Bacteria Colonization: Exploring Salmon Bacteria Growth on Bread

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Abstract

Salmon farms use antibiotics to treat diseases caused by bacteria. This study investigates the growth of bacteria on bread that came in contact with farmed salmon, grown with antibiotics, and wild-caught salmon, free of antibiotics. The motivation of this study was to uncover the effect of antibiotics on the natural bacteria on salmon. The data for the experiment was collected by pressing a standardized size of salmon, three distinct wild-type salmon and three distinct farmed salmon, on bread for 60 seconds. The progression of mold on the bread was monitored daily over a span of two weeks. A one-way Analysis of Variance (ANOVA) test was used to perform the statistical analysis. The results revealed that the farmed salmon had a higher mean percent cover growth rate. However, the results were not statistically significant. Two types of microbes grown on the bread were identified. The first type was found on all bread with visible microbe growth. The second type was only found on the wild-caught treatments. These discoveries will contribute to enhancing the marketing of farmed salmon, impacting both sellers and consumers, while also providing valuable insights for predicting mold growth from various salmon bacteria.

Introduction

Salmon, a significant component of global fisheries, serves as a vital source of nutrition and economic value. In recent times, the raising of farmed salmon has become increasingly prevalent and the fish are often stored in high density open water pens, where they remain vulnerable to bacteria as well as parasitic activity (Miranda et al., 2018). Currently, antibiotics are being utilized to manage bacterial infections in aquaculture settings (Miranda et al., 2018; Morrison et al., 2013). However, the impact of these antibiotics on the natural bacterial composition of salmon remains a subject of investigation (Gupta et al., 2019). This study aims to assess the influence of antibiotics on the growth of bacteria on salmon, particularly focusing on the observable mold growth on bread as an indirect indicator. In this experiment, the null hypothesis (H_0) states that there is no significant difference in the percent cover growth rates between farmed and wild-caught treatments. The alternative hypothesis (H_A) states that there is

a significant difference in percent cover growth rates between treatments. We predict that the farmed salmon will have lower percent cover growth rates because antibiotics are administered to farmed salmon to treat diseases from bacteria (Gupta et al., 2019).

The motivation for this research stems from the need to evaluate the success of antibiotics in controlling bacterial populations on salmon (Avendaño-Herrera et al., 2022). Although antibiotics are commonly administered in farmed salmon to prevent and treat bacterial infections, their repercussions on the broader bacterial ecosystem, including potential impacts on human consumption, are not fully understood (Salgado-Caxito et al., 2022). By employing quantitative measurements and statistical analysis, we seek to discern any significant differences in mold growth patterns between wild-type salmon and farmed antibiotic salmon.

Methods

Our objective of our study is to determine whether the presence of antibiotics on *O. nerka* will influence the amount of bacteria on the surface of the fish. Nine samples of each farmed (contains antibiotics) and wild-caught salmon (does not contain antibiotics) were each pressed onto a piece of bread, which was then observed over the course of three weeks for bacterial and fungal growth. Several salmon samples were collected by our group members. For replicability, three whole samples of wild salmon and three whole samples of farmed salmon were collected. The farmed salmon species collected were of two different species, one being Atlantic Salmon, and two Sockeye Salmon. The wild-caught salmon species were also of two different species, two being Coho, and the third salmon being Atlantic Pink. In addition to salmon, 20 slices of organic white bread were purchased. Each sample was purchased either on November 6th or

November 7th, and was stored in a refrigerator or freezer prior to being brought into the laboratory on November 7th.

Prior to exposing the salmon samples to our bread, several preparation steps were made. Each sample of salmon was cut into 5.5 x 5.5 cm pieces using a sterile razor blade. Then, each sample was cut into three equally sized pieces, for a total of 18 samples (9 whole fish samples, multiplied by three). The individual samples were all the same size, measuring at 1.8 x 5.5 cm. Then, 21 Ziploc bags were labeled accordingly. The Ziploc bags were labeled by either (1) Antibiotic, (2) Wild or (3) Control. Additionally, in order to keep track of each salmon sample, each bag was labeled with the corresponding first, second or third piece of salmon from its original sample. On each Ziploc bag, a grid of 1 cm x 1 cm boxes was traced. This allows for the calculation of percent cover on each sample on the bread. As a final preparation step, each piece of bread was cut in half.

When exposing our salmon samples to our bread, the lab bench and all materials were kept sterile. The bench and all materials were disinfected in between rounds of exposure. Each whole salmon sample's three pieces were exposed to bread at the same time, for 60 seconds each. The surface of the salmon was pressed into a corner of the bread using a petri dish, with a firm hand to allow for bacteria transfer. Immediately after 60 seconds, the salmon sample was removed from the bread, and the bread was placed into a Ziploc bag that was sealed immediately. This process was repeated for each of the 9 whole samples of fish, each with three pieces, for a total of 18 pieces of bread. Each sample of fish was discarded into the animal waste disposal.

The objective of our controlled samples is to determine the bacterial and fungal growth on bread without the influence of salmon exposure. The controlled samples were created using a sterile cheesecloth that had been dampened with deionized water. The cheesecloth was pressed

onto a piece of bread for 60 seconds, and then the bread was placed into its appropriate Ziploc bag. This was repeated three times, for a total of three control samples. In total, we had 21 Ziploc bags, 18 from our salmon samples, and three from our controlled samples (Figure 1).

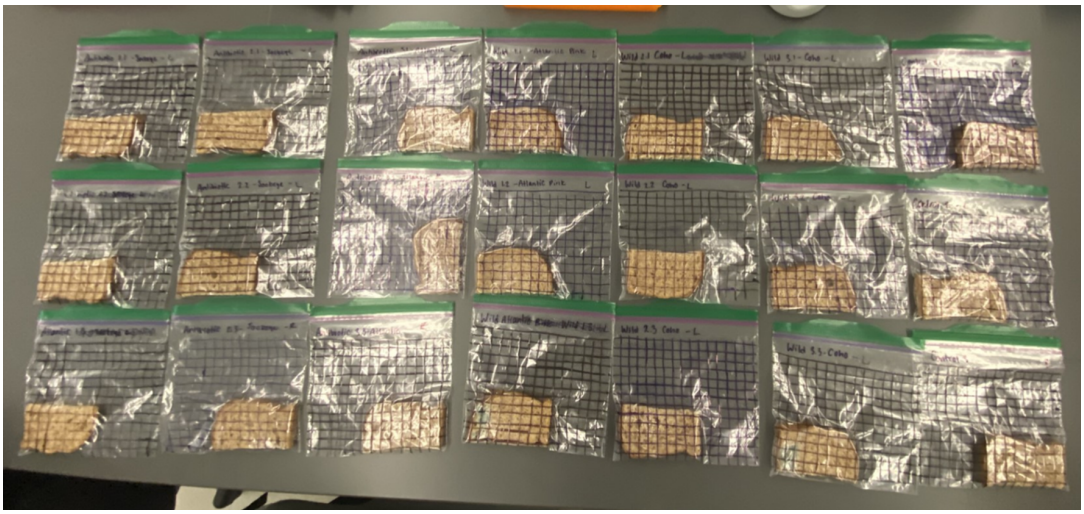

Figure 1. All 21 samples, including control treatments on Day 14.

The percent cover of the bacterial and fungal growth was determined by counting the number of individual squares on the ziploc bag that contained any bacterial or fungal colonies. This number was divided by the total number of squares on the grid, to determine percent cover (%). A one-way Analysis of Variance (ANOVA) test was conducted in order to analyze the variance between the percent cover growth rate means of the three wild-caught salmon, the three farmed salmon, and the control group.

Results

The graph of the growth rates (Figure 2) show that the Antibiotic treatment had a higher mean growth rate (2.60% cover per day) and larger variance (IQR = 6.50% cover per day and maximum of 8.62 % cover per day) compared to the Wild treatment, which had a mean growth

rate of 1.95% cover per day and an IQR of 1.49% cover per day, over a 14-day period. A one-way ANOVA test indicated that the growth rates are not statistically significant ($P > 0.05$).

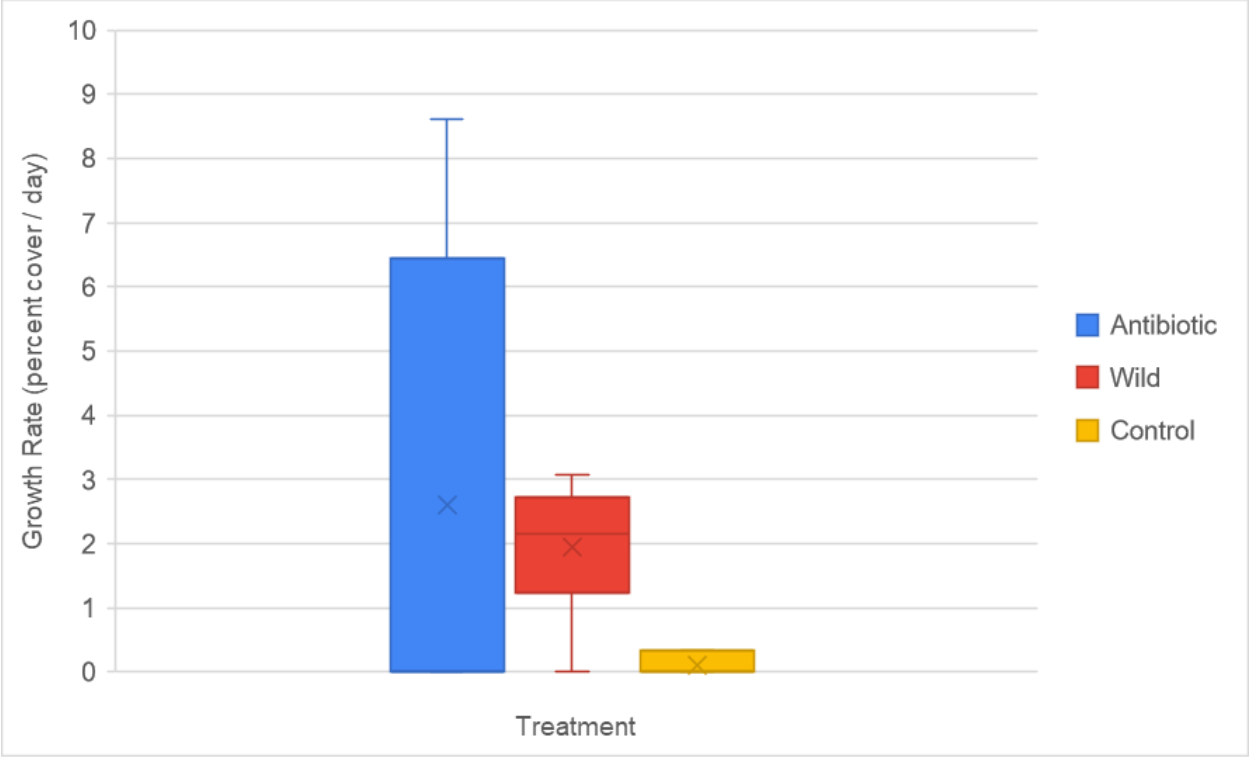

Figure 2. Growth rates (percent cover per day) over 14 days for the Antibiotic treatment (n = 9), the Wild treatment (n = 9), and the control (n = 3). Means are marked with an "X." The middle horizontal line in the Wild treatment shows the median. The upper (Q3) and lower (Q1) boundaries of the box represent the IQR. Whiskers extend to the maximum and minimum data points. A one-way ANOVA test revealed that the results are not statistically significant. The P-value of 0.61 is greater than 0.05, and the F value of 1.15 is smaller than the F critical value of 3.55.

There were two broad types of microbes growing on the bread which we defined based on their unique characteristics. We named the microbes Type I and Type II, based on their order of discovery.

Type I (Figure 3) appears on all bread with visible microbe growth. It was first identified on Day 3 on Antibiotic 3.1 Atlantic (one of the three Antibiotic 3 Atlantic replicates) and is small and white with defined edges.

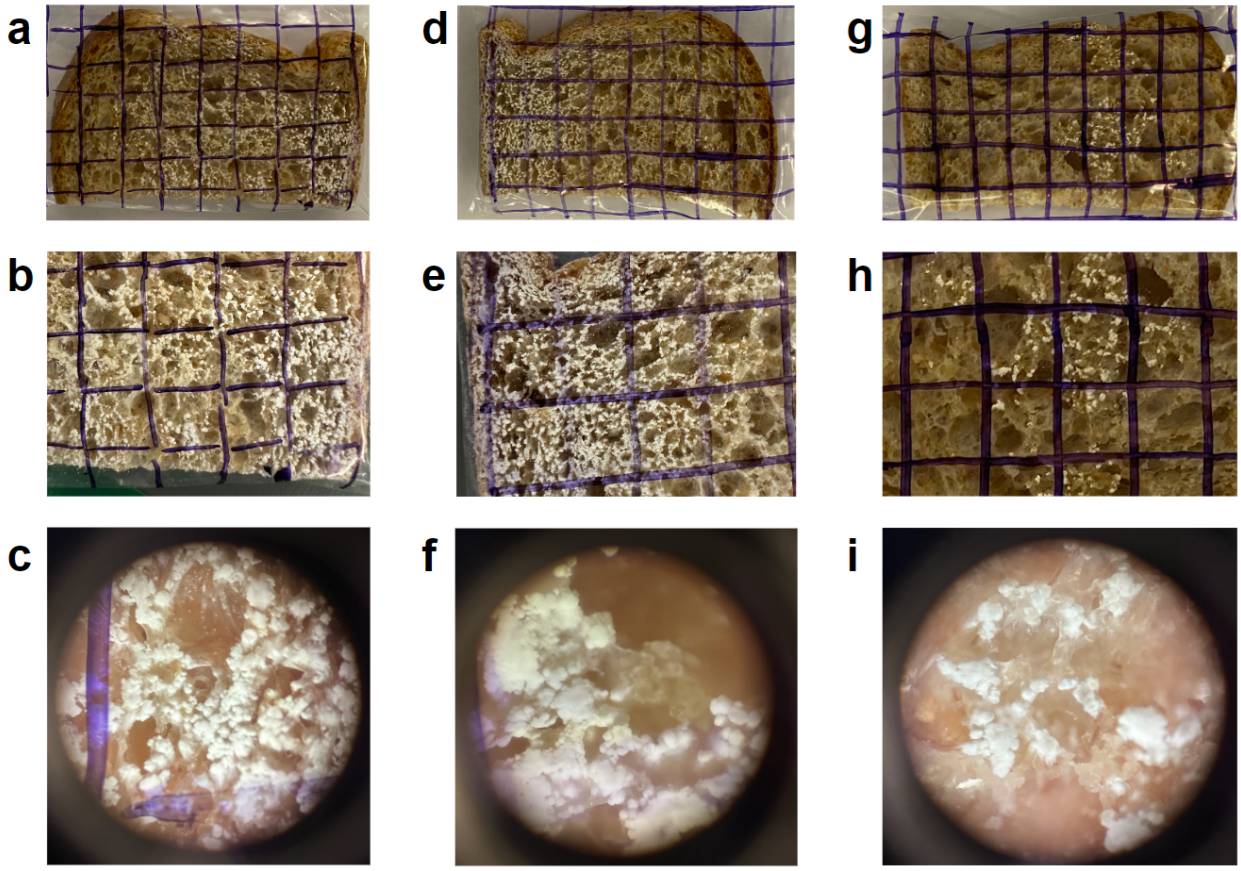

Figure 3. Type I on **(a)** Antibiotic 3.1 Atlantic, **(d**) Antibiotic 3.2 Atlantic, and **(g)** Wild 2.3 Coho. The middle row of images shows closer views of **(b)** Antibiotic 3.1 Atlantic, **(e)** Antibiotic 3.2 Atlantic, and **(h)** Wild 2.3 Coho. Images under the dissecting microscope of **(c)** Antibiotic 3.1 Atlantic, **(f)** Antibiotic 3.2 Atlantic, and **(i)** Wild 2.3 Coho are shown in the bottom row. Images were taken on Day 14.

Type II appears on Wild 3.3 Coho and Wild 1.3 Atlantic Pink. It was first identified on Wild 3.3 Coho on Day 7. Type II has a fuzzy appearance and a deep green coloured centre with white edges. Type II appears as one large spot of mold in Wild 3.3 Coho (Figure 4a-c), while Wild 1.3 Atlantic Pink contains smaller spots (Figure 4d-f).

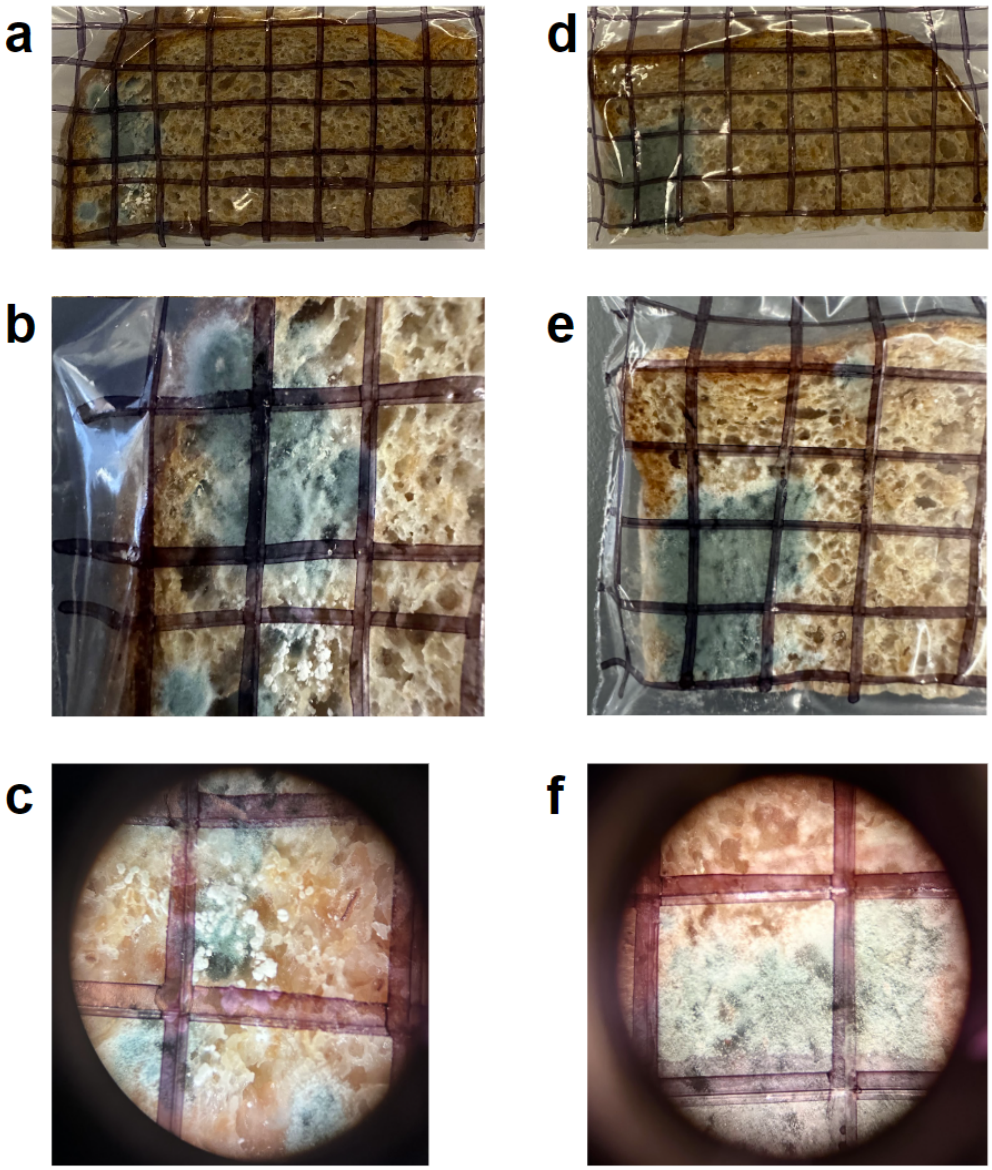

Figure 4. Type II on **(a)** Wild 1.3 Atlantic Pink and **(d**) Wild 3.3 Coho. The middle row of images shows closer views of **(b)** Wild 1.3 Atlantic Pink and **(e)** Wild 3.3 Coho. Images under the dissecting microscope of **(c)** Wild 1.3 Atlantic Pink and **(f)** Wild 3.3 Coho are shown in the bottom row. Images were taken on Day 14.

Discussion

Exploration of percent cover growth rate results

The Antibiotic treatment had a higher growth rate and larger variance compared to the Wild treatment (Figure 2). A one-way ANOVA test confirmed that the results were not

statistically different. We accept the null hypothesis (H_0) stating that there is no significant difference in the percent cover growth rates between treatments, and reject the alternative hypothesis (H_A) stating that there is a significant difference in percent cover growth rates between treatments. We predicted lower percent cover growth rates for farmed salmon because antibiotics are administered for preventing and curing diseases from bacteria (Gupta et al., 2019). The results were unexpected since the growth rate for the Antibiotic treatment was greater than the Wild treatment by 0.65% cover per day. However, results were not significant and the range for the Antibiotic treatment was large.

No mold growth was found on the Antibiotic 1 Sockeye replicates, while Antibiotic 3 Atlantic replicates had the highest mean growth rate. Zhang et al. (2022) found that antibiotic concentrations vary in different fish tissues, possibly due to the affinity of antibiotics to various biomacromolecules. Their findings may explain the large variance in the Antibiotic treatment. Since we used different parts of the salmon for each replicate, each piece may have contained varying antibiotic concentrations (Zhang et al., 2022). Zhang et al.'s (2022) research may also explain the absence of mold growth for Antibiotic 1 Sockeye: parts of the salmon used may have had high antibiotic concentrations.

Antibiotic 3 Atlantic's higher percent cover growth rate may be due to the salmon's storage. The Antibiotic 3 Atlantic salmon had a strong odor. Displeasing odors allow us to identify food spoilage to prevent consumption of toxins from microbes (Takahashi et al., 2004). Storing fish in higher temperatures promotes pathogen growth (Mercier et al., 2017). The Antibiotic 3 Atlantic salmon may have been poorly stored, contributing to its higher growth rate.

Possible microbe identifications

Two types of microbes on the bread were identified. Type I (Figure 3) may be the fungal species *Hypopichia burtonii*, as it matches the "chalky" description of *H. burtonii* (Berni & Scaramuzza, 2013). Type I resembles an image of *H. burtonii* grown on bread by Garcia et al. (2019), but has more defined edges.

Type II (Figure 4) matches an image of *Penicillium chrysogenum* grown on bread by Ollinger et al. (2022). It follows a similar growth pattern of *P. chrysogenum* described by Ollinger et al. (2022): it first appears as a white spot, then turns blue-green. As Type II is not present on Antibiotic treatments, antibiotics could potentially inhibit its growth.

Limitations and local antibiotic use

We assume wild-caught salmon do not have antibiotics. While one study did not detect antibiotics in wild salmon collected in major fishing areas (Chiesa et al., 2019), another study found antibiotics in wild salmonids in Chile, possibly due to antibiotic contamination of the water (Carrizo et al., 2021). Moreover, we assume the salmon were correctly labelled. According to Kroetz et al. (2020), Pacific salmon is often mislabelled as Atlantic salmon, and Atlantic salmon is often mislabelled as rainbow trout.

Although we cannot identify the antibiotics used, we will focus on antibiotic usage in Canadian farmed salmon, as we sourced our fish locally. Active ingredients in antibiotics approved by Health Canada for salmonids include formalin, emamectin benzoate, florfenicol, oxytetracycline hydrochloride, sulfadimethoxine and ormetoprim, and trimethoprim and sulfadiazine powder (Government of Canada, 2010). Salmon farms can administer trimethoprim and sulfadiazine powder to treat a disease by the pathogen, *Vibrio anguillarum*, but must wait a

minimum of 80 days before harvesting salmon (Government of Canada, 2010). Bronopol is administered to young Atlantic salmon to treat fungal infections by *Saprolegni* spp. (Government of Canada, 2010).

This study investigated the percent cover growth rate of bread that came in contact with wild-caught and farmed salmon. Farmed salmon had a higher mean percent cover growth rate, but the results were not statistically significant. This study can aid consumers in choosing how to source their salmon. We recommend future studies to use one salmon species and to use the same parts of the salmon across all replicates.

Conclusion

This research has provided valuable insights into the growth of bacteria on salmon, particularly emphasizing the impact of antibiotics on farmed salmon. Although the results were not significant, leading us to accept the null hypothesis stating that there is no significant difference in the percent cover growth rates between farmed and wild-caught treatments, our experiment showed the disparity in the type of mold growth between the treatments. These findings not only contribute to the scientific understanding of antibiotic influence on salmon but also have practical implications for the aquaculture industry.

The enhanced understanding of bacterial dynamics on salmon has the potential to improve the marketing of farmed salmon, benefiting both sellers and consumers. By demonstrating the significant impact of antibiotics on bacterial colonization, our research emphasizes the need for thoughtful antibiotic use in aquaculture, considering both the health of the salmon and the potential consequences for human consumption.

In conclusion, our research contributes to the broader scientific discourse on antibiotic use in aquaculture and its repercussions on salmon bacterial populations. As we move forward, these findings encourage further exploration into sustainable practices within the aquaculture industry, fostering a balance between the health of farmed salmon and the broader implications for human consumption.

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Appendix

Table 1

Percent cover shown as percentages (%) for each treatment with replicates from Day 1 to 14.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Antibiotic 1.1 Sockeye	0	0	0	0	0	0	0	0	0	0	0	0	0	2.2
Antibiotic 1.2 Sockeye	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Antibiotic 1.3 Sockeye	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Antibiotic 2.1 Sockeye	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Antibiotic 2.2 Sockeye	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Antibiotic 2.3 Sockeye	0	0	0	6	8	10	12	12	16	16	18	20	22	22
Antibiotic 3.1 Atlantic	0	0	19.6	27.5	27.5	33.3	47.1	52.9	58.8	62.7	68.6	68.6	68.6	68.6
Antibiotic 3.2 Atlantic	0	0	0	24	24	24	34	44	76	80	82	84	92	100
Antibiotic 3.3 Atlantic	0	0	0	7.7	7.7	15.4	17.9	33.3	41.0	48.7	59.0	69.2	71.8	89.7
Wild 1.1 Atlantic Pink	0	0	0	4.3	8.5	17.0	19.1	19.1	21.3	21.3	21.3	27.7	29.8	31.9
Wild 1.2 Atlantic Pink	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wild 1.3 Atlantic Pink	0	0	0	2.6	2.6	2.6	2.6	7.7	7.7	7.7	12.8	15.4	23.1	35.9
Wild 2.1 Coho	0	0	0	2.1	4.3	6.4	8.5	8.5	8.5	17.0	21.3	25.5	25.5	44.7
Wild 2.2 Coho	0	0	0	2.2	2.2	4.3	6.5	6.5	6.5	8.7	13.0	15.2	15.2	32.6

Wild 2.3 Coho	0	0	0	5.9	13.7	13.7	13.7	21.6	23.5	27.5	29.4	29.4	29.4	41.2
Wild 3.1 Coho	0	0	0	0	0	0	0	0	0	2.3	4.5	4.5	11.4	18.2
Wild 3.2 Coho	0	0	0	0	0	0	0	0	2.1	2.1	8.3	16.7	16.7	20.8
Wild 3.3 Coho	0	0	0	0	0	0	6.1	12.2	14.3	18.4	22.4	22.4	22.4	30.6
Control 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Control 2	0	0	0	0	0	0	0	0	0	0	0	4.7	4.7	4.7
Control 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0

For each of the 6 treatment groups and the control, a graph of the percent cover (%) from Day 1 to 14 was created using the 3 replicates in each treatment. An example is shown in Figure 1. A trendline was produced for each replicate and the mean of the slope of the 3 replicates was calculated to produce the growth rate mean.

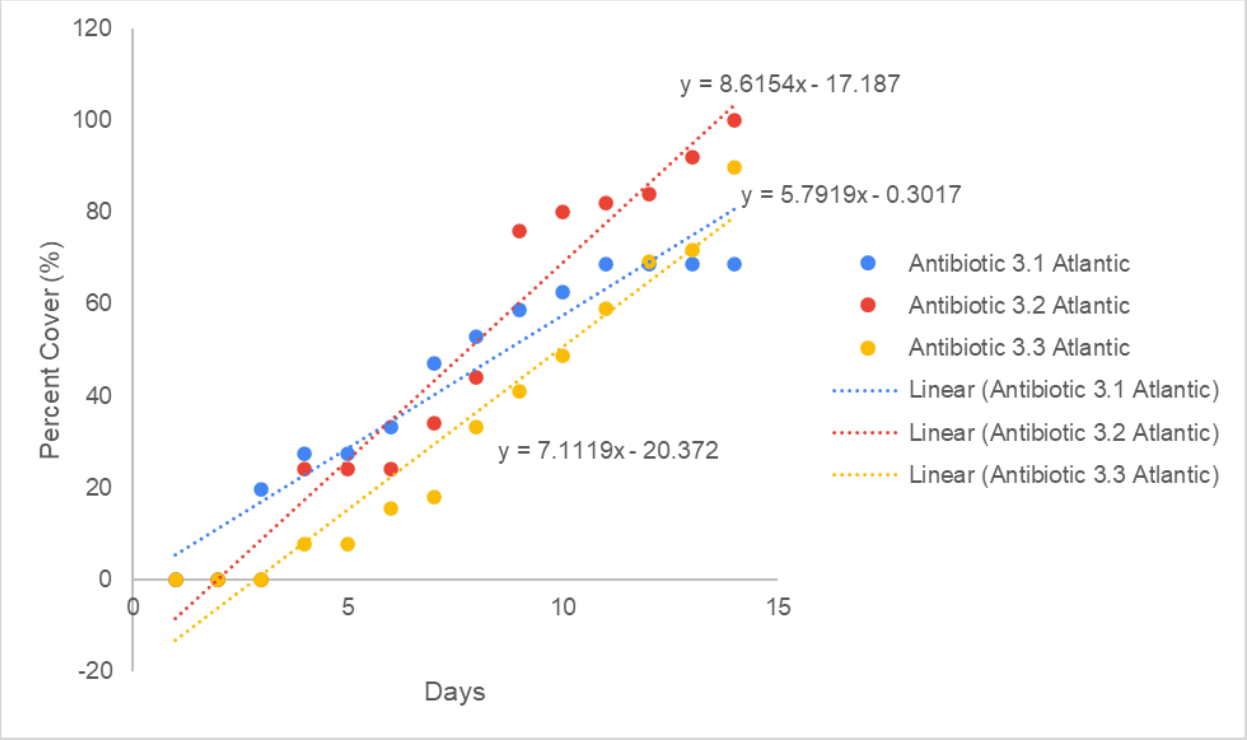

Figure 1. Percent cover (%) from Day 1 to 14 of Antibiotic 3 Atlantic. Replicates include: Antibiotic 3.1 Atlantic (blue), Antibiotic 3.2 Atlantic (red), and Antibiotic 3.3 Atlantic (yellow). Trendlines for each replicate are shown in their respective colours. The equation for each trendline is included next to the appropriate trendline.

Table 2

Growth rates for each replicate and mean growth rates.

Treatment:	Antibiotic 1 Sockeye	Antibiotic 2 Sockeye	Antibiotic 3 Atlantic	Wild 1 Atlantic Pink	Wild 2 Coho	Wild 3 Coho	Control
	0	0	5.79	2.61	2.85	0.98	0
	0	0	8.62	0	1.85	1.49	0.34
	0	1.87	7.12	2.15	3.06	2.52	0
Mean:	0	0.62	7.18	1.59	2.59	1.66	0.11

Table 3

Results after performing a one-way ANOVA test.

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	13.92	2	6.96	1.15	0.34	3.55
Within Groups	109.01	18	6.06			
Total	122.93	20				