Detection of The Red Sea Bream Iridovirus (RSIVD) and Quality of Frozen Mackerel (*Scomber japonicus*) Imported Through the Port of Tanjung Mas Semarang

A D Novitasari*, and Desrina2, dan T W Agustini3,

1Coastal Resource Management Postgraduate Program, Faculty of Fisheries and Marine Sciences, Diponegoro University
2Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Diponegoro University
3Department of Fisheries Product Technology, Faculty of Fisheries and Marine Sciences, Diponegoro University

Corresponding author: anita_dnov@yahoo.co.id and anita.dnov@gmail.com

Abstract. The imported frozen fish and fishery products can be a carrier of transboundary fish pathogen that subsequently may cause serious threat to the natural fish inhabitants, sustainability of aquaculture, human health and fisheries business in general. One of the transboundary fish diseases is Red Sea Bream Iridovirus Disease (RSIVD) caused by RSIV. The virus has been reported occurred in many countries that has active trading of fish and fisheries product with Indonesia such as Japan, China, Taipei, Hong Kong, Korea, Malaysia, Philippines, Singapore and Thailand. In the last four years, whole frozen mackerel (*Scomber japonicus*) is the most imported fisheries commodity through Tanjung Emas Port. The objective of this study were to detect the presence of RSIV using PCR method according to OIE and quality as food fish for human consumption by examining for Organoleptic test, TPC, *E. coli*, histamine test, and the presence of parasites *Anisakis* sp. according to Indonesian national standard (SNI). Fish samples (25 fishes/entries) were obtained by random sampling of 10 importing entries. Results showed that there was no RSIV detected. The results of quality testing include organoleptic test, TPC, *E. coli*, histamine meet the SNI standard of raw fish quality. *Anisakis* sp. was found with the prevalence between 12 - 100%. There is one import entry that does not meet the requirements of the minimum value of organoleptic and TPC values so that it does not meet the quality standards of raw materials.

1. Introduction

The importation of live fish and fishery products has the potential to become a carrier medium for the entry and the spread of harmful pests and fish diseases, endangering the sustainability of fish resources and the environment, human health, and the sustainability of fisheries. The mackerel (*S. japonicus*) is the most widely imported commodity through Tanjung Emas Sea Port for the past four years. One of the most dangerous fish pests is Red Sea Bream Iridovirus Disease (RSIVD). RSIVD caused by RSIV has been reported not only from Japan but also widely from other Eastern regions and Southeast Asian countries (China, Taipei, Hong Kong, Korea, Malaysia, Philippines, Singapore and Thailand) [1]. In Korea, RSIVD caused high mortality in rock bream fish culture (*Oplegnathus fasciatus*), it occured for the first in 1998. Since then, RSIVD has become a major cause of mass death in rock bream fish [2]. In Japan, the fish...
affected by RSIVD are yellowtail, sea bass, Japanese parrotfish, amberjack (*Seriola dumerili*), goldstriped amberjack (*S. aureovittata*), striped jacks (*Pseudocaranx dentex*), horse mackerel (*Trachurus japonicus*), Albacore (*Thunnus thynnus*), Japanese flounder (*Paralichthys olivaceus*), and tiger puffer (*Takifugu rubripes*) [3].

To ensure that fishery products circulating in the Indonesian territory are in good quality and safe for consumption, efforts to test the quality of fishery products imported into Indonesia are necessary. Quality tests conducted in accordance with the SNI standards include detection of the organoleptic tests, TPC, *E. coli*, histamine levels, and presence of *Anisakis* sp. parasites.

Parasites are organisms that live on or inside other organisms, taking food from the organism and they live in to multiply. Based on their habitat, parasites in the body of the fish are divided into two, namely ectoparasites (parasites that attack the surface of the fish's body or in cavities that are directly connected to the surface of the fish's body, for example the gills, fins and skin), and endoparasites (parasites that infect the internal organs of the body fish, for example intestines, kidneys and liver) [4].

Mackerel is an important commercial fish in East Asia, consisting of 10-25% of total marine fish caught in Korea [5]. Usually, it is consumed after boiled or roasted, but also often as raw dishes. Especially in Japan, it is consumed raw and has been confirmed as the main cause of Anisakiasis in humans. Almost all cases of anisakiasis in Japan are caused by *Anisakis simplex* [6]. One of the most commonly consumed fish in northern Morocco was mackerel fish which was detected a high prevalence of *Anisakis* spp. (67.9%) [7].

Scientific publications about marine fish diseases and quality testing of imported fish in Indonesia are still very limited. So, it is necessary to publish a research related to the import of mackerel (*S. japonicus*) in terms of the detection of RSIVD by PCR and sequencing methods, testing the quality of food safety by organoleptic test, TPC, *E. coli*, histamine, and detection and prevalence of *Anisakis* sp. conventionally, test based on the prevailing SNI standards. The purpose of this study is to detect and analyze the prevalence of Red Sea Br eam Iridovirus Disease (RSIVD) in imported mackerel fish, analyze the quality of imported mackerel fish products by organoleptic test, TPC, *E. coli*, histamine test, and analyzing the presence of *Anisakis* sp.

2. Research Methods

2.1. Fish Sampling.

This study analyzed 10 times imported mackerel entries and tested them with 2 replications. Samples were obtained frozen and stored at −18 °C. Before testing them, the samples were collected and the length and weight of fish were measured.

2.2. PCR Test To Detect RSIVD.

The targeted organs examined were the kidneys. PCR testing refers to the OIE (2017) - Manual of Diagnostic Test for Aquatic Animals method. Analysis was performed using a Polymerase Chain Reaction (PCR) method and specific primers 1-F: 1-F (5'-CTC-AAA- CAC-TCT-GGC-TCA-TC-3') and IR (5'-GCA-CCA-ACA-CAT-CTC-TA-TC-3'). Iridovirus amplification reaction was carried out with a total volume of 12.5 μL consisting of 0.5 μL forward primer, 0.5μL reverse primer, 6.25 μL master mix, 3.25 μL water PCR grade, and 2 μL DNA template obtained . The DNA was amplified using a thermal cycler based on the following program: 30 cycles at 94 °C for 30 seconds, 58 °C for 60 seconds, and 72 ° C for 60 seconds, and held at 72 ° C for 5 minutes. The amplification results were then verified through an electrophoresis process by placing 1 - 2 μl of each sample and control on 2% agarose gel. The PCR product was then observed using UV transilluminator and documented using a camera. Positive IVIV at 570 bp.
2.3. **Total Plate Count Test (SNI 2332.3-2015).**

The Total Plate Count (TPC) basically is used to grow the aerobic and anaerobic microorganisms (psychrophilic, mesophilic, and thermophilic) after incubation in agar medium at a temperature of 35°C ± 1°C for 24-48 hours. The TPC is determined in two ways, namely the agar plate pouring method and the plate method so that the spread plate is based on SNI 2332.3-2015. In the TPC test, Butterfield's Phosphate Buffered (BFP) solution was used as a sample diluent and PCA (Count Plate Agar) was used as a solid medium. A special reactant for Tri Phenyl tetrazalim Chlotide 1% (TTC) was also used [8].

The procedure of TPC testing according to SNI 2332.3-2015 is described that sample is weighed aseptically as much as 25 grams into a sterile stomacher bag. After that 225 ml of BFP was added, and it was homogenized with a stomacher for 30 seconds so that the suspension was obtained with a 10-1 dilution. Five (5) tubes or more were prepared and each of them had been filled with 9 ml of BFP. The results of the homogenization in the preparation of the sample which was the dilution of 10^-1 was obtained. Subsequent dilutions are up to 10^-6 or according to the required dilution. From each 1ml pipette dilution was put into a petri dish and duplo was made, into each cup 12-15 ml of PCA medium was poured which had been added 1% TTC temperature of 45°C ± 1°C. The petri dish was rotated forwards back and left to right so that it was fully mixed. To find out the sterility of the medium and diluent, a control test (blank) is made. In one cup, it was filled with 1 ml of diluent and agar medium, and another cup was filled with medium. After the medium solidified, the dish is incubated at 35°C for 48 hours ± 2 hours with the reversed position. All the growing colonies are calculated and the dilution value was recorded. The total plate count per gram is the average yield obtained from each dilution. The TPC calculations are as follows:

\[
N = \frac{\Sigma C}{[(1 \times n1) + (0,1 \times n2)]x (d)}
\]

Notes:

- N is the number of product colonies, expressed in colonies per ml or colony per g.
- \(\Sigma C\) is the number of colonies in all plates counted
- \(n_1\) is the number of plates at the first dilution calculated
- \(n_2\) is the number of plates on the second dilution calculated
- \(d\) is the first calculated dilution.

2.4. **Escherichia coli test (SNI 2332.1: 2015).**

*E. coli* testing procedure based on SNI 2332.1: 2015 is described as follows: The sample preparation is made by weighing 25 g of sample that is put into sterile plastic and adding 225 mL of Butterfield's Phosphate Buffered (BFP) solution. It was then homogenized for 2 minutes, this homogenate is a solution with dilution 10^1. The Stage of Coliform Estimation Test: prepare 10^-2 dilution by dissolving 10^-1 solution into 9 ml diluent solution (BFB). The further dilution according to the estimation of sample population density was then made. At each dilution, shake is made at least 25 times. Transfer was done by using a sterile pipette, as much as 1 ml of solution from each dilution into 3 series or 5 series of lauryl tryptose broth (LTB) tubes containing durham tubes. Incubation of the tubes was conducted for 48 hours ± 2 hours at 35oC ± 1oC. Careful attention was given to the gas formed after 24 hours of incubation and re-incubate negative tubes for 24 hours. A positive tube is marked by gas turbidity in a durham tube. The coliform affirmation test was made for positive tubes [9].

*Escherichia coli* estimation test: the inoculation of each positive LTB tube is punt into EC broth tubes containing durham tubes by using an oleum extract. Incubation in the EC broth in a circulating waterbath was done in 48 hours ± 2 hours at 45°C ± 0.5°C. The waterbath must be clean, the water in it must be higher than the height of the liquid in the tube to be incubated. The EC broth tubes that produce
the gas for 24 hours ± 2 hours must be tested, if negative, incubation has to be conducted again for another 48 hours ± 2 hours. A positive tube is characterized by turbidity and gas in a Durham tube. Most probable number (MPN) must be determined based on the number of positive EC tubes using the Most Probable Number (MPN). The value are stated as "MPN/g faecal coliform. Escherichia coli" affirmation test: from positive EC broth tubes by using an osegores needle to the L-EMB agar. The incubation must be conducted for 24 hours ± 2 hours at a temperature of 35°C ± 1°C. An unexpected Escherichia coli colony gives a characteristic (typical) that is black in the middle with or without metallic green. Take more than one (typical) Escherichia coli colony from each cup L-EMB and scratch onto PCA media tilt using a planting needle. The incubation must be conducted for 24 hours ± 2 hours at 35°C ± 1°C. If there is no typical colony, move 1 or more typical Escherichia coli colonies to oblique PCA medium.

Morphology tests were conducted by gram staining of each Escherichia coli colony that has been incubated for 24 hours. By using a microscope, Escherichia coli bacteria include negative gram bacteria, in the form of short rods or coccus. Biochemical tests were carried out by testing Indol production, voga proskauer (VP) test, methyl red (MR), Citrat (C) test, and gas production from lactose. Based on the interpretation of the results above, state the coliform and Escherichia coli in MPN/g using the Most Probable Number (MPN).

2.5 Organoleptic Test (SNI 2729: 2013).

Organoleptic testing was carried out by using a score sheet to test the quality of fresh fish. Assessment specifications according to SNI 2729: 2013 provide an assessment of the score sheet based on appearance in a frozen state, drying (dehydration), discoloration (discoloration), appearance after thawing, odor, meat, and texture. According to SNI No. 2729: 2013 minimum requirements for organoleptic values for fresh fish are 7 (score 1 - 9) [10].

2.6 Histamine Level Test (SNI 2354.10: 2016).

The principle of histamine testing by spectrophotometry is that histamine is extracted from the sample meat tissue using methanol and simultaneously converts histamine into OH form. The histamine substances are then purified through ionizing resins and converted to their derivative form with OPT compounds. The magnitude of histamine fluorescence was measured by fluorometry at an excitation wavelength of 350 nm and an emission of 444 nm. The histamine level test was carried out using a spectrophotometer (Cary Eclipse) with the method referring to SNI 2354.10: 2016 which was stated in μg/g or mg/kg based on the calculation [11]:

\[
\text{Histamine concentration (μg/g) example } = A \times \frac{\text{final volume(ml)} \times \text{fp}}{\text{gram sampled}}
\]

(1)

Notes:
A = The concentration (X) obtained in calculation (μg/ml)

2.7 Anisakis sp. Examination.

Fish samples were measured in length and weight and placed on a tray. The fish’s abdominal cavity was cut opened with scissors pointing anteriorly to the ventral fin, afterwards, it was cut to the dorsal direction of the fish to the side of the line and cut to the anal part of the fish. The anterior part of the fish’s side was cut up to the posterior part of the intestine, it was then examined in a petri dish and incised. Muscle and stomach tissues were examined for the presence of Anisakis sp. Prevalence and intensity are calculated using the following formula [12].

\[
\text{Prevalence } = \frac{\Sigma \text{infected fish}}{\Sigma \text{examined fish}} \times 100\%
\]

(2)
Intensity (ind/fish) = \frac{\sum \text{parasite found}}{\sum \text{infected fish}} \times 100\% \tag{3}

2.8. Data Analysis.
This research is a non-experimental exploratory research with the sampling method of "random sampling" that is the sample taken randomly [13]. The data obtained are qualitative and quantitative data. Qualitative data is the result of PCR test. While quantitative data is as the result of the organoleptic test, ALT, \textit{E.coli} test, histamine, and test worm examination data \textit{Anisakis} sp.. Data were analyzed using Microsoft Office Exel and descriptively based on literature.

3. Result and Discussion
3.1. Morphometric Measurement.
Morphometric measurements (weight and length) are categories of index of condition and energetic size of fish [14]. This study analyzes 10 (ten) imported items that entered through the Tanjung Emas Sea Port of Semarang in the period of April - June 2018. The imported mackerel fish were from X, Y and Z countries. Morphometric data of the mackerel fish can be seen in Table. 1.

| No. | Code | Length (cm) | Weight (gram) | Country of Origin |
|-----|------|-------------|---------------|------------------|
| 1.  | A    | 23 – 27     | 110 - 149     | X                |
| 2.  | B    | 21 – 22.5   | 91 – 97.7     | X                |
| 3.  | C    | 26 – 28     | 135 – 213.5   | X                |
| 4.  | D    | 25 – 28     | 107.6 – 178.2 | X                |
| 5.  | E    | 20 – 26.5   | 87 - 181      | X                |
| 6.  | F    | 37 – 38     | 424 – 452.4   | Y                |
| 7.  | G    | 37.5 – 38   | 444 – 446.5   | Z                |
| 8.  | H    | 22.5 – 25.5 | 81.5 – 144.2  | X                |
| 9.  | I    | 20 – 25     | 51.2 – 123.5  | X                |
| 10. | J    | 29 – 35     | 241 - 595     | X                |

The length and weight of imported mackerel depends on the purpose of import. Fish originating from Country X had the purpose of import for injection salting, namely the sample code A, B, C, D, E, H, I, and J code. While the F code and code G is a fish originating from Y and Z countries, the fish is filleted and then re-exported. The import of fishery products carried out by importers is used for the FPU (Fish Processing Unit) as raw materials for fish canning industry, the FPU raw materials are for re-exporting purpose and not to be traded within the territory of the Republic of Indonesia, the traditional processing raw materials in the form of injection salting (pemindangan), certain food fortification/ enrichment raw materials; and/or consumption of modern hotels, restaurants and markets [15]. The observation of the length and weight of fish becomes the supporting parameter. The length of the fish is distinguished according to the category of size of mackerel fish (\textit{S. japonicus}) which states that young fish have a size of 15-28 cm and adult fish have a size of more than 28 cm. [16].

3.2. Laboratory Test Result.
The PCR testing to detect RSIVD, the test of \textit{Anisakis} sp. Parasite, TPC test, and \textit{E.coli} test were carried out at the Testing Laboratory of KIPM Semarang. The organoleptic testing was carried out at the Fishery
Product Quality Testing and Application Center (BP2MHP) Semarang, and the histamine testing was carried out at Pekalongan BP2MHP in Cilacap. RSIVD testing results, TPC test, organoleptic, *E.coli*, histamine levels, and the prevalence and intensity of *Anisakis* sp. parasites, can be seen in Table 2.

### Table 2. Results of RSIVD Testing, TPC, *E.coli*, Organoleptic, Histamine Levels, and Prevalence and Intensity of Parasites *Anisakis* sp.

| Code | RSIVD | TPC (colony/gram) | Organoleptic (APM/gram) | Histamine (μg/kg) | Prevalence (%) | Intensity |
|------|-------|-------------------|-------------------------|------------------|----------------|-----------|
| A    | Negative | 175,000           | 7±0                     | <3.0             | 0.016±0.003   | 88        | 3.4       |
| B    | Negative | 630,000*          | 6±0*                    | <3.0             | 0.030±0.002   | 72        | 4.9       |
| C    | Negative | 47,000            | 8±0                     | <3.0             | 0.016±0       | 52        | 13.5      |
| D    | Negative | 48,000            | 7±0                     | <3.0             | 0.023±0.002   | 60        | 20        |
| E    | Negative | 6,600             | 7,5±0,5                 | <3.0             | 0.015±0.001   | 12        | 25        |
| F    | Negative | 24,000            | 8±0                     | <3.0             | 0.016±0.003   | 100       | 14.6      |
| G    | Negative | 5,000             | 8±0                     | <3.0             | 0.030±0.002   | 60        | 17.3      |
| H    | Negative | 5,300             | 7,5±0,5                 | <3.0             | 0.016±0       | 64        | 8.8       |
| I    | Negative | 48,000            | 7±0                     | <3.0             | 0.023±0.002   | 72        | 3         |
| J    | Negative | 115,000           | 7±0                     | <3.0             | 0.015±0.001   | 80        | 13.9      |

Notes: * does not meet the SNI threshold
Source: Research Data (2018)

4. Discussion

#### 4.1. RSIV test (OIE, 2017).

The PCR method has a significant role in the development of aquaculture, one of which is to detect RSIV infections. The sensitivity and speed of the RSIVD test by PCR is that the infection can be found at an early stage with high sensitivity and it can detect the virus before the appearance of disease symptoms and allow to get the final results in one day [17]. The RSIVD test in PCR way was conducted towards 10 times imported sample with two replications.

The infected fish will look lethargic, show severe anemia, gill bleeding and spleen enlargement [18]. This disease is characterized by the appearance of enlarged cells that are deeply colored with Giemsa solution on microscopic observations of the infected parts of the spleen, heart, kidneys, liver and gills of the fish. The RSIVD transmission goes horizontally through water [1].

Based on the PCR test results, all imported mackerel samples were not detected with RSIVD. As seen in Figure 1.
Figure 1. Results of the RSIVD virus PCR test in imported mackerel

Notes:
• Line A1 - J1: Samples (Negative RSIVD)
• K+: Positive Control (+) RSIVD at 570 bp
• M: DNA Marker
• K-: Negative (-) RSIVD Control
• Line A2-J2: Samples (Negative RSIVD)

The RSIV infections are generally characterized by swelling and damage to the spleen and kidneys [19] and [20]. The fish infected with RSIVD are characterized by a darker body color and accompanied by severe anemia that can be seen in the gills [21]. The liver is darker due to heavy bleeding or becomes pale and swollen. It is contrastly seen in the spleen organ that has swelling and is very dark in color which is almost black. But in this study, no mackerel fish with clinical symptoms infected with RSIVD was found.

A contaminated environment of poor water quality triggers an increase in RSIVD infection. This is mainly caused by direct contact between the gills and the digestive tract of fish with the environment. The spread of the virus between fish in the same production system will occur very quickly if the fish do not have a good immune system and are in a weak condition. In general, this virus spreads between regions/countries due to the introduction of imported fish that have been infected by previous RSIV or in nature becomes carrier of it [22]. In this study, mackerel fish were not infected with RSIVD.

4.2. Organoleptic Test (SNI 2729: 2013).
Organoleptic test is a way of evaluating product quality based on five human senses [23]. Organoleptic assessment is the most common way to determine signs of freshness of fish because it is easier and faster to do, it does not require a lot of equipment and is cheap. Organoleptic tests on imported mackerel fish are the appearance (eyes, gills, slime surface), meat, odor, and texture [24].The number of panelists in the organoleptic test are 6 standard panelists. Eyes are the main indicator to determine the freshness of fish. Increasing temperatures cause a significant decrease in organoleptic values [25]. Based on the results of organoleptic testing obtained from the results of the research ranged from 6-8 in the laboratory, there is
one imported item that has an organoleptic value of 6, so it does not meet the standard quality of raw materials. According to SNI No. 2729: 2013 minimum organoleptic value is 7 (score 1 - 9).

Based on the results of organoleptic testing, there was one import of imported mackerel fish (code B) which had an organoleptic value below the standard, that is 6. The results of field research still found fish that were not intact and broken in the stomach (code B). This could be due to physical collisions and handling that is not good. Physical pressure and impact of fish should be avoided because it can cause physical damage to the body of the fish such as bruised flesh, wounds, and a broken stomach. The fish in rigor condition were treated badly such as stacked too much, thrown, get collision, trampled, then the fish decay will take place more quickly.

4.3. **TPC Test (SNI 01-2332.3-2015)**

Total Plate Count (TPC) is one method used to calculate the number of microbes in foodstuffs [8]. The cup count method (TPC) is the most widely used method in analysis, because the colonies can be seen directly with the eye without using a microscope. According to the requirements of quality and safety of fresh fish in SNI 01-2332.3-2015 the maximum TPC value is 500,000 colonies/gram. Based on the results of ALT testing, there is one import of imported mackerel (code B) which exceeds the threshold of 650,000 colonies/gram. The decrease and increase in TPC occurs because fish meat is a medium suitable for bacterial growth [26].

Fresh fish that have just been caught are given ice crush so that the fish is in good condition when it is marketed and inhibits or stops the activity of harmful substances and microorganisms, storage with cold and frozen temperatures can also destroy spoilage microbes [29]. The use of low temperatures of 0°C after dead fish can prolong the rigor mortis period, reduce enzymatic, bacterial, chemical and physical changes in fish [30]. The use of low temperatures will inhibit microbial growth in fish [31]. An increase and decrease in TPC can occur because the fish meat is a medium that is suitable for bacterial growth [28].

4.4. **E. Coli Test SNI (01-2332.1-2015)**

*Escherichia coli* (*E. coli*) is not a natural microbe in fish, it can be isolated from the intestine and its presence in contaminated water from the environment [28]. Bacteria that are most widely used as indicators of sanitation are *Escherichia coli* because these bacteria are commensal bacteria in the human intestine and are generally not disease-causing pathogens. The results of laboratory testing showed the same results for all imported mackerel samples, namely <3.0 MPN/gram. According to the requirements of quality and safety of fresh fish in SNI 01-2332.1-2015 the maximum value of E. coli is <3.0 MPN/gram. In the process of dissecting the fish and releasing all the contents of the stomach can contaminate the muscles. Surgery for fish should be done in aseptic conditions so that muscle sample is carefully separated from the intestine [28].

*Escherichia coli* can enter the human body mainly through the consumption of contaminated food, such as raw meat, half-cooked meat, raw milk, and faecal contamination of water and food [9]. Over the years *Escherichia coli* is suspected as one of the causes of diarrhea that arises in humans, especially in children which results in death [30].

4.5. **Histamine Level Test (SNI 2354.10: 2016)**

Histamine is a chemical that is toxic if found in large quantities in the body of fish. Fish from the Scombridae family naturally contain histamine, histidine changes will become histamine if the fish species die [31]. Histamine has been identified as a chemical that is significantly dangerous in the implementation of HACCP in fish processing [32]. Histamine functions as a chemical indicator of fish decay [33]. Histamine is formed by decarboxylation of microbes from histidine as a result of the long storage time and improper storage temperature in certain fish species. Histamine poisoning is often referred to as
"scombrotoxin poisoning" because of the frequent association of disease with consumption of scombroid, such as tuna, bonito and mackerel.

Histamine poisoning caused by consumption of fish products that contain high histamine and occurs when the human body's metabolic capacity is saturated [34]. In most cases, the histamine level in these fish is more than 200 mg / kg and is often greater than 500 mg / kg. Histamine is produced by free histidine decarboxylation and results in activation of endogenous histidine or bacterial decarboxylase (HDC) [34]. Histamine poisoning can be prevented by immediately cooling the fish for consumption. Ideally fish are stored at 0°C or less to prevent bacterial growth and activation of histidine decarboxylase. Cooking or freezing contaminated fish can destroy bacteria but not destroy toxins [35].

Test of histamine content refers to SNI 2354.10: 2016 using a spectrofluorometer. Maximum histamine levels were 100 mg / kg. The results of histamine levels in imported mackerel fish ranged from 0.011 to 0.030 mg/g. Based on the results of the laboratory testing, the histamine levels in imported mackerel fish were still within the threshold so that they were feasible and safe for consumption. Preventive measures for histamine formation are primarily based on preventing or delaying the growth of histamine-forming bacteria (Morganella morganii, Klebsiella pneumoniae, Proteus vulgaris, and Hafnia alvei) and also slowing down the enzyme activity produced by the bacteria. Because of this, storage time and temperature control are mainly used for limiting criteria to monitor histamine formation. The histamine-forming bacteria are able to grow and produce histamine over a wide temperature range. Histamine is formed more commonly as a result of high temperature and long-term deterioration.

There are several ways to control histamine formation of fish products. The freezing can deactivate enzyme-forming bacteria. However, once the histidine decarboxylase enzyme has been formed, it can continue to produce histamine in fish even if the bacteria are inactive. The enzymes can be active at or near the temperature coolant. The enzymes tend to remain stable while frozen and can be reactivated very quickly after thawing. Both enzymes and bacteria can be inactivated by cooking. For this reason, the development of histamine is more likely to occur in raw fish and frozen fish. Because of this, it is important to control the formation of previous histamine processing, namely at the raw material stage [36].

4.6. Endoparasitic examination.

Examination of endoparasites was carried out visually and using a microscope. The identification of worm parasites from 250 samples of fish that had been examined in the interior and internal organs of the imported mackerel (S. japonicus). In this study one species was found, namely Anisakis sp. The worm from the Order Ascaridida is found in cysts and attached to the surface of the walls of the stomach, liver, stomach, muscles, and intestines (mucosa and lumen). The research parameters observed were the prevalence and intensity of parasites. The results showed that the lowest prevalence rate of Anisakis sp. in imported mackerel was 12% and the highest was 100%. The prevalence value of 12 means that the infection is classified as a frequent occurrence and that the value of 100% has a very severe infection (always). The smallest intensity is 3 and the greatest intensity value is 25. The intensity value of 3 is categorized as low intensity category and the value of 25 is classified as moderate intensity [12].

Anisakis sp. worm indirectly transmitted through food contaminated with eggs and larvae. Worm eggs hatch and become larvae that live in free waters and are eaten by intermediary hosts I (arthropods, copepods), and if the host between I is consumed by the intermediary host II then indirectly the fish will be infected by the Anisakis simplex worms [37]. At the time of examination in the laboratory, the mackerel fish stomach that was examined was a lot of acetes (rebon shrimp) which were the intermediary hosts I. The result Anisakis sp. worm found in organs in imported mackerel examined in this study can see in the Figure 2.
Figure 2. *Anisakis* sp. worm found in organs in imported mackerel

Description:

a. *Anisakis* sp. worm found stuck in the intestine of mackerel
b. *Anisakis* sp. worm in physiological NaCl solution
c. Morphology of *Anisakis* sp. anterior part with boring tooth1 (magnification of 100 X)
d. Morphology of *Anisakis* sp. posterior to mucron2 (magnification of 100 X)

The parasites that enter the human body are the third-stage larvae that enter with fish meat that is eaten and not cooked properly. In the human body, the larvae will live and generally remain as third-stage larvae, but sometimes also develops to the fourth stage larvae or larvae changing skin. In this case humans act as paratenic hosts. The larvae attack the sub mucosa but can also reach organs in the abdominal cavity [37]. The preventive measures to avoid transmission of *Anisakis* sp. to humans by avoiding the consumption of raw or undercooked fish, including salted, smoked, sauced or marinated fish or preparations for processed fish that are not sufficiently cooked (microwave oven or grill) [38]. Each cooking fish or squid must reach a core temperature of at least 60ºC. The fish should be frozen at -20ºC for at least 72 hours before preparation for consumption. Fish that have been captured must be prioritized and immediately frozen as it is feared that the parasites have entered the meat

Parasitic microhabitat is an environment or place that supports parasite life. The environment or place of residence must be available for food, oxygen and other factors including inter-species competition. *Anisakis* spread in several organs, to complete its life cycle. The stomach and intestine are preferential locations for *Anisakis* sp. These preferential factors can be influenced by ease of access to nutrients that is a place to process food and absorb nutrients. The small intestine provides a nutritional source for the nematode including blood, tissue cells, body fluids and food extracts contained in the lumen of the small intestine and is a place to process food and absorb nutrients. The digestive tract of fish is the organ most attacked by the *Anisakis* sp. Habitat and spread of parasitic worms in the intestine can be
affected by the structure and the physiology of the intestine, which affects the presence and number of parasites. Therefore, \textit{Anisakis} sp. is found more in the intestine areas to utilize the remains of organic matter in the body of the fish[39].

5. Conclusion

The test results in laboratories of the imported mackerel fish that enter through Tanjung Emas Port:

1. The imported mackerel were not detected with RSIVD so that it fulfills the requirement because it does not have the potential to spread harmful fish pests and diseases, endangering the sustainability of fish resources and the environment, human health, and fisheries business continuity.

2. The imported mackerel fish were detected with \textit{Anisakis} sp. with a prevalence of 12-100%, so it does not fulfill the requirements of the quality of the raw material because of the potential to spread harmful fish pests and diseases, endangering the sustainability of fish resources and the environment, human health, and the sustainability of fisheries.

3. The results of product quality analysis with organoleptic tests obtained the results value of 6-8 (Scale 1-9). From the 10 imported entries, there was 1 imported entry that did not meet the raw material quality requirements, with the score of 6.

4. TPC testing obtained a value of 4,200 - 650,000 colonies/gram. Of the 10 import entries that enter, there is one imported entry that does not meet the raw material quality requirements, which got the value of 650,000 colonies / gram.

5. \textit{E. coli} test obtained the same value which is 3.0 APM/gram, so that it meets the quality requirements standard for raw materials and it is safe for consumption.

6. The histamine level testing of the imported mackerel fish obtained a value of 0.011 to 0.030 mg/kg, thus it fulfills the requirements for standard quality of raw materials and safe for consumption.

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