Toxicity of Alkyl Sulfate Surfactant Detergent (AS) to Larvae of Vannamei Shrimp (Litopenaeus vannamei) From Marine Waters, District of Lingga Timur, Lingga Regency

Eddiwan*, Sukendi, Y. I. Siregar, Z. Saam
Environmental Sciences Study Program. Postgraduate of Universitas Riau.
*Corresponding author : eddiwan@lecturer.unri.ac.id

Abstract. The coastal area of East Lingga Subdistrict, Lingga Regency has been designated by the Lingga Regency government as the center for the development of shrimp pond cultivation. This study aims to determine the level of toxicity of the surfactant alkyl sulfate (AS) detergent to post larvae (PL) of Vannamei shrimp (Litopenaeus vannamei). An acute test was performed on PL10 samples exposed to AS media for 96 hours. Then the Median Lethal Concentration (LC50) value was calculated for 4 times (24 hours, 48 hours, 72 hours, and 96 hours). Furthermore, the PL-15 sample was AS in the sub chronic test, then the sample was exposed to AS media for 24 hours, then the growth rate and changes in the gill organ structure of the PL samples were observed. From the test results, it is known that the LC50 values in the AS test for the 24th, 48th, 72th, and 96th times of the shrimp larvae samples obtained LS50 values were 33.6 mg/l, 29.4 mg/l, respectively. 24.3 mg/l, and 22.8 mg/l. Subsequently, an increase in AS concentration by 17.11 mg/l in the rearing medium resulted in a decrease in the growth rate of post larvae. The acute and sub chronic testing period showed changes in the behavior of post-larvae samples. The histopathological observations found damage to the structure of the sample gill organs and hepatopancreas. Symptoms of damage to the structure of the gills began to appear at AS concentrations of 25.58 mg/l at time 96 and 72 with an LC50 value of 34.99 mg/l. Furthermore, it was also seen at an AS concentration of 9.78 mg/l at time 24. The results of this study found that AS toxicity to Vannamei larvae increased with increasing concentration and exposure time.

1. Introduction
The coastal area of East Lingga District has been designated by the Lingga Regency government as the center for the development of shrimp pond cultivation. This area is adjacent to a fairly dense residential area. The impact of uncontrolled waste and AS hold waste, especially those originating from washing residue, triggers the increase in the concentration of Alkyl Sulfate Surfactant Detergent (AS) in waters, especially those near the coast and shrimp ponds.

AS is a type of anionic detergent surfactant which is an active ingredient for products such as shampoo, toothpaste, and cosmetics [1]. Detergent production in Indonesia averages 380 tons AS per year, while the average per capita consumption rate in Lingga Regency in 2019 was 8,232 kg[2]. Disposal of waste into open waters which will lead to the sea as a lot of AS surfactants to accumulate in marine waters[3]. Surface detergent waste, including the AS, has the potential to become one of the pollutants that can reduce water quality in general and especially culture media in shrimp farming in the sea and coastal areas[4]. In fact, so far, the water source for shrimp pond cultivation has been taken from the coastal waters in East Lingga District which have been polluted by the AS.

Vaname Shrimp is an aquaculture commodity with high economic value, both for local and international markets [5]. This animal is a type of aquatic animal that is very sensitive to changes in water quality [6], especially for larvae (post larvae) [7]. Poor water quality will affect the survival, growth [8], and development of shrimp [9]. For larvae, poor water quality can make death or inhibit larval growth [5, 10]. Many cases of shrimp culture show that a decrease in water quality causes a decrease in pond shrimp production [11], and can damage aquatic resources [12] and water fertility [10, 13]. One of the causes of the decline in water quality is the increasing concentration of AS in the waters[14]. The purpose of this study was to determine the acute toxicity value of AS to Vannamei larvae (post larvae) as seen from the LC50
value and changes in the structure of the gills, as well as to observe the conditions of death, growth, behavior, and damage to the structure of the gill organs at the acute and sub chronic levels.

2. Materials and Methods
This research was conducted from January to March 2020. The activity of Vannamei shrimp larvae sampling was carried out in coastal waters of East Lingga District, Lingga Regency. Furthermore, to test the effect of AS concentration on samples of Vannamei shrimp larvae and data analysis was carried out at the Laboratory of Fisheries Biology, Faculty of Fisheries and Marine Affairs, Riau University Pekanbaru. This study used 3 stages of testing, namely (1) Range Search Test; (2) acute test; and (3) sub-chronic testing.

2.1. Range Search Test
A range value test or Range Search Test is performed to determine the upper threshold level (the concentration that causes death in the test animal within a certain time period) and the lower threshold (the AS concentration that causes the relative survival of the test animal to be more than 95% over a certain time period). The test animals used in this study were Vannamei shrimp post larvae (PL).

A total of 20 Vannamei larvae (PL size 10) were put in an aquarium containing 4 liters of seawater with a salinity of 25 ppt. It’s this experiment, the AS concentrations tested were 0, 10, 19, 26, and 35 mg/l, with two replications of each. Replacement of seawater media is carried out 100% every 12 hours (AS stability test results). To see the mortality rate for larvae, time limits of 0, 2, 4, 6, 12, and 24 hours are used, and so on is carried out every 24 hours to 96 hours. From the mortality value obtained through the range-finding test, it will be known that the upper and lower thresholds in determining the concentration range in the acute toxicity test of these larvae.

2.2. Acute Tests
Acute testing aims to determine the ability of AS to kill larvae. The toxicity of the test material is stated in the Median Lethal Concentration (LC50), which is the toxicity content of the test material that kills 50% of the test sample during a certain period of time [15]. Acute AS-level testing was performed following of the procedure [16]. Four levels of test material at the upper and lower threshold intervals were obtained from equations (1) and (2), and one treatment as a control (test substance content 0 mg/l) [17]. Each treatment was repeated twice[18]. The formula used is Log N/n = k (log a/n), and a/n = b/a = c/b = d/c. Where “N” is upper threshold concentration, “n” is lower threshold concentration, “k” is the number of concentration intervals tested between the upper threshold concentration, and the lower threshold concentration, and “a” is the smallest concentration within the specified concentration range.

The equation above, the treatment for AS concentrations is 0 mg/l, 13.68 mg/l, 18.71 mg/l, 25.58 mg/l, and 34.99 mg/l. In each treated container, 20 samples of shrimp larvae (PL) were placed in this study of PL10 size larvae (10-day old post larvae). Furthermore, observations were made at 0 hours, 2 hours, 4 hours, 6 hours, 8 hours, 10 hours, 12 hours, 24 hours, 36 hours, 48 hours, 60 hours, 72 hours, 84 hours, and 96 hours. In this test, the observed behavior and mortality of Vannamei larvae samples.

In the preparation stage, the test sample (Vannamei larvae) was prepared first by referring to the research procedures [19]. Where to see changes in the structure of the gill organs (histopathology) of the sample, observations were made on the sample of larvae that kept the upper waters from the start of maintenance to the end of maintenance [20]. Replacement of water media for larval sample rearing was carried out as much as 100% with time intervals every 12 hours. Water samples were measured before and after the replacement of the maintenance water media, namely at 0 hours, 12 hours, 84 hours, and 96 hours. The samples have then analyzed [21] the MBAS method, which follows the procedures contained in the Standard Method for Examination of Water and Wastewater [22]. During acute testing, the larvae were not fed.

2.3. Chronic Sub-Test
The sub chronic test is performed after the acute test is complete. A chronic test was used to determine the effect of AS detergent surfactant on samples of Vannamei larvae over a long period of time. Sub-chronic testing was carried out in conjunction with the procedure [23]. In this test, the concentrations used were 10%, 40%, and 70% of the LC50-96 hours’ values obtained from acute testing with two repetitions. This test was carried out for 24 days on a sample of larvae with an initial size of PL15. When sub-chronic testing is performed, changes in behavior, growth, and mortality of larvae samples are observed. In the preparation stage, first, the test larvae sample is prepared. Then, observations were made at the beginning and end of the rearing larvae samples. Replacement of seawater media was carried out as much as 100% with an interval of
12 hours. Seawater media samples were measured at the time before and after water replacement on days 1, 10, and 24. In this sub-chronic test, larvae samples were treated in the form of pellet feed 10% of the weight of larvae biomass sample, with a frequency of feeding 3 times per day.

2.4. Data analysis
The method used to analyze the AS toxicity test results data to determine the LC50 was carried out using the Probit Analysis Method [24]. The principle is the cumulative mortality from the sample data of Vannamei larvae. For each treatment, the data was compiled into a table and then converted again into a logarithmic form[ 25]. Then the research data is corrected, then transformed into probits, and weighting is carried out to determine the relationship between the mortality of the test larvae sample (in probity), and the level (in the logarithm of x), which is assumed by the regression equation \( y = (y - bx) + bx \). The x value is calculated by entering the \( y = 5 \) value in the regression line equation above[ 26]. The antilogies of the x value is LC50 [27, 28].

2.5. Research design
This research is an experimental study, with a completely randomized design model. The treatments tested were AS concentrations with levels of 0 mg/l, 10 mg/l, 19 mg/l, 26 mg/l, and 35 mg/l, and treatment time with levels of 0 hours, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, and 96 hours. Observation data were collected in the form of growth rates and mortality. Furthermore, the data obtained were analyzed statistically and presented in graphical form. Meanwhile, to see the effect of treatment on mortality and growth rate of larvae samples used a completely randomized design with 2 replications, then analyzed using the ANOVA test. If an effect is found on the sample of test larvae, it will be continued with the BNJ test. For the mortality parameter calculated using the method based on [29, 30], while to see the growth rate of the test larvae samples used the [31, 33].

3. Results and Discussion
3.1. Range Finding Test (RFT)
From the experiment looking for the RFT value, it was found that at a concentration of 10 mg/l, 5% of the larvae died. Meanwhile, at a concentration of 35 mg/l, the larvae died of 97.5% (Figure 1). From these data, AS concentration values were taken with a threshold value below 10 mg/l acute test. From the threshold value, AS concentration values obtained for the acute test were 13.68 mg/l, 18.71 mg/l, 25.58 mg/l, and 34.99 mg/l. Other researchers have also conducted Range Finding Tests such as those used by [34, 40].

3.2. Acute Tests
The response of the test larvae sample to the given treatment showed a fairly high mortality rate, due to the toxic strength of the AS detergent surfactant (Figure 2). Based on statistical tests, it was found that all treatments gave significantly different results to the control (p <0.05). So it can be said that giving AS treatment to the larval sample rearing media starting from a concentration of 13.68 mg/l has had a significant effect on the mortality of the Vannamei shrimp larvae sample. Shrimp larvae sample mortality occurred because the shrimp body had absorbed water from the rearing media that had been dissolved with AS surfactants [41], causing cell breakdown [42], and interactions with proteins[ 43] and semi-permeable membranes [44, 49] In addition, the occurrence of mortality of larvae samples can also be caused by breathing difficulties or asphyxia of shrimp larvae samples as a result of their damage [50]. gill epithelial tissue which results in disruption of the function of the gills of the larvae sample [51, 53].
Figure 1. Relationship between mortality of PL Vannamei Prawn Mortality and AS Concentration during Range Finding Test (96 hours).

Figure 2. Graph of Relationship between Mortality of Old Vannamei Prawn PL and AS Concentration during Acute Test (96 hours).

Based on the results of the calculation of the AS LC50 value of the Vannamei larvae sample test at the exposure time of 24 hours, 48 hours, 72 hours, and 96 hours (Figure 3), it shows that the LC50 value decreases with the length of time that increases with the respective LC50 value at the concentration. The AS as much as 33.6 mg/l, 29.4 mg/l, 24.3 mg/l, and 22.8 mg/l. The results of this study are in line with those found by [52], which showed that the effect of toxicity on samples of Vannamei larvae continued to increase with increasing exposure time. The results of this study are also in line with [54, 56] that the effect of pollutants on organisms depends on the concentration and duration of exposure to pollutants [57].

The 96-hour LC50 value of AS detergent surfactant for Vannamei larvae samples was equivalent to the LC50 value for small fish and for adult shrimp Fathead of 22.5 mg/l [58], much higher than the juvenile stage of Fathead minnows. (17 mg/l), Fathead small fish larvae stage (10.2 mg/l) and rainbow trout larvae stage (4.5 mg/l). So young Vannamei appear to be more tolerant of AS detergent surfactants and based on the classification proposed by [59, 60].

Based on observations of the behavior of the test shrimp larvae samples in the aquarium that were given different treatments and in the control aquarium, it shows that in the aquarium treatment the shrimp larvae samples were seen swimming near the water surface with an oblique motion (abnormal), and the movements were irregular. Where a small part of the sample of shrimp larvae test appeared unable to move around the water surface. Samples of shrimp larvae swimming near the surface of the water media have shown that they have experienced difficulty obtaining oxygen. Mang quote from [61, 62] stated that the detergent surfactants at high concentrations can cause an increase in the frequency of breathing two to three times the normal condition. Then there will be damage to the respiratory system of the shrimp, especially the epithelial organs of the gills [63]. The tilted and irregular swimming motion of the shrimp larvae indicates that the shrimp larvae have lost their balance. The change in behavior of shrimp larvae that he shows indicates that the
concentration of AS can be classified as a pollutant that can act on one or all of the receptors, and is able to affect the central nervous system of shrimp larvae [64].

Figure 3. AS LC50 values at 24, 48, 72, and 96 hours of exposure time.

Observations on the morphology of several samples of Vannamei shrimp larvae found gill damage and hepatopancreas when treated with AS concentrations of 25.58 mg/l and 34.99 mg/l, when compared to controls (Figure 6.1a and Figure 6.2a). In the treatment with an AS concentration of 34.99 mg/l at 72 hours of observation, condensation, fusion, necrosis, and hypertrophy was found in the gill filaments of the shrimp larvae sample, while at a lower Ad concentration of 25.58 mg/l The observed time was 96 hours, indicating that fusion, condensation, and hypertrophy had occurred (Figure 6.1d).

From the observation of the hepatopancreas, the sample of test shrimp larvae showed that there had been necrosis, fat degeneration, and hypertrophy. In the treatment with an AS concentration of 34.99 mg/l at 72 hours, from the observation that the hepatopancreatic cells had experienced necrosis, hypertrophy (Figure 6.2b), and fat degeneration (Figure 6.2c). Meanwhile, the AS concentration of 25.58 mg/l at the 96th observation time had hypertrophy and necrosis. Fusion and condensation of gill organ structures were also found in samples of Vannamei shrimp larvae when exposed to Alkyl benzene Sulphonate linear detergent surfactant (LAS) at an acute level [65, 66]. Damage to the structure of the gill epithelial organs of the shrimp larvae sample resulted in damage to gill organ function and is thought to be the main cause of death in vannamei shrimp larvae, and this is in line with the results [67, 70].

3.3. Chronic Sub-Test
The results of sub-chronic tests on samples of shrimp larvae have shown that the percentage of mortality of samples of test larvae has increased with increasing AS concentrations (Figure 4). The mortality of vannamei larvae in the control aquarium was thought to be because the larvae were cannibals due to hunger, thus allowing the larvae to attack each other while other shrimp were shedding their skin. Meanwhile, the mortality of larvae samples in the rearing aquarium was due to their cannibalistic nature, but also because the shrimp larvae had absorbed water media containing AS surfactants. Water containing surfactant AS can cause damage to cells and interact with semi-permeable proteins and membranes [71, 72]. According to [73, 74], the occurrence of mortality in shrimp larvae can be caused by more than one causative factor, but all of them are caused by stimulation of damage to the gill organs. At low toxic concentrations, the death of larvae can be caused by the action of the substance on functions other than those of gas exchange or osmoregulation[75, 76].
Based on the results of statistical tests showed that only the AS concentration of 17.11 mg/l was able to give significantly different results from the control (p <0.05). So it can be concluded that the treatment of giving AS concentrations to the maintenance media with low doses of 2.44 mg/l and 9.78 mg/l did not give significantly different results, but had a significant effect on the mortality rate of shrimp larvae. The growth rate of the sample of test shrimp larvae appears to decrease with increasing concentration of AS (Figure 5). From the statistical test on the growth rate of the sample of test shrimp larvae, it was shown that only the AS concentration of 17.11 mg/l gave significantly different results to the control (p <0.05). So it can be concluded that the treatment of giving AS to the maintenance water media will significantly reduce the growth rate from shrimp larvae, starting from a AS concentration of 17.11 mg/l.

**Figure 4.** Relationship between PL Vannamei Shrimp Mortality and AS Concentration during the Chronic Sub-Test (24 days).

**Figure 5.** Growth Rate of vannamei Post Larvae during Chronic Sub-Test (24 days)
Figure 6. Gills and Hepatopancreas Preparation of Vannamei Larvae Prawn during Acute and Chronic Sub-Tests.

Information:
1. Gills (1a = normal gills on control, showing gill filaments (200x magnification); 1b = hyperplasia of gill filaments (AS 9.78 mg/l, day -24, 100x magnification); 1c = atrophy of gill filaments (AS 9.78 mg/l, day 24, magnification 200x); 1d = condensation (1), hypertrophy (2), and fusion (3) on gill filaments (AS 25.58 mg/l 96th hour, magnification 100x)); 2. Hepatopancreas (2a = normal hepatopancreas in control, showing hepatopancreas cells (200x magnification); 2b = hyper trophy (1) and necrosis (2) in hepatopancreas cells (AS 34.99 mg/l 72 hours, 1000x magnification (immersion): 2c = fat degeneration in hepatopancreas cells (AS 17.11 mg/l, day 24, 200x magnification); 2d = fat degeneration in hepatopancreas cells (AS 34.99 mg/l hour to -72, 100x magnification).

The increase in AS concentration in water media treatment is thought to have affected the decrease in appetite in the sample of test shrimp larvae so that it directly affects the growth rate of the sample of test shrimp larvae [77]. The decreased appetite in shrimp is thought to be due to damage to the structure of the organ in shrimp larvae, especially damage that occurs in the sense of taste, because detergent surfactants have the potential to damage sensory organs [75, 78] thus making it difficult for the shrimp to carry out feeding activities [79].
The behavior of shrimp during the rearing period in the test water medium in the aquarium shows that the greater the concentration of AS contained in the water medium in the aquarium, the more larvae of the test shrimp show swimming motion closer to the surface[80]. This is due to damage to the gill organs of the shrimp larvae (Figures 6.1b and 1c) which can cause the shrimp larvae to experience asphyxia, namely getting more oxygen by increasing the frequency of breathing and swimming to the surface, as explained by [81, 83].

In the control aquarium water media, the test Vannamei shrimp larvae showed active movement, whereas in the treated aquarium given AS concentrations it showed that the greater the AS concentration, the test shrimp larvae sample tended to be stationary and inactive. This behavior is considered as an adaptation pattern of shrimp larvae to minimize the biochemical processes of the toxin that has been absorbed into the body of the Vannamei shrimp larvae. So that, the shrimp larvae can still survive or slow down the deadly effects for themselves [84].

In sub-chronic testing, changes in the gill organs and hepatopancreas were seen when treated with AS concentrations of 9.78 mg/l, and 17.11 mg/l on day 24. Observations of the organ structure of the test shrimp larvae were found to have atrophy, hypertrophy, necrosis, and degeneration. The fat is as shown in Figure 6 1b, 1c, and 2c. There are several similarities with the tissue damage in the Vanamei shrimp larvae due to exposure to the LAS detergent surfactant (linear Alkyl benzene sulfonate). The results of the study by [85] reported that sub-chronic concentrations of 0.042 mg/l C12 LAS caused necrosis of the gill filament tips of kuruma (L. Vannamei) on the 14th day of observation, and most of the cases atrophy of the filaments. Kuruma gills (L. Vannamei) at an acute concentration of 0.75 mg/l C12 LAS at the 96th hour of observation. The ability of detergent surfactants to interact with proteins and impair cell membrane permeability [86], [87] [88] is thought to have been the cause of damage to larval organ tissue of the Vannamei shrimp. Damage that occurs in the structure of the gill organs and hepatopancreas in shrimp larvae can result in disruption of the metabolic process in shrimp larvae, which can result in disruption of growth activity in vannamei shrimp larvae.

4. Conclusion
The LC50 value of the concentration of Alkyl Sulfate against Vannamei shrimp larvae (PL 10) at the observation time of 24, 72, 48, and 96 hours was 33.6 mg/l, 29.4 mg/l, 24.3 mg/l, and 22.8 mg respectively. In the acute test, AS concentrations can cause death and behavior changes as well as damage to the structure of the gill organs and hepatopancreas in the larvae of tested shrimp. The effect of AS concentration began to appear when given AS concentrations of 25.58 mg/l at 96 hours, and as much as 34.99 mg/l after 72 hours with exposure time. From the sub-chronic test, the concentration of AS significantly decreased the growth rate caused changes in behavior, and damage to the structure of the gill organs and hepatopancreas in the test shrimp larvae. The effect of AS concentration began to appear at a concentration of 9.78 mg/l at the 24th hour of observation. The toxicity of AS concentration (C12 AS) to Vannamei shrimp larvae was seen to increase with increasing exposure time.

References
[1] B. T. Arachea, Z. Sun, N. Potente, R. Malik, D. Isailovic, and R. E. Viola, “Detergent selection for enhanced extraction of membrane proteins,” Protein Expr. Purif., 2012.
[2] R. Ranganathan, L. Tran, and B. L. Bales, “Surfactant- and Salt-Induced Growth of Normal Sodium Alkyl Sulfate Micelles Well above Their Critical Micelle Concentrations,” J. Phys. Chem. B, 2000.
[3] R. Liu et al., “Beyond the detergent effect: A binding site for sodium dodecyl sulfate (SDS) in mammalian apo ferritin,” Acta Crystallogr. Sect. D Biol. Crystallogr., 2012.
[4] R. Ranganathan, M. Peric, and B. L. Bales, “Time-resolved fluorescence quenching measurements of the aggregation numbers of normal sodium alkyl sulfate micelles well above the critical micelle concentrations,” J. Phys. Chem. B, 1998.
[5] A. I. Choeronawati, S. B. Prayitno, and . Haeruddin, “STUDI KELAYAKAN BUDIDAYA TAMBAK DI LAHAN PESISIR KABUPATEN PURWOREJO,” J. Ilmu dan Teknol. Kelaut. Trop., 2019.
[6] A. A. Hindayani, A. C. Malina, B. R. Tampangalo, and A. F. Fathurahman, “Deteksi distribusi white spot sindrome virus pada berbagai organ udang vannamei (Litopenaeus
vannamei),” *J. Ilmu Kelaut. dan Perikan.*, 2015.

[7] M. Umiliana, Sarjito, and Desrina, *Pengaruh salinitas terhadap infeksi Infectious myonecrosis virus (IMNV) pada udang vaname Litopenaeus vannamei* (Boone, 1931). 2016.

[8] R. Syah, M. Makmur, and M. Fahrur, “BUDIDAYA UDANG VANAME DENGAN PADAT PENEBARAN TINGGI,” *Media Akuakultur*, 2017.

[9] M. Mangampa and H. S. Suwoyo, “BUDIDAYA UDANG VANAME (Litopenaeus vannamei) TEKNOLOGI INTENSIF MENGGUNAKAN BENIH TOKOLAN,” *J. Ris. Akuakultur*, 2016.

[10] L. Hakim, S. Supono, Y. T. Adiputra, and S. Waluyo, “PERFORMA BUDIDAYA UDANG VANAME (Litopenaeus vannamei) SEMI INTENSIF DI DESA PURWOREJO KECAMATAN PASIR SAKTI KABUPATEN LAMPUANG TIMUR,” *e-Jurnal Rekayasa dan Teknol. Budid. Perair.*, 2018.

[11] A. Farras, G. Mahasri, and H. Suprapto, “Prevalensi dan Derajat Infestasi Ektoparasit pada Udang Vaname (Litopenaeus vannamei) di Tambak Intensif dan Tradisional di Kabupaten Gresik,” *J. Ilm. Perikan. dan Kelaut.*, 2017.

[12] B. Romadhona, B. Yulianto, and S. Sudarno, “FLUKTUASI KANDUNGAN AMONIA DAN BEBAN CEMARAN LINGKUNGAN TAMBAK UDANG VANAME INTENSIF DENGAN TEKNIK PANEN PARSiAL DAN PANEN TOTAL Fluctuations of Ammonia and Pollution load in Intensive Vannamei Shrimp Pond Harvested Using Partial and Total Method,” *SAINTEK Perikan. Indones. J. Fish. Sci. Technol.*, 2016.

[13] “The Improvement of the Survival, Growth and Production of Vaname Shrimp (Litopenaeus vannamei) and Seaweed (Gracilaria verucosa) based on Polyculture Cultivation,” *Improv. Surviv. Growth Prod. Vaname Shrimp (Litopenaeus vannamei) Seaweed (Gracilaria verucosa) based Polycult. Cultiv.*, 2014.

[14] D. M. Wildan, R. Affandi, N. T. M. Pratiwi, M. Krisanti, I. P. Ayu, and A. Iswantari, “Evaluation of karst water quality as an early reference of land suitability mapping for vaname shrimp (Litopenaeusvannamei) culture media,” in *IOP Conference Series: Earth and Environmental Science*, 2017.

[15] Developed by the National Collaborating Centre for Acute Care, “Preoperative Tests: The use of routine preoperative tests for elective surgery,” *Nhs*, 2003.

[16] S. S. Dickerson and M. E. Kemeny, “Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research,” *Psychological Bulletin*, 2004.

[17] FDA, “Chapter IV . Guidelines for Toxicity Tests,” *Guid. Ind. Other Stakeholders Toxicol. Princ. Saf. Assess. Food Ingredients Redb.* 2000, 2000.

[18] T. A. Woreta and S. A. Alqahtani, “Evaluation of abnormal liver tests,” *Medical Clinics of North America*. 2014.

[19] A. W. Bannon and A. B. Malmberg, “Models of nociception: hot-plate, tail-flick, and formalin tests in rodents.,” *Curr. Protoc. Neurosci.*, 2007.

[20] C. Guideline, “Preoperative tests,” *Acute Care*, 2016.

[21] Anon, “METHODS FOR ACUTE TOXICITY TESTS WITH FISH, MACROINVERTEBRATES, AND AMPHIBIANS.,” *Ecol Res Ser EPA*, 1975.

[22] American Public Health Association (APHA), “Standard Methods for the Examination of Water & Wastewater 21st Edition,” *American Public Health Association: American Water Works A ssociation, Water Environment Federation*, 1999.

[23] E. G. E. Brooks *et al.*, “Global impacts of energy demand on the freshwater resources of nations,” *Biol. Conserv.*, vol. 21, no. 2, p. n/a-n/a, 2010.

[24] G. J. S. Ross and D. J. Finney, “Probit Analysis.,” *Stat.*, 1972.

[25] D. J. Finney, “Statistics for biologists,” *Nature*, 1974.

[26] L. C. Bernard, M. Mills, L. Swenson, and R. P. Walsh, “An evolutionary theory of human motivation,” *Genet. Soc. Gen. Psychol. Monogr.*, 2005.

[27] M. J. R. Healy and D. J. Finney, “Statistical Method in Biological Assay.,” *J. R. Stat. Soc. Ser. A*, 1979.
[28] C. Thornton and K. K. Yin, “Impact of elastic spheres with and without adhesion,” *Powder Technol.*, 1991.

[29] P. Duarte and J. G. Ferreira, “A methodology for parameter estimation in seaweed productivity modelling,” *Hydrobiologia*, 1993.

[30] C. L. McIntyre, M. W. Collopy, and M. S. Lindberg, “Survival Probability and Mortality of Migratory Juvenile Golden Eagles from Interior Alaska,” *J. Wildl. Manage.*, 2006.

[31] C. Aluminum *et al.*, “Standard Practice for Slow Strain Rate Testing to Evaluate the Susceptibility of Metallic Materials to Environmentally Assisted Cracking 1,” *Test*, 2006.

[32] A. Drews, “Standard Test Method for,” in *Manual on Hydrocarbon Analysis, 6th Edition*, 2008.

[33] F. Test-, “Standard Test Method for Determination of Slow Crack Growth Parameters of Advanced Ceramics by Constant Stress-Rate Flexural,” in *Ceramics*, 2001.

[34] T. F. Pettigrew and L. R. Tropp, “A meta-analytic test of intergroup contact theory,” *J. Pers. Soc. Psychol.*, 2006.

[35] F. Range and Z. Virányi, “Social learning from humans or conspecifics: Differences and similarities between wolves and dogs,” *Front. Psychol.*, 2013.

[36] M. A. Whooley, A. L. Avins, J. Miranda, and W. S. Browner, “Case-finding instruments for depression: Two questions are as good as many,” *J. Gen. Intern. Med.*, 1997.

[37] P. J. Bentley and J. P. Wakefield, “Finding Acceptable Solutions in the Pareto-Optimal Range using Multiobjective Genetic Algorithms,” in *Soft Computing in Engineering Design and Manufacturing*, 1998.

[38] J. He, H. Yang, T. Q. Tang, and H. J. Huang, “An optimal charging station location model with the consideration of electric vehicle’s driving range,” *Transp. Res. Part C Emerg. Technol.*, 2018.

[39] M. M. Fleck, D. A. Forsyth, and C. Bregler, “Finding naked people,” in *Lecture Notes in Computer Science (including subseries Lecture Notes in Artificial Intelligence and Lecture Notes in Bioinformatics)*, 1996.

[40] W. Johnson, J. te Nijenhuis, and T. J. Bouchard, “Still just 1 g: Consistent results from five test batteries,” *Intelligence*, 2008.

[41] OECD, “Earthworm, Acute Toxicity Tests,” *OECD Guidel. Test. Chem.*, 1984.

[42] C. Basnayake and D. Ratnam, “Blood tests for acute pancreatitis,” *Aust. Prescr.*, 2015.

[43] E. Lammer, G. J. Carr, K. Wendler, J. M. Rawlings, S. E. Belanger, and T. Braunbeck, “Is the fish embryo toxicity test (FET) with the zebrafish (Danio rerio) a potential alternative for the fish acute toxicity test?,” *Comp. Biochem. Physiol. - C Toxicol. Pharmacol.*, 2009.

[44] K. F. Liu, C. H. Chiu, Y. L. Shiu, W. Cheng, and C. H. Liu, “Effects of the probiotic, Bacillus subtilis E20, on the survival, development, stress tolerance, and immune status of white shrimp, Litopenaeus vannamei larvae,” *Fish Shellfish Immunol.*, 2010.

[45] B. Gomez-Gil, A. Roque, and J. F. Turnbull, “The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms,” *Aquaculture*, 2000.

[46] M. Ravi *et al.*, “Studies on the occurrence of white tail disease (WTD) caused by MrNV and XSV in hatchery-reared post-larvae of Penaeus indicus and P. monodon,” *Aquaculture*, 2009.

[47] P. A. W. Robertson, J. Calderon, L. Carrera, J. R. Stark, M. Zherdmant, and B. Austin, “Experimental Vibrio harveyi infections in Penaeus vannamei larvae,” *Dis. Aquat. Organ.*, 1998.

[48] R. Sudhakaran, S. Syed Musthaq, P. Haribabu, S. C. Mukherjee, C. Gopal, and A. S. Sahul Hameed, “Experimental transmission of Macrobrachium rosenbergii nodavirus (MrNV) and extra small virus (XSV) in three species of marine shrimp (Penaeus indicus, Penaeus japonicus and Penaeus monodon),” *Aquaculture*, 2006.

[49] J. S. Zhang, S. L. Dong, X. L. Tian, Y. W. Dong, X. Y. Liu, and D. C. Yan, “Studies on the rotifer (Brachionus urceus Linnaeus, 1758) as a vector in white spot syndrome virus (WSSV) transmission,” *Aquaculture*, 2006.
[50] S. Thitamadee et al., “Review of current disease threats for cultivated penaeid shrimp in Asia,” Aquaculture. 2016.
[51] C. Gambardella et al., “Effects of selected metal oxide nanoparticles on Artemia salina larvae: Evaluation of mortality and behavioural and biochemical responses,” Environ. Monit. Assess., 2014.
[52] B. M. Roth, K. A. Rose, L. P. Rozas, and T. J. Minello, “Relative influence of habitat fragmentation and inundation on brown shrimp Farfantepenaeus aztecus production in northern Gulf of Mexico salt marshes,” Mar. Ecol. Prog. Ser., 2008.
[53] P. Piña, D. Voltolina, M. Nieves, and M. Robles, “Survival, development and growth of the Pacific white shrimp Litopenaeus vannamei protozoa larvae, fed with monoalgal and mixed diets,” Aquaculture, 2006.
[54] B. T. Nga, R. Roijackers, T. T. Nghi, V. N. Ut, and M. Scheffer, “Effects of decomposing Rhizophora apiculata leaves on larvae of the shrimp Penaeus monodon,” Aquac. Int., 2006.
[55] S. E. Hook et al., “The impacts of modern-use pesticides on shrimp aquaculture: An assessment for north eastern Australia,” Ecotoxicol. Environ. Saf., 2018.
[56] J. Li et al., “Comparative study between probiotic bacterium Arthrobacter XE-7 and chloramphenicol on protection of Penaeus chinensis post-larvae from pathogenic vibrios,” Aquaculture, 2006.
[57] P. B. Key and M. H. Fulton, “Correlation between 96-h mortality and 24-h acetycholinesterase inhibition in three grass shrimp larval life stages,” Ecotoxicol. Environ. Saf., 2006.
[58] M. A. Rimmer, A. W. Reed, M. S. Levitt, and A. T. Lisle, “Effects of nutritional enhancement of live food organisms on growth and survival of barramundi, Lates calcarifer (Bloch), larvae,” Aquac. Res., 1994.
[59] R. Zhang et al., “A novel surfactant-, NaCl-, and protease-tolerant β-mannanase from Bacillus sp. HJ14,” Folia Microbiol. (Praha), 2016.
[60] Y. Wang et al., “An alkaline and surfactant-tolerant lipase from Trichoderma lentiforme ACCC30425 with high application potential in the detergent industry,” AMB Express, 2018.
[61] G. Razmah, I. Siti Afida, A. M. Zulina, Z. Noorazah, and A. H. Hazimah, “A comparative study of the ecotoxicity of palm-based Methyl ester sulphonates (MES) to Tilapia and Daphnia magna,” J. Oil Palm Res., 2016.
[62] T. Satsuki, Y. Nagoh, and H. Yoshimura, “Effect of Calcium Ions on Detergency. Part 2: Interactions between a surfactant, a calcium-sequestering builder and calcium ions,” Tenside, Surfactants, Deterg., 1998.
[63] S. S. Soleimani, H. Nadaroglu, and Z. Kesmen, “Lactobacillus brevis lipase: Purification, immobilization onto magnetic florosil NPs, characterization and application as a detergent additive,” Tenside, Surfactants, Deterg., 2017.
[64] P. De Marco, C. C. Pacheco, A. R. Figueiredo, and P. Moradas-Ferreira, “Novel pollutant-resistant methylotrophic bacteria for use in bioremediation,” FEMS Microbiol. Lett., 2004.
[65] D. Martinez, G. Orozco, S. Rincón, and I. Gil, “Simulation and pre-feasibility analysis of the production process of α-methyl ester sulfonates (α-MES),” Bioresour. Technol., 2010.
[66] P. Jaiswal, S. N. Jha, J. Kaur, and A. Borah, “Detection and quantification of anionic detergent (lissapol) in milk using attenuated total reflectance-Fourier Transform Infrared spectroscopy,” Food Chem., 2017.
[67] A. Shukla and S. P. Trivedi, “Anionic Surfactant, Linear Alkyl Benzene Sulphonate Induced Oxidative Stress and Hepatic Impairments in Fish Channa punctatus,” Proc. Zool. Soc., 2018.
[68] C. Lee, N. J. Russell, and G. F. White, “Modelling the kinetics of biodegradation of anionic surfactants by biofilm bacteria from polluted riverine sites: A comparison of five classes of surfactant at three sites,” Water Res., 1995.
[69] F. Rios, M. Olak-Kuchareczyk, M. Gmurek, and S. Ledakowicz, “Removal efficiency of anionic surfactants from water during UVC photolysis and advanced oxidation process in H2O2/UVC system,” Arch. Environ. Prot., 2017.
12

Science of the Total Environment, 1996.

[70] P. Kloepper-Sams, F. Torfs, T. Feijtel, and J. Gooch, “Effects assessments for surfactants in sludge-amended soils: A literature review and perspectives for terrestrial risk assessment,” in

[71] S. T. Method, “Standard Test Method for Testing Rock Slabs to Evaluate Soundness of Riprap by Use of Sodium Sulfate or Magnesium Sulfate 1,” Current, 1997.

[72] V. Lehtola, H. Luomajoki, V. Leinonen, S. Gibbons, and O. Airaksinen, “Efficacy of movement control exercises versus general exercises on recurrent sub-acute nonspecific low back pain in a sub-group of patients with movement control dysfunction. protocol of a randomized controlled trial,” BMC Musculoskel. Disord., 2012.

[73] S. I. Sherwani, H. A. Khan, A. Ekhzaimy, A. Masood, and M. K. Sakarker, “Significance of HbA1c test in diagnosis and prognosis of diabetic patients,” Biomarker Insights. 2016.

[74] R. D. Rogers et al., “Dissociable deficits in the decision-making cognition of chronic amphetamine abusers, opiates abusers, patients with focal damage to prefrontal cortex, and tryptophan-depleted normal volunteersEvidence for monoaminergic mechanisms,” Neuropsychopharmacology, 1999.

[75] H. M. Abella, G. Z. Reus, and J. Quevedo, “Animal models as tools to study the pathophysiology of depression,” Rev. Bras. Psiquiatr., 2013.

[76] D. Carroll, “A quantitative test of upper extremity function,” J. Chronic Dis., 1965.

[77] K. Steyn and A. Damasceno, Lifestyle and Related Risk Factors for Chronic Diseases. 2006.

[78] R. A. Deyo and R. M. Centor, “Assessing the responsiveness of functional scales to clinical change: An analogy to diagnostic test performance,” J. Chronic Dis., 1986.

[79] S. J. Singh, M. D. L. Morgan, S. Scott, D. Walters, and A. E. Hardman, “Development of a shuttle walking test of disability in patients with chronic airways obstruction,” Thorax, 1992.

[80] W. K. Fitt, “The role of chemosensory behavior of Symbiodinium microadiaticum, intermediate hosts, and host behavior in the infection of coelenterates and molluscs with zooxanthellae,” Mar. Biol., 1984.

[81] W. Wasilewsky, H. Atwood, A. Stokes, and C. L. Browdy, “Effect of natural production in a zero exchange suspended microbial floc based super-intensive culture system for white shrimp Litopenaeus vannamei,” Aquaculture, 2006.

[82] J. L. D. Davis, W. J. Metcalfe, and A. H. Hines, “Implications of a fluctuating fish predator guild on behavior, distribution, and abundance of a shared prey species: The grass shrimp Palaemonetes pugio,” J. Exp. Mar. Bio. Ecol., 2003.

[83] F. J. Martinez-Cordero and P. S. Leung, “Sustainable aquaculture and producer performance: Measurement of environmentally adjusted productivity and efficiency of a sample of shrimp farms in Mexico,” Aquaculture, 2004.

[84] M. Hernández R., L. F. Bückle R., E. Palacios, and B. Barón S., “Preferential behavior of white shrimp Litopenaeus vannamei (Boone 1931) by progressive temperature-salinity simultaneous interaction,” J. Therm. Biol., 2006.

[85] M. C. Mattson, J. A. Thomas, and D. St. Aubin, “Effects of Boat Activity on the Behavior of Bottlenose Dolphins (<I>Tursiops truncatus</I>) in Waters Surrounding Hilton Head Island, South Carolina,” Aquat. Mamm., 2005.

[86] S. Reiser, J. P. Herrmann, and A. Temming, “Thermal preference of the common brown shrimp (Crangon crangon, L.) determined by the acute and gravitational method,” J. Exp. Mar. Bio. Ecol., 2014.

[87] P. O. Moksnes and H. Wennhage, “Methods for estimating decapod larval supply and settlement: Importance of larval behavior and development stage,” Mar. Ecol. Prog. Ser., 2001.

[88] P. P. P. Rubinoff et al., “The convergence of Integrated Coastal Zone Management and the ecosystems approach,” Ocean Coast. Manag., vol. 54, no. 1, pp. 990–1001, 2010.