Correlation between water quality with prevalence of black tiger shrimp (*Penaeus monodon*) infested by ectoparasite protozoa in traditional ponds of Wonorejo, Surabaya

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Abstract. This research is intended to know the prevalence of black tiger shrimp (*Penaeus monodon*) were infested with ectoparasites protozoa and correlation with water quality in traditional ponds Wonorejo Surabaya. The method of this research is survey method with simple random sampling at five different locations ponds. The main parameters observed was the prevalence of ectoparasites that infested black tiger shrimp (*Penaeus monodon*). The supporting parameters was observed water quality parameters included pH, temperature, DO, salinity, brightness and ammonia content. The results of research showed that the prevalence of black tiger shrimp (*Penaeus monodon*) infested by protozoa ectoparasite in traditional ponds subdistrict Wonorejo Surabaya was 16.67% included in the category often. Correlation was shown indicated in the parameters of temperature, ammonia content and turbidity, that the higher the temperature, the ammonia content and turbidity, the higher the prevalence of black tiger shrimp (*Penaeus monodon*) infested by protozoa ectoparasite. Correlation was shown in the parameters of dissolved oxygen and pH, that the lower the dissolved oxygen and pH the higher the prevalence of black tiger shrimp (*Penaeus monodon*) infested by protozoa ectoparasite. Parameter salinity did not show any correlation because the correlation coefficient of R = 0.064, less the correlation coefficient (R) of less than 0.2.

1. Introduction

Tiger shrimp (*Penaeus monodon*) is one of the leading cultivated commodities in Asia [1]. This is because tiger prawns are the mainstay of the fisheries sub-sector with a very high selling price [2]. Along with the development of cultivation technology, several obstacles have emerged in the hatchery and enlargement sectors which have resulted in decreased of production [3].

One of the main problems in shrimp ponds is that the environmental conditions of the pond must match the living needs of shrimp [4]. Pond environmental conditions are closely related to pond water quality which is reflected in several water quality parameters. Poor water quality affects the entire environment, causing disease infections [5]. This can occur due to high stocking density, residual feed and feces and accumulation of waste on cultivated land.

The emergence of shrimp disease in ponds is due to the interaction between disease, host and the environment [6]. One of the disease agents that can attack tiger shrimp is parasites [7]. Ectoparasites that often attack tiger prawns (*P. monodon*) are protozoa groups, including *Vorticella* sp., *Epistylis* sp.,
and *Zoothamnium* [8]. Changes in water quality and disease attacks on tiger prawns greatly affect the production of tiger prawns (*Penaeus monodon*) [9].

Several cases of ectoparasite infestation in shrimp, one of which is in Gresik Regency, including in Bungah District, Manyar District, Ujung Pangkah District and Duduk Sampeyan District, the results of the examination show the prevalence of ectoparasite infested with *Zoothamnium* spp., *Epistylis* spp., *Vorticella* spp. was around 54.2% of the examined shrimp, namely 240 shrimps [10]. Another case is in the area of Lamongan Regency, where *Zoothamnium penaei* was found in tiger prawns, the results of the study showed a prevalence rate of up to 87% with a predilection for swimming feet, walking legs, carapace and gills [11].

Some traditional aquaculture ponds in Wonorejo Surabaya use a polyculture system by combining milkfish and tiger prawns. Polyculture cultivation is a type of cultivation that uses more than one type of commodity in an integrated pond or traditional pond. Decrease in water quality is usually caused by pollutants entering the ponds [12]. The Wonorejo river flow has been polluted since 1997 by textile dye industry waste, and the Wonorejo floodgate area has been contaminated by detergent waste with a concentration of 5 mg/L which is the result of washing machine waste from industrial activity [13].

Various efforts to prevent and control disease in tiger prawns (*P. monodon*) using drugs and antibiotics are highly discouraged by the government because they have an impact on consumers and the environment [3]. Based on the above background, it is necessary to conduct research to determine the correlation between water quality and the prevalence of tiger prawns (*Penaeus monodon*) infested with protozoan ectoparasites.

2. Material and methods

2.1. Place and time of research

The research was conducted in October-November 2016. Sampling and measuring water quality activities were carried out in Traditional Ponds, Wonorejo Village, Surabaya. Examination of protozoa ectoparasites in tiger prawns was carried out at the Educational Laboratory of the Faculty of Fisheries and Marine Universitas Airlangga, Surabaya.

2.2. Tools and material

The tools used in this study were a digital thermometer, Dissolved Oxygen (DO) meter, ammonia test kit, pH meter, refactometer, sachi disc, sample tube, sterofoam box, transport plastic, oxygen supplier, section set, ruler, scale and microscope.

The materials used in this study were 150 tiger prawns (*Penaeus monodon*) 15-20 cm in length, taken from Wonorejo Traditional Pond, Surabaya, pond water samples and ice cubes.

2.3. Working procedure

Preparation of tools were done by calibrating water quality tools (DO meter, pH meter and digital thermometer). While in the field, tiger prawn were taken from 5 ponds for 30 individuals, sampling was carried out 5 times with 6 individuals at each time so that the total sample was 150 individuals. The minimum number of samples for correlational descriptive research is 27-30 individuals per population [14]. Each sampling on each plot is divided into three points of collection locations, this is in accordance with the National Standardization Agency for the entry of river water, in the middle and the place where the water comes out of these waters.

Samples of tiger prawns that had been taken were put in a plastic bag filled with water and oxygen, the amount of water entered is 2 liters every 5-10 individuals with an average size of 15-20 cm. Then the plastic bag was labeled and finally put into a stereofoam filled with ice to keep the temperature inside cool [15]. The measurement of water quality was carried out in the location (in situ) of the traditional aquaculture ponds of Wonorejo Village, Surabaya. Measurement of water quality together with sampling of tiger prawns (*Penaeus monodon*) were scheduled at 09.00, sampling was carried out at a certain depth that is close to the tiger shrimp habitat (*Penaeus monodon*). Water sampling was carried out three times. Water quality measurements are carried out directly in the morning for stagnant waters [16].

Dissolved oxygen was measured using a digital DO meter. It is worked by inserting a probe containing a cathode and anode immersed in an electrolyte solution at the bottom of the pond.
compartment waters until the digital numbers stop changing [17]. Brightness of water was measured using a secchi disc by slowly dipping a circular plate with four black and white quadrants in the water until it is not visible and then lifted back up to be seen, the magnitude of the brightness is seen from the average length of the rope in centimeters from the two processes [18]. PH was measured using a pH pen that has been calibrated by dipping an electrode (the tip of the pH pen) into the waiting water until the digital numbers mstop [19]. Salinity was measured using a refractometer by dropping some of the pond water into a certain area on the refractometer, then the refractometer is pointed at a light source to see the line on the refractometer bar scale. Ammonia was measured using an Ammonia test kit by filling the sampling tube or sample tube with 10 ml of pond water and then dropping the ammonia salicylate reagent and ammonia cyanurate reagent, then depositing for 5 minutes, then comparing the water color of the test results with the color chart printed on the paper instruction [20].

The method used in ectoparasite examination is by means of scraping [21]. The shrimps taken first were measured for their length and weight and observed for their clinical symptoms. Scrapping is carried out on the body surface, swimming legs, walking legs and gills, then followed by observation under a microscope with a magnification of 100x. Inspection of shrimp gills is done natively, namely by directly examining the gill lamellae using a 100x magnification microscope.

2.4. Data analysis
Statistical analysis uses correlation to determine the strength of the relationship and the direction of the relationship between water quality and the prevalence of tiger prawns (Penaeus monodon) infested with protozoan ectoparasites. If the data has been stated to have a relationship, then continue by measuring the linear regression equation to determine the effect of a variable in predicting other variable (Anthoni and Iswati, 2009). Data were processed using SPSS version 16.00.

3. Result and discussion
3.1. Water quality of traditional pond in Wonorejo Surabaya
Water sampling and quality measurement were carried out in traditional aquaculture of tiger shrimp in Wonorejo Village, Rungkut District, Surabaya, which were selected from 5 randomly selected ponds, namely Tambak A, Tambak B, Tambak C, Tambak D, and Tambak E. Measurement of water quality includes temperature, degree of acidity (pH), solubility of oxygen (DO), salinity, ammonia content and brightness. The table of the results of measuring the quality of tiger shrimp pond water in Wonorejo Village is presented in Table 1.

| Pond | Temperature (°C) | pH | Dissolved Oxygen (mg/L) | Salinity (ppt) | Ammonia (mg/L) | Brightness (cm) |
|------|------------------|----|-------------------------|---------------|----------------|----------------|
| A    | 32.65            | 8.09 | 5.358                   | 19.35         | 0.5            | 29.1           |
| B    | 32.34            | 8.01 | 5.736                   | 19.43         | 0.5            | 29.83          |
| C    | 33.02            | 7.91 | 4.849                   | 19.1          | 0.6            | 29.53          |
| D    | 33.21            | 7.92 | 4.528                   | 20.08         | 0.62           | 30.5           |
| E    | 32.1             | 8.4  | 6.251                   | 19.81         | 0.5            | 28.63          |
| Normal Range (Subachir, 2014) | 29 – 32 | 7.6 – 8.8 | > 3 | 15 – 25 | < 0.1 | 30 – 40 |

Based on Table 1, it can be seen that the water quality measurements at the entire location of the tiger prawn pond sampling area resulted in the following data, temperature ranges from 32.1 - 33.21 °C, pH ranges from 7.91 - 8.4, dissolved oxygen 4.528 - 6.251 mg/L, salinity 19.1 - 20.08 ppt, ammonia 0.5 - 0.62 mg/L and brightness 28.63 - 30.5 cm. Water quality measurements are carried out directly on site (in situ).

3.2. Prevalence of tiger shrimp infested with protozoa ectoparasites
The results showed that the prevalence rate of ectoparasites in tiger prawns from each sampling location in traditional ponds in Wonorejo Village showed varied results. The results of the prevalence data examination are shown in Table 2.

Table 2. Prevalence of Tiger Shrimp (*Penaeus monodon*) Infested with Protozoa Ectoparasites in Wonorejo Traditional Ponds, Surabaya

| Pond | Number of Infested Shrimp (individuals) | Number of Inspected Shrimp (Individual) | Prevalence (%) | Prevalence Category |
|------|-----------------------------------------|-----------------------------------------|----------------|-------------------|
| A    | 6                                       | 30                                      | 20             | Often             |
| B    | 2                                       | 30                                      | 6.67           | Occasional        |
| C    | 7                                       | 30                                      | 23.33          | Often             |
| D    | 9                                       | 30                                      | 30             | Commonly          |
| E    | 1                                       | 30                                      | 3.33           | Occasional        |
| **Total** = 25 | **Total** = 150 | **Average** = 16.67 |                |                   |

Based on Table 2, it can be seen that the calculation of the prevalence of tiger prawns (*Penaeus monodon*) infested with protozoan ectoparasites has an average result of 16.67% which is included in the frequent category, this means that the incidence of protozoa ectoparasite infestation is quite frequent in tiger prawns. The highest prevalence of tiger prawns in traditional ponds in Wonorejo Surabaya is found in pond D, which is 30% (commonly category). Generally, this means that the incidence of protozoan ectoparasite infestation often occurs in tiger prawns. While the lowest prevalence is found in pond E, with the percentage of 3.33%, and included in the occasional category. This means that the incidence of protozoan ectoparasite infestation occasionally occurs in tiger prawns.

The prevalence of positively infested tiger prawns with protozoa ectoparasites was 16.67%. Protozoan ectoparasite infestation will increase if the surrounding water quality environment is suitable. Poor water quality conditions are the optimal environment for ectoparasite life. One of the water quality parameters that shows an effect on the growth of protozoan ectoparasites in tiger prawns is dissolved oxygen. Ectoparasite attacks caused by *Zoothamnium* spp., *Vorticella* spp. and *Epistylis* spp. increases when water quality decreases, especially when dissolved oxygen content is less than 3 mg/L [12]. In addition, the accumulation of organic matter in water can also affect ectoparasite infestation in tiger prawns. High organic matter is an environment favored by ectoparasites [21]. The discovery of *Zoothamnium* in this study indicated the presence of organic matter in the water. *Zoothamnium*, *Vorticella* and *Epistylis* were used as indicators of water quality in various biomonitoring studies [22].

The greatest prevalence was found in plot D pond, which was 30% and the lowest prevalence was in plot E ponds which was 3.33%. The greatest prevalence in pond D of 30% has the lowest dissolved oxygen water quality parameters, namely 4.528 mg/L and has the highest salinity, which is 20.08 ppt. Decrease in dissolved oxygen content in water can increase salinity [9]. Low oxygen is a suitable habitat for protozoan life, for example ciliates [23]. The lowest prevalence in E plot of 3.33% has the highest dissolved oxygen water quality parameter, namely 6.251 mg/L and has the lowest brightness of 28.63 cm. High brightness will reduce the supply of dissolved oxygen content in the water [24]. High brightness is closely related to the lack of abundance of phytoplankton and the photosynthetic activity carried out by phytoplankton, because phytoplankton accounts for most of the dissolved oxygen content in the waters from photosynthesis.

3.3. Protozoan ectoparasites found

Total examined tiger prawns (*Penaeus monodon*) were 150 from 5 different ponds. The protozoa ectoparasites found in tiger prawns (*Penaeus monodon*) was from the genus *Zoothamnium*. The *Zoothamnium* found in this study is included in the protozoa phylum, ciliate class, order peritricha and vorticellidae family. *Zoothamnium* spp. has a characteristic of living as colony, with a morphological characteristic of a globular zooid consisting of ciliated globular-shaped peristomial stalks, has a food vacuole, macronucleus and micronucleus. The existence of *Zoothamnium* spp. was found on the gills, body surface, swimming legs and tail, which infest 16.67% of 150 tiger prawns taken from traditional
Wonorejo ponds in Surabaya. The results of protozoan ectoparasite observations in tiger shrimp can be seen in Figure 1.

![Figure 1. Zoothamnium spp. trophozoid phase in the tail of tiger prawns scala bar 371.66 µm (magnification 400x)](image)

Note: a. Zooid; b. Pedicle or Stalk; c. Tiger Shrimp Tail

Zoothamnium spp. is included in the phylum protozoa, class ciliate, order peritricha, family vorticellidae and genus *Zoothamnium* [25]. *Zoothamnium* spp. lives as a colony, globular are zooids or rounded shaped, and have cilia consisting of peristomial stalks, contractile vacuoles, macronucleus, food vacuoles and micronucleus [26]. The myoneme of all stalks in the colony is connected to each other [27]. All stems in the colony can expand and contract simultaneously due to the presence of myoneme which is fused to one another [28].

*Zoothamnium* spp. can be found in several parts of the tiger prawns body, including on the legs of swimming, walking legs, body surface, tail and gills. *Zoothamnium* spp. has a predilection on body surfaces, swim feet, walkers, rostrum and gills [11]. *Zoothamnium* spp. found in tiger prawns has a stem that attaches to the substrate and develops by forming many branches which are included in the characteristics of the adult stage of *Zoothamnium* spp. *Zoothamnium* spp.‘s adult stage (trophozoid) will multiply their branches by producing microzooid and macrzooid [29].

### 3.4. Correlation of water quality and prevalence of tiger shrimp (*Penaeus monodon*) infested with protozoa ectoparasites in Wonorejo traditional ponds Surabaya

The correlation between water quality and the prevalence of protozoan ectoparasites in tiger prawns (*Penaeus monodon*) in traditional Wonorejo Surabaya ponds is differentiated based on each parameter measured. The correlation between water quality and the prevalence of protozoa ectoparasites in tiger prawns is presented in graphical form in Figure 2.
Figure 2. Graph of the correlation of water quality to the prevalence of protozoan ectoparasites in tiger shrimp (*Penaeus monodon*)

Note: correlation coefficient (a) R = 0.978; (b) R = -0.750; (c) R = -0.971; (d) R = 0.064; (e) R = 0.826; (f) R = 0.637

The correlation between temperature and prevalence shows that high temperature is followed by high prevalence. High temperatures can be caused by the penetration of sunlight into the waters and also other external factors. The intensity and angle of sunlight affects temperature, the smaller the radiation angle the greater the heat energy received by the waters [24]. The correlation between pH and
prevalence shows that high pH is followed by lower prevalence. pH does not directly affect protozoan ectoparasites but can affect other activities such as decomposition of organic matter in water, and high organic matter in ciliates such as *Zoothamnium* spp., *Epistylis* and *Vorticella* will develop well in water with high levels of organic matter. The decomposition maximization process of organic matter in water needs a high or alkaline pH [30]. In this study, the pH range is between 7.91 - 8.4 which is not high enough or alkaline to maximize the decomposition process, so the waters still contain high organic matter, which causes protozoan ectoparasites to infest tiger shrimp.

The correlation between dissolved oxygen and prevalence shows that high dissolved oxygen is followed by a low prevalence. Low oxygen is a suitable habitat for protozoa, *Ciliates* [23]. *Zoothamnium* spp. colony is higher in waters that have low dissolved oxygen [11]. Low dissolved oxygen is directly proportional to the need for dissolved oxygen in the waters, dissolved oxygen is not only needed for the respiratory activity of tiger prawns but also for decomposition of organic matter in waters [30]. Increasing in decomposition activity can reduce dissolved oxygen supply in the water. Increasing in decomposition of organic matter does not only cause low dissolved oxygen, but also a reduction in the abundance of phytoplankton which later causes a reduction in photosynthetic activity [31]. Water with lower photosynthetic activity may not be able to supply dissolved oxygen needs. High temperatures can also be the cause of low dissolved oxygen in water. The coefficient value between salinity and the prevalence of protozoan ectoparasites in tiger prawns is R = 0.064, which is a positive correlation but indicates that the level of relationship in the category is negligible because it is less than 0.2. The optimum salinity for protozoan ectoparasite attack is 30-35 ppt [32]. The distribution of salinity data shows slight differences between pond plots, this difference is likely because some measurements are influenced by high tides on water quality measurements on the fourth day and high rainfall in the Wonorejo area, Surabaya.

The correlation between ammonia content and prevalence shows that high ammonia content is followed by high prevalence. The ammonia content in the waters shows a number greater than 0.1 mg/L, which causes the growth of tiger prawns to be not optimal. The normal range of ammonia content for tiger prawn cultivation for optimal growth is > 0.1 mg/L [33]. The research also showed that the ammonia content above 0.1 mg/L causes the prevalence of *ciliate* protozoan ectoparasite infestation of 62%. The result of this study showed that the ammonia content ranged from 0.5 to 0.62 mg / L and resulted in protozoan ectoparasite infestation in tiger prawns. The correlation between brightness and prevalence shows that high brightness is followed by high prevalence as well. The low brightness of the research results indicates the presence of TSS (Total Suspended Solid) or high plankton abundance [33]. Abundance of phytoplankton supplies most of the dissolved oxygen in the waters through its photosynthetic activity [34]. This shows a relationship between brightness and dissolved oxygen but not directly related to protozoan ectoparasites. Brightness is also related to temperature through the intensity of sunlight in the waters so that the higher the brightness the higher the temperature in the waters.

The relationship between water quality parameters indicates that there is a relationship between environmental factors and the occurrence of disease in tiger prawns. The emergence of shrimp disease in ponds is due to the interaction between the disease agent, host and the environment [6] In this case the disease agent is *Zoothamnium* spp., the host is tiger shrimp and the environment is a parameter of water quality. *Zoothamnium* spp. has the ability to survive in a certain water quality to survive. One of the parameters has a correlation coefficient value close to -1/1, this indicates a very strong significant relationship between dissolved oxygen, temperature and ammonia content with prevalence of protozoan ectoparasites in tiger shrimp. The effect of distance on water sources in the Wonorejo river which is influenced by the tides of sea water is also one of the factors supporting the high prevalence in plot D. This distance allows the presence of particles of organic matter, heavy metals and material suspension carried by the Wonorejo river into plot D to be more than in plot E. High organic matter accumulation in waters is in accordance with the environment that *Zoothamnium penaei* needs [35].

4. Conclusion
Based on the research entitled the relationship between water quality and the prevalence of tiger prawns (*Penaeus monodon*) infested with protozoan ectoparasites in the traditional Wonorejo ponds in Surabaya, it can be concluded that the prevalence is 16.67%. It is in the category of “Often”, which means ectoparasites are often found infesting tiger prawns in the traditional ponds of Wonorejo.
Surabaya. The correlation is shown in the parameters of temperature, ammonia content and brightness. It means that the higher the temperature, ammonia content and brightness, the higher the prevalence of protozoan ectoparasites in tiger prawns. The opposite correlation is shown in the dissolved oxygen and pH parameters, which means that the lower the dissolved oxygen and pH the higher the prevalence of protozoan ectoparasites in tiger prawns. The salinity parameter does not show any correlation because the correlation coefficient is $R = 0.064$, the correlation coefficient ($R$) is less than 0.2.

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