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Water quality changes in the coastal area of intensive whiteleg shrimp brackish water pond aquaculture

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Abstract: Whiteleg shrimp (*Litopenaeus vannamei*) culture with more advanced technology has been developed in the coastal regions of Southeast Asia, including Indonesia, to catch up with the increasing worldwide demand for shrimp. If left unchecked, the effluent from this high-density shrimp farming could have irreversible impacts on the receiving environment and the shrimp industry. This study was carried out to determine changes in water quality status before and post-development of the intensive whiteleg shrimp industry in the coastal area of Je'neponto, a regency located in the south of South Sulawesi Province, Indonesia. The water quality parameters were measured *in situ* and *ex situ* before the farming cycle started and after harvesting. Temperature, salinity, pH, dissolved oxygen, nitrate, nitrite, ammonia, and phosphate were measured using standardised methods. The data were statistically analysed using Kruskal–Wallis, Mann–Whitney, and principal component analysis. Water quality status was determined using the storage and retrieval approach. The potential for waste from the intensive whiteleg shrimp ponds was estimated at 7,408 kg of total nitrogen (*TN*) per cycle and 1,748 kg of total phosphorus (*TP*) per cycle. The study also found that the wastewater treatment plant pond was only about 1.45% of the total pond volume and is classified as a low-capacity wastewater treatment plant for intensive whiteleg shrimp farming. The water quality was classified in the class B category (good or slightly polluted) prior to the operation of the shrimp farm to class C (moderate or moderately polluted) afterwards.

Keywords: brackish water pond, intensive technology, sustainable development, waste, water quality status, whiteleg shrimp

INTRODUCTION

The Indonesian government has pledged medium to long-term strategies to develop five primary aquaculture sub-sectors: brackish water, freshwater, marine, rice-fish, and offshore aquaculture (KKP, 2015). Among these aquaculture sub-sectors, pond-based brackish water aquaculture currently contributes the highest number in terms of production value. The main seafood products produced by the pond-based farming system range from whiteleg shrimp or vannamei shrimp or Pacific white shrimp (*Litopenaeus vannamei*), tiger shrimp (*Penaeus monodon*), milkfish (*Chanos chanos*), mud crab (*Scylla* sp.) to seaweed (*Gracilaria verrucosa*). Different technologies are used in brackish water pond aquaculture, in which traditional and traditional plus are the dominant ones, followed by semi-intensive, intensive, and super-intensive. Among these products, shrimp is considered the primadonna of the brackish water aquaculture products to support food security and foreign exchange that needs to be fully supported (Mustafa, Ratnawati and Undu, 2020). The Government of Indonesia has targeted to double shrimp production from 0.3–0.4 mln Mg to 0.6–1.0 mln Mg by 2030, citing the availability of potential areas of 0.9–1.2 mln ha suitable for brackish water pond aquaculture (KKP, 2015). However, the land availability alone will not be sufficient to achieve the target. Hence, the government vowed to increase the percentage of shrimp farming using intensive farming technology. Such a technological shift from traditional to intensive and super-intensive farming of whiteleg shrimp aquaculture could have dire pollution consequences for the environment (Mustafa *et al.*, 2022; Mustafa *et al.*, 2023). Uncontrolled organic waste from intensive shrimp ponds can give rise to environmental deterioration and unnecessary capital expenditure (Dauda *et al.*, 2019). Highly concentrated organic material in shrimp effluent poses severe threats to small inland waterways, which generally have low assimilative capacity (Jiang *et al.*, 2019). Algal blooming and hypoxia in the waters usually occur in these nutrient-saturated seascapes (Mustafa *et al.*, 2022), leading to organisms' decreased immunity and mass die-off. In severe cases, hazardous waste renders the receiving environment inhabitable for aquatic organisms, particularly benthic biota (Nguyen, Nguyen and Jolly, 2019).

Growing whiteleg shrimp in brackish water ponds at intensive and super-intensive levels affects the characteristics of the surrounding coastal waters. Solid wastes released from shrimp culture contain highly concentrated organic carbon (*OC*), total nitrogen (*TN*), and total phosphorus (*TP*) if not treated properly (Mustafa *et al.*, 2022). The amount of organic waste produced to culture whiteleg shrimp in a brackish water pond sized 1,000 m^2 and having a stocking density of 500 $ind \cdot m^{-2}$ was 50.12 g of *TN* per kg of shrimp, 15.73 g of *TP* per kg of shrimp, and 126.85 g of *OC* per kg of shrimp (Syah, Makmur and Fahrur, 2017). The feed used is the primary source of waste in an intensive shrimp culture (Olusegun, Babatunde and Abiodun, 2016). The waste flowed into the receiving aquatic environment as sediments and suspensions.

Different wastes have different characteristics due to their dynamic nature, size, potential long-term or intergenerational impacts, and distribution pattern. Above a certain concentration threshold, the waste will negatively affect water quality status. For example, shrimp effluent could severely impact other economic activities occupying the same coastal areas, such as milkfish, seaweed brackish water farms, mariculture, and shrimp hatcheries. The effluent is released in different farming stages, such as before stocking and after harvesting (Mustafa *et al.*, 2022). It alters the water quality attributes upon which these competing activities rely (Mustafa, Ratnawati and Undu, 2020; Mustafa *et al.*, 2022).

Water quality is defined as specific water characteristics to support a specific application or to sustain a particular use (Uddin, Nash and Olbert, 2021). Changes in water quality characteristics can immediately indicate declining water quality, particularly in coastal regions. The term "water quality status" relates to the water quality situation that indicates whether a water supply has been contaminated within a specific time. Different methods have been developed to measure these changes, including those in Indonesia. Indicators of water quality in Indonesia were adopted from various established standards. The core objective of the Indonesian government in developing these water quality standards was partially to address some of the sustainable development goals (SDGs), particularly objective 14.1, to prevent severe marine and coastal pollution caused by land-based activities, such as marine debris and nutrient runoff. The State Ministry of the Environment (Ind.: Menteri Negara Lingkungan Hidup) published the Minister's Decree Number 115 of 2003 about Guidelines on Determining Water Quality Status in Indonesia (Ind.: Keputusan Menteri Negara Lingkungan Hidup Nomor: 115 Tahun 2003 Tentang Pedoman Penentuan Status Mutu Air) (Keputusan, 2003). The guidelines cover various aspects of water quality, including regulating the maximum acceptable impact of aquaculture shrimp brackish water ponds, the standard water quality, and its surrounding area.

One of the shrimp production centres in South Sulawesi Province is located in Je'neponto Regency. The regency has at least 2,460 ha of brackish water ponds (BPS, no date). Anecdotal evidence has been reported that whiteleg shrimp brackish water pond aquaculture in Bulobulo and Palajau Villages, Arungkeke Subdistrict of Je'neponto Regency, discharge the wastewater into the surrounding waters causing damage to the nearby seaweed culture and increased fish mortality of farmed fish. This study aimed to determine the altered water quality status in the coastal waters of Je'neponto Regency, South Sulawesi Province, before and after the whiteleg shrimp brackish water farming activities. To achieve this goal, the overarching research question was focused on the current water quality status and how to devise alternative solutions to reduce the load of waste from the shrimp farm industry in the area. This is the first study that comprehensively assesses various water quality variables to determine changes in water quality status in the region's coastal waters before and after an intensive shrimp farm operated. The results of this study are expected to provide critical information to develop a mitigation plan for managing the coastal region as a response to the rapid development of intensive shrimp farms and serve as a study case for other areas in Southeast Asia experiencing similar situations.

MATERIALS AND METHODS

STUDY AREA

Two surveys were conducted in the coastal waters of Arungkeke Subdistrict, Je'neponto Regency, South Sulawesi Province, where the whiteleg shrimp brackish water farming was located. The first survey was carried out a week before the preparation of the brackish water pond to start a new farming cycle (July 2019), hereafter called before farming cycle. The second survey was done after the harvesting day (November 2019), hereafter called after farming cycle. Five line transects were set perpendicular to the coastline; each has four sampling points (Fig. 1). The line transects were positioned at distances of 0, 500, and 1,000 m from the pond wastewater outlet to the northeast and southwest. The sampling points perpendicular to the shorelines had distances starting from 100, 300, 500, and 700 m outward. The position of each sampling station was marked by a global positioning system (GPS).

COLLECTION OF FIELD DATA

Water quality variables measured *in situ* and *ex situ* were based on the list stipulated in the Ministerial Decree Number 51 of 2004, enacted by the Minister of the Environment about the

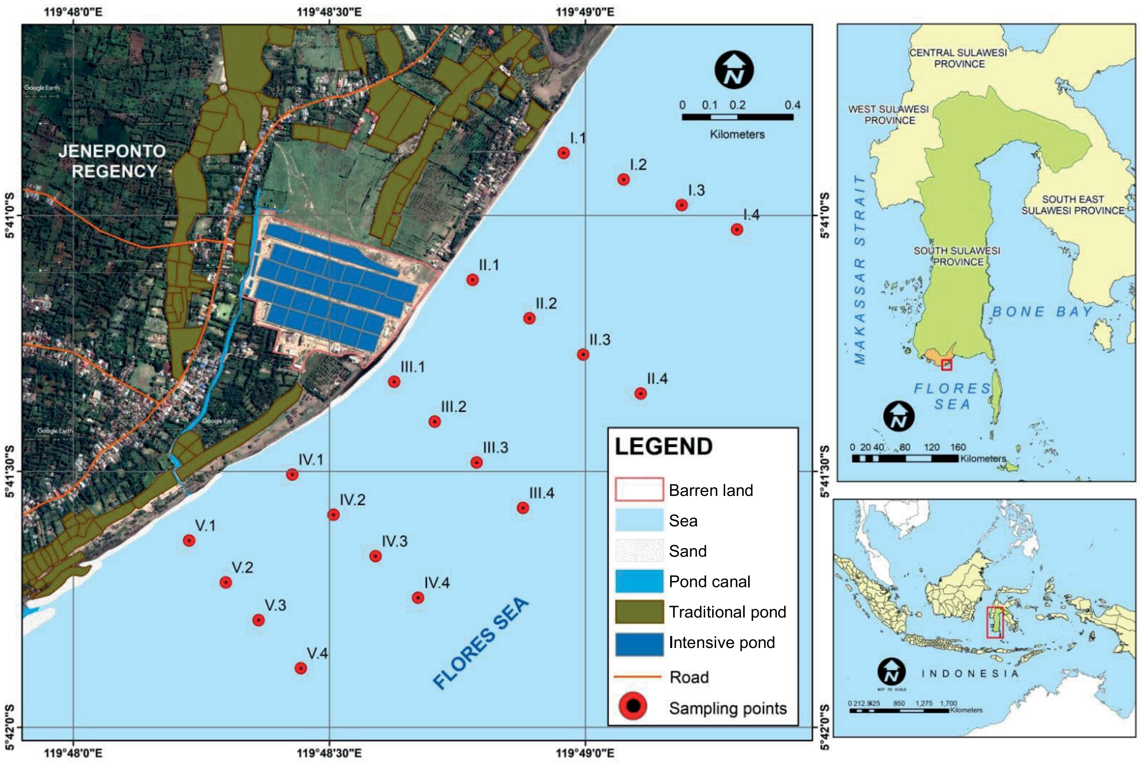

Fig. 1. The location of the study area and the sampling points in the coastal waters of the Arungkeke Subdistrict, Je'neponto Regency, South Sulawesi Province, Indonesia; I–V, 1–4 = transects; source: own elaboration

Standard Quality of Seawater for Marine Organisms (Keputusan, 2004). The water quality variables measured directly in the field were salinity, temperature, pH, and dissolved oxygen (*DO*) using YSI Pro Plus. Water samples were collected between 0–0.2 m depth from the sea surface using a Kemmerer water sampler for *ex situ* measurement of water quality variables. The measurement and water samplings were consistently carried out from 09.30 am to 1.00 pm local time. Water samples were preserved during the transportation to the laboratory following the standard guidelines (APHA, 2005) for the subsequent analyses.

Structured interviews using a semi-closed-ended questionnaire were conducted with the owners and managers of the whiteleg shrimp brackish water pond aquaculture to determine the shrimp farming practices. The other technical farming information (including survival rate (*SR*) and food conversion ratio (*FCR*)) was obtained from the farming records that the owners and managers voluntarily provided. *SR* and *FCR* were calculated using the following equations.

$$SR = \frac{Nh}{Ns} \tag{1}$$

where: *Nh* = number of harvested whiteleg shrimp, *Ns* = number of stocked whiteleg shrimp.

$$FCR = \frac{tf}{ts} \tag{2}$$

where: *tf* = total weight of feed consumed by whiteleg shrimp, *ts* = total weight of whiteleg shrimp produced.

Monthly rainfall data for Je'neponto Regency from 2016 to 2020 was collected from the Maros Class I Climatology Station (Ind.: Stasiun Klimatologi Kelas I Maros) in Maros Regency, South Sulawesi Province. This data was produced by the Agricultural Extension Service (Ind.: Balai Penyuluhan Pertanian – BPP) Benteng (05°34'46.2" S, 119°33'50.1" E), Pakkaterang (05°40'17.5" S, 119°44'00.3" E), and Tarowang (05°36'07.6" S, 119°51'41.1" E) stations.

WATER QUALITY AND WASTE LOAD ANALYSIS

Water quality variables consisting of nitrate (NO_3), nitrite (NO_2), ammonia (NH_3), and phosphate (PO_4) were analysed in the Water Laboratory of the Research Institute for Coastal Aquaculture and Fisheries Extension (Ind.: Balai Riset Perikanan Budidaya Air Payau dan Penyuluhan Perikanan) in Maros Regency, South Sulawesi, Indonesia. Sodium reduction method was used to examine NO_3. The colourimetric method was used to determine NO_2. Standard phenate method was used to measure NH_3. The ascorbic acid ($C_6H_8O_6$) method was used to evaluate PO_4. All water quality parameters were examined based on the guidelines from APHA (2005). For the analysis of NO_3 and NO_2 concentrations, compound manganese sulfate ($MnSO_4$), potassium permanganate ($KMnO_4$), and sodium nitrite ($NaNO_2$) were used. The compounds of the sulfuric acid (H_2SO_4), antimony potassium tartrate ($K(SbO)C_4H_4O_6 \cdot ½H_2O$), ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$), and $C_6H_8O_6$ were used in PO_4 analysis.

Estimation of the effluent total nitrogen (*TN*) and total phosphorus (*TP*) based on data on the content of N and P in whiteleg shrimp feed and whiteleg shrimp carcass results of previous studies (Paena *et al.*, 2020). Estimation of total waste *TN* and *TP* refers to the methods of Ackefors and Enell (1990) and Barg (1992), calculated using the following equation.

$$TN = (A \cdot Cdn) - (B \cdot Cfn) \tag{3}$$

where: *TN* = total nitrogen waste, *A* = wet weight of whiteleg shrimp feed used, *B* = wet weight of whiteleg shrimp produced, *Cdn* = content of N in feed expressed as % wet weight, *Cfn* = content of N from whiteleg shrimp carcass expressed as % wet weight.

$$TP = (A \cdot Cdp) - (B \cdot Cfp) \tag{4}$$

where: *TP* = total phosphorus waste, *Cdp* = content of P in feed expressed as % wet weight, *Cfp* = content of P from whiteleg shrimp carcass expressed as % wet weight.

DATA ANALYSIS

In the dry months, rainfall of less than 60 mm·mo^{-1} was segregated from the rainfall data. A nonparametric statistics test using the Kruskal–Wallis test was employed to compare water quality indicators of the samples from the sampling points (Fig. 1). The Mann–Whitney test (nonparametric statistics) explored the differences between water quality variables taken before and after the farming cycle of the whiteleg shrimp. A nonparametric normality test using the Shapiro–Wilk test was conducted before applying nonparametric statistics. The criteria of water quality assessed by principal component analysis (PCA) are predicated on the requirement that every variable kept has an eigenvalue of less than 1.0 (Jollife and Cadima, 2016). The varimax rotation approach reduces the number of variables to the greatest extent possible. It can contribute information about the dominant water quality variables in charge of the variability of water quality data. The validity of the PCA results was determined using the Kaiser–Meyer–Olkin (KMO) and Bartlett tests. High values (close to 1.0) in the KMO test and small values (less than 0.05 of the significance level) in the Bartlett test show that the PCA can be applied to water quality data (Zhou *et al.*, 2007). The Mann–Whitney, Shapiro–Wilk, and PCA tests were conducted using the statistical package for the social sciences (SPSS) Statistics version 25 application from the International Business Machines (IBM). In data analysis, descriptive statistics were employed to know the minimum and maximum values and the average of each examined water quality variable.

The results of the water quality analyses were correlated to the standard values for the water quality variables provided in the decree of Minister of the Environment Number 51 of 2004 about the Standard Quality of Seawater for Marine Organisms (Ind.: Keputusan Menteri Negara Lingkungan Hidup Nomor 51 Tahun 2004 Tentang Baku Mutu Air Laut) (Keputusan, 2004) and Regulation of the Ministry of Marine Affairs and Fisheries Number 75/Permen-KP/2016 about the General Guidelines for Culturing Tiger Shrimp (*Penaeus monodon*) and Whiteleg Shrimp (*Litopenaeus vannamei*) (Ind.: Peraturan Menteri Kelautan dan Perikanan Nomor 75/PERMEN-KP/2016 Tahun 2016 tentang Pedoman Umum Pembesaran Udang Windu (*Penaeus monodon*) dan Udang Vaname (*Litopenaeus vannamei*)) (Peraturan, 2016). The storage and retrieval (STORET) was applied to determine the water quality status before and after the farming cycle of the intensive shrimp farming ponds. The determination scores were based on Canter (1982), i.e., on sampling size as well as physical and chemical variables (Tab. 1). Water quality status was categorised into four classes: 1) score = 0: class A, excellent, in compliance with the standard quality, 2) score from –1 up to –10: class B, good, slightly polluted, 3) score from –11 up to –30: class C, moderate, moderately polluted, 4) score ≤–31: class D, poor, heavily polluted (Keputusan, 2003).

Table 1. The scoring system used to determine the water quality status of whiteleg shrimp ponds before and after the farming cycle

Number of variables	Score	Variable		
		physical	chemical	biological
<10	maximum	–1	–2	–3
	minimum	–1	–2	–3
	average	–3	–6	–9
≥10	maximum	–2	–4	–6
	minimum	–2	–4	–6
	average	–6	–12	–18

Source: own elaboration based on Canter (1982).

RESULTS AND DISCUSSION

The whiteleg shrimp brackish water pond culture in the Je'neponto Regency has been operated for at least four farming cycles up to this study. The first three cycles were carried out in 11 ponds, while the fourth was conducted in 23 ponds. Each pond has a dimension of 60 × 60 m^2 (3,600 m^2) and is made of concrete (Tab. 2). Wastewater from each brackish water ponds is disposed of via a central drain to the wastewater treatment plant (WWTP). The wastewater flowed out from the WWTP facility without enough duration for water retention due to the small WWTP capacity to retain wastewater in comparison to the wastewater volume from the brackish water ponds. This practice reiterates the report published by IWA (2018) that about 80% of all wastewater is discharged into the world's waterways. Such minimal control of waste discharge creates health, environmental, and climate risks, leading to changes in the waters' assimilative capacity (Teichert-Coddington *et al.*, 1999). Whiteleg shrimp ponds in Je'neponto Regency have a WWTP facility with an area of 0.12 ha (Tab. 2). A simple calculation indicates that the capacity of the WWTP facility is only around 1.45% of the total pond volume. The minimum capacity (15,560 m^3) of the WWTP facility required for an intensive shrimp operation is estimated at 20% of the total volume of the rearing media with a minimum residence time of two days (Syah, Makmur and Fahrur, 2017). For super-intensive technology, the required minimum capacity of the WWTP pond should be at least 30% of the total volume of rearing media, with the water residence time of not less than five days (Syah, Makmur and Fahrur, 2017). In Vietnam, intensive whiteleg shrimp ponds must have a minimum WWTP facility of 0.25 ha for 1.0 ha of rearing ponds or 25% of the total pond capacity (Nguyen, Nguyen and Jolly, 2019). Therefore, the WWTP facility of shrimp culture in Je'neponto Regency is classified as having a lower capacity than the recommended one.

Table 2. Performance of intensive technology of whiteleg shrimp farming in Je'neponto Regency, Indonesia

Description	Value
Pond area[1)] (m^2)	3,600
Number of ponds[1)] (pcs)	23
Total pond area[1)] (ha)	8.28
Wastewater treatment plant facility[1)] (ha)	0.12
Percentage of wastewater treatment plant capacity to total pond water volume[1)]	1.45
Stocking density[1)] (ind·m^{-2})	250
Partial harvest age[1)] (DOC)	65–70
The average weight of partial harvest[1)] (g·ind^{-1})	12.5
Partial harvest production[1)] (kg·pond^{-1}·cycle^{-1})	2,000–2,500 2,250
Total harvest age[1)] (DOC)	125–135
Total harvest production[2)] (kg·pond^{-1}·cycle^{-1})	9,956–28,558 16,113
The average weight of total harvest[1)] (g·ind^{-1})	35.5
Total production[2)] (kg·pond^{-1}·cycle^{-1})	12,205–30,808 18,363
Survival rate[1)] (%)	90–95
Feed[2)] (kg·pond^{-1}·cycle^{-1})	19,407–47,136 27,584
Feed conversion ratio[2)] (–)	1.44–1.59 1.503

[1)] Data were analysed from interviews with owners and managers of whiteleg shrimp farms.
[2)] Data were analysed from record keeping of whiteleg shrimp farms (2023).
Explanations: DOC = days of culture, values over the line = range, value below the line = average.
Source: own study.

Syah, Makmur and Fahrur (2017) suggested that most fish/shrimp farming systems have not yet applied a fully functioning WWTP. A WWTP facility must supposedly have sedimentation, aeration, and equalisation ponds (Areerachakul and Kandasamy, 2022). However, the WWTP facility under study primarily served as a sedimentation pond to receive wastewater from the brackish water ponds. It deviates from a standard design and fully functioning WWTP facility with a sufficient volume, specific duration of wastewater retention time, supporting infrastructure, waste treatment technology, and post-waste utilisation.

WHITELEG SHRIMP FARMING PRACTICES

Based on the interview data, the whiteleg shrimp fries stocked in the fourth cycle were obtained from a hatchery in Lampung Province in western Indonesia. The shrimp were stocked at 250 ind·m^{-2}, classified as a high stocking density in intensive shrimp culture (Mustafa *et al.*, 2022). Partial harvest was carried out within each cycle before the total harvest. Reducing the whiteleg shrimp biomass was done in the fourth cycle by carrying out partial harvest on 60–65 days of culture (DOC) and producing 2,000–2,500 kg·pond^{-1} of shrimp with an average weight of 12.5 g·ind^{-1}. In super-intensive shrimp farming, partial harvesting enhances the final production by allowing better individual growth and survival rates of shrimp. The total harvest was done after 125–135 DOC, resulting in 9,956–28,558 kg of shrimp per pond. The total shrimp production reached 12,205–30,808 kg per pond per cycle, with an average production of 18,363 kg per pond per cycle. The average weight of the harvested whiteleg shrimp was 35.5 g·ind^{-1}, with a survival rate of 90–95% (Tab. 2). The feed conversion ratio (*FCR*) of the whiteleg shrimp culture in this study ranged between 1.44 and 1.59 with an average of 1.503, which indicated that the amount of feed used in this study was between 19,407 and 47,136 kg per pond per cycle with an average of 27,584 kg per pond per cycle. The findings of this study are similar to that of Mustafa *et al.* (2022), who reported that *FCR* in whiteleg shrimp culture ranged between 1.4–1.8 and 1.5–2.6 for intensive and super-intensive systems, respectively. Mustafa *et al.* (2023) added that *FCR* in the whiteleg shrimp brackish water pond aquaculture in Pesawaran Regency, Lampung Province was 1.66 ±0.208. The low *FCR* value in whiteleg shrimp culture can be attributed to the shrimp's efficient ability to utilise the given feed and bacterial flocs (Mustafa *et al.*, 2022) applied in most intensive shrimp farming. The low *FCR* value (whiteleg shrimp need feed containing protein between 18–35% compared with 36–42% for tiger shrimp) and feed cost of whiteleg shrimp, which is generally cheaper than that of more carnivorous shrimp species, such as tiger shrimp, are the main reasons why whiteleg shrimp aquaculture develops rapidly in the past two decades.

This study showed that the shrimp farm produced 422.349 Mg of shrimp per cycle from 23 ponds, generating 17.54 kg of *TN* per Mg of whiteleg shrimp and 4.14 kg of *TP* per Mg of whiteleg shrimp. The discharged *TN* and *TP* can be attributed to the use of artificial feed used in the intensive technology, which contains 32–36% protein, of which the whiteleg shrimp did not consume 24.32% (Paena *et al.*, 2020). It is estimated that the shrimp farm has the potential to discharge 7.408 Mg of *TN* per cycle and 1.748 Mg of *TP* per cycle. These values are well within the *TN* and *TP* discharges range from most intensive shrimp farms of 12.6–21.0 kg of *TN* per Mg of shrimp and 1.8–3.6 kg of *TP* per Mg of shrimp reported by Teichert-Coddington *et al.* (1999). Muqsith *et al.* (2019) reported slightly lower *TN* and *TP* discharges from a whiteleg shrimp brackish water farm of 13.84 kg of *TN* per Mg of whiteleg shrimp and 8.09 kg of *TP* per Mg of whiteleg shrimp due to a lower stocking density of 150 ind·m^{-2} cultured in a pond sized 2,500 m^2.

WATER QUALITY CHARACTERISTICS

Before the farming cycle of whiteleg shrimp brackish water pond aquaculture

The water quality in the coastal waters of Je'neponto Regency before the farming cycle of the whiteleg shrimp culture is provided in Tables 3 and 4.

Generally, seawater temperatures in the tropics have minimum variations among locations. This study showed that the water temperatures measured from near the coastline outward were relatively the same (probability (p) = 0.410) (Tab. 3). However, water temperatures in the sampling points parallel to the coastline were significantly different (p = 0.012) (Tab. 4). It is argued that such differences were caused by the relatively close

Table 3. Significance of Kruskal–Wallis test results of water quality variables measured in 20 sampling points along the five line transects perpendicular to the coastline in Je'neponto Regency, Indonesia, before the whiteleg shrimp culture cycle

Variable	Transect					Significance
	I	II	III	IV	V	
Temperature (°C)	26.675	26.925	26.850	26.900	26.850	0.410
Salinity (ppt)	33.820	33.838	33.865	33.878	33.885	0.867
pH	8.305	8.360	8.405	8.428	8.415	0.005
Dissolved oxygen (mg·dm^{-3})	6.648	6.500	6.183	6.173	6.383	0.029
Nitrate (mg·dm^{-3})	0.32455	0.19435	0.24500	0.20973	0.16483	0.281
Nitrite (mg·dm^{-3})	0.00080	0.00080	0.00080	0.00080	0.00080	1.000
Ammonia (mg·dm^{-3})	0.10130	0.08243	0.09875	0.09545	0.10070	0.355
Phosphate (mg·dm^{-3})	0.05735	0.00470	0.05023	0.01320	0.00100	0.234

Explanations: transect I = around 1,000 m from the drain/outlet to the northeast, transect II = around 500 m from the drain/outlet to the northeast, transect III = 0 m from the drain/outlet, transect IV = around 500 m from the drain/outlet to the southwest, transect V = around 1,000 m from the drain/outlet to the southwest.
Source: own study.

Table 4. Significance of Kruskal–Wallis test results of water quality variables in sampling sites located parallel to the coastline of Je'neponto Regency, Indonesia, before the whiteleg shrimp culture

Variable	Transect				Significance
	1	2	3	4	
Temperature (°C)	27.200	26.780	26.680	26.700	0.012
Salinity (ppt)	33.692	33.874	33.926	33.936	0.008
pH	8.370	8.404	8.390	8.366	0.597
Dissolved oxygen (mg·dm^{-3})	6.318	6.280	6.404	6.506	0.502
Nitrate (mg·dm^{-3})	0.20478	0.23712	0.21018	0.25868	0.837
Nitrite (mg·dm^{-3})	0.00080	0.00080	0.00080	0.00080	1.000
Ammonia (mg·dm^{-3})	0.09762	0.08926	0.09946	0.09656	0.782
Phosphate (mg·dm^{-3})	0.02448	0.06004	0.00750	0.00916	0.568

Explanations: transect 1 = around 100 m from the coastline, transect 2 = around 300 m from the coastline, transect 3 = around 500 m from the coastline, transect 4 = around 700 m from the coastline.
Source: own study.

distance of some of the sampling sites and the shallowness of the surrounding waters. The temperature variations measured in the study are slightly lower than that of the optimal temperature suggested for higher tropic organisms, which ranges from 28 to 30°C (Keputusan, 2004). Similarly, Peraturan (2016) prescribed that the optimal water temperature should range from 28 to 30°C in intensive and super-intensive whiteleg shrimp brackish water ponds. Since the temperature measurements were done in sampling points in the coastal waters outside the ponds, the temperature in the shrimp pond will likely reach the suggested temperature due to warming up in the ponds.

Temperature variation significantly affects the physiological conditions of whiteleg shrimp. Low temperature leads to low metabolic processes and vice versa, which eventually influences the whiteleg shrimp appetite. The temperature also affects the growth, survival rate, *DO* consumption, molting cycle, and immune response of whiteleg shrimp. A sudden drop in temperature can cause the whiteleg shrimp to become stressed, leading to death, especially for the whiteleg shrimp infected with *Vibrio*. Increased water temperature can escalate the shrimps' sensitivity to toxins produced by cyanobacteria or blue-green algae. Low temperatures also affect the immunity of whiteleg shrimp, causing whiteleg shrimp to be more susceptible to diseases.

The suggested value of water salinity for intensive and super-intensive whiteleg shrimp brackish water pond aquaculture ranges from 26 to 32 ppt (Peraturan, 2016). In this study, insignificant salinity differences were observed in the sampling sites perpendicular to the coastline (p = 0.867) (Tab. 3). In contrast, significant salinity differences were observed in sampling sites parallel to the coastline (p = 0.008) (Tab. 4). This significant difference in salinity may have been influenced by the freshwater mass discharge from the land, reducing the salinity of seawater at sampling sites close to the coastline. Since no large river mouth existed in the study area, the effects of freshwater did not reach farther from the shorelines, leading to consistent high salinity in the open seawaters (Nybakken and Bertness, 2004).

The range of pH values deemed suitable for intensive and super-intensive whiteleg shrimp brackish water ponds is 7.5–8.5 (Peraturan, 2016). Keputusan (2004) similarly published that a pH value between 7.0–8.5 is suitable for marine organisms. The recorded pH values of the coastal waters in Je'neponto Regency ranged between 8.305 and 8.428. These values indicate that the seawater pH is within the optimum value for whiteleg shrimp aquaculture. The pH of the seawater surface in Indonesia generally ranges between 6.0 and 8.5 (Tanjung, Hamuna and Alianto, 2019). Despite that, the study found significant differences in the water pH values among sampling sites perpendicular to the coastline (p = 0.005). In contrast, the distribution of pH values in sampling sites parallel to the coastline showed no significant differences (p = 0.597). The lower pH values in seawater closer to the coastline may have been influenced by the higher amount of organic material brought from the land through runoff and small rivers along the coastline. The effect of this organic material is reduced due to dilution as it goes farther into the open sea (Kusumaningtyas *et al.*, 2014).

Dissolved oxygen (*DO*) concentration is one of the primary limiting factors in shrimp culture. It strongly affects shrimp growth and *FCR*, as well as the carrying capacity of the rearing media to support shrimp farming. Keputusan (2004) stipulates that the suitable *DO* concentration for marine organisms is over 5.0 mg·dm^{-3}, while Peraturan (2016) established a specific *DO* concentration of at least 4.0 mg·dm^{-3} for intensive whiteleg shrimp farming. This study found that all sampling sites had a *DO* concentration over 5.5 mg·dm^{-3} (Tabs. 3, 4). Differences in *DO* concentration were observed in sampling sites located perpendicular to the coastline (p = 0.029). Such differences were not observed in sampling sites parallel to the coastline (p = 0.502). Considering the high stocking density of whiteleg shrimp in intensive technology, aerators must be installed to maintain sufficient *DO* concentration in the shrimp ponds. Paddle wheels, jet aerators, and root blowers can maintain sufficient *DO* concentration in the ponds, each with different characteristics and efficiencies. Regular water exchange is often combined with aerators to increase *DO* concentration in the shrimp pond. Both approaches are essential to be simultaneously done considering that *DO* concentration is affected by temperature, gas partial pressure, salinity, and the presence of easily oxidised compounds in the water (Tran *et al.*, 2022). The higher the temperature, salinity, and pressure of the gas dissolved in water, the lower the oxygen solubility.

In the substance of the water quality, nutrients are defined as molecules of water that marine organisms can precisely consume for cellular growth (Landau, 1992). The most consumed nutrients are NO_3, NO_2, NH_3, and PO_4 (Olanrewaju, Tee and Kader, 2015). In the seawater ecosystem, NO_3 is the most critical nutrient affecting plankton growth and other higher plants, such as seaweeds. NO_3 concentrations in all sampling points set in the coastal water of Je'neponto Regency were relatively the same, both in sampling points set in the perpendicular transects (p = 0.281) and the parallel transects to the coastline (p = 0.837).

NO_2 is toxic to marine organisms because NO_2 can convert haemoglobin into methaemoglobin. Methaemoglobin, a bonding between NO_2 and haemoglobin, is highly effective in preventing the haemoglobin from bonding with oxygen. Shrimp only has hemocyanin, whose function is to transport oxygen and nutrition. When NO_2 bonds with haemocyanin, the transport of oxygen and nutrients to the shrimp's body will be hindered to the point that the shrimp could be deprived of oxygen and nutrition. NO_2 concentrations measured in all sampling sites were relatively the same, i.e., 0.0008 mg·dm^{-3} (Tabs. 3, 4). Peraturan (2016) states that the suitable NO_2 concentration for intensive and super-intensive whiteleg shrimp brackish water aquaculture should be less than 1 mg·dm^{-3}. This means that the concentration of NO_2 in the seawater of the study sites is still considered safe before the new shrimp farming cycle.

NH_3 can be found as molecule NH_3 or ion NH_4, where the former is more toxic than the latter. NH_3 can enter the cell membrane faster than NH_4. NH_3 concentration of 0.05–0.20 mg·dm^{-3} is sufficiently enough to harm the growth of aquatic organisms. NH_3 concentration suitable for intensive and super-intensive whiteleg shrimp brackish water pond aquaculture is less than 0.1 mg·dm^{-3} (Peraturan, 2016). The measured NH_3 concentrations in all sampling sites of the coastal water of Je'neponto Regency were relatively the same, both in parallel (p = 0.355) and perpendicular directions to the coastline (p = 0.782).

Up to a certain concentration, P is not harmful to fish and shrimp. However, excessive concentrations of P and N can stimulate toxic or oxygen-deprived algal blooms in the waters. PO_4 concentrations in the study area ranged from 0.00100 to 0.09946 mg·dm^{-3}, with no significant differences between sampling sites, either perpendicular or parallel directions to the coastline (Tabs. 3, 4). The suitable PO_4 concentration in the water supply and whiteleg shrimp culture area is between 0.01 and 5.0 mg·dm^{-3} (Peraturan, 2016), which indicates the study area's relatively good condition before the new shrimp farming cycle starts.

After the farming cycle of whiteleg shrimp brackish water pond aquaculture

Results of *in situ* and *ex situ* water quality measurements in the study site after the whiteleg shrimp culture farming cycle are presented in Tables 5 and 6. Temperature (p = 0.487) and salinity (p = 0.347) in sampling sites located perpendicular to the coastline were relatively the same (Tab. 5). However, water temperature and salinity differences were observed in sampling points parallel to the coastline (Tab. 6). Significant water temperature and salinity differences were observed when comparing the variables before and after the farming cycle. The water temperature after harvesting time (measured in November) was higher than before the whiteleg shrimp culture started (measured in July). These temperature variations agreed with a report by Yuda, Sulistya and Sopaheluawakan (2011) stating that the surface water temperature in Indonesia in November ranged from 25.68 to 30.10°C, slightly higher than in July, i.e., 24.59–29.86°C. In some sampling sites, the water temperature reached more than 30°C, exceeding the optimum water temperature limit for marine organisms, especially shrimp.

Higher water salinity after the harvesting time of the whiteleg shrimp culture (in November) may have been caused by the dry season in the Flores Sea region. Gordon, Ffield and Ilahude (1994) argued that the dry season increased the salinity of 34.4–34.6 ppt in the study site due to water mass flow from the Banda Sea with a salinity of 34.5–34.6 ppt. The high salinity was also exacerbated by the lower rainfall measured in November, as shown in Figure 2. Before November, the data shows two dry

Table 5. Significance of Kruskal–Wallis test results of water quality variables in the sampling sites located perpendicular to the coastline in Je'neponto Regency, Indonesia, after the harvesting time of whiteleg shrimp culture

Variable	Transect					Significance
	I	II	III	IV	V	
Temperature (°C)	30.000	29.925	29.825	29.800	30.025	0.487
Salinity (ppt)	34.835	34.965	34.943	34.890	34.775	0.347
pH	8.230	8.368	8.333	8.345	8.340	0.024
Dissolved oxygen (mg·dm^{-3})	5.643	5.520	6.225	5.590	6.020	0.056
Nitrate (mg·dm^{-3})	0.48853	0.64878	0.51055	0.60205	0.58393	0.832
Nitrite (mg·dm^{-3})	0.00080	0.00080	0.00080	0.00080	0.00080	1.000
Ammonia (mg·dm^{-3})	0.12978	0.12078	0.09430	0.09435	0.15753	0.016
Phosphate (mg·dm^{-3})	0.28030	0.48105	0.25583	0.24380	0.23060	0.715

Explanations as in Tab. 3.
Source: own study.

Table 6. Significance of Kruskal–Wallis test results of water quality parameters in sampling sites located parallel to the coastline of Je'neponto Regency, Indonesia, after the harvesting time of whiteleg shrimp culture

Variable	Transect				Significance
	1	2	3	4	
Temperature (°C)	30.220	29.880	29.840	29.720	0.005
Salinity (ppt)	35.004	34.820	34.810	34.892	0.025
pH	8.384	8.312	8.306	8.290	0.228
Dissolved oxygen (mg·dm^{-3})	6.126	5.684	5.818	5.570	0.257
Nitrate (mg·dm^{-3})	0.57460	0.57222	0.55720	0.56304	0.944
Nitrite (mg·dm^{-3})	0.00080	0.00080	0.00080	0.00080	1.000
Ammonia (mg·dm^{-3})	0.13442	0.11318	0.17272	0.33396	0.992
Phosphate (mg·dm^{-3})	0.19604	0.49010	0.00750	0.00916	0.076

Explanations as in Tab. 4.
Source: own study.

months, August and September, in Je'neponto Regency (Fig. 2). The drier condition within these months is needed to better prepare the ponds before stocking whiteleg shrimp fries. Better pond preparation will limit the transmission of disease organisms from one farming cycle to the next via contaminated water (Boyd *et al.*, 2020).

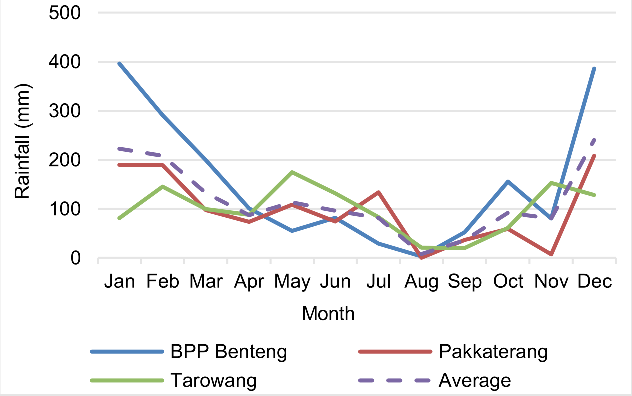

Fig. 2. Monthly rainfall from 2016 to 2020 in Je'neponto Regency, South Sulawesi Province, Indonesia; BPP = Agricultural Extension Service (Ind.: Balai Penyuluhan Pertanian), source: own elaboration based on data from the Maros Class I Climatology Station (Ind.: Stasiun Klimatologi Kelas I Maros)

Significant pH and *DO* differences were in sampling sites perpendicular to the coastline (Tab. 5). Such differences were not observed in sampling sites parallel to the coastline (Tab. 6). Significant pH and *DO* decreases were observed after the harvesting time of the whiteleg shrimp culture (Tab. 7). The lower *DO* levels could be attributed to the aerobic decomposition process of accumulated organic materials from wasted feed, shrimp's faecal matter, detritus, phytoplankton, zooplankton, and bacteria (Casillas-Hernández *et al.*, 2007). Waste accumulation can increase carbon dioxide (CO_2) concentration resulting from the respiration of decomposing microorganisms, leading to decreased water pH (Delgado *et al.*, 2003).

An increase in the average NO_3 concentration was observed from 0.22769 mg·dm^{-3} (before the shrimp culture) to 0.56676 mg·dm^{-3} (after the shrimp harvest), which was significant based on Mann–Whitney test ($p = 0.001$) (Tab. 7). The increase in NO_3 may have been caused by the accumulation of faecal matter from the cultured shrimp. Several sampling sites had NO_3 concentra-

Table 7. Significance of Mann–Whitney test results of water quality parameters (average from the all of the sampling sites) before and after the farming cycle of whiteleg shrimp in Je'neponto Regency, Indonesia

Variable	Before the shrimp culture	After the shrimp culture	Significance
Temperature (°C)	28.840	29.915	0.000
Salinity (ppt)	33.857	34.882	0.000
pH	8.391	8.323	0.000
Dissolved oxygen (mg·dm^{-3})	6.377	5.799	0.000
Nitrate (mg·dm^{-3})	0.22769	0.56676	0.001
Nitrite (mg·dm^{-3})	0.0008	0.0008	1.000
Ammonia (mg·dm^{-3})	0.09573	0.11933	0.011
Phosphate (mg·dm^{-3})	0.25295	0.29832	0.000

Source: own study.

tions that surpassed the suggested upper threshold of 0.5 mg·dm^{-3} (Peraturan, 2016). In most aquaculture systems, 30% of the total feed is not consumed by cultured fish/shrimp, while 25–30% is excreted as faecal matter (McDonald *et al.*, 1996). Around 25–30% of N and P concentrations in the feed will be retained in the fish/shrimp meat, while the rest will be discharged into the surrounding waters (Susetyaningsih *et al.*, 2020). On average, the total nutrients wasted in the form of *TN* from the whiteleg shrimp culture in the study area could be as high as 7.408 Mg of *TN*.

The concentration of NH_3 shows a tendency to rise from 0.09573 mg·dm^{-3} (before the shrimp culture) to 0.11933 mg·dm^{-3} (after the harvest time), which was significant based on the Mann–Whitney test ($p = 0.011$) (Tab. 7). The increase was highly likely caused by NH_3 input originating from the feed residues and faecal matter from the cultured whiteleg shrimp. Despite that, the rise in NH_3 concentration has not exceeded the limit suggested by Keputusan (2004). High NH_3 concentration was observed in sampling sites near the shrimp ponds' drainage/outlet. The outlet has been closed due to suspicions of wastewater flow from agriculture and household activities to the coastal waters near transect V. The waste sources observed by this study indeed originate from pond aquaculture, agricultural, and household activities. However, much of the waste is produced from the whiteleg shrimp ponds. The research results carried out by Paena *et al.* (2020) using stable isotope analysis show similar results where whiteleg shrimp pond waste was more dominant (95%) compared to agricultural and household wastes.

The NO_2 concentration in this study was relatively the same (0.0008 mg·dm^{-3}) before the start of the next farming cycle and after the harvesting time. From the three forms of N analysed in this study, only NO_3 and NH_3 increased after harvesting. The wasted shrimp feed caused the increase of P concentration along the coastline of Je'neponto Regency from 0.25295 mg·dm^{-3} (before the start of the subsequent culture cycle) to 0.29832 mg·dm^{-3} (after harvesting time).

The result of PCA analysis shows that the dominant water quality variables responsible for determining the water quality status of the study site both before the shrimp culture and after the shrimp culture are temperature, salinity, pH, and NO_3, which are incorporated in factor or component 1. *DO*, NH_3, and PO_4 are the second dominant group variable (component 2) (Fig. 3). The component 1 variables (temperature, salinity, pH, and NO_3) can contribute 57.82% (Fig. 3) of the total variance of the seven water quality variables resulting from the waste discharge of intensive technology of whiteleg shrimp. Thus, *TN*, NH_3, NO_3, and *TP* are the primary factors influencing pollution levels (Kupiec, Staniszewski and Kayzer, 2022).

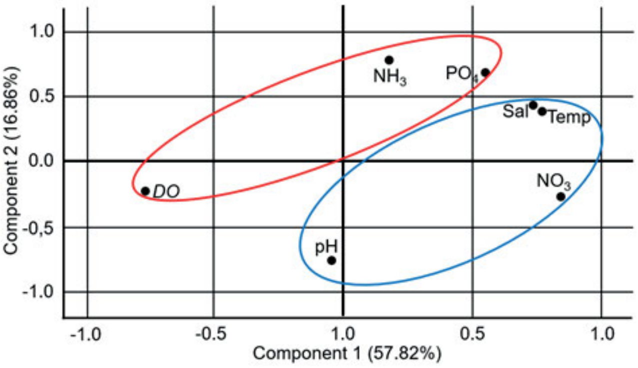

Fig. 3. Placement of components in rotated space for two components of principal component analysis of water quality variables before the start and after the farming cycle of whiteleg shrimp in Je'neponto Regency, Indonesia; Temp = temperature, Sal = salinity, *DO* = dissolved oxygen; source: own study

WATER QUALITY STATUS

The STORET analysis conducted to determine the water quality status before the start of the farming cycle resulted in a total score between –2 and –10 in sampling sites based on distances from the outlet (Fig. 4) and between –2 and –8 based on distances from the coastline (Fig. 5). These scores indicated that all sampling points have relatively the same water quality status, which was class B (good or slightly polluted). The only water quality variable with a value range outside the suggested threshold for marine organisms was the PO_4 concentration. The other water quality

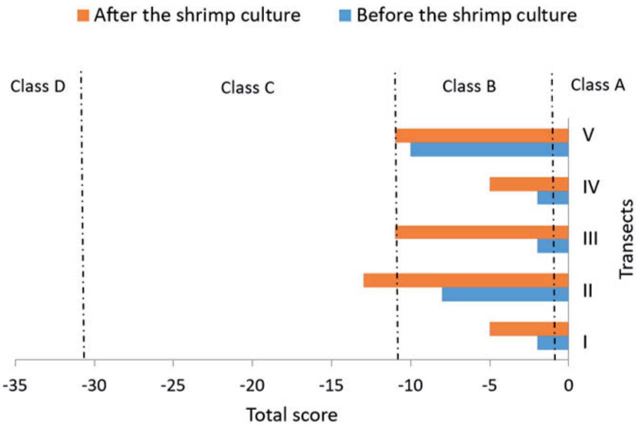

Fig. 4. Water quality status before and after the farming cycle of the intensive whiteleg shrimp culture in sampling sites perpendicular to the coastline in Je'neponto Regency, Indonesia; class A = excellent or fulfilling standard quality, class B = good or slightly polluted, class C = moderate or moderately polluted, class D = poor or heavily polluted; source: own study

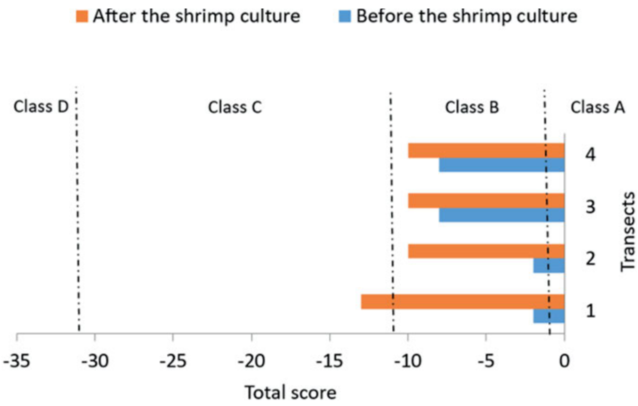

Fig. 5. Water quality status before and after the whiteleg shrimp culture of water samples taken from sampling points in transects parallel to the coastline in Je'neponto Regency, Indonesia; class A, class B, class C, class D as in Fig. 4; source: own study

variables, such as temperature, salinity, pH, *DO*, NO_3, NO_2, and NH_3, were all within the applicable limit for marine organisms.

The low PO_4 concentration in Je'neponto Regency may have been caused by PO_4 utilisation in the nearby seaweed culture area. Seaweed requires N and P, where the latter is absorbed as PO_4. A key element for higher plants and aquatic algae is P, it is also the limiting factor for the growth of plants and aquatic algae and affects open-water productivity (Jones and Bachmann, 1976). Seaweed needs P to grow because it is the primary ingredient in ribonucleic acid (RNA), leading to protein synthesis (Douglas, Haggitt and Rees, 2014). Excessive P intake in aquatic algae happens when the surrounding waters contain enough P, so the aquatic algae collect P in their cells, outstanding the required amount (Ren *et al.*, 2017). An excessive amount of P will be used when the surrounding waters lack P; therefore, the seaweed can still grow for a specific time despite the lack of P in the surrounding waters. In this study, 65% of sampling sites (13 of 20) had PO_4 concentrations lower than 0.02 $mg \cdot dm^{-3}$, indicating that the waters have low fertility. After the farming cycle, water quality status in sampling sites perpendicular to the coastline showed a total score between −5 and −13 (Fig. 4). In contrast, sampling sites parallel to the coastline showed a total score between −10 and −13 (Fig. 5). Changes in water quality status occurred in several sampling points perpendicular to the coastline (transects II, III, and V). These sampling points along transects II, III, and V were categorised as class C (moderately polluted) from class B before the new farming cycle started (Fig. 4). The other sampling stations located along transects I and IV had the same water quality status before the start of the farming cycle and after the shrimp culture.

Change in water quality status after the shrimp culture also happened in transect 1 (parallel to the coastline), from class B to class C (Fig. 5). Transects 2, 3, and 4 had no changes in water quality status after the shrimp culture (Fig. 5). However, this study showed that transects near the pond's outlet have a low-class C water quality status. The dominant water quality variable causing the reduced water quality status was NO_3, most likely caused by the cultured shrimp's feed residues and faecal matter. In the intensive shrimp culture, N causes the dynamic system between nutrition and toxicity (Burford and Lorenzen, 2004). The primary source of N is an artificial feed containing a protein of 13–60% (2–10% N) (Kar, Salam and Rana, 2017). Compared to NO_2 and NH_3, NO_3 is a less toxic form of N. At high concentrations, NO_3 can be toxic for shrimp. Several anomalies are experienced by shrimp exposed to high NO_3 concentrations, such as having shorter antennae, abnormal gills, and blisters in the hepatopancreas. Kuhn, Smith and Flick (2011) reported that antennae shortened and gills deformity are often regarded as initial clinical symptoms of shrimp's health deterioration. NO_3 concentration in water sources for intensive and super-intensive shrimp culture should not exceed 0.5 $mg \cdot dm^{-3}$ (Peraturan, 2016). It is essential to manage and control the healthy condition of the water source when culturing shrimp. Denitrification is an important mechanism to control any form of N, including NO_3, by converting NO_3 to nitrogen gas (N_2) (Morrissey and Franklin, 2015). Denitrification is the main path to evaporating N from the water body (Laverman *et al.*, 2010). Strong, McDonald and Gapes (2011) showed that water temperature, pH, *DO*, *OC*, and C:N ratio can affect denitrification efficiency. N can also be controlled through NH_3 and NO_3 absorption by phytoplankton, proper feeding, water change, recirculation system, and bioremediation using heterotroph bacteria through probiotic administration. Many studies and direct evidence in the field show the benefits of using probiotics, including reducing the content of NH_3, NO_3, NO_2, hydrogen sulfide (H_2S), organic waste (derived from leftover feed, faecal, and other organic material), and the potential for disease. It is expected that by controlling N, the sustainable intensification of shrimp culture in Je'neponto Regency can be achieved. Godfray *et al.* (2010) expressed sustainable intensification as a production technique that administers more feed from the same land area without adverse environmental impact. Figures 4 and 5 show that the quality of the water source for whiteleg shrimp culture is still considered suitable in Je'neponto Regency before the start of the farming cycle. After whiteleg shrimp culture, the quality of the water supply for the shrimp ponds decreased and was classified as moderately polluted in locations close to pond water drain or outlet. Therefore, taking water for whiteleg shrimp culture at a distance greater than 300 m from the coastline is recommended.

The findings of this study are expected to provide basic information regarding the area's characteristics and water quality status. The findings of this study indicate that greater awareness of the environmental impact of intensive whiteleg shrimp ponds is pivotal to ensuring the sustainability of whiteleg shrimp aquaculture industry. Presenting the best option for managing coastal area resources is challenging without regular monitoring of water quality temporally and spatially (Mustafa *et al.*, 2022; Mustafa *et al.*, 2023). The study suggests that subsequent water quality monitoring should be carried out regularly to ensure a healthy aquatic ecosystem and sustainability of intensive technology whiteleg shrimp aquaculture.

CONCLUSIONS

Intensive whiteleg shrimp culture was conducted in coastal waters of Arungkeke Subdistrict, Je'neponto Regency, with a stocking density of 250 $ind \cdot m^{-2}$ in 23 ponds, each sized 3,600 m^2. The total production of the shrimp ponds was between 9,956 and 28,558 kg per pond per cycle after being reared for 125–135 days with a food conversion ratio (*FCR*) of 1.44–1.59. The potential waste produced from the intensive whiteleg shrimp ponds can reach

7,408 kg of total nitrogen (*TN*) per cycle and 1,748 kg of total phosphorus (*TP*) per cycle. Despite the shrimp farm's high production, the wastewater treatment plant was only 1.45% of the total pond volume. It is classified as a wastewater treatment plant with low capacity and capability for intensive technology whiteleg shrimp ponds. Temperature, salinity, pH, and NO_3 are the primary variables that can represent the overall variables by simplifying the data from multiple highly correlated variables. By transforming the data linearly, obtained variables with smaller dimensions with maximum variability are independent. By transforming a large set of water quality variables into a smaller one that still contains most of the information in the large set. Values of pH and dissolved oxygen (*DO*) had decreased after the harvest time, while at the same time, the values of NO_3, NH_3, and PO_4 increased. It is evidently clear that the presence of shrimp farming has significantly affected the water quality status in the area. The water quality status of the site was categorised in class B but then changed to class C after the shrimp operated, especially in locations less than 300 m from the coastline. Among other variables, NO_3 is a water quality variable that dominantly affects the decline in water quality status in the coastal areas receiving waste from the intensive technology whiteleg shrimp aquaculture. It is, therefore, essential to manage and control the application of feed, water quality, and shrimp health to ensure that the shrimp is economically profitable and environmentally sustainable. This study recommends the improvement of the existing wastewater treatment plant and conducting regular monitoring of water quality temporally and spatially in the coastal waters. Taking water supply for whiteleg shrimp culture should be done at a distance greater than 300 m from the coastline is also recommended.

AUTHOR CONTRIBUTIONS

1st Author (contribution – 25%): study design, data collection, statistical analysis, data interpretation, manuscript preparation, literature search. 2nd Author (contribution – 15%): study design, data collection, statistical analysis, manuscript preparation, literature search. 3rd Author (contribution – 15%): manuscript preparation. 4th Author (contribution – 4%): data collection. 5th Author (contribution – 4%): data collection. 6th Author (contribution – 4%): data collection. 7th Author (contribution – 4%): data collection. 8th Author (contribution – 4%): data collection. 9th Author (contribution – 4%): data collection. 10th Author (contribution – 4%): literature search. 11th Author (contribution – 4%): data collection. 12th Author (contribution – 4%): data collection. 13th Author (contribution – 4%): data collection. 14th Author (contribution – 5%): data interpretation.

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CONFLICT OF INTERESTS

All authors declare that they have no conflict of interests.

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