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Dinamika Populasi Bakteri Pada Budidaya Ikan Koi (*Cyprinus rubrofuscus*) Sistem Resirkulasi

Dynamics Changes of Total Bacteria in the Recirculating Aquaculture System (RAS) of Koi Fish (*Cyprinus rubrofuscus*)

I Made Dedi Mahariawan[1*], Sri Andayani[1], Heny Suprastyani[1], Feni Tri Sa'adati[1]

[1]Department of Aquaculture, Faculty of Fisheries and Marine Science, University of Brawijaya, Malang, Jalan Veteran, Ketawanggede, Lowokwaru, Malang, Jawa Timur, 65145, Indonesia
*Korespondensi: imd_mahariawan@ub.ac.id

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ABSTRAK

Ikan koi (*Cyprinus rubrofuscus*) merupakan salah satu komoditas akuakultur yang memiliki daya jual yang tinggi karena memiliki corak warna dan keindahan bentuk tubuhnya. Budidaya ikan koi di masyarakat terdapat permasalahan karena hadirnya mikroba seperti parasit dan patogen lainnya yang menyebabkan timbulnya berbagai jenis penyakit. Manajemen kualitas air dilakukan sebagai upaya mencegah masuknya berbagai mikroorganisme penginfeksi. Penerapan sistem resirkulasi digunakan untuk meningkatkan kualitas air dan resistensi ikan koi terhadap penyakit. Tujuan dari penelitian ini yaitu untuk mengevaluasi teknologi sistem resirkulasi pada budidaya ikan koi dengan media filter yang berbeda terhadap populasi bakteri. Penelitian ini menggunakan 4 perlakuan yaitu perlakuan A (RAS dengan filter dakron dan batu apung), B (RAS dengan filter dakron dan pasir), C (RAS dengan filter pasir dan batu apung), dan K (kontrol tanpa sistem resirkulasi). Parameter yang diamati yaitu kepadatan bakteri di media pemeliharaan ikan koi. Hasil penelitian menunjukkan bahwa perbedaan filter yang digunakan mempengaruhi kepadatan bakteri yang dihasilkan. Kepadatan bakteri tertinggi diperoleh pada perlakuan C (RAS dengan filter pasir dan batu apung) yang memiliki nilai rata-rata pada minggu pertama sampai minggu ketiga yaitu lebih dari 3,4 log CFU/ml. Peningkatan kepadatan bakteri nitrifikasi di sistem budidaya ikan koi didukung dengan kualitas pemeliharaan yang juga berada dalam kondisi optimal.

Kata kunci: ikan hias; kepadatan bakteri; resirkulasi;

ABSTRACT

Koi fish (*Cyprinus rubrofuscus*) is one of the aquaculture commodities that is highly marketable because of its unique color pattern and body shape. The prevalence of parasites and pathogenic agents in Koi fish among farmers poses challenges and leads to the occurrence of numerous illnesses. Water quality management is implemented in an effort to prevent several infectious microorganisms. The application of a recirculation system is used to enhance water quality and bolster the disease resistance of Koi fish. The objective of this study was to evaluate the application of RAS technology in the koi fish culture under different filter media on bacterial density. This study used four treatments, namely treatment A (RAS with a dacron filter and pumice), B (RAS with a dacron filter and sand), C (RAS with a sand and pumice filter), and K (control without a recirculation system). The parameter observed in this study was the density of bacteria in the koi fish rearing-media. These findings demonstrated that the utilization of various filters had an impact on bacterial density. Treatment C (RAS with sand and pumice filter) exhibited the greatest bacterial density with an average value exceeding 3.4 log CFU. mL^{-1} from the first week to the third week. The density of nitrifying bacteria in the koi fish production system is positively influenced by the high-quality maintenance practices that are maintained under optimal conditions.

Keywords: bacterial density; ornamental fish; recirculation;

INTRODUCTION

Aquaculture is one of the fisheries sectors that contributes to the development of global industry, particularly in terms of raising societal economic standards. Fisheries resources in Indonesia are oversufficient which is supported by an overabundance of biodiversity. In Indonesia, there are also a lot of attractive opportunities for the development of ornamental fish enterprises (Sinansari & Priono, 2019; Mulyati *et al*., 2022). This condition is caused by ornamental fish having a high selling value. One type of ornamental fish that is quite popular with consumers is the koi fish (*Cyprinus rubrofuscus*) which represents its unique color pattern and body shape (Qur'ania & Verananda, 2017; Amin *et al*., 2023). Based on these advantages of this species, this fish gains appeal and increases its sales value (Maulana *et al*., 2021).

The development of koi fish culture in society presents various kinds of issues. The primary challenge frequently encountered in fish farming is the emergence of diseases induced by parasites (Elisafitri *et al*., 2021; Hailu & Mitiku, 2021). The parasitic diseases that commonly affect koi fish are caused by the presence of several microorganisms such as *Trichodina* sp., *Chilodonella* sp., *Myxobollus* sp., *Dactylogyrus* sp., and *Gyrodactylus* sp. (Prasetya *et al*., 2013; Elisafitri *et al*., 2021). The consequences of this infection encompass impaired fish movement and the detachment of fish scales (El-Deen & Elkamel, 2015). Disease infections could adversely affect some external organs such as the fins, skin, and gills which would decline the overall quality of farmed fish. In addition, the significant frequency of disease outbreaks adversely affects fish productivity which causes economic losses for farmers as a result of high mortality rates (Elisafitri *et al*., 2021).

Controlling fish farming systems is the method used to prevent the spread of harmful microorganisms. The presence of alterations in water quality which serves as a habitat for fish is impacted by the high stocking density and the frequency of feeding (Abe *et al*., 2019; Wijayanto *et al*., 2022). An effective measure to address water quality issues and promote sustainable fish culture involves the implementation of Recirculating Aquaculture System (RAS) technology (Yang *et al*., 2023; Lindholm-Lehto, 2023). This technology has the capability to enhance water quality that is unsuitable, thereby increasing its resistance to disease outbreaks (Dalsgaard *et al*., 2013).

The Recirculating Aquaculture System (RAS) is a technology applied in intensive cultivation systems that involves the continuous utilization of water. This technology offers prospects for enhancing waste management, minimizing water consumption, and reutilizing nutrients (Espinal & Matulic, 2019; Taufik *et al*., 2023). The productivity of recirculating aquaculture systems is influenced by various factors such as the kind of cultivated species, stocking density, feeding rate, the duration of the production cycle, and other aspects of management (Ahmed & Turchini, 2021). It is necessary to conduct a study on the application of RAS technology in koi fish farming in order to assess the health status of koi fish. The density of bacteria in fish living media could be determined by examining the presence of filters as a substrate for the growth of nitrifying bacteria (Lukmantoro *et al*., 2020). Therefore, the objective of this study was to evaluate the application of RAS technology in the koi fish culture under different filter media on bacterial density.

RESEARCH METHODS

Research design

This research was carried out in 2021 at the koi fishpond with recirculation aquaculture system located in Garum, Blitar, Jawa Timur. The duration of this study was one month of maintenance. This research consisted of four treatments and was repeated two times. The treatments used consisted of treatment A (RAS with a dacron filter and pumice), B (RAS with a dacron and sand filter), C (RAS with a sand and pumice filter), and K (control treatment with a non-recirculating pond). The koi fishpond provided an area of 4x4 m^2 with a depth of 50 cm. Each treatment had a stocking density of 50 koi fish per treatment pond.

Water Sampling Method

Water samples were collected from treatment ponds A, B, C, and K of koi fish (*C. rubrofuscus*) culture. Sampling occurred weekly for each treatment. Water samples were collected in each maintenance pond as much as 20 ml with 2 repetitions per treatment. The water sample was transferred into a 20 ml sample bottle and subsequently stored in a refrigerator at a temperature of 4˚C until it was ready for further analysis.

Preparation of Bacterial Culture Media

The bacterial growth medium used in this study was nutrient agar (NA) based on the method of Wijimulyati *et al*. (2019) to determine the total plate count (TPC). 1 ml of water sample was prepared and then multilevel dilutions were carried out using a physiological sodium chloride solution of 0.9%. 1 ml of the diluted sample was inoculated onto Nutrient Agar (NA) media using the pour technique. Incubation was carried out in an incubator for a duration of 24 hours at 37°C.

Bacterial Enumeration

Bacterial colonies were counted on a colony counter after the bacteria were planted on agar media and incubated in the incubator for 24 hours. The number of bacterial cells could be counted by using the total plate count (TPC) method. The results of calculating the number of bacterial cells were referred to as colony-forming units (CFU) per mL of sample (Said and Ahmed, 2022). The requirement for calculating bacterial colonies was in the range of 25-250 and was calculated into a formula based on the Indonesian National Standard (SNI, 2006).

Data Analysis

Data analysis of the bacterial count was tested using SPSS software (IBM Corp., Armonk, NY, USA) with a confidence level of 95% ($P<0.05$).

RESULTS AND DISCUSSIONS

Total Bacteria Count

The application of RAS technology in koi fish farming had a significant impact on the bacterial density observed during each sampling ($p<0.05$) (Figure 1). The highest results were obtained in treatment C with the application of RAS technology using sand and pumice filters for a duration of one to three weeks. The mean bacterial density in treatment C in the first week was 3.41 log CFU. mL^{-1}. This trend increased in the second and third weeks with a bacterial density of 3.56 log CFU. mL^{-1} and 3.72 log CFU. mL^{-1}, respectively. These results were influenced by the application of RAS combined with a filter which played an important role in maintaining water quality and thus ensuring its proper maintenance. As a result, it also decreased fish stress and enhanced fish appetite. The diversity of the microbial community in the RAS was also affected by the filter materials used (Sugita *et al*., 2005; Rodriguez-Leal *et al*., 2023). The stability and efficiency of filters in maintaining the dynamic balance of bacterial groups should be adapted to the specific conditions of each recirculating aquaculture system (Schreier *et al*., 2010). Pungrasmi *et al*. (2016) reported that the application of pumice stone was denoted as a nitrification biofilter under aerobic conditions. The porous structure of the pumice stone provided a suitable habitat for bacteria. Pumice stone as a fundamental substrate was used to remove nitrogen from fish farming systems. The pumice stone utilized was approximately 3 millimeters in diameter.

The nitrifying bacteria that attached to the pumice cavities had the ability to rapidly convert ammonia into nitrites and nitrates (Pungrasmi *et al*., 2016). Meanwhile, the sand filter acts as a physical filter to filter dirt from leftover feed and fish feces that were sucked in through the pump (Hastuti *et al*., 2020). Furthermore, sand was exhibited as the primary medium for filtration during the water filtration process especially in removing suspended solids from the water. The sand acts as a medium for retaining fine particles and sediments, typically capturing solid materials and mud from water that has been filtered through it (Huguenin & Colt, 2002; de Oliveira *et al*., 2019). Lindholm-Lehto *et al*. (2021) observed that sand infiltration proved to be a cost-effective method for removing particulate matter from the water removed from the system. Water infiltration through sand eliminates dissolved and particulate matter and improves water quality.

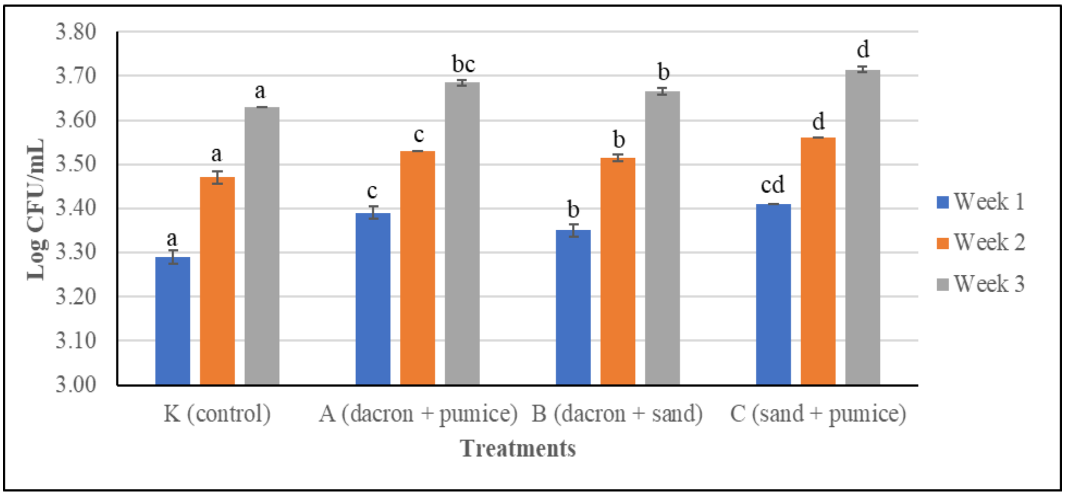

Figure 1. The mean total bacterial count in Koi fish culture (*C. rubrofuscus*)

The implementation of RAS technology involves several crucial processes, including solid waste disposal, biofiltration, carbon dioxide gas removal, oxygenation, and disinfection (Ebeling, 2000; Boaventura *et al*., 2018). Solid waste disposal efficiency is an essential process in RAS applications which could enhance the performance of other components within the cultivation system (Dauda *et al*., 2019). Generally, these solids originate from leftover feed and feces which could lead to several issues such as biofouling, ammonia production, and oxygen depletion. This condition will result in the development of a disease within the system.

Biofiltration uses living microorganisms to eliminate ammonia (through nitrification) from water. The biofilter will provide a suitable environment for the growth of microorganisms. Microorganisms convert inorganic solids such as ammonia and nitrite into nitrate through the nitrification process. The composition of microorganisms consists of ammonium and nitrite-oxidizing bacteria. It is crucial to eliminate excessive carbon dioxide gas, as it frequently induces stress in fish

(Srithep *et al*., 2015; Neissi *et al*., 2022; Almeida *et al*., 2022). The primary and crucial factor that determines water quality in RAS is the concentration of dissolved oxygen. The oxygenation in RAS enhances the oxygen supply in the water to fulfill the fish's requirements (Seginer & Mozes, 2012).

The utilization of RAS technology combined with various filter media improves the water quality and makes it more conducive and beneficial for the life of cultivated fish (Kelabora & Sabariah, 2010). Water circulation is usually combined with filter media to treat fish production waste or leftover feed to prevent any detrimental effects on the fish. This study demonstrated that the recirculating system had a significant impact on the development of nitrifying bacteria in both the filter media and water. Consequently, the bacterial density in the water would increase, particularly due to the presence of nitrifying bacteria that utilized the nitrogen in the water as a nutrient. These results were associated with the reduction of ammonia and nitrite in the system through nitrification.

The bacteria that grow in a recirculation system combined with filter media would transform compounds that are harmful to fish (ammonia and nitrite) into harmless compounds (nitrate). The occurrence of the nitrification process is strongly correlated with the level of dissolved oxygen in the water (Tanjung *et al*., 2019). Thus, the total bacterial density of nitrifying bacteria would impact the nitrification process in the implementation of RAS under various filter media. Nitrification plays a crucial role in the nitrogen cycle. In RAS technology, the accumulation of unconsumed feed and fecal matter results in the production of toxic ammonia and nitrite compounds which have detrimental effects on the health of fish. Nitrification is the process of converting ammonia into nitrite, and then further into nitrate facilitated by the collective action of ammonia and nitrite-oxidizing bacteria (Hupeden *et al*., 2020).

Correlation between Water Quality and Bacterial Density

Water quality is an important indicator of the life and growth of bacteria. The water quality range in this study was in the optimal range as reported in previous research (Table 1). The water quality influenced total bacteria because the water was a living medium for bacteria. Therefore, bacterial growth would be hampered if environmental condition parameters were not optimal for their lives, such as temperature, dissolved oxygen concentration, total ammonia nitrogen, nitrite, and nitrate concentrations. Optimal water quality usually includes balanced nutrient availability. If nutrients are available in sufficient quantities, bacteria will grow and reproduce quickly (Auffret *et al*., 2013). The use of filters plays an important role in improving water quality. Water filters play a role in removing solid particles such as food waste, feces, and dissolved organic matter which can maintain water clarity and prevent the deposition of organic remains at the bottom of the pond (Tanjung *et al*., 2019).

Table 1. The mean water quality in the recirculation pond

No	Parameters	Treatment Ponds			
		A	B	C	K
1	Ammonia (ppm)	0.08	0.06	0.05	0.24
2	Nitrite (ppm)	0.41	0.45	0.39	0.71
3	Nitrate (ppm)	3.06	4.07	2.42	5.31

(Source: Andayani *et al*., 2022)

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The pumice filter in this study become a place for nitrifying bacteria to attach and grow due to the presence of pores. Nitrifying bacteria formed colonies on filter media which played a role in maintaining water quality by carrying out the nitrification process. This process occurred by converting ammonia (NH3) produced from leftover feed and feces into nitrite compounds (NO2-) and then into nitrates (NO3-). Apart from that, many water filters have an aeration or oxygenation system which could help provide sufficient oxygen for bacteria which influences the nitrification process to take place properly (Oktavia *et al*., 2021).

The abundance of bacteria and their activity in water is considered a crucial factor for RAS management performance (Michaud *et al*., 2014). These bacteria-inhabited particles will degrade further until they eventually become part of the dissolved organic matter in the water and support the growth of more bacteria (Rojas-Tirado *et al*., 2018). Several studies have demonstrated that the bacterial community in RAS is predominantly composed of heterotrophic bacteria (Michaud *et al*., 2014; Rud *et al*., 2017). In addition, these bacteria derive energy from the degradation of organic carbon compounds (Prest *et al*., 2016).

CONCLUSION

According to the findings of this study, the application of RAS technology with sand and pumice filters (treatment C) exhibited the most effective filter media for total bacteria density in the Koi fish rearing media.

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