Prediction Model For Luminecent Dinoflagellate And *Pseudomonas* Sp. In White Leg Shrimp (*Litopenaeus Vannamei*) Pond

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Abstract

Mohamad Fadjar, Sri Andayani, Arning W. Ekawati, Yunita Maemunah, Rani Yuanita. Prediction Model For Luminecent Dinoflagellate And *Pseudomonas* Sp. In White Leg Shrimp (*Litopenaeus Vannamei*) Pond. Aquacltura Indonesiana, 22 (1) : 24-32. The occurrence of luminescent shrimp pond water can be prevented from the conditions of existing environmental factors, including water quality and microorganisms. One of the preventive efforts that can be done is to use a prediction model to prevent the occurrence of cases of luminescent shrimp pond water, by combining the parameters that play a role in the shrimp pond water with STELLA (System Thinking, Experimental Learning laboratory with Animation) application system. The occurrence of cases of luminescent ponds which are still often found in East Java, Indonesia; inspire to create predictive models for the emergence of luminescent shrimp pond water cases. The target of this research was a prediction model to determine when the phenomenon of shrimp pond water is luminescence which can be used by white leg shrimp (*L. vannamei*) farmers by using the STELLA 9.1.4 application. This research was an experimental descriptive study based on bioluminescence in white leg shrimp farm. Measurement of the daily water quality sampling was dissolved oxygen, pH, salinity and temperature. Meanwhile, weekly measurements were made for nitrate, nitrite, alkalinity, Total Organic Matter (TOM), and plankton. Meanwhile, the bacterial test was only carried out when the pond luminescent. The result of this research was a prediction model for prevention of luminescent shrimp pond water. The bacteria found were *Pseudomonas* sp. and phytoplankton was dominated by diatom, chlorella, and. *Peridinium* sp., a dinoflagellate, was a suspect causing of luminescent shrimp water pond.

Key words : luminescence, *Peridinium* sp., *Litopenaeus vannamei*, dinoflagellate

Introduction

One of the problems that arise is the phenomenon of glowing pond water which is still found in some vaname shrimp (*Litopenaeus vannamei*) aquaculture ponds. Bioluminecent in shrimp ponds is particularly predominant in the marine environment, in members of planktonic bacteria and protozoa, many invertebrates, and vertebrates with specialized light producing organs that harbour symbiotic bioluminescent bacteria (Haddock et al., 2010).

A chemical-physical transduction was found in bioluminescence process which comes from chemical energy into light energy. Highly exothermic process involving enzymatically catalysed chemical reactions transformation in which the energy of chemical bonds of organic compounds is converted preferably into visible light. In these molecular reactions, oxygen oxidized luciferins which catalysed by luciferase, producing electronically excited molecules that decay by emitting light.

Bioluminescence in shrimp ponds can be caused by plankton or bacteria. The luminescent bacteria caused reduction to shrimp production which is connected to bacterial disease (Ruangpan 1987, Songserm *et al*. 1990, Ruangpan *et al*. 1997), and it has also been reported to cause economic losses to the shrimp industry in the Philippines, Vietnam, India, and Indonesia (Nguyen & Le Trong 1994, Fernandez & Mayo 1994; Raju 1994, Sulasmi *et al*. 1994, Taslihan & Wijayati 1994) .

Fluorescent bacteria that are often found in in shrimp ponds included *Vibrio harveyi* and *Pseudomonas flurescent* (Golas *et al*., 2019). They are ubiquitous in aquatic environments, and they are capable of decomposing various organic compounds due to a broad range of enzymatic activities (Sugita et al., 2005; Kawahara et al., 2009). Development of luminous disease in hatchery was dominated by *V. harveyi* during the early shrimp larval stage (Azizunnisa and Sreerumulu, 2013). The percentage incidence and

intensity of luminous bacteria which was found in shrimp pond was 84% (9.1 X 10- 1.2 x 10^2 cfu/ml) and 92% from ponds effluent while in hatchery was 92 % (1,5 X 10 - 1.0 x 10^2 cfu/ml) while in hatcheries effluent were 67% (1,5 X 10 - 1.0 x 10^2 cfu/ml) (Sodthongkong, 1996).

Some dinoflagellates in aquaculture form Harmful Algal Blooms (HABs) own the remarkable genetic, biochemical, and cellular machinery to produce bioluminescence and toxins (Hacckett et al., 2004; Valiadi and Iglesias-Rodriguez, 2013; Cusick and Widder, 2020).

Some alternative methods were used to avoid and control luminous disease such as using probiotics or biological control, i.e. *Bacillus* sp., *Lactobacillus casei*, *L. acidophilus*, and *P. aeruginosa* (Phianpark *et al.*, 1997; Jiravanichpaisal *et al*. 1997; Janakiram *et al*., 2014) as probiotics feed, have also been used to hamper *V, harveyi* growth. Beneficial microorganisms found to be effective for growth inhibition of *V. harveyi* on the laboratory scale include *V. alginolyticus* (Ruangpan et al. 1998) *Chlorella* sp. (Direkbusarakom et al. 1997) and *Skeletonema costatum* (Panichsuke et al. 1997) or using freshwater .

Although this case has been found for a long time, the incident of luminescent shrimp pond water has been found to date. Therefore, prevention efforts can be carried out by monitoring environmental conditions, including water quality, plankton and microorganisms in the pond by applying prediction models so that preventive actions can be taken immediately in order to avoid dangerous effect..

Materials and Methods

The research was an experimental description research. Research was done in a shrimp farming in Situbondo, East Java, Indonesia. Sample was taken from 3 shrimp ponds which one of it got a luminescent water phenomena. Data were used to build a prediction model of luminescent water ponds.

2.1 Phytoplankton

The phytoplankton amount was calculated by the formula according to APHA (2005) as follows:

$$N = n + \frac{a}{A} X \frac{v}{vc} x \frac{1}{V}$$

Note :
N: abundance of phytoplankton (cell / L)
n: Number of phytoplankton found (cells)
a: Broad sedickick rafter (mm^2)
v: Volume of filtered water (mL)
A: Observed rafter sediment area (mm^2)
VC: Volume of water on Sedgwick Rafter (mL)
V: Volume of filtered water (L)

The types of phytoplankton was identified based on Davis (1995)), using a microscope with 100 X magnification.

2.2 Bacteria

Sample of glowing water pond was analysed in BKIPM Lab, Surabaya; based on methods by Mac Faddin (2004)

2.3 Water Quality

Water sample was taken for five days when the luminescence appear at the 5^{th} day. Measurement for daily water quality sample i.e. dissolved oxygen (DO), temperature, pH, salinity were taken using DO meter, pH meter and refractometer. While ammonia, nitrite, nitrate, and orthophosphate was checked every week using test kits (Prodac test®).

2.4 Model

In this research, there were activities in the form of dynamic system modelling. The water quality data that has been obtained, such as dissolved oxygen, temperature, pH, salinity, ammonia, nitrite, nitrate, and orthophosphate will be entered into the model. Dynamic system modelling was done using STELLA 9.1.4 software. The use of symbols for applications and dynamic model analysis in Stella software is listed in Table 1.

Construct a system that simulates dynamically depending on time and with a number of different variables and functions. Each variable is associated with a real or self-created unit. Existing variables have numeric values

that are part of these variables. In the system, each variable is described in several symbols (Muhammadi. et al, 2001).

Table 1. Research components and object-oriented modeling categories

No.	Component	Object Oriented Model Category
1	Bacteria, Plankton	*Stock*
2	Temperature pH, Salinity, DO, Ammonia, Nitrite, Nitrate, Ortophospat, Feed, SGR, Culture time, Density , Glowing water pond, Metabolism	*Converter*
3	Organic compound, Mortality, Biomass total	*Flow*
4	Functional relationships and correlations	*Connector*

Results and Discussions

3.1 Phytoplankton

Phytoplankton identification from 3 shrimp ponds can be seen in Table 2.

Table 1. Problem structure in daily plankton report sub system

Variabel	Species	Amount (cell/mL)	Plankton group	Total (cell.mL)	Percentage (%)
1st Pond Water Quality	*Chlorella*	60.000	green algae	60.000	44
	Oscilatoria	2.500	blue green algae	2.500	1,85
	Navicula	2.500	diatomae	67.500	50
	Nitzchia	2.500			
	Rizhosolenia	62.500			
	Peridinium	5.000	dinoflagellata	5.000	3,7
			protozoa	0	0
			zooplankton	0	0
2nd Pond Water Quality	*Chlorella*	30.000	green algae	30.000	46
			blue green algae	0	0
	Navicula	7.500	diatom	30.000	46,15
	Bidhulpia	2.500			
	Rizhosolenia	2.500			
	Coscinodiscus	7.500			
	Cyclotella	10.000			
	Gymnodinium	5.000	dinoflagellata	5.000	7,69
			protozoa	0	0
		5.000	zooplankton	0	0
3rd Pond Water Quality	*Chlorella*	20.000	green algae	20.000	35
			blue green algae	0	0
	Chaetoceros	2.500	diatomae	32.500	56,52
	Coscinodiscus	7.500			
	Cyclotella	22.500			

Gymnodinium	2.500	dinoflagellata	5.000	8,7
Peridinium	2.500			
		protozoa	0	0
		zooplankton	0	0

The plankton density in the ponds was dominated by diatom in the 1st , 2nd , and 3rd ponds with a percentage of 50%, 46.15%, and 56.52% of the total plankton or at a density of 6.75 x 10^4, respectively. ; 3 x 10^4, and 3.25 x 10^4 cells / mL. The 1st pond was dominated by *Rhizosolenia* sp. with a density of 6.25 x 10^4 cells / mL, while in 2nd pond was dominated by *Chlorella* sp. with density of 3 x 10^4 cells / mL. Whereas in 3rd pond was dominated by *Cyclotella* sp. with a density of 2.25 x 10^4 cells / mL

The presence of dinoflagellates is suspected to be one of the causes of the ignition of pond water become bright blue bioluminescent (Harvey, 1957). Fluorescence is used to discriminate between autotrophic and heterotrophic dinoflagellates, and to identify other autotrophic plankton (Messié et al., 2019). Several bioluminescent species are cosmopolitan in both coastal and open ocean regions and include important heterotrophs (e.g., *Noctiluca* and *Protoperidinium*) and toxic (e.g., *Alexandrium*), or generally harmful species (e.g., *Noctiluca*, *Lingulodinium*, and *Ceratium*) (Valiadi and -Rodriguez,

Bioluminescence and toxin production, are connected to the ecological significance of either. Although dinoflagellate species that form some of the most widespread and frequent HAB are bioluminescent, the molecular and eco-evolutionary associations between these two traits has received little attention. Of the 17 major classes of dinoflagellate toxins, only two are produced by bioluminescent species: saxitoxin (STX) and yessotoxin (Cusick and Wieder, 2020).

The substrate in the bioluminescence reaction of dinoflagellates is luciferin, which is an open-chain tetrapyrrole similar in structure to chlorophyll. While the synthesis pathway for dinoflagellate luciferin is not known, the structural similarities between dinoflagellate luciferin and chlorophyll indicate luciferin formation via chlorophyll catabolism: *P. lunula* was shown to incorporate radioactively labelled chlorophyll precursors into chlorophyll and luciferin, suggesting biosynthesis of the two are linked (Topalov and Kishi, 2001; Wu et al., 2003; Cusick and Wieder, 2020).

The evidence of bioluminescence has a relation with nutritional condition ,such as food concentration and growth rate. The rhythm of bioluminescence was under endogenous control (Buskey et al., 1994).

3.2 Microorganism

Sample of bacteria was taken from 1st shrimp pond luminescent water , there were 4 different colonies which grown in the agar culture and identified all as *Pseudomonas* sp.

Appearance of a yellow-green, fluorescent, water-soluble pigment combination by . *fluorescens* occurred only when the bacteria were iron-deficient and was not directly influenced by the nature of the organic carbon source (Meyer and Abdallah, 1978). In this study, a bright blue bioluminescence was appeared in the shrimp po is a sign owned by dinoflagellates, so that the alleged cause of bioluminescence in shrimp pond can be ruled out.

3.3 Water Quality

Water quality was measured during the evidence of bioluminescent shrimp pond water as mentioned in Table 3.

Table 3. Water quality measurement

Parameter	**Value**			**Reference**
	1st pond	**2nd pond**	**3rd pond**	
Nitrate (ppm)	0.00 - 200	0.00-168	0.00-160	< 0.01 (SNI, 2006)
Nitrite (ppm)	0.00 -	0.00	0.05	0.11-0.54 (SNI, 2006)
Ammonia (ppm)	0.00	0.077	0.068	< 0.1 (SNI, 2006)
TOM (ppm)	71-81	63-81	61-68	< 55 ppm (Parlina, *et al*., 2018)
Phosphate (ppm)	3	3	3	1 ppm (SNI, 2006)
TAN (ppm)	0	1.6	1.6	< 0.1 ppm (Boyd,

				1998)
pH	7.8-8.3	7.8-8.3	7.8-8.2	7.5-8.5 (SNI, 2006)
DO (ppm)				➢ 3.5 (SNI, 2006)
Temperature (^{o}C)	30-32	30-32	30-32	28.5-31.5 (SNI, 2006)
Salinity (ppt)	24	26-27	23-26	15-25 (SNI, 2006)
Turbidity (cm)	25-35	30-55	30	≤ 40 cm (Malik, 2014)
Water colour	Dark brown	Dark brown	Light brown	

Primavera (1998) reported that artificial feed contributed 92% of the total nitrogen input and 51% phosphorus. The organic matter that settles at the bottom of the pond is a source of NO3, NO2, TAN and PO

3.4 Modelling

3.4.1 Causal Loop Diagram

Causal loop diagram is another meaning of cause and effect diagram. This diagram illustrates the good and bad relationship between the components and the object of research. This linkage will affect the system created (Ismail, 2019). In this study, there are three causal diagrams. The first diagram, which is the water quality sub-system, is aimed at an explanation of the relationship between the concentration of various water quality parameters on the pond conditions. Then the results of the first diagram are used as input to operate the second diagram, namely the daily plankton report sub system aimed at explaining the relationship between various types of plankton species to the number of each type of plankton. After that, the results of the second diagram are used as input to operate the third diagram, namely the micro-organism sub-system. The final result that will be aimed at the system is the stock or amount of plankton and the number of *Pseudomonas* sp. bacteria based on different water quality for each pond.

The interaction pattern in the causal loop is represented by the positive (+) and negative (-) signs. The interaction between components which states a positive sign (+) is intended as an increase or increase in effect. Meanwhile, the negative sign (-) means that the interaction is reducing or decreasing.

The causal diagram in the water quality sub-system starts from the relationship of each water quality parameter variable to water conditions. In ponds with water quality that can invite various species of plankton into the water, it is indicated by the results in the flow of 1. If the water quality does not support inviting various plankton species, then the flow results are 0. In modelling, 1st, 2nd, and 3rd ponds produced a value of 1 in the flow of water conditions. This shows that the modelling process can be continued towards the causal loop diagram in the next sub-system.

The causal loop diagram in the daily plankton report sub system starts from the relationship between water conditions and various types of plankton species. Water conditions with different concentrations of each parameter for each pond have different impacts on the diversity and density of plankton. It is connected using connectors with positive polarity, which means that it will increase the number of each plankton species. Each of these species is linked to the flow of plankton species (dinoflagellates, diatoms, green and blue green algae, protozoa, and zooplankton). The connectors to the flow are given positive polarity, which means that this type of plankton is an accumulation of several plankton species.

The causal loop diagram in the microorganism sub-system starts from the link between the types of plankton and the plankton stock, the water conditions with the *Pseudomonas* sp. stock. Plankton stock is an accumulation of various types of plankton in the daily plankton report sub system. The plankton stock was given reciprocal connectors or negative polarity to the previous sub-system, this was done to determine the percentage of each type of plankton. So that it can make it easier to find out what types of plankton dominate the pond. Stock plankton is a dividing factor for each type of plankton. In *Pseudomonas* sp. stock, it was related to the colour of water variable. This was done to determine the number of *Pseudomonas* sp. based on the colour of pond water. The cause and effect diagram can be seen in Figure 1 below.

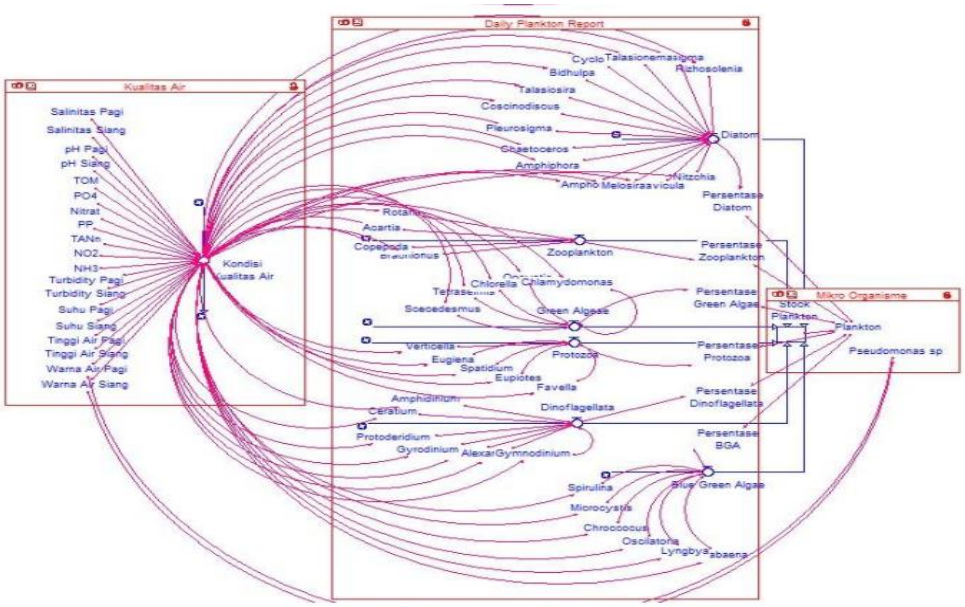

Figure 1. Causal Loop Diagram

3.4.2 Stock and Flow Modelling

Stock and Flow is a compiled model in this study. The model created described the conditions in accordance with the observed problem. In other words, modelling is the representation of a real-world system into a simulation. In this case, it is the condition of the water against plankton density and *Pseudomonas* sp.. The modelling explains that the number of microorganisms was influenced by various water quality parameters with different concentrations of each pond.

The modelling equation to achieve these results can be seen in Figure 2.

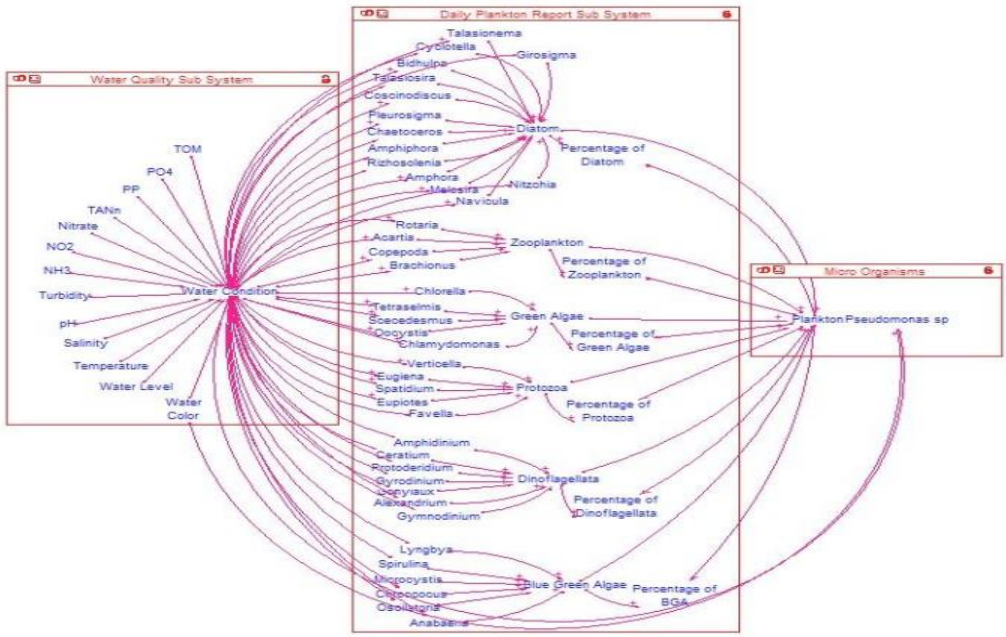

Figure 2. Modeling of dynamic system of stock and flows of water quality condition to quantity phytoplankton and bacteria.

In the stock and flow modelling shows that the number of micro-organisms can change either increase or decrease due to the influence of pond waters conditions. In the 1st pond, there were plankton with a density of 1.35 x 10^5 cells /mL and *Pseudomonas* sp. bacteria with a density of 10^7 CFU/mL on the fourth and fifth days. In the 2nd pond, there was plankton with a density of 6.5 x 10^4 cells /mL and *Pseudomonas* sp. with a density of 10^7 CFU/mL on the 3rd to 5th day when the pond water appeared to be lit. In the 3rd pond, there was plankton with a density of 5.75 x 10^4 cells / mL and *Pseudomonas* sp. bacteria with a density of 10^7 CFU/mL on the first to the fifth day when the pond water appeared to be on.

Stock and modelling can be used to arrange the water quality in order to avoid appearance of bioluminescence water. in this case, the bioluminance appears to be due to high dinoflagellates, *Peridinium* sp., which is associated with water quality conditions. In the three ponds, Nitrate and TOM levels were seen to be higher than the normal conditions desired in shrimp culture, especially in the first pond where bioluminance occurred (Nitrate = 200 ppm, TOM=71-81). .

Conclusions

Suspected bioluminescence in this shrimp pond caused by dinoflagellate (*Peridinium* sp.) in density of 5,000 cells/L but the prediction model can be used as prevention strategy to avoid bioluminescence by dinoflagellate and *Pseudomonas* sp

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