CHAPTER I
INTRODUCTION

1.1. Background

Aquaculture is an important sector in meeting global animal protein needs, considering that fish are a rich source of protein and essential nutrients (Boyd *et al*., 2022). Among aquaculture species, tilapia (*Oreochromis niloticus*) has an important role in the fisheries sector because it is able to well adapted, has a rapid growth rate, and has a high level of demand in the market (El-Sayed & Fitzsimmons, 2023). Globally, tilapia contributes 70–80% of total farmed tilapia production and ranks third in world farmed fish production (El-Sayed, 2020).

With a consistent annual production increase of 20% between 2004 and 2017, Indonesia has become the world's second largest tilapia producer after China (El-Sayed, 2020), Indonesia produced 1,506,156 tons of tilapia, generating an economic value of 34.7 trillion rupiah, highlighting its important role in supporting food security and the national economy (MMAF, 2021).

However, increasing the intensity of tilapia cultivation also increases the risk of disease outbreaks, which can cause significant economic losses. One of the main pathogens that poses a threat in cultivation is *Aeromonas hydrophila*, which causes *Motile Aeromonas Septicemia* (MAS), which is have some symptoms such as systemic bleeding, lethargy, and chronic

mortality in tilapia (Bekele *et al*., 2019). In addition, *Aeromonas veronii*, although rarely reported, has also been associated with cases of skin ulcers, septicemia, and mortality in farmed fish, including tilapia (Abdel Rahman *et al*., 2023). Both of these bacteria can thrive in the water quality decreation conditions and fish health productivity outbreaks and cause endanger fish health (Zaheen *et al*., 2022).

Early detection of pathogens is an important step in controlling aquaculture diseases. However, conventional methods to culture bacteria and biochemical tests are often take extra time, labor-intensive, and have limited specificity. Delays or misdiagnosis can worsen the spread of disease and hinder effective management. Therefore, molecular detection methods include *Polymerase Chain Reaction* (PCR) had been developed as faster, more sensitive, and more specific tools. Single PCR allows accurate detection of pathogen-specific genes, but to detect more than one pathogen simultaneously, duplex PCR approach is needed, a method that is able to amplify two target genes in a single reaction. Duplex PCR has proven to be more efficient in terms of time, cost, and labor, without sacrificing sensitivity and specificity. State by (Novita *et al.*, 2020), the duplex *polymerase chain reaction* (PCR) technique approach facilitates rapid diagnosis through simultaneous detection of two pathogens using two sets of specific primers targeting distinct genes.

The duplex PCR method has been successfully used in the detection of other pathogens, whereas Dong *et al*., (2016) research had implemented

two phatogen detection in other type of tilapia. Based on this, this study aims to develop a duplex PCR method to rapidly and specifically detect the *aerolysin* gene from *Aeromonas hydrophila* and the *rpoB* gene from *Aeromonas veronii* in tilapia (*Oreochromis niloticus*), and to evaluate the efficiency of the duplex PCR method compared to single PCR in detecting both pathogens simultaneously, so that it can be an effective tool in fish disease surveillance in the aquaculture sector.

1.2. Problem Statement

Problem statement of this research are:

1. How morphological, biochemical, and molecular characterization appear due to *Aeromonas hydrophila* and *Aeromonas veronii* infection in Nile tilapia (*Oreochromis niloticus*).
2. How do Single and Duplex PCR methods be utilized to rapidly and specifically detect the presence of *A. hydrophila* and *A. veronii* in nile tilapia (*O. niloticus*)?
3. Is Duplex PCR more efficient than Single PCR in simultaneously detecting both bacteria?

1.3. Objectives

Objective of this research are:

1. Characterized morphological, biochemical, and molecular *A. hydrophila* and *A. veronii* that infect Nile tilapia fish (*O. niloticus*).
2. To develop Duplex PCR methods for the rapid and specific detection of *A. hydrophila* and *A. veronii* in Nile tilapia (*O. niloticus*).

3. To evaluate the efficiency of Duplex PCR compared to Single PCR in simultaneously detecting both *A. hydrophila* and *A. veronii*.

1.4. Benefits

This research is scientifically beneficial as it develops rapid, specific, and accurate molecular methods based on Duplex PCR to detect *A. hydrophila* and *A. veronii* in Nile tilapia (*O. niloticus*), while expanding knowledge on the effectiveness of simultaneous detection using Duplex PCR. Practically, this study aids aquaculture practitioners and diagnostic laboratories in identifying pathogens more efficiently, supporting fish health management, and reducing economic losses caused by pathogen infections through early detection.