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Parasitic and bacterial diseases in aquarium-reared Indonesian snakehead (*Channa micropeltes*)

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Abstract. Toman or *Channa micropeltes* has high economic potential to be cultured because of their high albumin content. This study aims to obtain information about what kinds of pathogens can infect toman fish in high-density stress (10, 20, 30 fish with an average weight of 3 - 15 g and length of 8-14 cm in 20-liter aquarium) and temperatures between 26-34°C that were treated with artificial infections of *Aeromonas hydrophila*. The results showed that stress treatments with high density and temperature did not cause significant death in toman, or *C. micropeltes*. The bacteria that can be isolated from toman fish were *Aeromonas* sp., *Moraxella lacunata*, *Vibrio vulnificus*, and *Brevundimonas vesicularis*. The parasites found in toman were *Trichodina* sp. (Prevalence (P) = 25%, Intensity (I) = 1), *Gyrodactylus* sp. (P= 35%, I= 1.4), *Myxosporea* sp. (P= 25%, I= 1.2), and *Oodinium* sp. (P= 20%, I= 7.5), which infected gills, mucus, gut, and skin. Being treated with artificial infection of *A. hydrophila*, toman fish were very susceptible and 100% death occurred after 24 hours of treatment. The treatment of predisposition factors in toman fish, which can prove adaptation and survival from stress conditions and the presence of the pathogen, will have an impact on the aquaculture development of toman fish so that health management will be applied in aquaculture.

Keywords: Toman, predisposition factors, stress, high density, temperature, artificial infection

1. Introduction

Toman or *Channa micropeltes* is predator fish known by the name giant snakehead because the adult toman can reach about 150 cm length and is the second-largest fish in the Channidae family. *Toman* fish is native to Southeast Asia. They can be found in Thailand, Mekong River, Vietnam, Laos, Malaysia, and northern Sumatra. This fish has a very attractive body-color with elongated cylindrical posture. Toman is a freshwater fish that can live in various habitats, such as swamps, lakes, ponds, rivers, and others. Toman lives in freshwater and brackish water. This fish can live in oxygen-deprived waters because it can take oxygen directly from the air. This is because the fish also breathes using primitive lungs that are located behind the gills [1].

Toman, which is related to snakehead, have high economic potential to be cultured because of their high albumin content. In Malaysia, omega fatty acids and albumin of this fish have been extracted for use in the pharmaceutical field. This fish is classified as a carnivorous fish belonging to the Channidae family. There are a total of 30 *Channa* spp., which are identified throughout the world, but only 7 species are known to originate from Malaysia. One type of *Channa* is toman [2, 3].



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Disease is a condition that occurs in living organisms that experience physiological dysfunction due to changes in the body's system and usually shows signs and symptoms. Disease arises due to pathological factors in the form of pathogens in the aquaculture environment, weak immune system, and culture media that are not beneficial [3]. In line with the aquaculture development of toman, some disturbing problems such as diseases also emerge. This study aims to obtain information on types of pathogens that can infect toman that was treated with artificial infections in high-density stress and high temperatures. This is based on the allegation that high density and temperature are triggering factors for disease in toman, so that action or control strategies should be taken.

2. Materials and methods

2.1. Natural conditions

Parasites and bacterial isolates were screened for toman during the acclimation period and before the fish were treated.

2.2. High density conditions

The treatments in this study were: (A) Seed size of 15 g (\pm 14 cm) with a density of 10 fish/aquarium; (B) Seeds measuring 15 g (\pm 14 cm) with a density of 20 fish/aquarium; (C) Seed size of 15 g (\pm 14 cm) with a density of 30 fish/aquarium and two replications. Each aquarium was filled with 20 L of water. During the maintenance, the tested fish were given commercial feed twice a day on an ad libitum basis. Then, observation on parasites, bacteria, behavior, clinical symptoms, and mortality of the tested fish was carried out every day until the end of the trial for 21 days [4].

2.3. Temperature stress conditions

The treatments used in this research were: seed size 3-5 g (\pm 7-8 cm), the density of 10 fish/container that was filled with 20 L water with temperatures ranging from 20°C to 34°C. The temperature treatments were (1) 20°C, (2) 26°C, (3) 30°C, and (4) 34°C. Water temperature stress on treatment (1) was conducted by placing it in the test container and the room with Air conditioner (AC), the treatment (2) was conducted by using two water heaters per container. Each container was equipped with a minimum-maximum thermometer that was reset every 24 hours. The experimental treatment was conducted with two replications. Parasites, bacteria, behavior, clinical symptoms, and mortality of the tested fish were observed every day for 21 days [4].

2.4. Artificial infection in toman

Preparation of the suspension of pathogenic bacteria was conducted by culturing *A. hydrophila* on TSA media and incubating the culture at 25°C for 24 hours. Then, the bacterial suspension was made by a dilution method with a density of 10⁶CFU/mL in sterile physiological salts (0.85% NaCl). Each of the 15 experimental fish with two replications was injected with 0.1 mL intramuscular bacterial suspension [5]. All injected fish were transferred to the tested tank and observed for seven days from the trial period. No feed was given during this time. Injected fish were observed for clinical symptoms, death, and pathogenicity of the tested pathogens. To confirm the infection of the tested fish, re-isolation of the infected fish was carried out and culture was prepared for TSA.

3. Results

The results of laboratory-scale observations during the study identified microbes in toman during the acclimatization period and before stress treatments were presented in Table 1.

Table 1. Type of parasites isolated from *toman* fish before high-density stress, thermal stress, and artificial infection

Toman	Parasite		
	Gill	Fin	Mucus
Toman fish 1	<i>Trichodina</i> sp.	<i>Trichodina</i> sp.	<i>Trichodina</i> sp.
Toman fish 2	<i>Trichodina</i> sp.	-	-
Toman fish 3	<i>Trichodina</i> sp.	-	<i>Trichodina</i> sp.
Toman fish 4	<i>Gyrodactylus</i> sp.	-	-

The types of bacteria that can be isolated from *toman* fish before treatment, namely, *Aeromonas hydrophila* and *A. Salmonicida*, can be identified based on biochemical tests that have been carried out.

3.1. Stress stocking conditions

The parasites found in toman samples were from the genus of *Gyrodactylus* sp., *Trichodina* sp., *Myxosporea* sp., and worms. The parasite *Gyrodactylus* sp. infected only on the surface of a fish's body (fins, operculum, and body surface). The kind of parasite is called an ectoparasite. Therefore, *Gyrodactylus* sp. can move from gill to body surface or vice versa. Infection from *Gyrodactylus* sp. is not too dangerous, but if these parasites are in large numbers, it can cause damage to the gills. This is due to the gills containing many blood capillaries which are food for *Gyrodactylus* sp. [6]. Other general effects of the parasitic attack of *Gyrodactylus* sp. are slow growth and weight loss. As can be seen in Figure 1, the parasites found before the treatment were *Trichodina* sp. and *Gyrodactylus* sp. Observation of parasites from mucus on the body of toman has identified *Trichodina* sp. (Table 2). The results showed the prevalence (P) and intensity (I) of the parasites of toman, such as *Trichodina* sp. in gill, fin, and mucus (P= 25%, I=1), *Gyrodactylus* sp. in mucus (P=35%, I=1.4), *Myxosporea* sp. in fin (P=25%, I=1.2), and *Oodinium* sp. in mucus (P= 20%, I=7.5).

Table 2. Parasites in toman after stress treatment of stocking densities

Toman	Parasite		
	Gill	Fin	Mucus
Toman fish 1	<i>Trichodina</i> sp.	<i>Trichodina</i> sp.	<i>Trichodina</i> sp.
Toman fish 2	-	<i>Trichodina</i> sp.	<i>Oodinium</i> sp.
Toman fish 3	-	<i>Myxosporea</i> sp.	<i>Trichodina</i> sp.
Toman fish 4	-	-	<i>Gyrodactylus</i> sp.

The results of API 20 NE for identification of the bacterial isolates obtained *Brevundimonas vesicularis*, *Aeromonas* sp, *Vibrio vulnificus*, and *Moraxella lacunata*.

**Figure 1.** The results of identification of parasites before toman were given stress treatment (a). *Trichodina* sp., (b). *Gyrodactylus* sp.

3.2. Temperature stress

Toman in each treatment for 21 days of maintenance did not experience a lot of mortality in the first week and began to show symptoms of death at 20°C because the fish is susceptible to temperature.

Biochemical test results and API 20 NE and API 20 E for pathogens that were isolated from toman treated with temperature and the identification results of bacteria and parasites isolation were shown in Table 3. Identified bacteria were *Aeromonas hydrophila*, *Pseudomonas* sp., and *Brevundimonas vesicularis*, while the parasites that were successfully isolated were *Trichodina* sp., *Gyrodactylus* sp., and *Oodinium* sp.

Table 3. Results of bacterial isolation in toman after thermal stress

Isolate	Bacteria
TG 3	<i>Aeromonas hydrophila</i>
TM2	<i>Pseudomonas</i> sp.
TM 3	<i>Brevundimonas vesicularis</i>
TH4	<i>Aeromonas hydrophila</i>
	Parasite
TM1	<i>Trichodina</i> sp.
TM 2	<i>Gyrodactylus</i> sp.
TM4	<i>Oodinium</i> sp.

3.3. Artificial infection in toman

This artificial infection aimed to test the virulence of *A. hydrophila* through intramuscular injection, showing that the bacterium was very virulent for toman. Because of bacterial infection with bacterial density of 10^6 cfu/mL, the tested fish experienced 100% death within 24 hours. The clinical symptoms were in the form of changes in fish behaviour. The fish became weak, inactive, and unresponsive. There was local haemorrhage at the base of the dorsal fin, base of the caudal fin, and operculum, followed by fish's death. Death occurred on the first day after the injection. This was due to higher bacterial density, with signs of injury that began to occur in the abdomen near the body and caudal fins. The circled part in Figure 2 was the location of artificial infection.



Figure 2. Toman in local haemorrhage infected with *Aeromonas hydrophila*

4. Discussion

Toman (*Channa micropeltes*) is a fish species from the family of Channidae. It is included in the genus of *Channa*. Fish of this genus are commonly known as snakeheads. It is one of the cultured fish that has very promising market potential and opportunity. This potential needs to be accompanied by the attention to improve the quality of the seedlings produced by hatcheries because hatching activities are the beginning of a series of fish farming activities. High-quality fish seedlings are one of the keys to the success of aquaculture activities.

The results of observations on high-density stress, thermal stress, and artificial infections in toman indicated that the effect of high density would result in low dissolved oxygen, whereas ammonia would increase due to fish metabolic waste and feed residue. This condition is an environmental pressure that can cause the comfort of the fish to be disturbed. Growth will be hampered because the energy for growth is used by fish to defend itself from environmental pressures [7]. Increased stocking densities will disrupt the physiological processes and behavior of fish in their range of motion, which

could ultimately reduce the health and physiological conditions of fish. A further consequence of this process is the decline in food appetite, growth, and survival [8].

Survival rate is one of the main parameters that showed success in the maintenance of an aquatic organism. The results obtained in treatments A, B, and C with stocking density of 10, 20, and 30 fish/20 L aquarium indicated that higher stocking density decreased the survival rates. This showed that the higher stocking density will increase the fish mortality. The cause of mortality was thought to be jostling fish, especially when they were competing over food. Meanwhile, mortality that occurred during maintenance was due to the increasingly narrow range of motion that put pressure on the fish. The impact of stress resulted in decreased fish endurance, so that fish easily got sick and even died.

An important factor influencing the growth and survival of fish besides feed is water quality, especially temperature. Temperature can affect important activities of fish, such as breathing, growing, and reproducing. High temperatures can reduce dissolved oxygen and fish appetite [9]. The survival rate of toman that were treated showed the same results, which is not many fish died at temperatures above 30°C. However, many fish died at temperatures below 30°C to 20°C, showing that the treatment of different temperatures gave a different effect on the survival of toman.

Temperature played the most important role in returning fish to normal or non-stressful conditions [10]. Temperature is the main environmental factor related to the stress of fish. Drastic and sudden changes in temperature can cause stress on fish. Stress is the inability of an organism to maintain the condition of homeostasis due to the disruption of the individual by external stimuli called stressors [10]. Stress on fish is caused by environmental factors (temperature, pH, high ammonia, and low DO), density, and disease. In addition, unstable temperatures may also result in slow fish growth. Changes in temperature or unstable temperatures can make toman become stressed and die. This is because the temperature is very influential in the metabolic process and metabolic processes will affect the growth of fish. Changes in water temperature can result in changes in fish habits. The cooler the temperature is, the lower the appetite and growth actually become.

The study of toman is something that still new. The current problem is that toman from their natural habitat in the river were moved to a pond or controlled container. Then, the fish experienced death because of stress. The causes of stress include external and internal changes. External changes that can cause stress responses include temperature fluctuations, lack of oxygen, and transport time. In accordance with the opinion of Scholz [11], Handisoeparjo [12] stated that basically the live fish transport to a new environment that is different from the original environment accompanied by sudden environmental changes, such as temperature that can cause stress.

5. Conclusion

Toman or *Channa micropeltes* in treatment of high-density stress and thermal stress did not experience significant mortality. Bacteria that can be isolated by toman were Gram-negative and rod-shaped bacteria, namely, *Aeromonas* sp., *Moraxella lacunata*, *Vibrio vulnificus*, and *Brevundimonas vesicularis*, while the identified parasites were *Trichodina* sp., *Gyrodactylus* sp., and *Myxosporea* sp. Whereas, in the treatment of artificial infections with *Aeromonas hydrophila*, toman were very vulnerable to 100% mortality after 24 hours of treatment.

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