Temperature differentiation of aquatic microflora of a closed water supply system by the example of incubation of microbiological crops at 21 and 37 °C

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Abstract. In this paper, the dependence of the temperature conditions of incubation of microbiological water cultures taken directly from the pool of a closed water supply system on the activity and species diversity of the resulting microflora is analyzed. The object of the study was clarias catfish (Clarias gariepinus), which was grown at a temperature of 22-24 °, Ph 6.5-7.5, which corresponds to the optimal requirements for breeding this type of fish. The study was conducted on the basis of the Russian-Norwegian Center for Research in the field of Innovation and Aquaculture of the MSUTM named after K.G. Razumovsky. As a result of the study, a reliable regularity of the activity of aquatic microflora in CFU / ml, as well as generic and species affiliation, was revealed.

1. Introduction
The results of this study clearly reflect the dependence of the incubation temperature of microbiological crops of water microflora of the selected recirculation aquaculture systems on the numerical and specific activity of water microflora [1-2]. The authors experience and the information obtained because of this study can be useful in the field of applied Microbiology for more efficient and accurate selection of thermophilic and non-thermophilic genera and species of water microflora representatives separately from each other [3]. This will also help predicting the activity of certain genera of water microflora under conditions of changing water temperature conditions of the studied objects. Also, for the sanitary and bacteriological analysis of the water of the studied object based on the results obtained under conditions close to the temperature conditions of the human body, which can be useful in the sanitary assessment of the safety or insecurity of the studied objects, which can also help to adjust the breeding conditions [4].

In addition, the high prevalence recirculation aquaculture systems fish farming in the Russian Federation and abroad complement the relevance of this work. The technology of fish breeding in this way greatly facilitates the maintenance of hydrobiont breeding, monitoring the state of the system, and allows you to install complexes of aggregates that meet certain economic needs [5]. In addition, this type of farm has to base in regions that initially do not have the necessary natural resources for breeding the desired species of fish [6]. However, these systems are susceptible to the appearance of various potentially dangerous pathogens of aquatic microflora [7], which requires constant monitoring of water quality and safety, where the experience of this study can be applied.
2. Materials and methods
The object under study is a system for growing Clarias catfish (*Clarias glariepinus*) in a closed water supply, with an approximate temperature of 22-24 °C, Ph 6.5-7.5. The density of fish landing in the pool is 1000 individuals/m³; the feed and mineral additives used do not exceed the norms of bacterial contamination, meet the declared characteristics and are stored in conditions acceptable by the manufacturer. Among other conditions, night and day lighting modes observed in the room, environmental stress factors minimized, and the health of fish regularly monitored and sick individuals are isolated in others. That the combination of factors corresponds to the optimal requirements for breeding this type of fish.

As microbiological growth media, it decided to use Endo-Agar (Endo-GRM agar) for the selection of intestinal microflora and Soyabean Digest Casein Agar (Trypton-Soy medium) with universal selective properties for the selection of aquatic microflora:

- Water seeding carried out by the direct method without dilution, immediately after water sampling, in accordance with the established methodology. Water for crops taken directly from the fish pool [8];
- After sowing, the microbiological growth media placed for incubation separately in two Binder FD 115 thermostats, with the creation of two parallel "branches" of temperatures, respectively at 21 and 37 °C for a total of 160 hours;
- After 20-24 hours, the cultures were selected for further study for daily results of Gram staining, tests for catalase and oxidase, as well as for determining morphology by microscopy and other necessary studies;
- During incubation, the temperature regime did not change, except for the moment when the cultures was transferred for further research and for calculating the TMN, due to the need to extract microbiological media from the thermostat for the above operations.

3. Results
B According to the results of the tests, the presence of the following genera was determined (table 1).

| Movement | Morphology     | Gram color test (+/−) | Catalase test (+/−) | Oxydase test (+/−) | Genre                  |
|----------|----------------|-----------------------|---------------------|--------------------|------------------------|
| +/-*     | rod-shaped     | −                     | +                   | +                  | *Aeromonas**           |
| +        | rod-shaped     | −                     | +                   | +                  | *Pseudomonas**         |
| −        | grouping coccus| +                     | +                   | −                  | *Staphylococcus*       |
| +/-*     | grouping rod-shaped | −               | +                   | −                  | *Escherhia*            |
| +        | rod-shaped     | +                     | +                   | −                  | *Salmonella*           |
| −        | grouping coccus| −                     | +                   | −                  | *Acinetobacter*        |

* During microscopy of various samples known as the genera Aeromonas and Escherhia, both mobile and stationary bacteria were detected, which is a consequence of the presence of several different species in the samples, respectively mobile and non-mobile; ** According to the test results, some cultures had identical test results, and it was determined from the totality of all known facts that the same test result belongs to two genera.

Based on the data obtained, the following genera identified: *Escherhia, Salmonella, Staphylococcus, Pseudomonas, Aeromonas, and Acinetobacter*:

- E. cloacae identified as belonging to the genus *Escherichia*. As well as the presence of two undefined species of *Escherhia spp*, this species is an indicator of water pollution and a
conditional pathogen and has only a veterinary and sanitary hazard factor. Highly pathogenic species such as *Escherichia coli* O104:H4 and *Escherichia coli* O157:H7 not detected;

- S. salmonella, which is pathogenic to humans, was definitely proved to belong to the genus *Salmonella*, but its number was relatively small (approximately 10-15% of the OMH);
- The genus *Pseudomonas* definitely proved to belong to *P. fluorescens*; *P. aeroginosa*. Fluorescent Pseudomonas is more of a fish pathogen;
- The genus *Aeromonas* definitely proved to belong to *A. hydrophilia*, possibly the presence of *A. eucrenophilia*;
- The genus *Staphylococcus* designated by one representative - *S. aureus*;
- The species belonging of the detected bacteria of the genus *Acinetobacter* could not be determined for certain (hereinafter referred to as *Acinetobacter spp*);
- The genus *Vibrio* not found;
- Traces of the presence of representatives of the genus *Proteus* were also been found, tests showed the presence of *Proteus vulgaris*. Nevertheless, it was not possible to take into account their species quality and, later, their quantitative value reliably.

Based on the results of incubation for 160 hours, the following TMN/Ml results obtained (figures 1 and 2).

According to the results of incubation, the following TMN/Ml results obtained (figures 1-4 and table 2).

**Figure 1.** Average TMN*10^3/ml at 21 °C on Endo-GRM Agar and Trypton-soyabean agarn for 1-7 days.
The graphs clearly show the temperature dependence of TMN/mL on the incubation temperature. The decrease in the number of colony-forming units on all types of media during the transition from 21 to 37 °C was obviously predictable due to the inhibitory effect of temperature increase.

During the study of microflora, the average ratio of detected and counted species was determined (figures 1 and 2).

**Figure 2.** Average TMN*10^3/mL at 37 °C on Endo-GRM Agar and Trypton-soyabean agar for 1-7 days.

**Figure 3.** Ratio of detected species to genera based on incubation results at 21 °C for 160 hours.
Figure 4. Ratio of detected species to genera based on incubation results at 37 °C for 160 hours. * The empty area on the diagram indicates a decrease in TMN/ml relative to the temperature of 21°C.

Along with the decrease in population, the diagrams clearly show a decrease in the species diversity of the detected crops. This moment was also obviously predictable, due to the "cutting off" of non-thermophilic cultures by inhibiting their activity by increasing temperature, as well as the General depressing effect due to changing conditions.

Table 2 provides information on COE/ml by birth:

| Genre              | TMN*10^3/Ml at 21°C | TMN*10^3/ML at 37°C |
|--------------------|---------------------|---------------------|
| Aeromonas          | 18                  | 15                  |
| Pseudomonas        | 19                  | 8                   |
| Acinetobacter      | 5                   | 0                   |
| Escherichia        | 59                  | 20                  |
| Salmonella         | 7                   | 3                   |
| Staphylococcus     | 6                   | 9                   |

4. Discussion

The decrease in the number of colonies is due to the limiting factor of temperature, when non-thermophilic cultures do not show activity or die. However, Tripton-Soy medium reduction of almost 25% is not super-critical, on top of Tripton-soy medium is versatile enough selective properties, allowing it to be active and a certain number of coliforms that can be taken for the error and subtract from this value the average TMN/ml of Endo medium. With the result that it appears that at Tripton-soy environment KOE/ml averaged 55 from a source 114, and on Agar of Endo-timing 59 TMN*10^3/ml, in General, we can assume the identical value (9% difference). At the same time, the number of Endo-GRM Agars fell to 66 % (from 59 to 20 TMN*10^3/ml), which is due to the deterioration of environmental conditions due to increased temperature and selective properties of the medium that cut off other species. Thus, the results of COE*10^3/ml Tripton-soy medium when the
temperature fell from 59 to 35, given the subtraction results with Endo agar (a decline of 39%), without subtracting from 114 to 65 TMN*10^3/ml (35%, a difference of 4% can be taken as the error of the study). The disappearance of the genus *Acinetobacter* was also observed on microbiological media that were incubated at a temperature of 37°C, and the suppression of the genera *Escherchia*, *Salmonella*, and *Aeromonas*, among which *A. eucrenophilia*, *P. fluorescense*, and all representatives of the genus *Escherichia* [9], *P. aegidinosa* [10], in turn, as well as *A. hydrophila* [11-12], showed some resistance to increased temperature and they retained the dominant activity in the samples, compared to the remaining microflora. However, the increase in temperature did not have a noticeable inhibitory effect on the genus *Staphylococcus*, as evidenced by a slight increase in its population. In addition, during the study of the results obtained, it found that traces of the presence of *Proteus vulgaris* also disappeared from microbiological media that incubated at a temperature of 37 °C.

In addition to the above, focusing on the graphics, author say that at elevated temperatures the growth progresses less rapidly, as evidenced by the smaller slope of the graph line temperature 37°C in relation to the schedule must be 21 °C. Efficiency increase in the number colony in the first 2 days at a temperature of 37 °C was less than 50% in the next 5 — 75-90% [13].

5. Conclusion
Thus, according to the results of the study, the following conclusions were formulated:

- The temperature conditions closest to the water conditions of the USV system basin (21°C) showed the greatest results of the breadth of the species diversity of microflora, as well as greater activity and more rapid growth (on average by 50-75%), compared to the conditions of increased temperature (37°C), which was predictable;
- The decrease in the activity of the microflora under consideration under conditions of increasing temperature is clearly observed both in quantitative and species States, which was also obviously predictable;
- *Escherchia* (reduction of COE/ml more than 3 times), *Salmonella* (reduction of COE/ml about 2 times) and *Aeromonas* (almost complete disappearance of representatives of *A. tucrenophilia*) were the MOST oppressed at elevated temperatures);
- The extinction of the genus *Acinetobacte* was observed when the temperature increased to 37 °C;
- The increase in temperature did not have a noticeable effect on the activity of the genus *Staphylococcus*, the COE/ml practically did not decrease, even some growth can be noted;
- The genera *Aeromonas* and *Pseudomonas* retained the dominant abundance at both temperatures compared to other microflora, however, their species diversity was somewhat changed and depleted.

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