental tanks also became and remained alkaline, each tank fluctuating up and down. No meaningful pattern was observable. The two gallon sizes were the most alkaline throughout, while all others maintained similar pH values.

This increase in pH is at least partially explainable by three factors. 1) Brown (1960) reports that the excretion of most tropical fish is alkaline in nature. While no quantitative data are available for the guppy either in the literature or from this experiment, this factor should be considered. 2) It was discovered that when food was placed in 2, 5, and 10 gallon aquaria containing water only, the pH rose and remained at 7.3 - 7.3 pH throughout a 24 hour period. The fish were fed twice a day and always had as much as or more than they could eat. 3) In 2 and 5 gallon tanks with water and sand only, the pH rose to 7.1 - 7.2 pH in a 24 hour period.

The effect of the pH was most clearly evident in the 10 and 20 gallon sizes (controls vs. experimentals) (Table 2; Figure 1). It is assumed that its effect was operating in a similar manner in the 2 and 5 gallon sizes, but it is impossible to determine to what extent since the volume restrictions produced extreme stunting.

Mortality was highest in tank 2B where 10 fish died.

Only in the ten gallon tanks were there no mortalities. As expected an increase of growth occurred in the smaller sizes when some fish died. The rate leveled off again when the new population had reached its maximum attainable growth (Tables 3 and 4).

Each control tank grew more than its experimental counterpart (Table 2; Figure 1). This was most evident in the ten gallon size where the controls grew 2.5 mm. more than the experimentals and in the twenty gallon size where the controls grew 2.1 mm. more than the experimentals. This was less obvious in the two and five gallon sizes where the controls exceeded the experimentals by only 0.5 mm. and 0.4 mm. respectively. The two and five gallon sizes were much too small, leading to extreme stunting.

The fish in tank 10A attained the greatest average growth, (8.6 mm.), followed by tanks 20A and 10B, (Table 2 and Figure 1). It would seem then that the optimum size in this experiment was ten gallons, although the fish in the twenty gallon size grew almost as much. The difference in total growth between tanks 10A and 20A was 1.1 mm. and between tanks 10B and 20B was 0.7 mm. This would indicate that the size difference was somewhat less important than the pH difference, for these sizes. However, in the two and five gallon sizes the reverse was true. The difference between tanks 5A and 2A was 1.8 mm., and between 5B and 2B it was 1.9 mm., indicating that the size factor was dominant in

these sizes.

In the ten gallon size, both control and experimental, the initial growth was rapid, (3-10 weeks), decelerating in the later period, (10-17 weeks, Figure 3 and 4). The total growth rate curves were similar in experimental tanks 20, 5, and 2; the initial growth rate was slower but accelerated in the later period (10-17 weeks). The same pattern was true for the control tank 2. Growth rates for the five and twenty gallon sizes of control tanks were nearly constant through the entire experiment.

For tanks 10A and 10B, and even for 5A, the greatest percentage of growth occurred in the 3-10 week period (Table 3; Figure 1). For all other tanks the greatest percentage of growth occurred in the 10-17 week period. In fact, the fish in the ten gallon tanks grew about two thirds of their total length in the 3-10 week period, about one third or less of their total length in the 10-17 week period; all others, except the five gallon control tank, grew two thirds of their total length during the 10-17 week period.

There seemed to be a wide divergence between percentages per period in tanks 2A and 2B. This was at least partially due to the fact that only 4 fish died in the 10-17 week period in 2A, while 6 fish died during the same period in 2B. A difference of two fish may not be significant enough to explain a difference of 14% in the 10-17 week period. However, the smaller the volume of water per fish, the greater the influence of a single fish on the population as

a whole. In other words, the death of one fish in the two gallon tank has more of an effect on the population than a death in, for example, a twenty gallon tank.

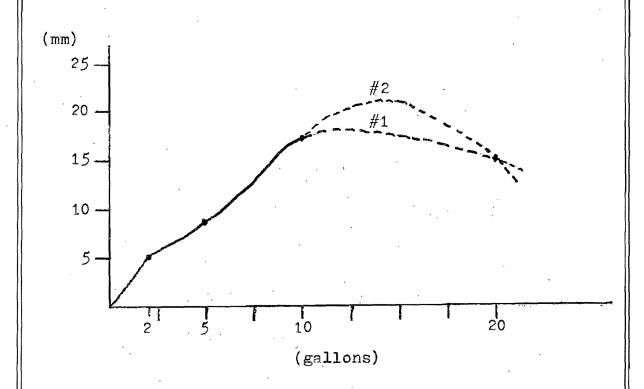
Similarly, there was a wide divergence between the five gallon sizes; a difference of 32% for the 10-17 week period. Once again this is partially explainable by the fact that eight fish died in tank 5B during that period while only one fish died in 5A for the same period. This divergence can be observed more graphically in Figure 1.

It would seem that there is an optimum volume per unit length ratio for Lebistes reticulatus. In this experiment, that of the ten gallon tanks, 47 cc of water per mm. of fish, appeared to be the optimum (Table 4). Apparently this ratio becomes more ideal with an increase of volume, up to a point, where it begins to decrease. The ten gallon tanks more closely approximates this optimum than did the others. From the data, the lower limit of this ratio was more certain. The difference in growth between tanks 5A and 10A (a difference in volume of only five gallons) was 4.3 mm. The upper limit was less certain, however; the difference in growth between tanks 10A and 20A (a difference in volume of 10 gallons) was only 1.1 mm. It is impossible to be certain whether the ten gallon size (or more precisely the 50 cc/mm ratio) is indeed the optimum, or whether the optimum lies between the 50 cc/mm and the 120 cc/mm ratios.

The following figure suggests two possibilities. 1) If the actual growth curve resembles #1, then the 50 cc/mm

ratio is very near the optimum. It could be concluded from this, that too much space has less effect than overcrowding, since an increase of ten gallons produced a decrease in total length of only 1.1 mm. while a decrease in volume of five gallons produced a decrease in total length of 4.3 mm. 2)

If, however, the actual growth curve resembles #2, then the optimum actually lies between the 50 cc/mm and the 120 cc/mm ratios, perhaps around the 75-80 cc/mm point. In this case the effects of too much space and of overcrowding were operating in a similar manner. Further research would be needed to determine which is actually the case.



#### Summary

Both pH and space factors affect the growth rate of Lebistes reticulatus. The effects of pH are most obvious when space conditiones are near the optimum. Lebistes reticulatus clearly shows increased growth when the pH is 7.0 pH. There is also an optimum volume per unit length of fish ratio. The 50 cc/mm ratio (that of the ten gallon tanks) most closely approximated this optimum. Further research would be necessary to determine if this is, in fact, the optimum, or whether the optimum lies between the ten and twenty gallon sizes.

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