Chapter 16
The Intestine Microbiota Community and Enzyme Activity in *Trachinotus ovatus* After Short-Time Antibiotic Bath Administration

Xing Zheng, Siqi Lin, Zhifeng Gu, and Zhenhua Ma

Abstract The control of microbiota is essential for the prevention of bacterial and fungal diseases in aquaculture. Antibiotic is often used as an effective strategy for health management in fish farming. This chapter reviews and updates the recent research outcomes in preventing and treating bacterial infections in golden pompano *Trachinotus ovatus*. A short-time antibiotic bath administration was used with 5 mg enrofloxacin/L for 24 h. The results indicate that 5 mg/L enrofloxacin bath administration for 24 h did not induce mortality and affect the gut bacterial richness of golden pompano, but dramatically reduced pathogen bacteria. Furthermore, the short-time antibiotic bath administration is unlikely to result in a dysfunction of the anti-oxidative system or a digestive system disorder. Thus, 5 mg/L enrofloxacin bath administration is safe to prevent bacterial diseases in *T. ovatus* farming. This chapter sheds light on bacterial disease prevention and treatment to optimize the use of enrofloxacin in the *T. ovatus* farming to improve health management in the aquaculture of this economically important fish species.

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16.1 Introduction

It is well known that the control of microbiota is essential in aquaculture as bacterial and fungal diseases are a severe challenge to aquaculture enterprises (Buchmann 2015). There is a need to develop effective methods for disease control. Furthermore, a hatchery operation is different from the grow-out facility in general, and juvenile fish would be disinfected before being pooled into reared tanks or cement pools.

Golden pompano (*Trachinotus ovatus*) is an economically important warm-water marine fish species (25–32 °C), widely distributed in the tropical and temperate seas of China, Japan, Australia, and other countries. In recent years, it has become a popular cultured species in the Asia-Pacific region for its fast growth and high flesh quality (Li et al. 2006; Ma et al. 2014, 2016). However, high stocking density and low water quality can make fish susceptible to microbial and parasitic infections. There are severe economic losses in *T. ovatus* due to disease outbreak in the last decade (Guo et al. 2018; Harikrishnan et al. 2011; Kumari and Sahoo 2006). Vibriosis, viral necrosis, and cryptocaryon are the primary diseases in *T. ovatus* farming (Guo et al. 2018; Xia et al. 2012). Thus, there is a need to identify a method to control infectious diseases successfully.

To prevent fish disease outbreak, antibiotics, vaccines, chemical medicine, and immunostimulants have been widely used in aquaculture. Particularly, antibiotics and chemicals have traditionally been used to control pathogens in hatcheries (Rico and Van den Brink 2014). Furthermore, the method of treatment is vital to develop a cost-effective management strategy to mitigate microbial infections. Oral administration with feed, direct injection, and immersion in antibiotic bath solutions are commonly used for fish health management (Fang et al. 2018). The advantage and disadvantage of different methods are listed in Table 16.1. The addition of antibiotics in fish feed is the most common application method, but the infected fish often have a reduced appetite making oral uptake less efficient. Antibiotic injections are a

Table 16.1 Advantage and disadvantage of different modes for antibiotics administration (Armstrong et al. 2005; Haya et al. 2005)

Administration modes	Advantage	Disadvantage
Oral with feed	Time-saving; cost-effective	Less efficient due to low appetite
Intramuscular/intraperitoneal injection	Direct and efficient Less antibiotic used and losses	High labor costs
Bath administration	Easy to use and control	Antibiotics need to be physically removed or destroyed before discharge; stressful to the fish

direct and efficient way to administer medicine, but it incurs a high labor cost. Bath administration is the most convenient way and ease of use and effective for bacterial infected skin diseases, but antibiotics in the solution need to be physically removed or destroyed before discharge (Armstrong et al. 2005; Haya et al. 2005).

However, previous studies have indicated that antibiotics and administration modes to control fish diseases can stress the host. It may induce drug-resistant pathogens, suppress aquatic animals' immune system, and change intestinal bacteria community composition (Cabello et al. 2013; Xu et al. 2018).

This chapter reviews and updates the recent research outcomes in preventing and treating bacterial infections in *T. ovatus* farming. Enrofloxacin bath administration was chosen under 5 mg/L for 24-h, and this dose has been used to treat bacterial diseases by farmers. This chapter aims to provide fundamental knowledge and improve the health management for golden pompano farming.

16.2 Changes of Digestive Enzyme Activity in the Stomach After Enrofloxacin Bath Administration

The digestive system in marine fish is likely to be affected by reactive oxygen species (ROS) induced by environmental stress to disturb normal physiological function (Deng et al. 2010). The activity of digestive enzymes (amylase, pepsin, trypsin, and lipase) is used to indicate the digestive processes and nutritional condition of fish (Abolfathi et al. 2012). Various digestive enzymes are involved in digestive and absorptive processes. Evidence has suggested that the availability of digestive enzymes is essential for fish growth and development and is also essential to cope with the stress from the environment (Yufera et al. 2000; Yufera and Darias 2007).

According to Lin et al. (2019), the significant changes of specific enzyme activities (e.g., pepsin and trypsin) were not observed in *T. ovatus*' stomach ($P > 0.05$, Fig. 16.1). The specific activity of pepsin was 34.58 ± 19.96 U/g protein after 5 mg/L enrofloxacin bath administration for 24-h and was 38.89 ± 14.48 U/g protein in control (Fig. 16.1a). The trypsin-specific activity was 0.83 ± 0.24 U/mg protein after 5 mg/L enrofloxacin bath administration for 24 h but was 1.16 ± 0.04 U/mg protein in control (Fig. 16.1b). This study indicates that 5 mg/L enrofloxacin bath administration for 24 h is unlikely to cause a disorder of the digestive system.

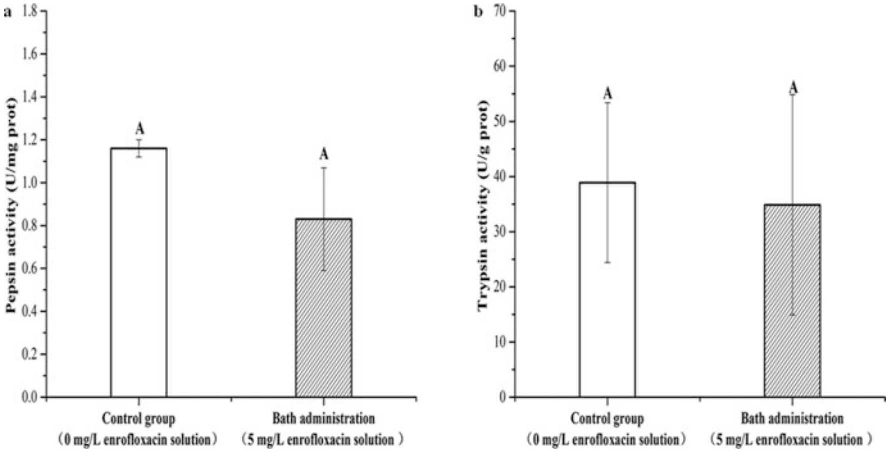

Fig. 16.1 The total activity of pepsin (**a**) and trypsin (**b**) in *Trachinoutus ovatus* stomach after 5 mg/L enrofloxacin bath administration for 24-h. Fish in the natural condition from recirculating system was used as control. (Reproduced from Lin et al. (2019), mean ± SD ($n = 10$) with the same superscript letter are not significantly different (increase the letter size of the *x*-axis label))

16.3 Changes of Antioxidant Enzyme Activity in the Liver After Enrofloxacin Bath Administration

Reactive oxygen species (ROS) normally increases when animals are subjected to stress, and this process will induce oxidative stress. To copy with ROS stress, physiological responses normally happen, especially the antioxidant defense system (Martínez-Álvarez et al. 2005). The antioxidant system protects cells by maintaining ROS at low levels and attenuating damages related to their high reactivity. All organisms have their own cellular antioxidant defense system, comprising both enzymatic and nonenzymatic components. Antioxidant enzyme activities are usually used as potential indicators of oxidative stress in fish (Lesser 2006; Xu et al. 2014), consisting of alkaline phosphatase (AKP), superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and glutathione peroxidase (GPX), which provide cellular defense against endogenous and exogenous ROS (Winston 1991).

The liver tissue is the major metabolic organ assisting in digestion by secreting enzymes that break down fats and storage carbohydrates and plays a vital role in digestion, metabolism, immunity, and the storage of nutrients inside the body. All other metabolic pathways depend upon the efficiency of liver for their energy supply (Satyaparmeshwar et al. 2006). Thus, the function of the liver is important to evaluate the antioxidant defense systems.

According to Lin et al. (2019), the significant change of antioxidant enzyme activities was not observed in the liver of *T. ovatus* ($P > 0.05$, Fig. 16.2), including AKP, POD, SOD, GPX, and CAT. The results suggest that enrofloxacin bath

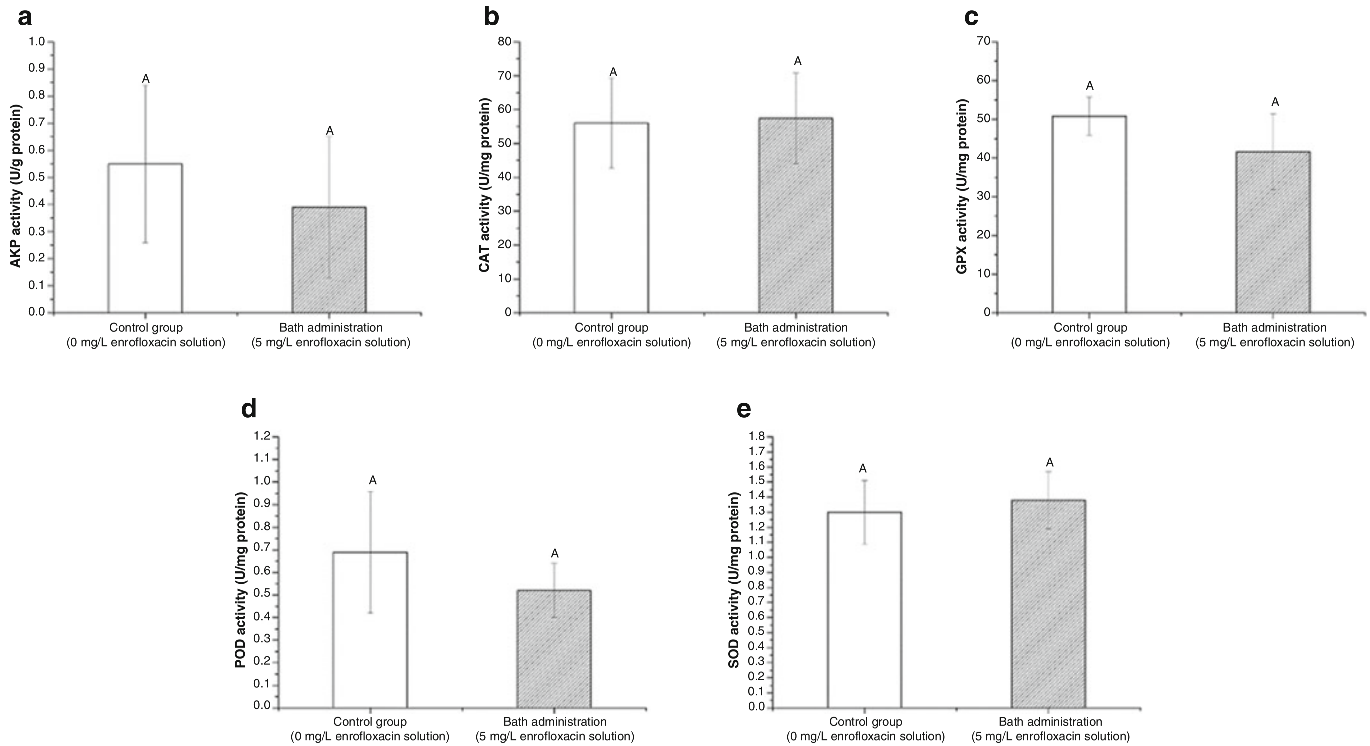

Fig. 16.2 Antioxidant enzyme activity of *Trachinotus ovatus* liver after 5 mg/L enrofloxain bath administration. (Reproduced from Lin et al. (2019), mean ± SD ($n = 10$)). (**a**) Alkaline phosphatase (AKP) activity, (**b**) catalase (CAT) activity, (**c**) glutathione peroxidase (GPX) activity, (**d**) peroxidase (POD) activity, and (**e**) superoxide dismutase (SOD) activity

administration under 5 mg/L for 24 h did increase the production of reactive oxygen species or cause dysfunction of the anti-oxidative system in the liver.

To reduce the damage of ROS, O_2^- are dismutated by SOD to H_2O_2 which is reduced to water and molecular oxygen by CAT or is neutralized by GPX that catalyzes the reduction of H_2O_2 to water and organic peroxide to alcohols using glutathione (GSH) as a source of reducing equivalent (Verlecar et al. 2007). CAT provides the first line of defense to clean up ROS, while GPX is involved in detoxification of hydroperoxides (Farombi et al. 2007). Phosphatases remove phosphate groups from the substrates by hydrolyzing phosphoric acid monoesters into phosphate ions and molecules with free hydroxyl groups. ACP and AKP are two important phosphatases in marine organisms, participating in the degradation of foreign protein, carbohydrate, and lipid, as well as immune regulation, ion secretion, and other important physiological functions (Foss et al. 2009; Liu et al. 2004). ACP is used as a marker for detecting lysosomes within cell fractions and is also a reliable tool for assessing environmental pollution (Blasco et al. 1993; Mazorra et al. 2002; Rajalakshmi and Mohandas 2005). AKP is an intrinsic plasma membrane enzyme in the cell membranes (Blasco et al. 1993; Jing et al. 2006; Mazorra et al. 2002).

16.4 Changes of Gut Bacterial Diversity, Evenness, and Community Composition

The gut is the home of trillions of microbial cells known as gut microbiota (Zhang et al. 2016). The gut microbiota, which comprises a diverse and vast population of microorganisms, plays critical functions in host nutrient and physiology (Ray et al. 2012; Tremaroli and Bäckhed 2012). The gut microbiota composition and interactions affect energy extraction efficiency and are essential in metabolism and immunity (Moore et al. 2011; Tremaroli and Bäckhed 2012). In comparison to mammals, the gut microbial composition in fish is more likely to be affected by the environment, such as diet (De Filippo et al. 2010), drug (Zwolinska-Wcislo et al. 2011), and stress (Galley et al. 2014; Xia et al. 2014). An altered microbiota in the gut can change host immune function and increase disease risk (Morgan et al. 2012). Evidence has demonstrated that antibiotic exposure, including oral, intramuscular, or bath administration, would stress the treated animals and change the gut bacteria community composition, diversity, and evenness (Cabello et al. 2013; Xu et al. 2018; Zhou et al. 2018). Antibiotic exposure can adversely affect the health of the host. Therefore, maintaining a functional and steady gut microbiota is vital to the host.

The fish microbiome with rich biodiversity can predictably react to the changing gut condition (Hennersdorf et al. 2016). In *T. ovatus* gut bacteria community, the community richness (estimators by ACE and Chao1) was not significantly affected by 5 mg/L enrofloxacin bath administration ($P > 0.05$, Fig. 16.3a, b), but the Shannon index for diversity decreased significantly from 4.00 ± 0.12 to

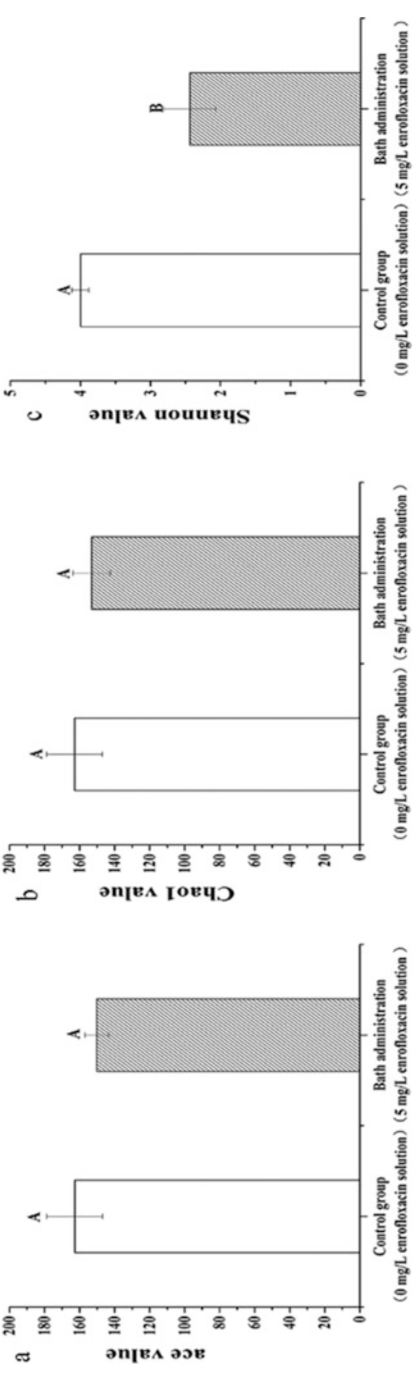

Fig. 16.3 The gut bacterial community richness (ACE and Chao1) and diversity (Shannon) in *Trachinotus ovatus* after 5 mg/L enrofloxacin bath administration. (**a**) ACE estimator. (**b**) Chao1 estimator. (**c**) Shannon estimator. Mean ± SD (n = 5) with the different superscript letter are significantly different

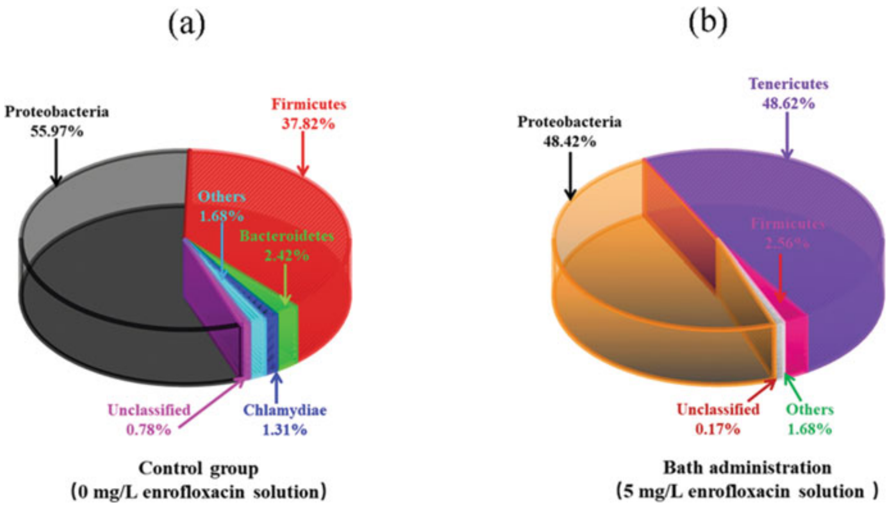

Fig. 16.4 The gut bacteria communities in *Trachinotus ovatus* at phyla level. "Others" meant the sum of bacteria relative abundance were less than 1%. $N = 5$. (**a**) Control group and (**b**) bath administration

2.44 ± 0.37 ($P < 0.05$, Fig. 16.3c). Alpha diversity shows the richness of *T. ovatus* gut bacteria was not affected significantly by the administration of low concentrations of enrofloxacin. Similarly, a previous study has also demonstrated that the gut microbiota of aquatic animals is not affected significantly by the environment (Zhang et al. 2016). In contrast, diversity was negatively affected. Bacterial diversity or composition may be more susceptible to low administration concentrations of enrofloxacin, which is consistent with the results of zebra fish gut bacteria composition after oxytetracycline exposure (Zhou et al. 2018).

The composition of the gut bacterial community varies with a unique core microbiome in each specific host species. Firmicutes and Bacteroidetes are the most dominant phyla in mammals (Qin et al. 2010), whereas Proteobacteria, Firmicutes, Fusobacteria, Actinobacteria, and Bacteroidetes are the major phyla in the intestine of carnivorous marine fish (Rückert et al. 2008). Our research found a total of 12 phyla that were detected in the gut bacterial community of *T. ovatus*. Proteobacteria, Tenericutes, and Firmicutes were the most common phyla (Fig. 16.4). The result is similar to the study of the woody forage effect on golden pompano intestinal bacteria diversity (Chen et al. 2018). This finding is also in agreement with the studies of the Atlantic salmon parr (Dehler et al. 2017), rainbow trout (Lyons et al. 2015), and East African cichlid (Baldo et al. 2015). It is speculated that Proteobacteria and Firmicutes are the common gut microbes in fish and play an important role in intestinal function. Proteobacteria could catabolize feedstuff components (Jumpertz et al. 2011), and Firmicutes may be involved in energy resorption (Komaroff 2017) and have demonstrated probiotic properties in fish (Bøgwald and Dalmo 2014). We found that the relative abundance of Proteobacteria was not

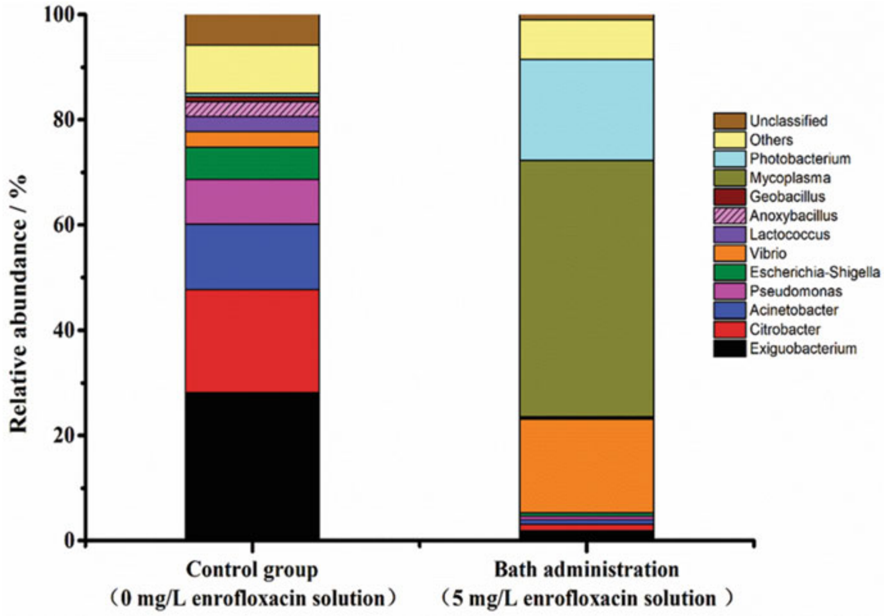

Fig. 16.5 The intestinal bacteria communities in *Trachinotus ovatus* intestine at the genus level. "Others" meant the sum of bacteria relative abundance was less than 1%. $N = 5$

significantly affected by the dose of 5 mg/L enrofloxacin bath administration for 24 h. It is consistent with the zebra fish results with sulfamethoxazole bath (Zhou et al. 2018), indicating that a short-term enrofloxacin bath administration has no significant effect on Proteobacteria.

Microbial identification is meaningful only when microbiota can be classified at the genus or species concerning animal husbandry (Petrosino et al. 2009). A total of 84 genera were detected in the *T. ovatus* gut microbiota composition from the control group (0 mg/L enrofloxacin solution) in our research, including *Exiguobacterium*, *Citrobacter*, *Acinetobacter*, *Pseudomonas*, and *Escherichia-Shigella* as the dominant genera. It is similar to previous results, showing that *Aeromonas*, *Vibrio*, *Micrococus*, *Alteromonas*, and *Acinetobacter* are the main genera in marine fish (Blanch et al. 1997; Newman et al. 2011). Moreover, 33 genera were detected in the *T. ovatus* gut microbiota from the treatment of 5 mg/L enrofloxacin bath administration, including the dominant genera of *Mycoplasma*, *Photobacterium*, *Vibrio*, and *Desulfovibrio* (Fig. 16.5). *Escherichia-Shigella* and *Vibrio* were conditional pathogens (Hao et al. 2017; Tan et al. 2014). Our result found the relative abundance of *Escherichia-Shigella* was significantly decreased after bathing with 5 mg/L enrofloxacin, indicating 5 mg/L enrofloxacin bath administration for 24 h is useful to control the quantity of conditional pathogen in the *T. ovatus* gut microbiota composition.

Antibiotics can cause adverse effects on animal physiology by affecting host tissues or confusing commensal microbiota (Morgun et al. 2015). A disturbance in gut microbial community can lead to changes in the microbial diversity and abundance of certain bacteria, resulting in beneficial or harmful effects in fish (Gómez and Balcázar 2008). The altered microbiota in the intestine can lead to the change of the host immune functions and increase the risk of disease infection (Morgan et al. 2012). Antibiotic treatment can stress the treated animal, and the animal physiological response may occur, depending on the strength and duration of stress. The results indicate that the short-term enrofloxacin bath under a low concentration can help control the number of conditional pathogens, without significantly affecting the intestinal bacteria richness. It is useful for golden pompano farming and health management to prevent and treat bacterial diseases without significantly changing gut microbial community.

16.5 Conclusion

Golden pompano *T. ovatus* have harbored specific and core intestine microbiota, including the dominant phyla, Proteobacteria and Firmicutes, and the dominant genera, *Exiguobacterium*, *Citrobacter*, *Acinetobacter*, *Pseudomonas*, and *Escherichia-Shigella*, in a circulating aquaculture system. Short-time antibiotic bath administration (5 mg/L enrofloxacin bath for 24 h) did not induce mortality and affect the gut bacterial richness of golden pompano, but reduced the diversity, where the conditional pathogen declined dramatically. Furthermore, a short-time antibiotic bath is unlikely to result in dysfunction of the anti-oxidative system or disorder of the digestive system. Thus, the dose of 5 mg/L enrofloxacin bath may be a safe way to prevent bacterial diseases in *T. ovatus* in aquaculture.

References

Abolfathi M, Hajimoradloo A, Ghorbani R, Zamani A (2012) Effect of starvation and refeeding on digestive enzyme activities in juvenile roach, *Rutilus rutilus caspicus*. Comp Biochem Physiol A 161:166–173

Armstrong SM, Hargrave BT, Haya K (2005) Antibiotic use in finfish aquaculture: modes of action, environmental fate, and microbial resistance. In: Hargrave BT et al (eds) Environmental effects of marine finfish aquaculture. Handbook of environmental chemistry, vol 5M. Springer, Berlin

Baldo L, Riera J, Toomingklunderud A, Albà M, Salzburger W (2015) Gut microbiota dynamics during dietary shift in eastern African cichlid fishes. PLoS One 10:e127462

Blanch A, Alsina M, Simón M, Jofre J (1997) Determination of bacteria associated with reared turbot (*Scophthalmus maximus*) larvae. J Appl Microbiol 82:729–734

Blasco J, Puppo J, Sarasquete MC (1993) Acid and alkaline phosphatase activities in the clam *Ruditapes philippinarum*. Mar Biol 115:113–118

Bøgwald J, Dalmo RA (2014) Aquaculture nutrition: gut health, probiotics and prebiotics. Wiley, London

Buchmann K (2015) Impact and control of protozoan parasites in maricultured fishes. Parasitology 142:168–177

Cabello F, Godfrey H, Tomova A, Ivanova L, Dölz H, Millanao A, Buschmann A (2013) Antimicrobial use in aquaculture re-examined: its relevance to antimicrobial resistance and to animal and human health. Environ Microbiol 15:1917–1942

Chen B, Gao L, Pan Q (2018) Woody forages effect the intestinal bacteria diversity of golden pompano *Trachinotus ovatus*. AMB Express 8:29–37

De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, Collini SPGL (2010) Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci U S A 107:14691–14696

Dehler CE, Secombes CJ, Martin SAM (2017) Environmental and physiological factors shape the gut microbiota of Atlantic salmon parr (*Salmo salar L.*). Aquaculture 467:149–157

Deng JM, Mai KS, Ai QH, Zhang WB, Tan BP, Xu W, Liu F (2010) Alternative protein sources in diets for Japanese flounder *Paralichthys olivaceus* (Temminck and Schlegel): II. Effects on nutrient digestibility and digestive enzyme activity. Aquac Res 41:861–870

Fang X, Zhou J, Liu X (2018) Pharmacokinetics and tissue distribution of enrofloxacin after single intramuscular injection in Pacific white shrimp. J Vet Pharmacol Ther 41:148–154

Farombi EO, Adelowo OA, Ajimoko YR (2007) Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African cat fish (Clarias gariepinus) from Nigeria Ogun River. Int J Environ Res Public Health 4:158–165

Foss A, Imsland A, Roth B, Schram E, Stefansson S (2009) Effects of chronic and periodic exposure to ammonia on growth and blood physiology in juvenile turbot (*Scophthalmus maximus*). Aquaculture 296:45–50

Galley JD, Nelson MC, Yu Z, Dowd SE, Bailey MT (2014) Exposure to a social stressor disrupts the community structure of the colonic mucosa-associated microbiota. BMC Microbiol 14:189

Gómez GD, Balcázar JL (2008) A review on the interactions between gutmicrobiota and innate immunity of fish. Pathog Dis 52:145–154

Guo S, Mo Z, Wang Z, Xu J, Li Y, Dan X, Li A (2018) Isolation and pathogenicity of Streptococcus iniae in offshore cage-cultured *Trachinotus ovatus* in China. Aquaculture 492:247–252

Hao Y, Wu S, Jakovlić I, Zou H, Li W, Wang G (2017) Impacts of diet on hindgut microbiota and short-chain fatty acids in grass carp (*Ctenopharyngodon idellus*). Aquac Res 48:5595–5605

Harikrishnan R, Balasundaram C, Heo M (2011) Impact of plant products on innate and adaptive immune system of cultured finfish and shellfish. Aquaculture 317:1–15

Haya K, Burridge L, Davies I, Ervik A (2005) A review and assessment of environmental risk of chemicals used for the treatment of sea lice infestations of cultured salmon. Springer, Berlin

Hennersdorf P, Kleinertz S, Theisen S, Abdul-Aziz MA, Mrotzek G, Palm HW, Saluz HP (2016) Microbial diversity and parasitic load in tropical fish of different environmental conditions. PLoS One 11:e151594

Jing G, Li Y, Xie L, Zhang R (2006) Metal accumulation and enzyme activities in gills and digestive gland of pearl oyster (*Pinctada fucata*) exposed to copper. Comp Biochem Physiol C 144:184–190

Jumpertz R, Le DS, Turnbaugh PJ, Trinidad C, Bogardus C, Gordon JI, Krakoff J (2011) Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans. Am J Clin Nutr 94:58–65

Komaroff A (2017) The microbiome and risk for obesity and diabetes. JAMA 317:355–356

Kumari J, Sahoo PK (2006) Non-specific immune response of healthy and immunocompromised Asian catfish (*Clarias batrachus*) to several immunostimulants. Aquaculture 255:133–141

Lesser MP (2006) Oxidative stress in marine environments: biochemistry and physiological ecology. Annu Rev Physiol 68:253–278

Li G, Zhao D, Huang L, Sun J, Gao D, Wang H, Tan Y, Liang L (2006) Identification and phylogenetic analysis of *Vibrio vulnificus* isolated from diseased Trachinotus ovatus in cage mariculture. Aquaculture 261:17–25

Lin S, Hu J, Zhou S, Yang R, Zheng X, Gu Z, Ma Z (2019) Effect of enrofloxacin treatment on antioxidant enzymes and digestive enzymes in *Trachinotus ovatus* (Linnaeus 1758). Fish Sci Technol Inf 46:96–102. (In Chinese)

Liu SL, Jiang XL, Hu XK, Gong J, Hwang HM, Mai KS (2004) Effects of temperature on non-specific immune parameters in two scallop species: *Argopecten irradia*ns (Lamarck 1819) a*nd Chlamys farreri* (Jones & Preston 1904). Aquac Res 35:678–682

Lyons P, Turnbull J, Dawson K, Crumlish M (2015) Exploring the microbial diversity of the distal intestinal lumen and mucosa of farmed rainbow trout *Oncorhynchus mykiss* (Walbaum) using next generation sequencing (NGS). Aquac Res 48:77–91

Ma Z, Guo H, Zheng P, Wang L, Jiang S, Qin JG, Zhang D (2014) Ontogenetic development of digestive functionality in golden pompano *Trachinotus ovatus* (Linnaeus 1758). Fish Physiol Biochem 40:1157–1167

Ma Z, Hu J, Liu Y, Yang R, Qin JG, Sun D (2016) Insulin-like Growth Factor (IGF) genes in golden pompano *Trachinotus ovatus* (Linnaeus 1758) larvae: molecular cloning and response to water temperature and nutrient manipulation. Isr J Aquacult Bamidgeh 68:1334–1341

Martínez-Álvarez R, Morales A, Sanz A (2005) Antioxidant defenses in fish: biotic and abiotic factors. Rev Fish Biol Fish 15:75–88

Mazorra MT, Rubio JA, Blasco J (2002) Acid and alkaline phosphatase activities in the clam *Scrobicularia Plana*: kinetic characteristics and effects of heavy metals. Comp Biochem Physiol B 131:241–249

Moore A, Christian M, Sommer M, Gautam D (2011) Functional metagenomic investigations of the human intestinal microbiota. Front Microbiol 2:188

Morgan X, Tickle T, Sokol H, Gevers D, Devaney K, Ward D, Reyes J, Shah S, LeLeiko N, Snapper S, Bousvaros A, Korzenik J, Sands B, Xavier R, Huttenhower C (2012) Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. Genome Biol 13:R79

Morgun A, Dzutsev A, Dong X, Greer R, Sexton D, Ravel J, Schuster M, Hsiao W, Matzinger P, Shulzhenko N (2015) Uncovering effects of antibiotics on the host and microbiota using transkingdom gene networks. Gut 64:1732–1743

Newman JJT, Cosenza BJ, Buck JD (2011) Aerobic microflora of the bluefish (*Pomatomus saltatrix*) intestine. J Fish Boa Can 29:333–336

Petrosino J, Highlander S, Luna R, Gibbs R, Versalovic J (2009) Metagenomic pyrosequencing and microbial identification. Clin Chem 55:856–866

Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto J, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J, Brunak S, Doré J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J, Bork P, Ehrlich SD, Wang J (2010) A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464:59–65

Rajalakshmi S, Mohandas A (2005) Copper-induced changes in tissue enzyme activity in a freshwater musse. Ecotoxicol Environ Safe 62:140–143

Ray A, Ghosh K, Ringø E (2012) Enzyme-producing bacteria isolated from fish gut: a review. Aquac Nutr 18:465–492

Rico A, Van den Brink PJ (2014) Probabilistic risk assessment of veterinary medicines applied to four major aquaculture species produced in Asia. Sci Total Environ 468–469:630–641

Rückert S, Palm HW, Klimpel S (2008) Parasite fauna of seabass (Lates calcarifer) under mariculture conditions in Lampung Bay, Indonesia. J Appl Ichthyol 24:321–327

Satyaparmeshwar K, Ravindra Reddy T, Vijaykumar N (2006) Study of carbohydrate metabolism in selected tissues of freshwater mussel, Lamellidens marginalis under copper sulphate toxicity. J Environ Biol 27:39–41

Tan W, Yunos N, Tan P, Mohamad N, Adrian T, Yin W, Chan K (2014) Characterisation of a marine bacterium *Vibrio brasiliensis* T33 producing N-acyl homoserine lactone quorum sensing molecules. Sensors 14:12104–12113

Tremaroli V, Bäckhed F (2012) Functional interactions between the gut microbiota and host metabolism. Nature 489:242–249

Verlecar XN, Jena KB, Chainy GBN (2007) Biochemical markers of oxidative stress in *Perna viridis* exposed to mercury and temperature. Chem Biol Interact 167:219–226

Winston GW (1991) Oxidants and antioxidants in aquatic animals. Comp Biochem Physiol C 100: 173–176

Xia L, Huang Y, Lu Y (2012) The diseases in Trachinotus ovatus culture and their research progress. Anhui Agric Sci Bull 18:140–143

Xia J, Lin G, Fu G, Wan Z, Lee M, Wang L, Liu X, Yue G (2014) The intestinal microbiome of fish under starvation. BMC Genomics 15:266

Xu X, Zhang Y, Yan B, Xu J, Tang Y, Du D (2014) Immunological and histological responses to sulfide in the crab Charybdis japonica. Aquat Toxicol 150:144–150

Xu G, Xing W, Li T, Ma Z, Liu C, Jiang N, Luo L (2018) Effects of dietary raffinose on growth, non-specific immunity, intestinal morphology and microbiome of juvenile hybrid sturgeon (*Acipenser baeri Brandt* ♀ × *A. schrenckii Brandt* ♂). Fish Shellfish Immun 72:237–246

Yufera M, Darias M (2007) The onset of exogenous feeding in marine fish larvae. Aquaculture 268: 53–63

Yufera M, Fernandez-Diaz C, Pascual E, Sarasquete M, Moyano F, Diaz M, Alarcon F, Garcia-Gallego M, Parra G (2000) Towards an inert diet for first-feeding gilthead seabream *Sparus aurata* L. larvae. Aquac Nutr 6(3):143–152

Zhang M, Sun Y, Chen L, Cai C, Qiao F, Du Z, Li E (2016) Symbiotic bacteria in gills and guts of Chinese mitten crab (*Eriocheir sinensis*) differ from the free-living bacteria in water. PLoS One 11:e148135

Zhou L, Limbu SM, Shen M, Zhai W, Qiao F, He A, Du Z, Zhang M (2018) Environmental concentrations of antibiotics impair zebrafish gut health. Environ Pollut 235:245–254

Zwolinska-Wcislo MKGP, Sliwowski Z, Urbanczyk K, Drozdowicz D, Konturek SJ, Pawlik WW, Brzozowski T (2011) Antibiotic treatment with ampicillin accelerates the healing of colonic damage impaired by aspirin and coxib in the experimental colitis. Importance of intestinal bacteria, colonic microcirculation and proinflammatory cytokines. J Physiol Pharmacol 62:357–368