

## Conference Paper

# Safety Analysis of Freshwater Fish

Nikolai Fedorov, Nikolai Soloviev, and Yuri Gak

Federal State Budgetary Educational Institution of Higher Education “Don State Agrarian University”, Persianovski, Russian Federation

**ORCID:**Nikolai Fedorov: <http://orcid.org/0000-0001-9451-5897>**Abstract**

The results presented in this paper demonstrate that the microbial population in the hydroecosystem has seasonal variations. The bacterial contamination of water and fish in November was 140 and 11 times less than in July respectively. It was found that fish caught in natural reservoirs in the autumn-winter period had different dynamics in the development of postmortem changes in comparison with those caught in the summer period. Postmortem secretion of mucus was not as pronounced in autumn-winter as in summer. Postmortem rigidity on the contrary was pronounced; gill covers were tightly fitting; and spinal muscles were tensed and of dense texture. Storing fresh river fish at a temperature of 3-5°C for 24 hours did not lead to a change in sensory evaluation. Physic-chemical indicators corresponded to the values characterizing fresh, healthy fish. During storage, the bacterial contamination of fish increased by more than 100 times, while the maximum permissible number of microorganisms ( $5.0 \times 10^4$  CFU / g) was not exceeded.

Corresponding Author:

Nikolai Fedorov

79298132049@yandex.ru

Published: 5 April 2021

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Selection and Peer-review under the responsibility of the DonAgro Conference Committee.

**Keywords:** fish, microbiological control, organoleptic and physic-chemical indicators.

## 1. Introduction

Fish and fish products are extremely important in human nutrition. Their range and quantity are growing from year to year [3]. However, fresh fish is a very unstable product and it is included in the category of perishable products [1]. Fresh fish begins to decay in 12 to 24 hours after death when it is stored without refrigeration [14,15].

It was found that the pollution of the reservoir has a direct effect on the native aquatic microflora and the microbiocenosis of fish, changing their quantitative and qualitative ratio [2, 9]. The causative agents of a number of fish infectious diseases are potentially pathogenic microbes that populate the skin integuments and mucous membranes as normal flora. These include, in particular, bacteria of the genus *Aeromonas*, *Pseudomonas*, *Streptococcus*, the representatives of the Enterobacteriaceae family, etc. [5, 7]. Under adverse conditions, the natural resistance of fish decreases and the bacteria that

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inhabit it penetrate into the internal organs; they start active reproduction, causing all kinds of negative phenomena, both in the internal organs and in muscle tissue [6].

Thus, the quality control of fish and fish products involves the fulfillment of sanitary and hygienic requirements for the cultivation, fishing, transportation, processing, storage and sale of aquatic organisms [4, 11, 13]. In this regard, the task was to study the dynamics of physic-chemical and bacteriological indicators of river fish during storage.

## 2. Methods and Equipment

The object of research was a carp grown in aquaculture and commercial fish caught in the waters of the Don River. The study involved more than 150 samples of pond and river fish stored at a temperature of 3-5 ° C.

The veterinary and sanitary examination of freshwater fish was carried out using generally accepted organoleptic, microbiological, physic-chemical and biochemical methods. The change in the main indicators characterizing the good quality of fish was evaluated in dynamics over 24 hours.

## 3. Results

During the analysis of the data in Table 1, a pronounced seasonal difference in the number of microorganisms, both in water and in fish caught in aquaculture, is of great interest. Enterobacteria reached their maximum development in summer, amounting to  $6.8 \times 10^3$  CFU / cm<sup>3</sup> in water, which was 10 times more than in autumn. A similar situation was during the assessment of total microbial contamination of water and fish, in which the number of microflora in November was 140 and 11 times less than in July.

TABLE 1: Sanitary indicators of water in pond and microbial contamination of fish flesh

| Indicators   | Research object             | Research month    |                   |
|--|-----------------------------|-------------------|-------------------|
|  |                             | July              | November          |
| Quantity of Mesophilic Aerobic and Facultative Anaerobic Microorganisms (QMAFAnM), | Water CFU / cm <sup>3</sup> | $6,9 \times 10^8$ | $4,9 \times 10^6$ |
|  | Fish CFU / g                | $4,9 \times 10^3$ | $4,4 \times 10^2$ |
| Coliform bacterias   | Water CFU / cm <sup>3</sup> | $6,8 \times 10^3$ | $5,1 \times 10^2$ |
|  | Fish CFU /0,01g             | absent            | absent            |

The safety of the flesh of carps grown in aquaculture was confirmed by physico-chemical and bacteriological studies (Table 2). The concentration of hydrogen ions in the flesh extract of carps in all samples corresponded to standard indicators; hydrogen sulfide and ammonia were not detected. Benzidine test, gave a positive reaction during the observation period.

TABLE 2: Physico-chemical and bacteriological indicators of fish grown in aquaculture (n=14).

| Indicators                | Observation time, hour |                     |                     |                     |                     |
|---------------------------|------------------------|---------------------|---------------------|---------------------|---------------------|
|                           | 2                      | 6                   | 12                  | 24                  |                     |
| pH of fish                | 6,93±0,11*             | 6,75±0,27           | 6,81±0,07*          | 6,86±0,13           |                     |
| Bacterial load            | Surface layers         | absent              | absent              | single cocci        | cocci +             |
|                           | Deep layers            | absent              | absent              | single cocci        | cocci +             |
|                           | QMAFAnM, CFU/g         | 2,7×10 <sup>2</sup> | 4,7×10 <sup>2</sup> | 3,3×10 <sup>3</sup> | 4,1×10 <sup>4</sup> |
| Peroxidase response       | +                      | +                   | +                   | +                   |                     |
| Hydrogen sulfide reaction | –                      | –                   | –                   | –                   |                     |
| Ammonia reaction          | –                      | –                   | –                   | –                   |                     |

Note: P<0,05\*; P<0,01\*\*; “single cocci” - up to 10 coccal forms in 25 per visual field; “cocci +” - up to 20-25 coccal forms in 25 per visual field

Microorganisms were not found in the course of bacterioscopy of smears of fingerprints from the surface layers taken before the implementation start. The underlying structures were sterile. No traces of muscle tissue decay were detected. A similar pattern was observed during twelve hours of observation. After 12 hours, the situation changed. Up to 20–25 cocci were found in surface and deep layers of muscle tissue. Decay of muscle tissue was not recorded.

The evaluation of the purity of fish caught in natural reservoirs in the autumn-winter period allowed establishing the changes in the dynamics of development of posthumous changes in comparison with the summer period. Thus, there was not pronounced post-mortem secretion of mucus as in summer. The mucus was transparent, non-sticky, it evenly covered fish flesh.

Post mortem rigidity on the contrary was pronounced; gill covers were tightly fitting, spinal muscles were tensed, the texture was dense. The deflection angle of the body of such fish as pikeperch, roach, was no more than 25-300, and these indicators persisted throughout the observation period (24 hours).

The results of the organoleptic research of the studied fish at all stages corresponded to the indicators of fresh fish.

All fish samples were characterized by the following: the body configuration was not broken, the anal ring was sunken, the eyes were convex, with a transparent cornea, the jaws were closed, the gill covers were tight and the gills were bright red, covered

with viscous, clean and transparent mucus and odorless. The scales were glossy, clean, firmly held in pockets, by the 24th hour of storage they lost their gloss due to drying out.

The flesh was dense, elastic and odorless, the muscles were tightly connected to the spine and ribs, the muscle pattern corresponded to the species of studied fish, the pit from finger pressure was quickly reformed. The abdominal cavity was dry, with a “fishy” smell, the intestines were not swollen, there was no gall staining around the gall bladder, the kidneys were dense, bright red in color.

During cooking, the broth was transparent, there were large traces of fat on the surface, a specific smell (pleasant, fishy), the meat was well divided into muscle bundles.

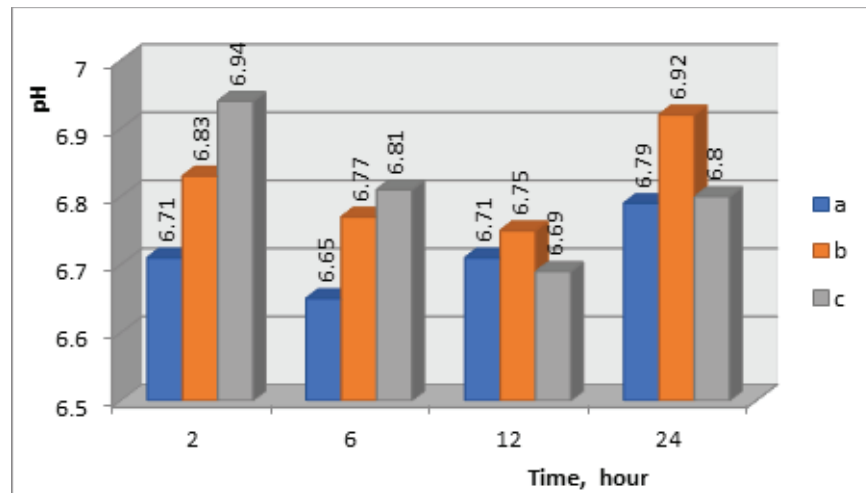
The assessment of the physic-chemical parameters of fish meat is reflected in (Table 3).

TABLE 3: Physic-chemical characteristics of fish flesh during storage at temperature 3-5°C.

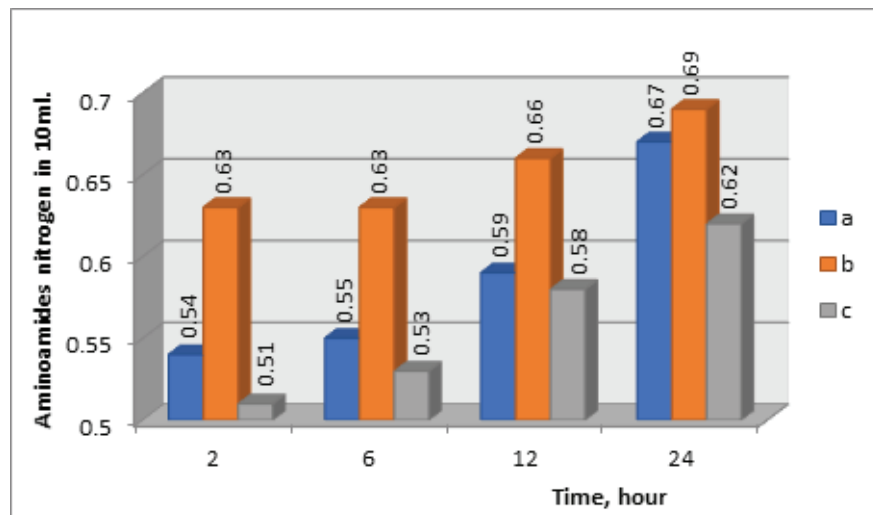
| Time, hours | Fish n=5     | Indicators  |                               |                     |                          |                           |
|-------------|--------------|-------------|-------------------------------|---------------------|--------------------------|---------------------------|
|             |              | pH of flesh | Of Ammonium nitrogen in 10 ml | Peroxidase response | NH <sub>3</sub> response | H <sub>2</sub> S response |
| 2           | pike perch   | 6,71±0,50   | 0,54±0,12*                    | +                   | –                        | –                         |
|             | bream        | 6,83±0,17*  | 0,63±0,27                     | +                   | –                        | –                         |
|             | crucian carp | 6,94±0,32   | 0,51±0,43                     | +                   | –                        | –                         |
| 6           | pike perch   | 6,65±0,08   | 0,55±0,51                     | +                   | –                        | –                         |
|             | bream        | 6,77±0,33   | 0,63±0,13*                    | +                   | –                        | –                         |
|             | crucian carp | 6,81±0,24*  | 0,53±0,04*                    | +                   | –                        | –                         |
| 12          | pike perch   | 6,71±0,19   | 0,59±0,22                     | +                   | –                        | –                         |
|             | bream        | 6,75±0,04*  | 0,66±0,08                     | +                   | –                        | –                         |
|             | crucian carp | 6,69±0,098  | 0,58±0,27                     | +                   | –                        | –                         |
| 24          | pike perch   | 6,79±0,21   | 0,67±0,41                     | +                   | –                        | –                         |
|             | bream        | 6,92±0,15   | 0,69±0,17*                    | +                   | –                        | –                         |
|             | crucian carp | 6,80±0,06*  | 0,62±0,31                     | +                   | –                        | –                         |

The pH values of muscle tissue of fish at the beginning of the study were in the range of 6.71-6.94 units (Figure 1). Subsequently, the pH curve had a classical character; it decreased to a maximum value of 6.65-6.75 units by 6-12 hours of the study and increased by 24 hours.

The graph (Figure 2) shows that the amount of amino ammonia nitrogen in fish flesh during 24 hours of storage increased in average by 20%, and its increased content in bream was most likely due to breed -specific features. In general, the studied indicator was within the boundaries characterizing fresh, pure fish.



**Figure 1:** Dynamics of pH changes of flesh extract during storage, pike perch (a), bream (b), crucian carp (c).



**Figure 2:** Dynamics of amino ammonia nitrogen in 10 ml of flesh extract during storage of pike perch (a), bream (b), crucian carp (c).

The Eber’s reagent response, the lead acetic acid and peroxidase responses corresponded to standard indicators for fresh fish at all stages of the study.

The number of microorganisms in fish flesh during the entire observation period did not exceed fresh fish standard (Table 4).

During the first six hours, the degree of contamination by microorganisms did not increase the underlying layers (Figure 3).

The exception was crucian carp, in the flesh of which the number of microorganisms increased by an order of magnitude over 6 hours of storage.

During next twelve hours of storage, the situation changed dramatically, there was a significant increase (100 times) in the population of microorganisms in muscle tissue, while the maximum allowable amount [12] ( $5.0 \times 10^4$  CFU / g) was not exceeded.

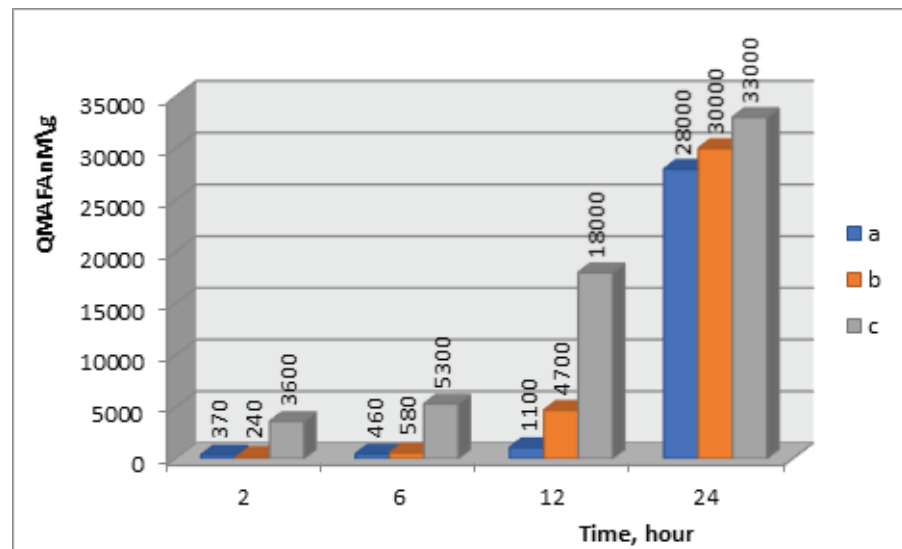
TABLE 4: Bacteriological indicators of fish flesh during storage at temperature 3-5°C.

| Time, hours | Fish n=5     | Indicators        |                               |                      |   |
|-------------|--------------|-------------------|-------------------------------|----------------------|---|
|             |              | QMAFAnM, CFU/g    | Coliform bacterias, in 0,01 g | S. aureus, in 0,01 g | Pathogenic, including salmonella and L.monocytogenes, in 25 g |
| 2           | pike perch   | $3,7 \times 10^2$ | absent                        | absent               | absent  |
|             | bream        | $2,4 \times 10^2$ | absent                        | absent               | absent  |
|             | crucian carp | $3,6 \times 10^3$ | absent                        | absent               | absent  |
| 6           | pike perch   | $4,6 \times 10^2$ | absent                        | absent               | absent  |
|             | bream        | $5,8 \times 10^2$ | absent                        | absent               | absent  |
|             | crucian carp | $5,3 \times 10^3$ | absent                        | absent               | absent  |
| 12          | pike perch   | $1,1 \times 10^3$ | absent                        | absent               | absent  |
|             | bream        | $4,7 \times 10^3$ | absent                        | absent               | absent  |
|             | crucian carp | $1,8 \times 10^4$ | absent                        | absent               | absent  |
| 24          | pike perch   | $2,8 \times 10^4$ | absent                        | absent               | absent  |
|             | bream        | $3,0 \times 10^4$ | absent                        | absent               | absent  |
|             | crucian carp | $3,3 \times 10^4$ | absent                        | absent               | absent  |

#### 4. Discussion

The seasonal dynamics of microflora extracted from water and fish has its own characteristics, due to a number of factors. One of the key is temperature, which activates or inhibits the growth of microflora. It is no coincidence that fishing out in aquaculture begins with cooling water, which leads to a significant decrease in the sanitary-indicative microflora in fish flesh (Table 1).

In our opinion, the high concentration of hydrogen ions in crucian fish is due to the increased viability of this fish species (they were alive when brought to the market) and therefore the decay of glycogen with the formation of lactic acid was at the initial stage. We should note that in the cold season, the acidity of fish flesh during the research did not exceed the normative indicators of fresh pure fish. The process of



**Figure 3:** Dynamics of microbial contamination of fish flesh during storage of zander (a), bream (b), crucian carp (c).

putrescence of meat proteins is accompanied mainly by the destruction of peptide bonds of protein molecules. Accordingly, the amount of nitrogen of amino groups and nitrogen of ammonia (ammonia nitrogen) increases, which can serve as one of the indicators of the development of putrescence of fish flesh. The initial high content of microorganisms in crucian meat is probably due to the place of fish catching- floodplain lakes, Don Rivers (stagnant water bodies) with a high content of bacteria in the water.

## 5. Conclusion

The results allow concluding that the microbial population in hydroecosystem has seasonal variations. The bacterial contamination of water and fish in November is 140 and 11 times less than in July respectively.

Short-term (24 hours) storage of nonrefrigerated river fish at a temperature of 3-5°C does not lead to significant changes in organoleptic, physic-chemical and bacteriological parameters.

## Funding

The research was carried out with the financial support of the state order of the Ministry of Agriculture of the Russian Federation as part of the research work on the topic: "Typical Regulation on the State Veterinary and Sanitary Expertise Laboratory" No. [AAAA-A20-120041490029-6].

## Acknowledgement

The authors express their gratitude to the administration of Don State Agrarian University for their support in this research. They are also grateful to all the reviewers who made a valuable contribution to this paper.

## Conflict of Interest

The authors have no conflict of interest to declare.

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