Acute toxicity of 4 algal toxins on 5 common fishes of the pearl river estuary

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Abstract. This study studied the common economic fish in the Pearl River Estuary, including Konosirus punctatus, Hypophthalmichthys nobilis, Oreochromis spp, dace and Carassius auratus. We evaluated the toxic effects using hydrostatic toxicity. The method is to expose the test organism to different concentrations of algal toxin, and obtain the LC50 by using the probability unit method. The results showed that the same algal toxin had significant differences in toxicity to five species of fish. 96h-LC50 of Anabaena-a toxins on 5 common fishes. is 38.1μg/L, 45.6μg/L, 68.2μg/L, 78.2 μg/L, 64.5 μg/L; MC-LR is 66.9μg/L, 226.8μg/L, 255.4μg/L, 317.5 μg/L, 184.3 μg/L; MC-RR is 91.2μg/L, 266μg/L, 285μg/L, 337.6 μg/L, 214.8 μg/L; MC-YR is 95μg/L, 299μg/L, 505μg/L, 709μg/L, 439 μg/L. Anabaena-a toxins is the most toxic to the test organism, while MC-YR is the weakest. The toxicity of different algal toxins is different for the tested organisms. In this study, we found that the toxicity of the four algal toxins was from strong to weak: Anabaena-a toxins>MC-LR>M C-RR>MC-YR, this research results can provide reference and basic data support for the water environment quality and water supply safety in Dawan District and Pearl River Estuary.

1. Introduction

The Pearl River Estuary is a typical brackish water boundary water area with abundant fish resources. With the rapid growth of the social economy, the eutrophication of the Pearl River estuary has become increasingly serious. It causes the breeding and reproduction of algae in water, and the competition of dominant species occurs. Algae secrete toxins during the competition, leading to water environmental problems. It may affect aquatic organisms to varying degrees and reach the human body through the food chain, affecting human life and health. At present, the toxicological effects of algal toxins on fish have become one of the research hotspots at home and abroad, trying to explain the effects of algal toxins on the water environment through animals. As early as 1878, it was found in Alexandrina Lake in southern Australia that livestock poisoning was caused by drinking water pollution [1]. Since then, there have been many reports of poisoning and death of animals such as birds, fish and livestock caused by water contaminated with algal toxins. For example, Puschner et al. [2] reported that there were 24 poisonings in 175 cattle after drinking pond water contaminated with cyanobacteria. death. The lyophilized extract of the cyanobacterial cells collected on site was induced by intraperitoneal injection to cause death and hepatocyte necrosis in mice. The on-site water samples were tested by HPLC and contained higher MC-LR. There have also been cases of death of wild birds due to microcystins poisoning in Japan [3]. Karjalainen reported [4-6] that nodular toxins can eventually
accumulate in the food chain through *Paralichthys olivaceus*, *Acipenser sinensis*, *Mylopharyngodon piceus* and *Oncorhynchus keta*, and produce toxic effects, resulting in a large number of fish deaths. Xu Guodong et al [7] reported that a large number of dead fish appeared in the fish pond in the base of the South Sharon Island in Guangzhou in 2003. After monitoring, it was found that the dead fish were overgrowth due to water alga, the fish clams were entangled by algae, and there were higher algal toxins in the water. Therefore, it is very necessary and meaningful to study the acute toxicological effects of different algal toxins on different fish species. In this paper, the economics of *Konosirus punctatus*, *Hypophthalmichthys nobilis*, *Oreochromis spp*, dace and *Carassius auratus*, which are common in the Pearl River Estuary, were studied. The acute toxicological effects and influencing factors of algae toxins in the waters were discussed, which were the Pearl River estuary MCs. Provide a basis for bioaccumulation and aquatic product health risks in the waters.

2. Materials and instrument reagents

2.1. Instrument reagents
High performance liquid chromatography (Agilent 1260), methanol (meck, HPLC), acetonitrile (meck, HPLC), anabaena-a toxin, MC-LR, MC-LR, MC-RR, MC-YR (purity 98%, Shanghai Anpu)

2.2. Biological collection
Test organisms include the common fish *Konosirus punctatus*, *Hypophthalmichthys nobilis*, *Oreochromis spp*, dace and *Carassius auratus*. *Konosirus punctatus* has found that there is no cultured fish species. Therefore, Nansha Port is selected for fishing near the Pearl River estuary. It weighs 15±1.0g and is 10-15cm long. *Hypophthalmichthys nobilis*, *Oreochromis spp*, dace and *Carassius auratus* are purchased in the market. The market purchased the same fish species fry, *Hypophthalmichthys nobilis* 10 ± 1.0g, length 10-12cm, *Oreochromis spp* 2 ± 0.5g, length 3-4cm, *Carassius auratus* weight 0.1 ± 0.05g, length 2-3cm and dace weight 0.1 ±0.05g, length 3-4cm.

2.3. Test conditions
The test adopts the static water test method. The laboratory window is covered with blinds. During the test, no bait is fed, no water is changed, continuous inflation, and the dead individual is cleared and recorded in time. The experimental seawater was taken from Nansha, Guangzhou, activated carbon filtration, pH 8.01, dissolved oxygen was 7.94 mg/L, salinity was 6.75 NTU, water temperature was 18-22 °C, Anabaena toxins, MC-LR, MC-RR, MC-YR was not detected in the collected seawater. All sampling and parameter detection methods refer to the “Marine Monitoring Specification” (GB17378.4-2007), in which pH is measured by pH method (detection time limit is 0.01), salinity is measured by salinity method (detection limit is 0.01NTU), water temperature Using the thermometer method, Anabaena toxins, MC-LR, MC-RR, MC-YR using HPLC

2.4. Test settings
2.4.1. Formal test concentration range. Pre-experimental determination of the highest survival concentration and the lowest total lethal concentration as the concentration range of the formal test. In the formal test, 6 to 8 concentration gradients were designed according to the equidistant group spacing, and another control group was set up(see Table 1). All the experiments were performed in groups of 3 groups.

The test vessel is 60 cm×45 cm ×35 cm glass aquarium. Each group was randomly placed into the initial number of test organisms is 10, and the water volume was 20L.
2.4.2. Test biological death criteria. Use a glass rod to tap the fishtail handle. If you find it doesn't move, prove it is dead.

2.4.3. Recording. Record the observed number of deaths and abnormal individuals, calculate the percentage of death by taking the average of 3 parallel groups; find the corresponding probability units according to the percentage of death, and calculate the LC_{50} according to the test concentration, and require the mortality of each parallel group. The difference is less than 20%, otherwise the test is repeated. The linear regression equation was drawn by using spss22.0 software, and the 96h semi-lethal concentration and 95% confidence interval were obtained.

### Table 1. Acute toxicological test algal toxin concentration.

| Fish                      | toxins            | concentration (μg/L) |
|---------------------------|-------------------|---------------------|
| Konosirus punctatus       | Anabaena-a toxins | 1 2 3 4 5 6 7       |
| Hypophthalmichthys nobilis| MC-LR             | 0 20 30 40 60 80 120|
| Oreochromis spp           | MC-RR             | 0 50 100 200 300 400600 |
| dace and Carassius auratus| MC-YR             | 0 50 100 200 300 400 600 |

3. Results and discussion

3.1. Test results

### Table 2. The acute toxicity results of Anabaena-a toxins for five fishes at 96h.

| Fish                      | Regression equation | LC_{50}(μg/L) | 95% Confidence interval(μg/L) |
|---------------------------|---------------------|---------------|------------------------------|
| Konosirus punctatus       | y=0.0703x+2.3198    | 38.1          | 13.5-99.3                    |
| Hypophthalmichthys nobilis| y=0.0601x+2.2633    | 45.6          | 16.8-146.7                  |
| Oreochromis spp           | y=0.0587x+0.9966    | 68.2          | 2.89-110.6                  |
| dace                      | y=0.0561x+0.6213    | 78.2          | 1.6-71.3                    |
| Carassius auratus         | y=0.0721x+0.3477    | 64.5          | 7.93-70.2                   |

### Table 3. The acute toxicity results of MC-LR for five fishes at 96h.

| Fish                      | Regression equation | LC_{50}(μg/L) | 95% Confidence interval(μg/L) |
|---------------------------|---------------------|---------------|------------------------------|
| Konosirus punctatus       | y=0.0127x+4.1535    | 66.9          | 98.5-546                    |
| Hypophthalmichthys nobilis| y=0.0178x+1.169     | 215.2         | 37.6-485                    |
| Oreochromis spp           | y=0.0163x+0.8372    | 255.4         | 25.8-475                    |
| dace                      | y=0.0159x-0.0791    | 317.5         | 20.2-471                    |
| Carassius auratus         | y=0.0166x+1.9411    | 184.3         | 45.8-495                    |

The behavior of the five tested fish in algae toxins is: rapid breathing - chaotic swimming - body rollover - loss of balance - swimming ability and respiratory function weakened - poisoning paralysis - death. As the concentration of algal toxins increases, the fish breathe faster (the bubbles on the water surface increase gradually), the bowel movements increase, the swimming speeds up, occasionally convulsions, and individual rollovers. The above indicates that the tested fish showed a sensitive stress response in the water containing algal toxins. The concentration-effect relationship, LC_{50} and 95% confidence intervals between the four algal toxins and the tested fish are shown in Tables 2-5. In the test, the mortality rate of the test fish control group was 0, which met the requirements of the standard
method of emergency toxicity test. It can be seen from Tables 2 to 5 that the toxicity of algal toxins on the tested fishes tends to increase with the extension of exposure time within 96 hours. The tolerance of the tested fish to different algal toxins was significantly different. Based on 96h-LC$_{50}$, the toxicity of the four algal toxins to the tested fish was as follows: Anabaena-a toxins>MC-LR>MC-RR>MC-YR. The order of tolerance of the 5 tested fish to 4 algal toxins was: dace >Oreochromis spp > Carassius auratus > Hypophthalmichthys nobilis > Konosirus punctatus.

Table 4. The acute toxicity results of MC-RR for five fishes at 96h.

| Fish            | Regression equation | LC$_{50}$(μg/L) | 95% Confidence interval(μg/L) |
|-----------------|---------------------|-----------------|------------------------------|
| Konosirus punctatus | y=0.0125x+3.862   | 91.2            | 88-539                       |
| Hypophthalmichthys nobilis | y=0.0171x+0.482   | 265.9           | 22.3-475                     |
| Oreochromis spp | y=0.0166x+0.2744   | 284.9           | 18.7-467                     |
| dace            | y=0.0133x+0.507    | 337.6           | 23.1-473                     |
| Carassius auratus | y=0.0133x+0.508   | 214.8           | 53-647                       |

The acute toxicity results of MC-YR for five fishes at 96h.

| Fish            | Regression equation | LC$_{50}$(μg/L) | 95% Confidence interval(μg/L) |
|-----------------|---------------------|-----------------|------------------------------|
| Konosirus punctatus | y=0.0098x+4.0737   | 94.9            | 77.6-776.5                   |
| Hypophthalmichthys nobilis | y=0.0128x+1.1719 | 299.2           | 11.7-689                     |
| Oreochromis spp | y=0.0096x+0.1474   | 505.5           | 19-679                       |
| dace            | y=0.0074x-0.2456   | 708.9           | 16.8-714                     |
| Carassius auratus | y=0.0109x+0.1684  | 439.2           | 24-679                       |

3.2. Analysis and evaluation

The results showed that the tolerance of five common economic fishes in the Pearl River Estuary to different algal toxins was anabaena toxin>MC-LR>MC-RR>MC-YR. Separately, anabaena is the most toxic, followed by MC-LR, and MC-RR and MC-YR are gradually reduced. According to the different algae, the toxic death of algae after death is not the same. Among the many isomers, the most common, high content, and toxic, the more detailed research is Anabaena toxin, MC-LR, MC-RR and MC-YR [8]. The reason may be that there are currently more than 80 variants of the MCs structure. Because of the different functional groups in the structure, they exhibit different toxicities. Among them, MC-LR, MC-RR and MC-YR (L, R, Y represent leucine, arginine and tyrosine, respectively), the toxicity change is MC-LR>MC-RR>MC-YR[8]. Anabaena toxin is neurotoxic and is stronger toxicity in the environment. Kenneth et al. [9] first reported that toxins produced by the decomposition of cyanobacterial blooms have certain toxicity to fish. At present, the toxicological studies of MCs on fish mainly focus on fish histopathology and accumulation in tissues caused by MCs, MCs on embryo hatching, fish behavior and growth, serum biochemical indicators, oxidase and detoxification enzyme activities, and immunotoxicity. aspect. Oberemm A et al [10-12] performed acute toxicological experiments on Zebrfish, Hypophthalmichthys molitrix and Cyprinus carpio seedlings at 0.5, 5, 50 μg/L MC-LR, MC-RR and MC-YR crude extracts, respectively. It was found that different concentrations of algal toxins have different degrees of toxicity and even death to the tested fish. Chen et al [13-15] used both intraperitoneal and hydrostatic contact methods to study acute toxicology of carp, and found that algal toxin can cause abnormal and pathological changes in carp. Yin Yiwei et al [16] studied that the LC$_{50}$ value of dinoflagellate to zebrfish is 5-30μg/L, and there are many factors affecting the biotoxicity of algal toxin. The tolerance of test organisms to algae toxin is one. Aspects may be caused by differences between species. On the other hand, it is related to the difference in individual size between the test organisms, the water environment and dissolved oxygen in water. Guo Xiaoohun [17]believe that most MCs are hydrophilic and generally cannot directly pass through the
cell membrane of vertebrates. Therefore, MC requires ATP for transport protein absorption. Bury et al.[18] demonstrated the use of $^3$H-labeled MC-LR. Through intestinal absorption, it is first delivered to the liver and muscles, not to the kidneys or spleen.

4. Conclusions
This study studied the common economic fish in the Pearl River Estuary, including Konosirus punctatus, Hypophthalmichthys nobilis, Oreochromis spp, dace and Carassius auratus. We evaluated the toxic effects using hydrostatic toxicity. Anabaena-a toxins, Konosirus punctatus, Hypophthalmichthys nobilis, Oreochromis spp, dace and Carassius auratus 96h-LC$_{50}$ is 38.1μg/L, 45.6μg/L, 68.2μg/L, 78.2 μg/L, 64.5 μg/L; MC-LR is 66.9μg/L, 226.8μg/L, 255.4μg/L, 317.5 μg/L, 184.3 μg/L; MC-RR is 91.2μg/L, 266μg/L, 285μg/L, 337.6 μg/L, 214.8 μg/L; MC-YR is 95μg/L, 299μg/L, 505μg/L, 709μg/L, 439 μg/L. Anabaena-a toxins is the most toxic to the test organism, while MC-YR is the weakest. The toxicity of different algal toxins is different for the tested organisms. In this study, we found that the toxicity of the four algal toxins was from strong to weak: Anabaena-a toxins>MC-LR>MC-RR>MC-YR, The results of this study can provide a basis for the Pearl River Estuary MCs to bioaccumulate the water and health risks of aquatic products.

Acknowledgement
Fund support: Guangdong Provincial Natural Science Foundation (No. 2017A030313329), Guangdong Science and Technology Plan Project (Project No.:2018B030320002), Open Fund of Chinese Academy of Sciences (No. SKLOG201919) and Guangzhou Science Technology Innovation Development Special (No. 201904010158).

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