BIOLOGY EXTENDED ESSAY

Reproduction of bacteria on the surface of different fish types

Research question:

"How bacteria reproduction differs on the surface of different types of fishes; Sparus aurata, Dicentrarchus labrax, Merlangius merlangus, Engraulis encrasicolus kept in refrigerator for four days at 4°C, indicated by counting the number of colonies reproduced on eosin methylene-blue lactose sucrose agar plate in 24 hours at 37°C under controlled conditions (temperature, light intensity, moisture etc.) in a culture incubator?"

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ABSTRACT

Fishes are considered as one of the healthiest foods because of omega-3, protein and vitamins that they contain. However, food poisoning from fish is seen commonly. Inadequate storage conditions are the main reason and bacteria growth that cause illness differs from species to species. The aim of this research is to observe, compare and contrast the bacterial growth on the surface of four different fish species after four days of storage duration in refrigerator.

The research question of this investigation is: "How bacteria reproduction differs on the surface of different types of fishes; Sparus aurata, Dicentrarchus labrax, Merlangius merlangus, Engraulis encrasicolus kept in refrigerator for four days at 4°C, indicated by counting the number of colonies reproduced on eosin methylene-blue lactose sucrose agar plate in 24 hours at 37°C under controlled conditions (temperature, light intensity, moisture etc.) in a culture incubator?"

The method used in this experiment is viable cell counting method. The fishes are bought freshly from the fish market and kept in the refrigerator for four days in stretch wrapped plates at 4°C. A sample is taken from each fish surface, incubation of the sample is done on EMB agar plates for 24 hours at 37°C. Bacterial colonies are counted after 24 hours.

The mean results of living bacteria colony number are as follows: 50 on *Merlangius merlangus,* 98,4 on *Dicentrarchus labrax,* 153,6 on *Engraulis encrasicolus ,* and 198,6 on *Sparus aurata.* These results showed that on *Sparus aurata* the bacteria reproduction was the highest. The least bacteria reproduction was observed on *Merlangius merlangus*. According to this result, *Merlangius merlangus* is the best one for storage at 4°C. The conclusion is that bacteria reproduces on each four species of fishes and not edible after such duration. Anova calculation showed that p value is $3{,}59197\text{x}10^{-21}$, which indicates there is a meaningful statistical difference in bacterial colony reproduction on the surface of different fish types.

Word count: 316

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BACKGROUND INFORMATION

Foodborne illness (foodborne disease and colloquially referred to as food poisoning) is any illness resulting from the spoiled food which is contaminated by pathogenic bacteria, viruses, or parasites, and it may also result due to chemical or natural toxins such as poisonous mushrooms.(1)

Fish is considered to be one of the healthiest foods and suggested by the experts to be consumed at least two times a week. It is an important source of protein, fat-soluble vitamins (A, D, E and K) and the omega-3 fatty acids.

Fishes may cause food poisoning if not properly prepared or stored. Sometimes, biotoxins caused by poor storage conditions may result in food poisoning. Fish can be spoiled in a short time period by virtue of the microorganisms that produce on it such as bacteria. Fishes are living organisms before being hunted and then interact with air, human beings or some surfaces, thus bacteria may be transferred to the fish. Fish contains nutrient, blood etc. which supplies bacteria an optimal growth medium.

Storage of fish in the market or home is vital to prevent any health issues. Several factors may affect the growth of bacteria on the surface of fish as temperature, moisture, pH, oxygen and available nutrients, surface texture of fish, minerals etc.

In this experiment, bacteria reproduction is expected on the surface of all fish. As they live in different seas, salt and mineral concentration of the sea various. Gilt-head bream (*Sparus aurata*) is found in the Mediterranean Sea, European anchovy (*Engraulis encrasicolusm*) is found in the Black Sea, Moronidae (*Dicentrarchus labrax*) is found in the Black Sea, Mediterranean Sea and the Sea of Marmara and Whiting (*Merlangius merlangus*) *i*s found in the Black Sea and the Sea of Marmara. The seas can be ordered according to salt concentrations in percentage from the most to the least: Mediterranean Sea(3.8), Sea of Marmara(2.2), Black Sea(1.7). This different mineral concentration may affect bacteria growth on the surface.(2)

Fat percentage is a physiological difference between the fishes. Approximately; *S. aurata* has 2 grams, *D. labrax* has 1 gram*, M. merlangus* has 1 gram*, E. encrasicolus* has 5 grams of fat in 100 grams. This difference may also affect bacterial growth on the surface.(3)

Another difference can be given as skin texture of the fishes, which is the surface area where bacteria grow. *M. merlangus* seems to have the smoothest skin which suggests the gaps where nutrients and warmth may stay in are less. So there won't be enough room for bacteria to hold and reproduce. On the other hand, *S. aurata* and *D. labrax* seems to have the roughest skins, increasing the surface area which is already bigger than the others, and provide extra room,

moisture, and nutrient for bacteria to grow. *S. aurata* is wide but *D. labrax* is plenty in length, surface area is nearly the same. However, the spiny dorsal fins of the *S. aurata* and the gaps between them enlarge the surface area not significantly but a little. *E. encrasicolus* is in between not smooth as *M. merlangus* and not rough as the others.

I have been through food poisoning caused by fish about a year ago when my family decided to eat out. My choice was different from them because of my habits. I chose to eat red mullet which made me ill. That incident made me think and wonder if the bacterial growth depends on or is affected by the fish type. My wonder is how I decided on my topic.

The aim of this experiment is to compare the reproduction of bacteria on four different fish species which are kept in refrigerator at the end of a four day period. I have decided to investigate on these types of fishes since they are the most commonly consumed types in Turkey, and they were easy to find freshly because of the season. The fishes are bought as freshly as possible from the fish market and kept in stretch wrapped plates in refrigerator for four days at 4 °C. Research question of this study is:

“How bacteria reproduction differs on the surface of different types of fishes; *Sparus aurata, Dicentrarchus labrax, Merlangius merlangus, Engraulis encrasicolus* kept in refrigerator for four days at 4°C, indicated by counting the number of colonies reproduced on eosin methylene-blue lactose sucrose agar plate in 24 hours at 37°C under controlled conditions (temperature, light intensity, moisture etc.) in a culture incubator?”

Figure1: Fish species used in the investigation

S. aurata	*D. labrax*
M. merlangus	*E. encrasicolus*

HYPOTHESIS

Food poisoning, especially from fish, is a serious problem. "According to the Centers for Disease Control and Prevention (CDC), raw foods of animal origin are the most likely to be contaminated."(4)

The bacterial growth can be affected by various factors as temperature, moisture, pH. Most importantly, type of fish may affect the growth of bacteria. Skin texture which creates room, nutrients and warmth for bacterial growth differs from fish to fish. Rougher skins are more likely to have more gaps which give bacteria an optimal growth medium. Bacterial growth is expected in all of the fish species.

The reasons given concluded to the hypothesis to be: The growth of bacteria on the surface should be the most on *S. aurata* followed by *D. labrax, E. encrasicolus.* The least growth of bacteria is expected to be on *M. merlangus.* So the main goal of this research is to compare the growth of bacteria on the surface of different fishes.

The null hypothesis states that bacteria reproduction will be same on all species.

METHOD DEVELOPMENT AND PLANNING

Throughout determining my research question, I went through some phases. The most important two phases were deciding on the topic and the method to investigate my research question.

When we were told about the extended essays we should write as an IB assignment I wanted a subject and a topic which I would enjoy writing and investigating instead of choosing the easy one. I want to be a forensic anthropologist therefore I thought my subject could be biology. I discussed with my supervisor about the topics I had in mind which were engaging to me.

In daily-life, we are in touch with bacteria all the time and they may affect human body in both negative and positive ways. I decided to combine bacteria with something in daily-life. As my mother has a colleague who is a medical microbiology specialist working on my mother's working place's hospital, he has access to microbiology lab. It eased to manage my experiment.

The challenging part was deciding on which daily-life situation to engage with bacteria. I thought that my investigation could include food because bacteria on food may cause food poisoning

Then it occurred to me that food poisoning from fish is almost the most common one, and I have experienced it about a year ago from eating red mullet at a restaurant. Also, we consume fish at least two times a week as suggested from the specialists. Poor storage conditions and expired fishes may result in serious health issues. It is difficult to understand in the market that if the fishes are fresh or not. As common knowledge, if the fish’s eyes are shiny, if you press the skin and it comes back to its initial form, if the gills are pink or reddish; it can be said that fish is fresh, but it’s hard to determine the freshness with these methods. When it came to deciding on which fish types I will study on, I listed the most commonly consumed fishes in Turkey and chose between them because at the time of this experiment hunting prohibition was starting and not all of the fishes were available freshly at the fish market. I decided on *E. encrasicolus*, *M. merlangus*, *D. labrax*, and *S. aurata.*

I decided to not to limit the experiment with one species of bacteria and instead observed the total bacteria growth. Focusing on only one species would not give me an expanded view. Beside the effort it needed, it was needless. Next part was choosing a bacteria counting method. There are several methods for counting alive bacteria found in samples; turbidimetric, direct microscopic & standard (viable cell) counts. (5)

I chose the viable cell (colony) counting due to its relevance to my investigation and accuracy. Number of living colonies were my interest, thus I chose to count the bacterial growth one by one and eliminated the other methods in which viable cell detection is hard. I decided using eosin methylene blue plate which is a rich medium and allows many different types of bacteria to grow. Since it contains methylene blue, living cells won't take methylene blue inside the cell, so counting the living colonies would be easy.

In my method, I cared about the fishes' freshness. While doing this I bought them at the same time from the same fish market, five of each four species. Because if there were a difference between freshness of the fishes, if some of their storage durations at the market differed, bacteria would have been grown before bought. Thus time period wouldn't be the same and controlled. As far as my conversation with the manager of the fish market, fishes were caught at the same day and the transportation was done in about 24-28 hours at 0°C. While transportation from the fish market to home, fishes were put in different paper-bags according to their species, and I used cooling bags they sell at the gross market's cool sections.

When reached home, I put sterile gloves on my hand to prevent any transfer from my hand to the fishes. The fishes were taken out the paper-bags and put into the sterilized plates, again according to the species, plate is strictly closed with stretch wrap in order to create a closed system and prevent any matter exchange with the environment. I labeled each plate with a post-it on which its species is written. Refrigerator's setting was 4°C as usual. If the fishes were frozen, bacteria growth wouldn't be significant enough to observe. I placed all of the plates in the same level to prevent any difference that may be caused by the fans or lights of the refrigerator. The fishes stayed in the refrigerator for four days.

For the cultivation of bacteria I used EMB agar plates as a medium and divided each plate to four sections with a wire, so we wouldn't waste too many plates. Each section was labeled and then done zig-zag movements on with warmed wire to prepare an appropriate medium for bacteria to grow (as shown in appendix 1). Then the spreader was rubbed on the fish from up to down for both sides (right and left) for 6 times (3 for each side) in a section of 2x2 square cm surface area (Appendix 2). The spreader was rubbed on the plate's matching section with zig-zag movements from up to down. These zig-zag incubations were repeated during rotation of the plate 90°.

Incubation was done at 37°C which is the most suitable temperature for bacteria growth. The cultures were kept 24 hours in the incubator to reproduce enough bacteria on to form visible and countable colonies. The optimal conditions for bacterial growth and reproduction were tried to be provided as much as possible. After the incubation, I counted the colonies one

by one with carefulness to not to miss one, with marking the each colony counted with an acetate pen, thus not a single colony would be counted again.

Five trials for each species were done in pursuance of decreasing the errors and increasing the accuracy of the investigation.

METHOD

Variables:

Controlled Variables: Buying time, duration of keeping the fishes in the refrigerator (4 days), temperature of the refrigerator (4°C), type of medium, number of rubbing the spreader on the fishes, duration of keeping the samples in the incubators (24 hours), the incubator; temperature (37°C), light intensity, moisture, nutrients.

Independent Variables: Type of fishes (*S. aurata, D. labrax*, *M. merlangus, E. encrasicolus*).

Dependent Variables: Reproduction amount of bacteria.

Materials:

Materials are listed in the appendix 3

Method:

Before the lab experiment (at home):

1. Buy four different types of fresh fishes from the fish market: *S. aurata*, *D. labrax*, *M. merlangus, E. encrasicolus*, five of each.
2. Use cooling bags and ice packs while transportation of fishes to the home.
3. Prepare and clean the plates that fishes are going to be put in.
4. Put the plastic gloves on.
5. Put the fishes in the plates. (different genus in different plate)
6. Wrap the plates with stretch wraps carefully to create a closed system and prevent the matter exchange.
7. Label the plates according to the genus.
8. Set the refrigerator's temperature to 4°C.
9. Put the fishes in the refrigerator at the same level.
10. Keep the fishes in the refrigerator for four days.

At the lab (inoculation)

1. Check if the air conditioning is on.
2. Put the plastic gloves on.
3. Open the emb- blood petri dish.
4. Divide the dish in four sections with wire, one for each genus.
5. Write the name of the genus in each section.
6. Turn the burner on.

7. Move the wire on the flame.
8. Do zig-zag movements in each section from top to bottom.
9. Rotate the petri dish 90° and repeat the zig-zag movement.
10. Open the spreader.
11. Rub the spreader on the middle of the right side of *S. aurata* 3 times in a section of 2x2 square cm.
12. Rub the spreader on the middle of the left side of *S. aurata* 3 times in a section of 2x2 square cm.
13. Rub the spreader on the section for *S. aurata* with zig-zag movements.
14. Rotate the petri dish 90° and repeat the zig-zag movement.
15. Repeat steps 3-14 four more times.
16. Put the fishes in the trash bins after the experiment to avoid the bad smell.
17. Repeat steps 3-16 with *D. labrax*, *M. merlangus,* and *E. encrasicolus*.
18. Put the dishes in the incubator.

(Appendix 4)

<u>After inoculation</u>

1. Take the dishes out of the incubator after 24 hours.
2. Count the number of colonies in each section from top to bottom.
3. Note the numbers.
4. Place the dishes in the appropriate rubbish bins.

RAW DATA TABLE:

Table 1: The number of bacterial colonies reproduced

Species of the Fish	Trials	Number of Bacteria colonies reproduced on agar plates
Merlangius merlangus	1	48
	2	52
	3	55
	4	46
	5	50
Dicentrarchus labrax	1	96
	2	100
	3	103
	4	95
	5	99
Engraulis encrasicolus	1	154
	2	157
	3	150
	4	145
	5	152
Sparus aurata	1	196
	2	204
	3	206
	4	198
	5	201

Table 1: This table shows the number of bacterial colonies reproduced on different types of fishes; *M. merlangus, D. labrax, E. encrasicolus, S. aurata* after a four day period under controlled conditions such as temperature, time and light intensity.

Table 1.2: Statistical Values

Species of the Fish	Trials	Number of Bacteria colonies reproduced on agar plates
Merlangius merlangus	1	49
	2	52
	3	53
	4	47
	5	50
Mean		50
Standard Deviation		2,387467277
Standard Error		1,067707825
Dicentrarchus labrax	1	96
	2	100
	3	102
	4	95
	5	99
Mean		98,4
Standard Deviation		2,880972058
Standard Error		1,288409873
Engraulis encrasicolus	1	154
	2	156
	3	157
	4	149
	5	152
Mean		153,6
Standard Deviation		3,209361307
Standard Error		1,435270009
Sparus aurata	1	194
	2	203
	3	196
	4	199
	5	201
Mean		198,6
Standard Deviation		3,646916506
Standard Error		1,630950643

Table 1.2: This table shows the calculated statistical values; mean, standard deviation, and standard error for number of bacterial colonies reproduced on different species of fishes; *M. merlangus, D. labrax, E. encrasicolus, S. aurata.*

Calculations for *M. merlangus*

- **Mean**= $\frac{\sum x}{N}$= $\frac{49+52+53+47+50}{5}$= $\mathbf{50}$
- **Standard Deviation** = $\sqrt{\frac{\sum(x-\bar{x})^2}{(n-1)}}$

  1: x-$\bar{x}$= 49-50= -1

  2: x-$\bar{x}$= 52-50= 2

  3: x-$\bar{x}$= 53-50= 3

  4: x-$\bar{x}$= 47-50= -3

  5: x-$\bar{x}$= 50-50= 0

  $$=\sqrt{\frac{(-1)^2+(2)^2+(3)^2+(-3)^2+(0)^2}{4}} = \sqrt{\frac{23}{4}} = \sqrt{5.75} = \mathbf{2.39}$$

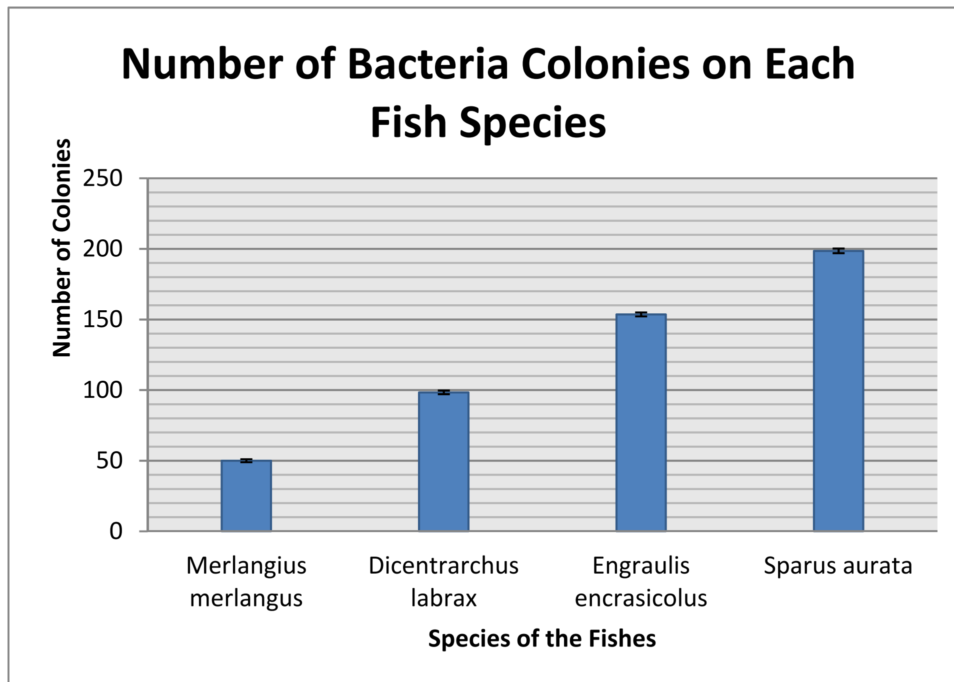
- **Standard Error**= $\frac{std.dev.}{\sqrt{n}} = \frac{2.39}{\sqrt{5}}$= $\mathbf{1.06}$

ANOVA CALCULATION:

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	62686,8	3	20895,6	2222,93617	**3,59197E-21**	3,238871517
Within Groups	150,4	16	9,4			
Total	62837,2	19				

Table: ANOVA results of calculated values

Graph 1: The number of bacterial colonies reproduced



Graph 1: This graph shows the mean numbers of reproduced bacterial colonies on different species of fishes. Most reproduction occurred in *S. aurata.* Error bars drawn according to standard errors are small.

DISCUSSION, EVALUATION AND CONCLUSION

DISSCUSSION

Fishes are recommended as one of the healthiest foods by scientists regarding to richness of omega-3, fat soluble vitamins and protein. Foodborne illness, as known as food poisoning, is mostly caused by infections origin from bacteria. Food poisoning can be serious and poisoning from fishes is very common. Expired fishes and poor storage conditions of the fishes are the main reason of fish poisoning.

In this experiment, the aim was to observe, compare and contrast the bacterial growth on four different fish species surface. Research question of this study is: "How bacteria reproduction differs on the surface of different types of fishes; Sparus aurata, Dicentrarchus labrax, Merlangius merlangus, Engraulis encrasicolus kept in refrigerator for four days at 4°C, indicated by counting the number of colonies reproduced on eosin methylene-blue lactose sucrose agar plate in 24 hours at 37°C under controlled conditions (temperature, light intensity, moisture etc.) in a culture incubator?". The hypothesis was that the reproduction of bacteria would be the most on *S. aurata* followed by *D. labrax, E. encrasicolus.* The least growth of bacteria was expected to be on *M. merlangus.*

The experiment's mean bacteria colony reproduction results show that; 50 on *M. merlangus* with the standard deviation of 2.38 and standard error of 1.06, 98,4 on *D. labrax* with the standard deviation of 2.88 and standard error of 1.28, 153,6 on *E. encrasicolus* with the standard deviation of 3.2 and standard error of 1.43, and 198,6 on *S. aurata* with the standard deviation of 3.64 and standard error of 1.63. These results support my hypothesis in aspects of order from the most to the least. All of the species had significant amount of bacteria growth on and difference between each other. The results show that the bacterial growth can be ordered from the most to the least as; *S. aurata, E. encrasicolus, D. labrax, M. merlangus.* Standard error and standard devation for each type and trial is not much to affect the accuracy or precision.

The graph shows that the bacteria reproduced least on *M. merlangus* since the bar which represents the reproduction on it seems to be the shortest significantly, followed by *D. labrax, E. encrasicolus,* and the highest column which represents the most bacterial growth belongs to *S. aurata.* The difference between the data from the species could be explained as the bacteria found a more appropriate medium to grow and reproduce on some than the others. This means that some aspects such as surface area, mineral and salt concentration, fat concentration affected the growth. As I stated initially the surface area is the roughest on *S. aurata* as a

consequence of squama and it is the smoothest on *M. merlangus.* In addition, *S. aurata*is mostly found in muddy areas which may contain bacteria more than clear water.

EVALUATION

The null hypothesis was that for α=0.05, the difference between fish types isn't significant. The p value of ANOVA test is $3{,}59197\text{x}10^{-21}$, less than α, so there's a meaningful difference between types. This impugns the null hypothesis, and proves my initial hypothesis.

There are some weaknesses of the experimental design. First of all, the fish types were limited because the hunting season was finished. The fishes that could be found fresh at the market were all used in the experiment. Also, only the fishes caught from the sea were used, not the fishes caught from the fresh water, in order to not to mix them to avoid a wrong comparison because the fresh water fishes are fed in pools. One way to overcome this problem is the timing of the experiment. If the experiment was done whilst the fishing season, there would be more fresh fish species at the fish market. So, more species would be examined and compared. In addition, the fishes were caught and brought to the market in the same day as told by the fishermen, but fishing with fishing rod if possible would make a more trustworthy data. However, since there's no sea in the city that I live, it is impossible. It could be done in further investigations in the future.

Also, the rubbing of the spreader on the agar plates is a weakness. Even though, the rubbing was tried to be done equally it was not controlled and limited with a number. If the inoculation was done and limited with a number (i.e. Rubbing the spreader on the agar plates with zig-zag movements from left to right and right to left 5 times each and repeating after rotation) it would have been controlled in a better way. If the spreader was firstly shaken in a liquid growth medium after taking the cultures from fishes, then a certain ml of that was taken and planted, the experiment would be better.

The time was kept constant by buying the fishes from the market at the same day. The light absorption and temperature tried to be kept constant by placing the fishes at the same level in the same refrigerator at 4°C to prevent any heat transfer from the fans of the refrigerator to the plates which fishes were placed in. Also the rubbing amount of spreader on the fishes were kept constant and limited with a number to keep the conditions same for each. The agar plates were placed in the same incubator at the same temperature for 24 hours. Also, since the value of the standard error is small, it can be said that the errors in this experiment is not much and the data is reliable.

If the time and the trial number were more, the data collected would be more significant and accurate, the results could be more trustworthy. Also, if the controlled variables were kept more constant, if the change, such as change in light absorption, was absolutely prevented or kept constant, the data collected would be more accurate. Therefore the modifications that can be done on the design and the method such as timing, limiting the number of rubbings and finding the bacteria species etc., would give more accurate data which could be interpreted in a more correct way.

CONCLUSION

The research question: “How bacteria reproduction differs on the surface of different types of fishes; Sparus aurata, Dicentrarchus labrax, Merlangius merlangus, Engraulis encrasicolus kept in refrigerator for four days at 4°C, indicated by counting the number of colonies reproduced on eosin methylene-blue lactose sucrose agar plate in 24 hours at 37°C under controlled conditions (temperature, light intensity, moisture etc.) in a culture incubator?”was answered in this investigation.

These results show that mean bacteria colony formation on *M. merlangus, D. labrax, E. encrasicolus,* and *S. aurata* are 50 , 98,4 , 153,6, and 198,6 respectively. The standard error in each is not much, so that the results are trustworthy. My hypothesis was that the growth would be the most on *S. aurata,* followed by *E. encrasicolus, D. labrax, M. merlangus.* As the graph above shows, my hypothesis was correct.

The general conclusion that can be done is that bacteria reproduce on fishes which are stored below standard storage conditions. If any of them must be chosen, *M. merlangus* has the most endurance but it doesn't indicate that it is edible after such poor storage conditions. The main reason I wanted to investigate this topic was to increase my awareness after an incident happened to me, food poisoning. The fishes should be stored at 4°C at the markets and freezers at home to prevent any health issues.

Appendices:

Appendix 1:

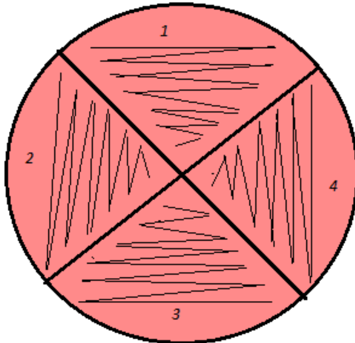


Figure 1: a diagram of zig-zag movements done on sections 1, 2, 3, and 4 labeled as; *Engraulis encrasicolus*, *Merlangius merlangus*, *Dicentrarchus labrax*, and *Sparus aurata*

Appendix 2:

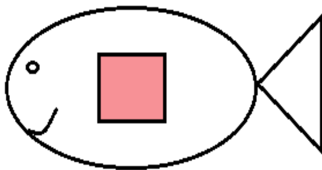


Figure 2: a diagram of 2x2 square cm section where the spreader was rubbed on.

Appendix 3:

1. Plates
2. Five Gilt-head bream (*Sparus aurata*)
3. Five Moronidae (*Dicentrarchus labrax*)
4. Five Whiting (*Merlangius merlangus*)
5. Five European anchovy (*Engraulis encrasicolus*)
6. Stretch wrap
7. Refrigerator
8. Wire
9. Burner

10. Spreader
11. Emb petri dishes
12. Incubator
13. Nose mask
14. Plastic gloves
15. Cooling bags

Appendix 4:

Bibliography:

(1) https://en.wikipedia.org/wiki/Foodborne_illness
(2)http://www.msxlabs.org/forum/soru-cevap/217248-denizlerimizin-tuzluluk-orani-nedir.html
(3) https://www.diyetkolik.com/kac-kalori/hamsi/
(4) http://seafoodhealthfacts.org/pdf/seafood-safety-pc-microbes.pdf
(5)http://fire.biol.wwu.edu/brodham/biol346_S07/labman_week4.pdf