The Killifish Muscle

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Killifish and other kinds of fish have fast muscles and slow muscles as same as humans.Tuna uses slow muscles which has many amount of oxygen and sugar content to swim longer.And sea bream use fast muscles which contain little oxygen because they don't need stamina.It is said that muscle hypertrophy occur by loading the muscles and consuming protein.In this research,we researched most efficient way of muscle hypertrophy in fishes by using Killifish and feed with different protein.

1 Background

We are all members of an athletic club. In the course of our daily strength training, we asked ourselves the question," How do fishes undergo muscle hypertrophy?" I found a paper on muscle hypertrophy in fish using goldfish in our school's previous research,and in the paper, there were experiments and results that examined the effects of water flow on muscle hypertrophy with and without exercise load. The paper did not provide any evidence for this due to the massive deaths of goldfish caused by excessive water flow. As a result of investigating the muscle structure of fish, it was found that some fish use their muscles for different purposes similar to humans, and killifish was one of them. Killifish are inexpensive and easy to keep, and their muscles are mainly divided into two parts, blood muscle and normal muscle, which are suitable for endurance exercise and instantaneous movement, respectively, similar to slow and fast muscles in humans.Based on the above, we decided to conduct this experiment using medaka(Japanese medaka) instead of goldfish, focusing on the hypertrophy of the blood muscle, which is a muscle mainly used for swimming, and also taking into consideration the amount of protein contained in the food,considering that protein also affects muscle hypertrophy in fish.

2.Hypothesis

Based on the above points, since the blood muscle is the muscle mainly used for swimming, we thought that individuals fed with water flow and protein-rich food would have the most muscle hypertrophy.

3 Method

We have 4ways of keeping to fish

1 protein rich food and presence of water flow

2 protein rich food and absence of water flow

3 normal food and presence of water flow

4 normal food and absence of water flow

We raised 20 Killifish each water tank,feed 0.05g each tank every day.

(3)Paraffin section production and staining process

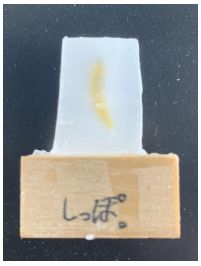
Paraffin sectioning process

We use paraffin sections of killifish for area measurement. The body of killifish contains water, and paraffin does not dissolve easily in water, so water cannot be replaced with paraffin as it is. Therefore, in order to smoothly replace water with paraffin, the sections are immersed in ethanol and xylene, which are compatible with each other, step by step. The process is described below.

1. Paralyze the killifish in ice water

and immerse them in Bouin's solution (about 1hour).

2. Soak in running water (several hours)
3. Ethanol (70%) (about 1hour)
4. Ethanol (80%) (about 1hour)
5. Ethanol (90%) (about 1hour)
6. Ethanol (95%) (about.1hour)
7. Ethanol (99.5%) (about 1hour)
8. Asb. ethanol (about 1hour)
9. Xylene 1 (about 1hour)
10. Xylene 2 (about. 2hours)
11. Paraffin 1 (about 1hour at 58° C)
12. Paraffin 2 (about 1hour at 70° C)
13. Paraffin 3 (about 1hour at 77° C)
14. Decompression treatment (about.30min)
15. Making paraffin blocks
16. Form paraffin blocks and attach to the base wood (fig.1)
17. Slice each block into 10 μm pieces using a microtome (fig.2) (fig.3) and make paraffin sections.



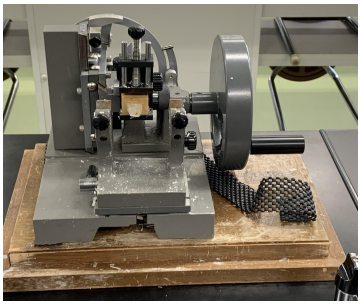


fig.1 Block fig.2 Microtome

fig.3 Paraffin sheet

Staining process of paraffin sections

Hematoxylin and eosin are used to stain the paraffin sections. Hematoxylin stains nuclei blue-violet, and eosin stains cytoplasm and cell membranes red. These two stains will be used in this study, but since there are many steps to soak paraffin sections before staining, they are shown below.

1. Xylene 2 (about 10 minutes)
Xylene 1 (about 10 minutes)
Abs. ethanol (about 5 minutes)
4. Ethanol (95%) (2 to 3 minutes)
5. Ethanol (90%) (2-3 minutes)
Ethanol (80%) (2 to 3 minutes)
Ethanol (70%) (2 to 3 minutes)
Ethanol (50%) (2 to 3 minutes)
9. Water (2 to 3 minutes)
10. Hematoxylin (15 minutes)
11. Rinse with water (10 to 20 minutes)
12. Eosin (30 seconds to 1 minute)
13. Water (up and down several times and soak)

(4) To evaluate muscle hypertrophy

1.Electron micrographs of killifish specimens are taken, and the ratio of the blood muscle area to the total cross-sectional area of the killifish is measured using image analysis software called image j. (fig.4)

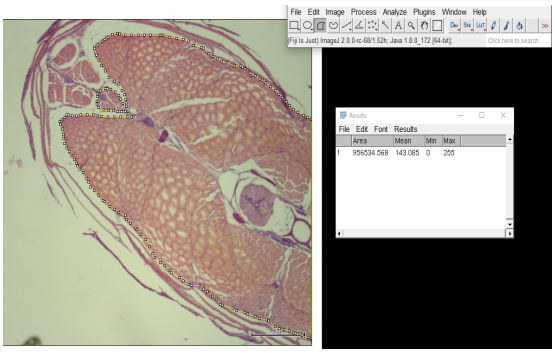


fig.4 The area of the inner part plotted by the dotted line is shown in the figure on the right, where the area is shown numerically.

2. In order to statistically examine whether there is really a difference in the mean values of the proportions obtained in this experiment, a T-test was conducted

The T-test assumes that the null hypothesis (*1) is correct and sets the significance level (*2), which is the probability of rejecting the null hypothesis, and calculates the value indicating whether there is a meaningful difference in the data to be compared. If the p-value is smaller than the significance level, the null hypothesis is rejected and the desired result is shown.

(*1) The statistical hypothesis that a variable is not related to another variable or a group of variables, or that there is no difference between two or more populations.

(*2) The probability of making an error in statistical hypothesis testing, and the criterion for a small p-value.
(*3) In statistical hypothesis testing, the probability that the test statistic will be the same under the null hypothesis, and the smaller the p-value, the less likely it is that the test statistic will be the same in the larges population.

3 Result & Discussion

Result 1(How many survived)

1.Protein 55% Presence of waterflow 4 survived	3.Protein 45% Presence of waterflow All died
2.Protein 55% Absence of waterflow All died	4.Protein 45% Absence of waterflow 5 survived

Because many individuals have died we have suspended the experiment.

Discussion 1
・The number of killifish decreased rapidly because of bad water quality.
→Over feeding and quantity of sunshine are cause of water polluting
・We gave too much water flow.

Improvement
・Change the feeding and place of the water tank once a week.
・Give water flow 15 minutes per 3 hours.
・Give medicine to improve water quality.

Result 2 (How many survived)

1.Protein 55% Presence of waterflow 22 survived	3.Protein 45% Presence of waterflow 4 survived
2.Protein 55% Absence of waterflow 7 survived	4.Protein 45% Absence of waterflow 16 survived

(Area)

1.Protein 55% Presence of waterflow 8.70% ※	3.Protein 45 % Presence of waterflow No data
2.Protein 55% Absence of waterflow No data	4.Protein 45% Absence of waterflow 7.68 %

※ This is the average value of individual

Significant differences by T-test
A T-test was conducted on the two tanks that produced the results. In this t-test, the null hypothesis was "there is no difference between the muscle hypertrophy of killifish in these two tanks" and the significance level was set at p=0.05 as is customary. The results are as follows.

	1.Protein 55% Presence of waterflow	4.Protein 45% Absence of waterflow
Number measured of killifish	13	10
Average of muscle area	8.672	7.654
Standard error	0.368	0.218
Standard deviation	1.275	0.655

Significance level	0.3930
Result	0.01<p<0.05

T-test showed that the p-value was less than 0.05, so the null hypothesis was rejected and there was a significant difference in the mean of the muscle area of the two tanks measured.

Discussion 2
・There was a large difference in the rate of enlargement in tank1 (protein55%,presence of waterflow)
→waterflow or protein affected for their muscle hypertrophy.

4 Connection for our society

We have researched the best way for muscle hypertrophy in killifish.
If our research is successful,we would like to use this research on other fish to help the field of aquaculture.
Then we will grow various fish at low cost.
Also,people will buy fish at a low price and they will be healthy by eating fish.
Now there are 800 million food shortages in the world.By using our research, we can contribute to solving food shortages.
Our research will save the world and people.

5 References

・Previous research in Sanko
・SHUGO WATABE, Functional diversity of muscle proteins from fish and shellfish and molecular mechanisms involved, NIPPON SUISAN GAKKAISHI, 2006, Volume 72, Issue 3, Pages 357-365, Released June 07, 2006, Online ISSN 1349-998X, Print ISSN 0021-5392,