PEER REVIEW

A3

LEMBAR
HASIL PENILAIAN SEJAWAT SEBIDANG ATAU PEER REVIEW
KARYA ILMIAH : JURNAL ILMIAH

Judul Jurnal Ilmiah (Artikel) : Genetic Variation of Hampala Fish (Hampala macrolepidota) Population in PB Soedirman Reservoir and Serayu River.

Penulis Jurnal Ilmiah *) :
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2 Sorta Basar Ida Simanjuntak
3 Sri Sukmaningrum

Jumlah Penulis : 3

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A3

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Genetic Variation of Hampala Fish (*Hampala macrolepidota*) Population in PB. Soedirman Reservoir and Serayu River

✉**Suhestri Suryaningsih, Sorta Basar Ida Simanjuntak, Sri Sukmaningrum**

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Abstract

Panglima Besar Soedirman waters reservoir and the Serayu River in Banjarnegara Regency, Central Java is one of the habitats of hampala fish . Hampala fish is a member of the Cyprinidae family, which has economic value but is fully captured from the wild. The study on the genetic diversity using approaches of isozyme analysis needed to support conservation and domestication of the fish in this area. This study was aimed at the genetic variation of the hampala fish population in PB. Soedirman water reservoir and the Serayu River in Banjarnegara Regency based on esterase (EST), acid phosphatase (ACP), peroxidase (PER), and aspartate aminotransferase (AAT). Visualization of the isozyme was carried out employing horizontal electrophoretic technique with potato starch. From the results of this study it can be concluded that the hampala fish from the reservoir of PB. Soedirman, Serayu River area before reservoir and after reservoir, all of which are in Banjarnegara Regency, can visualized isozymes EST, ACP, and AAT well, except PER isozyme. This finding can be used as based information on population genetics and finally can be used for conservation of this fish. The results of this study are expected to be utilized to evaluate the potential genetic condition of hampala fish, which is the basis for conservation strategy and domestication.

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INTRODUCTION

The hampala fish (*Hampala macrolepidota* Kuhl & van Hasselt, 1823) is a freshwater fish from the Cyprinidae family. Hampala fish have natural habitats in various types of waters such as rivers, lakes and reservoirs. Management of various types of waters is essential to ensure the sustainability of its ecological functions. Effective management policies will only be achieved if it is arranged based on support from various scientific information, including data on the fish species (Nuryanto *et al.*, 2017). Hampala fish is found in several rivers in Java, for examples in the Logawa River (Lestari, 2004), in the Serayu River midstream area that flows in the districts of Banjarnegara and Purbalingga (Suryaningsih, 2008), and downstream of the Serayu River in Cilacap (Murtiningsih *et al.*, 2009), in the downstream area of the Opak River, Yogyakarta (Djumanto *et al.*, 2013), in the PB. Soedirman Reservoir, and the Serayu River, Banjarnegara (Suryaningsih *et al.*, 2014). In Sumatra, it is found in the upper of Asahan River (Simanjuntak, 2012), at Lake Kerinci Jambi (Samuel & Suryati, 2014), Batang Toru River (Roesma *et al.*, 2016). In Borneo is found in Floodplain Batang Kerang, Sarawak (Rahim *et al.*, 2009). In other regions, it is found in China, on the Nanla River, Mengla County, Yunnan (Ming-Dian *et al.*, 2015). In Northern Thailand, found upstream of Wang River (Petsut & Kulabtong, 2015).

Hampala fish have important economic value, because many traded by society in fresh condition and processed. The meat is thick and without the thorny spines. The body length of hampala fish up to 33.80 cm with a weight of 477.32 grams (Suryaningsih *et al.*, 2014). Hampala fish include wild freshwater fish, potentially to be domesticated. According to Sukamsipoetro (2003), the high demand for economic fish such as baceman and kething in Klawing River Purbalingga, Central Java caused the arrests to continue with other economic fish, including hampala fish located in Klawing River and Serayu River. It is feared that the decline in populations of some important economical fish species, including hampala fish, makes it endangered. Declining populations of fish species in both quantity and quality can lead to a decrease in genetic variation, which may further lead to the declinee in the ability to adapt to the environment. Further impact of the species is vulnerable to extinction (Nuryanto & Solichin, 2006). *Hampala macrolepidota* has been included in the red list IUCN (Allen, 2013), whereas this species is germplasm that has economic value and not yet cultivated. Therefore, the initial step to prevent its extinction is needed, such as by doing conservation and domestication efforts against the species.

In order to support the effort of conservation and domestication of hampala fish, it is needed the availability of biological data from many aspects, such as by knowing the genetic variation. The study of genetic variation is a very important aspect in the conservation and utilization of germplasm. One possible approach to evaluate the condition of gene variation in a population of natural species is the application of biochemical techniques in the form of isozyme or allozyme analysis. Isozyme or allozyme is an enzyme having different molecular form but has the same catalytic activity of a tissue or organ (Suranto, 2000). According to Adams (*in* Mansyah, 1999), studies of genetic variation based on the polymorphism of some isozyme loci can give an idea the genetic variation condition of the species population studied. Different alleles will be inherited co-dominantly so that the heterozygotes individual can be distinguished from the homozygotes individual based on the appearance of the ribbon pattern. Isozymes can be separated by electrophoretic techniques on polyacrylamide gel or starch gel. The results are zymograms that can be genetically interpreted (Indriani *et al.*, 2002).

The most widely used isozymes for the study of genetic variation in fish include esterase (EST), peroxidase (PER), acid phosphatase (ACP), malate dehydrogenase (MDH), SOD (superoxide dismutase), alcohol dehydrogenase (ADH) and amino acids transfer (AAT). Of the seven isozymes used in the javanis barb fish (*Puntius orphoides*) population it is apparent that the visualized (emerging pattern of ribbon) is clearly only five isozymes i.e. EST, ACP, MDH, ADH and AAT (Suryaningsih, 2009). In the study of trout (*Salmo trutta*) used AAT, MDH, SOD, EST, and EST-D that can visualize a clear ribbon pattern. Zhigileva *et al.* (2010) in the study on genetic subdivision populations of roach *Rutilus rutilus lacustris*, ide *Leuciscus idus*, and dace *L. leuciscus baicalensis* living in rivers of West Siberia used NAD, MDH, LDH, SOD, AAT and EST.

This study aimed to determine the condition of genetic variation of the hampala fish population in the reservoir of PB. Soedirman, the Serayu River area before and after the reservoir, in Banjarnegara Regency, Central Java, by polymorphism isozyme esterase, peroxidase, acid phosphatase and aminotransferase. The results of this study are expected to be utilized to evaluate the potential genetic condition of hampala fish, which is the basis for conservation strategy and domestication.

METHODS

Fish sources

The research material is hampala fish with body length 20,7-33,2 cm and weight 93,37-398,50 gram totaled 15. The research method used was survey by group sampling. The fish caught by sein net mesh size 1 inch from three different location, i.e. the PB. Soedirman Reservoir, the Serayu River area before and after the reservoir, in Banjarnegara Regency, Central Java. Hampala fish identification based on Kottelat *et al.* (1993) and Binohlan (2017).

Procedure

Work procedures for the enzymes extraction, gel buffers and electrode buffers, starch gel, electrophoresis, staining and zymogram preparation follows Sugama *in* Suryani *et al.* (2001) and Nuryanto *et al.* (2003).The starch gel was prepared by dissolving 10% starch. The starch was dissolved in 1/3 of the gel buffer part and shaken until homogeneous, and then added the heated gel buffer 2/3 of part again the and shaken. Subsequently, the starch mixture was heated again in the microwave until it boils, until the gel is clear. The gel is vacuumed to remove air bubbles. The gel is poured into a mold previously vaselinee coated to avoid sticking to the bottom of the mold. Gel is covered in plastic and stored in the refrigerator for 24 hours. The gel is perforated according to the number of samples to be tested.

Enzyme extraction was done by grinding 5 grams of hampala fish meat until smooth using quartz sand and 0.5 ml buffer extract. The enzyme loading into the gel is done by inserting a piece of Whatman filter paper into the meat extract. The piece of filter paper is lifted and cleaned using paper towels, then the piece of filter paper is inserted into the prepared starch gel and given a hole. To control the enzyme migration rate, in one of the opening ports of the gel is inserted filter paper dipped into the bromphenol blue. The gel mold inserted with the sample paper is inserted into an electrophoresis tray containing the electrode buffer and connected to the electric field at 100 volts and a strong current of 18 milliamperes for approximately 4 hours, then the gel is dyed EST, ACP, PER and ATT. Next was washed with water to flow clean and fixed using a mixture of glycerol: ethanol (1: 1), then do the observation and shooting.

Data analysis

The observed data were obtained from the result of visualization of isozyme banding pattern based on horizontal starch gel electrophoresis technique from hampala fish meat. The obtained data was then transferred into the zymogram and the allele frequency calculation, the percentage of the polymorphic locus and the average heterozygosity according to Suryani *et al.* (1996), as follows:

Allele frequencies : $p = (2Ho + He) / 2N$

N = Number of individuals analyzed
Ho = the number of one homozygous genotype
He = the number of heterozygous genotypes

If the value $p \geq 0.095$, the locus is called monomorphic, if the value of $p < 0.95$ the locus is called polymorphic.

Calculating Percentage of Polymorphic Loci:
= number of polymorphic loci / total number of loci observed x 100%
Counting number of allele per locus:
= number of jumlah allele / number of locus
Average Heterozygosity :

Describing the proportion of the observed heterozygous locus, averaged over all the loci tested in a population, calculated by the formula :
He = number of genotype heterozygote / number of samples x number of loci

Data on the calculation of allele frequencies and heterozygosity were analyzed descriptively to evaluate the condition of genetic variation of hampala fish from the three sampling areas.

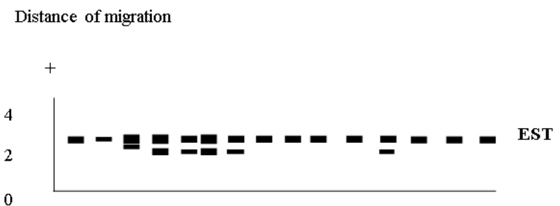
RESULTS AND DISCUSSION

Expression of Band Pattern Isozymes

From the four isozymes used, almost all are well exposed / emerging patterns of the bands, although there are some individual samples of hampala fish that do not appear in the pattern of the band, that is in PER isozyme. The absence of isozyme banding pattern according to Richardson *et al.* (2012), among others, can be caused by three possibilities. First, the isozyme molecule does not experience migration, so it stays in the well of electrophoresis. Second, isozymes migrate, but denaturation occurs, so it becomes inactive. Third, isozymes are not expressed on the tested sample tissue. The expression of the band pattern on the hampala fish of the isozymes EST, ACP, PER, and AAT are shown in Figure 1-3.

Isozyme EST is expressed by two band patterns of thick and thin bands , but almost all sample populations in the reservoir waters of the PB. Soedirman, in the waters of Serayu River before and after the reservoir, Banjarnegara Regency with less ideal band formation (Figure 1). According to Richardson (2012), ideal band formation is capable of expressing thin and sharp bands, while those experiencing irregularities will be expressed thickly. The thick band is thought to have a large molecular weight that has not been separated properly. Thick bands are thought to have formed due to the merging of bands that are very close.

The direction of sample migration with isozyme EST toward positive pole (anode), with different migration distance. This shows the difference in molecular weight of the isozyme. Nur and Adijuwana (1989) stated that molecules that have a greater molecular weight would move slowly. According Sugiri *in* Nuryanto (2001), that condition also provides information about a genetic variation on a chromosome locus and genetic variation at different loci.

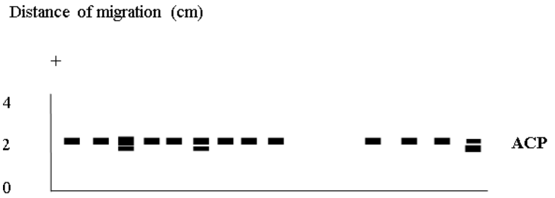


A	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
B	1	2	3	4	5	4	5	1	1	1	1	5	1	1	1
C	1	1	2	2	2	2	2	1	1	1	1	2	1	1	1

Figure 1. Isozyme esterase band pattern (EST) of hampala fish in PB.Soedirman reservoir (6-10), river area before reservoir (1-5), river area after reservoir (11-15) A: sample number, B: banding pattern, C: number of bands

The result of EST isozyme electrophoresis in the hampala fish shows that this isozyme is regulated by one locus, where the locus is polymorphic, both in the population of PB.Soedirman reservoir (sample number 6-10), river area before reservoir (sample number 1-5) and river area after reservoir (sample number 11-15) (Figure 2).

Zymogram EST in reservoir area populations (samples 6-10) expresses 3 groups of bands, the majority of which are medium-thick bands, and each of these bands has a sub-unit structure of monomers and dimers. In the reservoir area population (sample number 1-5) expressing 5 groups of bands, it also has the structure of monomer sub-units and dimers. Similarly in the river area after reservoir population (sample number 11-15), which expresses only two groups of bands, has a sub-unit structure of monomers and dimers. This is in accordance with Suryaningsih (2009) research on Javanis barb fish in the Klawing River which is a tributary of the Serayu River, which states that EST isozymes present monomorphic and polymorphic loci. However, EST isozymes can express more than one locus. Similarly, on the mangrove crab from Cilacap and Pemalang (Suryaningsih & Kusbiyanto, 2009) and the crab from Cilacap (Arnawati, 2003).



A	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
B	1	1	2	1	1	2	1	1	1	0	0	5	1	1	3
C	1	1	2	1	1	2	1	1	1	0	0	2	1	1	2

Figure 2. Isozyme acid phosphatase (ACP) of hampala fish in PB.Soedirman reservoir (6-10), area before reservoir (1-5) and after reservoir (11-15) A: sample number, B: banding pattern, C: number of bands

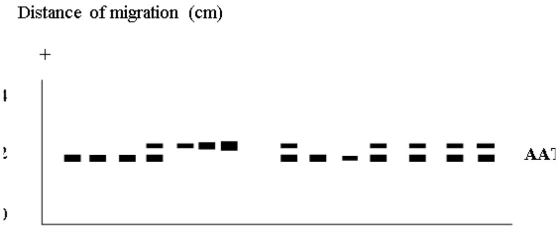
The result of ACP isozyme electrophoresis in the hampala fish indicates that this isozyme is regulated by one locus, where the locus is monomorphic, both in the PB.Soedirman reservoir population (sample number 6-10), the river area before the reservoir (sample number 1-5) and river area after reservoir on sample number 11-15) (Figure 2).

The ACP zymogram in the reservoir area population (samples 6-10) expresses two groups of bands, the majority of which are medium-thick bands, and each of these bands has a sub-unit structure of monomers and dimers. Similarly, in the reservoir area population (samples 1-5) and the post-reservoir area population (sample number 11-15), it also expresses only two groups of ribbon, with the structure of monomer sub-units and dimers. Similarly, the results of Suryaningsih (2009) research on javanis barb fish in the Klawing River which is a tributary of the Serayu River, that the ACP isozyme reveals the monomorphic locus. Similarly, on the mangrove crab from Cilacap and Pemalang (Suryaningsih & Kusbiyanto, 2009) and the crab from Cilacap (Arnawati,2003), on tiger shrimp from Brebes, Tegal and Cilacap ponds.

Isozim PER in hampala fish was not expressed by all individuals either in the reservoir area, river area before or after reservoir. Unlike the case with mangrove crabs (*Scylla serrata* Forsk) (Suryaningsih & Kusbiyanto, 2009), most individual samples express it even though it shows only one banding pattern with the same direction and distance of migration. The emerging band pattern indicates that PER in the mangrove crab is regulated by a homozygous locus, a similar condition to that occurring in fish *Oxyeleotris marmorata* from the Penjalin Reservoir (Susanto & Suryaningsih, 2006).

AAT isozyme is visualized with two groups of thick and thin bands with less ideal coverage. The result of the AAT isozyme electrophoresis in the hampala fish shows that this isozyme is regulated by one locus, the locus is monomorphic, both in the PB.Soedirman reservoir population (samples 6-7), the river area before the reservoir (samples 1-5) and after reservoir (sample number 11-15) (Figure 3).

The AAT zymogram in the hampala fish from three locations pointed toward the Anode pole and produced 3 band patterns in the reservoir population, and 3 ribbon patterns in river area before the reservoir population, and 2 in the river area after the reservoir. The isozyme is regulated by one polymorphic locus in all three populations, and all of which have monomer and dimer structures. The results of the Javaen barb fish from the Klawing River have 7, a much higher band pattern (Suryaningsih, 2009), with monomer and dimer structures. In *Penaeus monodon* shrimp AAT is governed by two loci (Sugama *et al.*, 2017) whereas in *Oxyeletris marmorata* fish is only regulated by one locus and not all samples are expressed (Susanto & Suryaningsih, 2006).



A	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
B	1	1	1	2	3	4	5	0	2	1	1	4	4	4	4
C	1	1	1	2	1	1	1	0	2	1	1	2	2	2	2

Figure 3. Isozyme aspartate amino transaminase (AAT) of hampala fish in reservoir PB.Soedirman (6-10), river area before reservoir (1-5) and after reservoir (11-15); A: sample number, B: banding pattern, C: number of bands

The genetic variation of hampala fish

Genetic variation is determined by the frequency of alleles, polymorphism, and average heterozygosity. The higher of the average value of heterozygosity, the higher genetic variation. Genetic variation is a reflection of the properties of heredity that will be passed from parent to offspring. The nature of heredity is reflected in growth, survival, disease resistance and feed conversion value (Leary in Arnawati, 2003). Based on the interpretation of the ribbon pattern on the starch gel, of 9 loci expressed from 4 isozymes (EST, ACP, PER, and AAT), the allele frequency values, the number of polymorphic loci and average heterozygosity are presented in Table 1.

Based on the observations in the sampling area (Table 1), it can be seen that the hampala fish from the reservoir of PB. Soedirman can express 3 loci from 4 isozymes EST, ACP, PER and AAT, all of which are polymorphic. PER isozyme cannot express locus. Thus the degree of polymorphism achieved is 1.00 or 100%. The value of the locus polymorphism depicts the genetic variation level of the hampala fish population from the PB Soedirman reservoir.

Next to the river area before the PB. Soedirman reservoir, also expresses 3 loci from 4 isozymes, i.e., EST, ACP, PER and AAT, but only 2 loci are polymorphic. The PER isozyme in this sampling area also can not express the locus. Thus the degree of polymorphism achieved in this area is 0.66 or 66%.

The latter, in the sampling area after the PB.Soedirman reservoir, express 3 loci from 4 isozymes, i.e., EST, ACP, PER and AAT, all of which are polymorphic. PER isozyme also remains unable to express the locus. Thus the degree of polymorphism achieved in this sampling area is 1.00 or 100%. The value of the locus polymorphism depicts the genetic variation level of the hampala fish population from the PB. Soedirman reservoir.

Observations have been made based on the degrees of polymorphism, indicating that the best genetic variation is the hampala fish population from the river area after the PB. Soedirman reservoir and waters of the PB. Soedirman reservoir , in the same value, followed by the river area before the PB. Soedirman reservoir. Based on the degree of polymorphism, it can be said that the genetic variation of hampala fish in Serayu River Kabupaten Banjarnegara is still good because the degree of polymorphism is approaching 90%. As a comparison of genetic variation of Javaen barb fish in the Klawing River which is a tributtary of the Serayu River is 39,9% (Suryaningsih, 2009). However, the fact that the hampala fish is difficult to obtain in the sampling area is feared that in a relatively short period it will be a decrease in genetic variation. This condition can threaten

Table 1. Data number of loci, number of genotypes, allele frequency, average heterozygosity and polymorphism on hampala fish from waters of PB Soedirman reservoir, river area before and after the reservoir, in the Serayu River, Banjarnegara Regency

Population	Locus	Number of Genotypes			N	Allele Frequency		P/M
		AA	Aa	aa		A	a	
PB. Soedirman reservoir	EST	2	3	0	5	0.7	0.3	P
	ACP	4	1	0	5	0.9	0.1	P
	PER	0	0	0	0	0	0	-
	AAT	1	2	2	5	0.4	0.6	P
Total		7	5	2	14			**1.0**
River area before PB. Soedirman reservoir	EST	3	2	0	5	0.8	0.2	P
	ACP	3	1	0	4	0.875	0.125	P
	PER	0	0	0	0	0	0	-
	AAT	2	0	2	4	1	0	M
Total		8	3	2	13			**0.66**
River area after PB. Soedirman reservoir	EST	4	1	0	5	0.9	0.1	P
	ACP	3	1	0	4	0.875	0.125	P
	PER	0	0	0	0	0	0	-
	AAT	1	4	0	5	0.6	0.4	P
Total		8	6	0	14			1.0

Note:
A= alleles with fast migration (*fast ellele*)
a= alleles with slow migration (*slow allele*)
N= the number of individuals expressing the ribbon
He-r = average heterozygous value
M= monomorphic, P = polimorphic
*) a locus is considered polymorphic if the most frequent allele appear < 0,95 (Suryani *et al.,* 2001)

the sustainability of hampala fish, so this species is urgent for conservation, although hampala fish is an important local commodity. Hadie *et al.* (2000) state that conservation efforts can be done in-situ and ex-situ. In-situ conservation is done by arranging a reservation and reconstruction system. Ex-situ conservation includes population maintenance that provides domestication efforts and cultivation and gene management about several factors including population size, inbreeding rates and genetic drift. However this research results supporting scientific information of population genetics of *hampala* and furthermore can be used as a tool for conservation and dometication.

CONCLUSION

Based on the results and the discussion it can be concluded that the hampala fish from the study area, can express isozyme EST, ACP, and AAT well, except PER isozyme.and the genetic variation of the hampala fish in all three locations observed is still good.. The results of this study are expected to be utilized to evaluate the potential genetic condition of hampala fish, which is the basis for conservation strategy and domestication.

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TURNITIN

Genetic Variation of Hampala Fish (Hampala macrolepidota) Population in PB. Soedirman Reservoir and Serayu River

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Genetic Variation of Hampala Fish (*Hampala macrolepidota*) Population in PB. Soedirman Reservoir and Serayu River

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Abstract

Panglima Besar Soedirman waters reservoir and the Serayu River in Banjarnegara Regency, Central Java is one of the habitats of hampala fish . Hampala fish is a member of the Cyprinidae family, which has economic value but is fully captured from the wild. The study on the genetic diversity using approaches of isozyme analysis needed to support conservation and domestication of the fish in this area. This study was aimed at the genetic variation of the hampala fish population in PB. Soedirman water reservoir and the Serayu River in Banjarnegara Regency based on esterase (EST), acid phosphatase (ACP), peroxidase (PER), and aspartate aminotransferase (AAT). Visualization of the isozyme was carried out employing horizontal electrophoretic technique with potato starch. From the results of this study it can be concluded that the hampala fish from the reservoir of PB. Soedirman, Serayu River area before reservoir and after reservoir, all of which are in Banjarnegara Regency, can visualized isozymes EST, ACP, and AAT well, except PER isozyme. This finding can be used as based information on population genetics and finally can be used for conservation of this fish. The results of this study are expected to be utilized to evaluate the potential genetic condition of hampala fish, which is the basis for conservation strategy and domestication.

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INTRODUCTION

The hampala fish (*Hampala macrolepidota* Kuhl & van Hasselt, 1823) is a freshwater fish from the Cyprinidae family. Hampala fish have natural habitats in various types of waters such as rivers, lakes and reservoirs. Management of various types of waters is essential to ensure the sustainability of its ecological functions. Effective management policies will only be achieved if it is arranged based on support from various scientific information, including data on the fish species (Nuryanto *et al*., 2017). Hampala fish is found in several rivers in Java, for examples in the Logawa River (Lestari, 2004), in the Serayu River midstream area that flows in the districts of Banjarnegara and Purbalingga (Suryaningsih, 2008), and downstream of the Serayu River in Cilacap (Murtiningsih *et al*., 2009), in the downstream area of the Opak River, Yogyakarta (Djumanto *et al*., 2013), in the PB. Soedirman Reservoir, and the Serayu River, Banjarnegara (Suryaningsih *et al*., 2014). In Sumatra, it is found in the upper of Asahan River (Simanjuntak, 2012), at Lake Kerinci Jambi (Samuel & Suryati, 2014), Batang Toru River (Roesma *et al*., 2016). In Borneo is found in Floodplain Batang Kerang, Sarawak (Rahim *et al*., 2009). In other regions, it is found in China, on the Nanla River, Mengla County, Yunnan (Ming-Dian *et al*., 2015). In Northern Thailand, found upstream of Wang River (Petsut & Kulabtong, 2015).

Hampala fish have important economic value, because many traded by society in fresh condition and processed. The meat is thick and without the thorny spines. The body length of hampala fish up to 33.80 cm with a weight of 477.32 grams (Suryaningsih *et al*., 2014). Hampala fish include wild freshwater fish, potentially to be domesticated. According to Sukamsipoetro (2003), the high demand for economic fish such as baceman and kething in Klawing River Purbalingga, Central Java caused the arrests to continue with other economic fish, including hampala fish located in Klawing River and Serayu River. It is feared that the decline in populations of some important economical fish species, including hampala fish, makes it endangered. Declining populations of fish species in both quantity and quality can lead to a decrease in genetic variation, which may further lead to the declinee in the ability to adapt to the environment. Further impact of the species is vulnerable to extinction (Nuryanto & Solichin, 2006). *Hampala macrolepidota* has been included in the red list IUCN (Allen, 2013), whereas this species is germplasm that has economic value and not yet cultivated. Therefore, the initial step to prevent its extinction is needed, such as by doing conservation and domestication efforts against the species.

In order to support the effort of conservation and domestication of hampala fish, it is needed the availability of biological data from many aspects, such as by knowing the genetic variation. The study of genetic variation is a very important aspect in the conservation and utilization of germplasm. One possible approach to evaluate the condition of gene variation in a population of natural species is the application of biochemical techniques in the form of isozyme or allozyme analysis. Isozyme or allozyme is an enzyme having different molecular form but has the same catalytic activity of a tissue or organ (Suranto, 2000). According to Adams (*in* Mansyah, 1999), studies of genetic variation based on the polymorphism of some isozyme loci can give an idea the genetic variation condition of the species population studied. Different alleles will be inherited co-dominantly so that the heterozygotes individual can be distinguished from the homozygotes individual based on the appearance of the ribbon pattern. Isozymes can be separated by electrophoretic techniques on polyacrylamide gel or starch gel. The results are zymograms that can be genetically interpreted (Indriani *et al*., 2002).

The most widely used isozymes for the study of genetic variation in fish include esterase (EST), peroxidase (PER), acid phosphatase (ACP), malate dehydrogenase (MDH), SOD (superoxide dismutase), alcohol dehydrogenase (ADH) and amino acids transfer (AAT). Of the seven isozymes used in the javanis barb fish (*Puntius orphoides*) population it is apparent that the visualized (emerging pattern of ribbon) is clearly only five isozymes i.e. EST, ACP, MDH, ADH and AAT (Suryaningsih, 2009). In the study of trout (*Salmo trutta*) used AAT, MDH, SOD, EST, and EST-D that can visualize a clear ribbon pattern. Zhigileva *et al.* (2010) in the study on genetic subdivision populations of roach *Rutilus rutilus lacustris*, ide *Leuciscus idus*, and dace *L. leuciscus baicalensis* living in rivers of West Siberia used NAD, MDH, LDH, SOD, AAT and EST.

This study aimed to determine the condition of genetic variation of the hampala fish population in the reservoir of PB. Soedirman, the Serayu River area before and after the reservoir, in Banjarnegara Regency, Central Java, by polymorphism isozyme esterase, peroxidase, acid phosphatase and aminotransferase. The results of this study are expected to be utilized to evaluate the potential genetic condition of hampala fish, which is the basis for conservation strategy and domestication.

METHODS

Fish sources

The research material is hampala fish with body length 20,7-33,2 cm and weight 93,37-398,50 gram totaled 15. The research method used was survey by group sampling. The fish caught by sein net mesh size 1 inch from three different location, i.e. the PB. Soedirman Reservoir, the Serayu River area before and after the reservoir, in Banjarnegara Regency, Central Java. Hampala fish identification based on Kottelat *et al*. (1993) and Binohlan (2017).

Procedure

Work procedures for the enzymes extraction, gel buffers and electrode buffers, starch gel, electrophoresis, staining and zymogram preparation follows Sugama *in* Suryani *et al*. (2001) and Nuryanto *et al.* (2003).The starch gel was prepared by dissolving 10% starch. The starch was dissolved in 1/3 of the gel buffer part and shaken until homogeneous, and then added the heated gel buffer 2/3 of part again the and shaken. Subsequently, the starch mixture was heated again in the microwave until it boils, until the gel is clear. The gel is vacuumed to remove air bubbles. The gel is poured into a mold previously vaselinee coated to avoid sticking to the bottom of the mold. Gel is covered in plastic and stored in the refrigerator for 24 hours. The gel is perforated according to the number of samples to be tested.

Enzyme extraction was done by grinding 5 grams of hampala fish meat until smooth using quartz sand and 0.5 ml buffer extract. The enzyme loading into the gel is done by inserting a piece of Whatman filter paper into the meat extract. The piece of filter paper is lifted and cleaned using paper towels, then the piece of filter paper is inserted into the prepared starch gel and given a hole. To control the enzyme migration rate, in one of the opening ports of the gel is inserted filter paper dipped into the bromphenol blue. The gel mold inserted with the sample paper is inserted into an electrophoresis tray containing the electrode buffer and connected to the electric field at 100 volts and a strong current of 18 milliamperes for approximately 4 hours, then the gel is dyed EST, ACP, PER and ATT. Next was washed with water to flow clean and fixed using a mixture of glycerol: ethanol (1: 1), then do the observation and shooting.

Data analysis

The observed data were obtained from the result of visualization of isozyme banding pattern based on horizontal starch gel electrophoresis technique from hampala fish meat. The obtained data was then transferred into the zymogram and the allele frequency calculation, the percentage of the polymorphic locus and the average heterozygosity according to Suryani *et al.* (1996), as follows:

Allele frequencies : $p = (2Ho + He) / 2N$

N = Number of individuals analyzed
Ho = the number of one homozygous genotype
He = the number of heterozygous genotypes

If the value $p \geq 0.095$, the locus is called monomorphic, if the value of $p < 0.95$ the locus is called polymorphic.

Calculating Percentage of Polymorphic Loci:
= number of polymorphic loci / total number of loci observed x 100%
Counting number of allele per locus:
= number of jumlah allele / number of locus
Average Heterozygosity :

Describing the proportion of the observed heterozygous locus, averaged over all the loci tested in a population, calculated by the formula : He = number of genotype heterozygote / number of samples x number of loci

Data on the calculation of allele frequencies and heterozygosity were analyzed descriptively to evaluate the condition of genetic variation of hampala fish from the three sampling areas.

RESULTS AND DISCUSSION

Expression of Band Pattern Isozymes

From the four isozymes used, almost all are well exposed / emerging patterns of the bands, although there are some individual samples of hampala fish that do not appear in the pattern of the band, that is in PER isozyme. The absence of isozyme banding pattern according to Richardson *et al*. (2012), among others, can be caused by three possibilities. First, the isozyme molecule does not experience migration, so it stays in the well of electrophoresis. Second, isozymes migrate, but denaturation occurs, so it becomes inactive. Third, isozymes are not expressed on the tested sample tissue. The expression of the band pattern on the hampala fish of the isozymes EST, ACP, PER, and AAT are shown in Figure 1-3.

Isozyme EST is expressed by two band patterns of thick and thin bands , but almost all sample populations in the reservoir waters of the PB. Soedirman, in the waters of Serayu River before and after the reservoir, Banjarnegara Regency with less ideal band formation (Figure 1). According to Richardson (2012), ideal band formation is capable of expressing thin and sharp bands, while those experiencing irregularities will be expressed thickly. The thick band is thought to have a large molecular weight that has not been separated properly. Thick bands are thought to have formed due to the merging of bands that are very close.

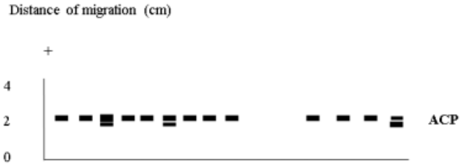
The direction of sample migration with isozyme EST toward positive pole (anode), with different migration distance. This shows the difference in molecular weight of the isozyme. Nur and Adijuwana (1989) stated that molecules that have a greater molecular weight would move slowly. According Sugiri *in* Nuryanto (2001), that condition also provides information about a genetic variation on a chromosome locus and genetic variation at different loci.

A	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
B	1	2	3	4	5	4	5	1	1	1	1	5	1	1	1
C	1	1	2	2	2	2	2	1	1	1	1	2	1	1	1

Figure 1. Isozyme esterase band pattern (EST) of hampala fish in PB.Soedirman reservoir (6-10), river area before reservoir (1-5), river area after reservoir (11-15) A: sample number, B: banding pattern, C: number of bands

The result of EST isozyme electrophoresis in the hampala fish shows that this isozyme is regulated by one locus, where the locus is polymorphic, both in the population of PB.Soedirman reservoir (sample number 6-10), river area before reservoir (sample number 1-5) and river area after reservoir (sample number 11-15) (Figure 2).

Zymogram EST in reservoir area populations (samples 6-10) expresses 3 groups of bands, the majority of which are medium-thick bands, and each of these bands has a sub-unit structure of monomers and dimers. In the reservoir area population (sample number 1-5) expressing 5 groups of bands, it also has the structure of monomer sub-units and dimers. Similarly in the river area after reservoir population (sample number 11-15), which expresses only two groups of bands, has a sub-unit structure of monomers and dimers. This is in accordance with Suryaningsih (2009) research on Javanis barb fish in the Klawing River which is a tributary of the Serayu River, which states that EST isozymes present monomorphic and polymorphic loci. However, EST isozymes can express more than one locus. Similarly, on the mangrove crab from Cilacap and Pemalang (Suryaningsih & Kusbiyanto, 2009) and the crab from Cilacap (Arnawati, 2003).



A	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
B	1	1	2	1	1	2	1	1	1	0	0	5	1	1	3
C	1	1	2	1	1	2	1	1	1	0	0	2	1	1	2

Figure 2. Isozyme acid phosphatase (ACP) of hampala fish in PB.Soedirman reservoir (6-10), area before reservoir (1-5) and after reservoir (11-15) A: sample number, B: banding pattern, C: number of bands

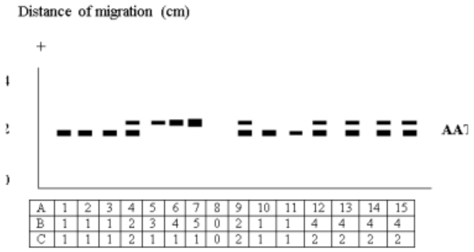
The result of ACP isozyme electrophoresis in the hampala fish indicates that this isozyme is regulated by one locus, where the locus is monomorphic, both in the PB.Soedirman reservoir population (sample number 6-10), the river area before the reservoir (sample number 1-5) and river area after reservoir on sample number 11-15) (Figure 2).

The ACP zymogram in the reservoir area population (samples 6-10) expresses two groups of bands, the majority of which are medium-thick bands, and each of these bands has a subunit structure of monomers and dimers. Similarly, in the reservoir area population (samples 1-5) and the post-reservoir area population (sample number 11-15), it also expresses only two groups of ribbon, with the structure of monomer subunits and dimers. Similarly, the results of Suryaningsih (2009) research on javanis barb fish in the Klawing River which is a tributary of the Serayu River, that the ACP isozyme reveals the monomorphic locus. Similarly, on the mangrove crab from Cilacap and Pemalang (Suryaningsih & Kusbiyanto, 2009) and the crab from Cilacap (Arnawati,2003), on tiger shrimp from Brebes, Tegal and Cilacap ponds.

Isozim PER in hampala fish was not expressed by all individuals either in the reservoir area, river area before or after reservoir. Unlike the case with mangrove crabs (*Scylla serrata* Forsk) (Suryaningsih & Kusbiyanto, 2009), most individual samples express it even though it shows only one banding pattern with the same direction and distance of migration. The emerging band pattern indicates that PER in the mangrove crab is regulated by a homozygous locus, a similar condition to that occurring in fish *Oxyeleotris marmorata* from the Penjalin Reservoir (Susanto & Suryaningsih, 2006).

AAT isozyme is visualized with two groups of thick and thin bands with less ideal coverage. The result of the AAT isozyme electrophoresis in the hampala fish shows that this isozyme is regulated by one locus, the locus is monomorphic, both in the PB.Soedirman reservoir population (samples 6-7), the river area before the reservoir (samples 1-5) and after reservoir (sample number 11-15) (Figure 3).

The AAT zymogram in the hampala fish from three locations pointed toward the Anode pole and produced 3 band patterns in the reservoir population, and 3 ribbon patterns in river area before the reservoir population, and 2 in the river area after the reservoir. The isozyme is regulated by one polymorphic locus in all three populations, and all of which have monomer and dimer structures. The results of the Javaen barb fish from the Klawing River have 7, a much higher band pattern (Suryaningsih, 2009), with monomer and dimer structures. In *Penaeus monodon* shrimp AAT is governed by two loci (Sugama *et al*., 2017) whereas in *Oxyeletris marmorata* fish is only regulated by one locus and not all samples are expressed (Susanto & Suryaningsih, 2006).



A	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
B	1	1	1	2	3	4	5	0	2	1	1	4	4	4	4
C	1	1	1	2	1	1	1	0	2	1	1	2	2	2	2

Figure 3. Isozyme aspartate amino transaminase (AAT) of hampala fish in reservoir PB.Soedirman (6-10), river area before reservoir (1-5) and after reservoir (11-15); A: sample number, B: banding pattern, C: number of bands

The genetic variation of hampala fish

Genetic variation is determined by the frequency of alleles, polymorphism, and average heterozygosity. The higher of the average value of heterozygosity, the higher genetic variation. Genetic variation is a reflection of the properties of heredity that will be passed from parent to offspring. The nature of heredity is reflected in growth, survival, disease resistance and feed conversion value (Leary in Arnawati, 2003). Based on the interpretation of the ribbon pattern on the starch gel, of 9 loci expressed from 4 isozymes (EST, ACP, PER, and AAT), the allele frequency values, the number of polymorphic loci and average heterozygosity are presented in Table 1.

Based on the observations in the sampling area (Table 1), it can be seen that the hampala fish from the reservoir of PB. Soedirman can express 3 loci from 4 isozymes EST, ACP, PER and AAT, all of which are polymorphic. PER isozyme cannot express locus. Thus the degree of polymorphism achieved is 1.00 or 100%. The value of the locus polymorphism depicts the genetic variation level of the hampala fish population from the PB Soedirman reservoir.

Next to the river area before the PB. Soedirman reservoir, also expresses 3 loci from 4 isozymes, i.e., EST, ACP, PER and AAT, but only 2 loci are polymorphic. The PER isozyme in this sampling area also can not express the locus. Thus the degree of polymorphism achieved in this area is 0.66 or 66%.

The latter, in the sampling area after the PB.Soedirman reservoir, express 3 loci from 4 isozymes, i.e., EST, ACP, PER and AAT, all of which are polymorphic. PER isozyme also remains unable to express the locus. Thus the degree of polymorphism achieved in this sampling area is 1.00 or 100%. The value of the locus polymorphism depicts the genetic variation level of the hampala fish population from the PB. Soedirman reservoir.

Observations have been made based on the degrees of polymorphism, indicating that the best genetic variation is the hampala fish population from the river area after the PB. Soedirman reservoir and waters of the PB. Soedirman reservoir , in the same value, followed by the river area before the PB. Soedirman reservoir. Based on the degree of polymorphism, it can be said that the genetic variation of hampala fish in Serayu River Kabupaten Banjarnegara is still good because the degree of polymorphism is approaching 90%. As a comparison of genetic variation of Javaen barb fish in the Klawing River which is a tributtary of the Serayu River is 39,9% (Suryaningsih, 2009). However, the fact that the hampala fish is difficult to obtain in the sampling area is feared that in a relatively short period it will be a decrease in genetic variation. This condition can threaten

Table 1. Data number of loci, number of genotypes, allele frequency, average heterozygosity and polymorphism on hampala fish from waters of PB Soedirman reservoir, river area before and after the reservoir, in the Serayu River, Banjarnegara Regency

Population	Locus	Number of Genotypes			N	Allele Frequency		P/M
		AA	Aa	aa		A	a	
PB. Soedirman reservoir	EST	2	3	0	5	0.7	0.3	P
	ACP	4	1	0	5	0.9	0.1	P
	PER	0	0	0	0	0	0	-
	AAT	1	2	2	5	0.4	0.6	P
Total		7	5	2	14			**1.0**
River area before PB. Soedirman reservoir	EST	3	2	0	5	0.8	0.2	P
	ACP	3	1	0	4	0.875	0.125	P
	PER	0	0	0	0	0	0	-
	AAT	2	0	2	4	1	0	M
Total		8	3	2	13			**0.66**
River area after PB. Soedirman reservoir	EST	4	1	0	5	0.9	0.1	P
	ACP	3	1	0	4	0.875	0.125	P
	PER	0	0	0	0	0	0	-
	AAT	1	4	0	5	0.6	0.4	P
Total		8	6	0	14			1.0

Note:
A= alleles with fast migration (*fast ellele*)
a= alleles with slow migration (*slow allele*)
N= the number of individuals expressing the ribbon
He-r = average heterozygous value
M= monomorphic, P = polimorphic
*) a locus is considered polymorphic if the most frequent allele appear < 0,95 (Suryani *et al.*, 2001)

the sustainability of hampala fish, so this species is urgent for conservation, although hampala fish is an important local commodity. Hadie *et al.* (2000) state that conservation efforts can be done in-situ and ex-situ. In-situ conservation is done by arranging a reservation and reconstruction system. Ex-situ conservation includes population maintenance that provides domestication efforts and cultivation and gene management about several factors including population size, inbreeding rates and genetic drift. However this research results supporting scientific information of population genetics of *hampala* and furthermore can be used as a tool for conservation and dometication.

CONCLUSION

Based on the results and the discussion it can be concluded that the hampala fish from the study area, can express isozyme EST, ACP, and AAT well, except PER isozyme.and the genetic variation of the hampala fish in all three locations observed is still good.. The results of this study are expected to be utilized to evaluate the potential genetic condition of hampala fish, which is the basis for conservation strategy and domestication.

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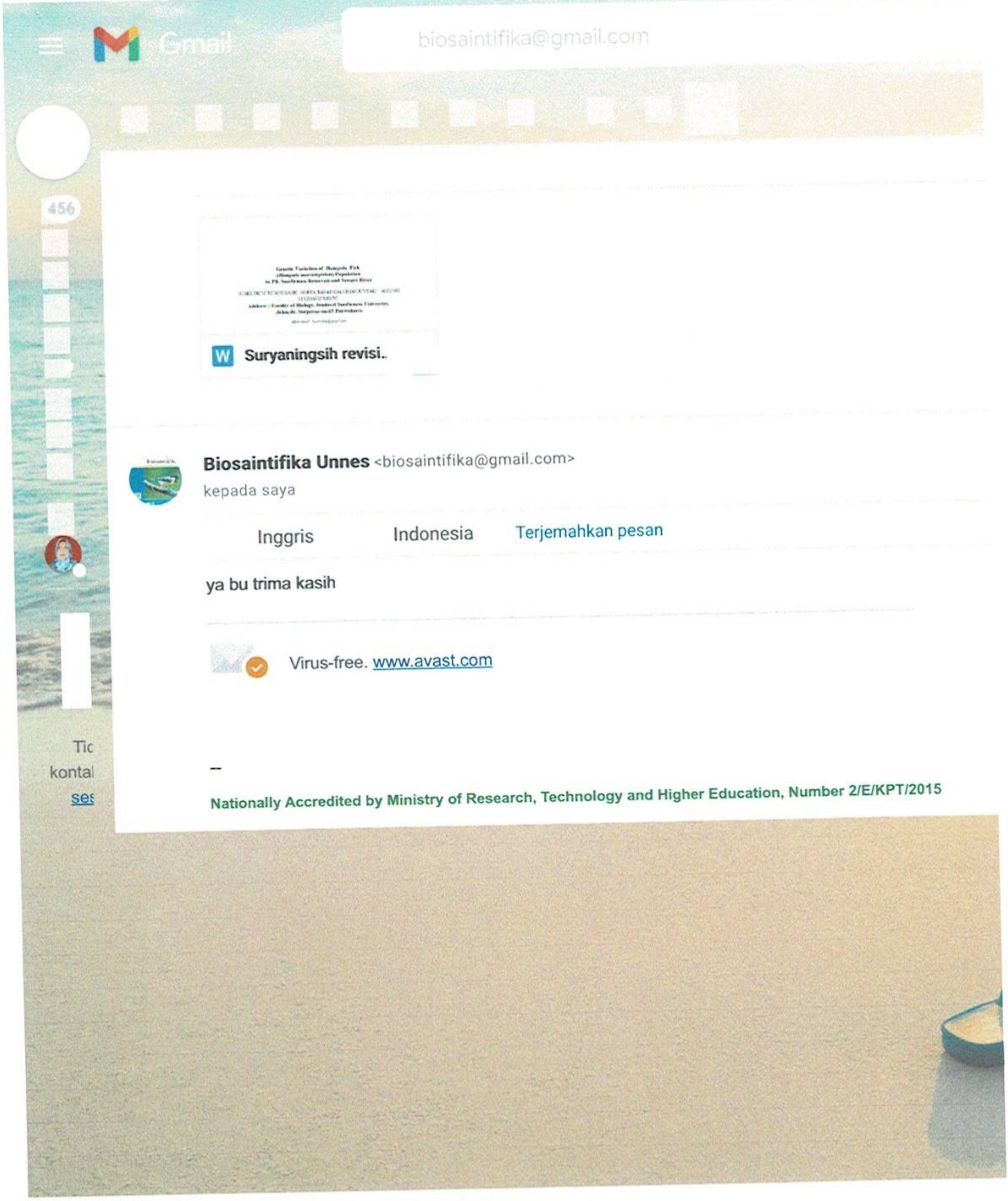

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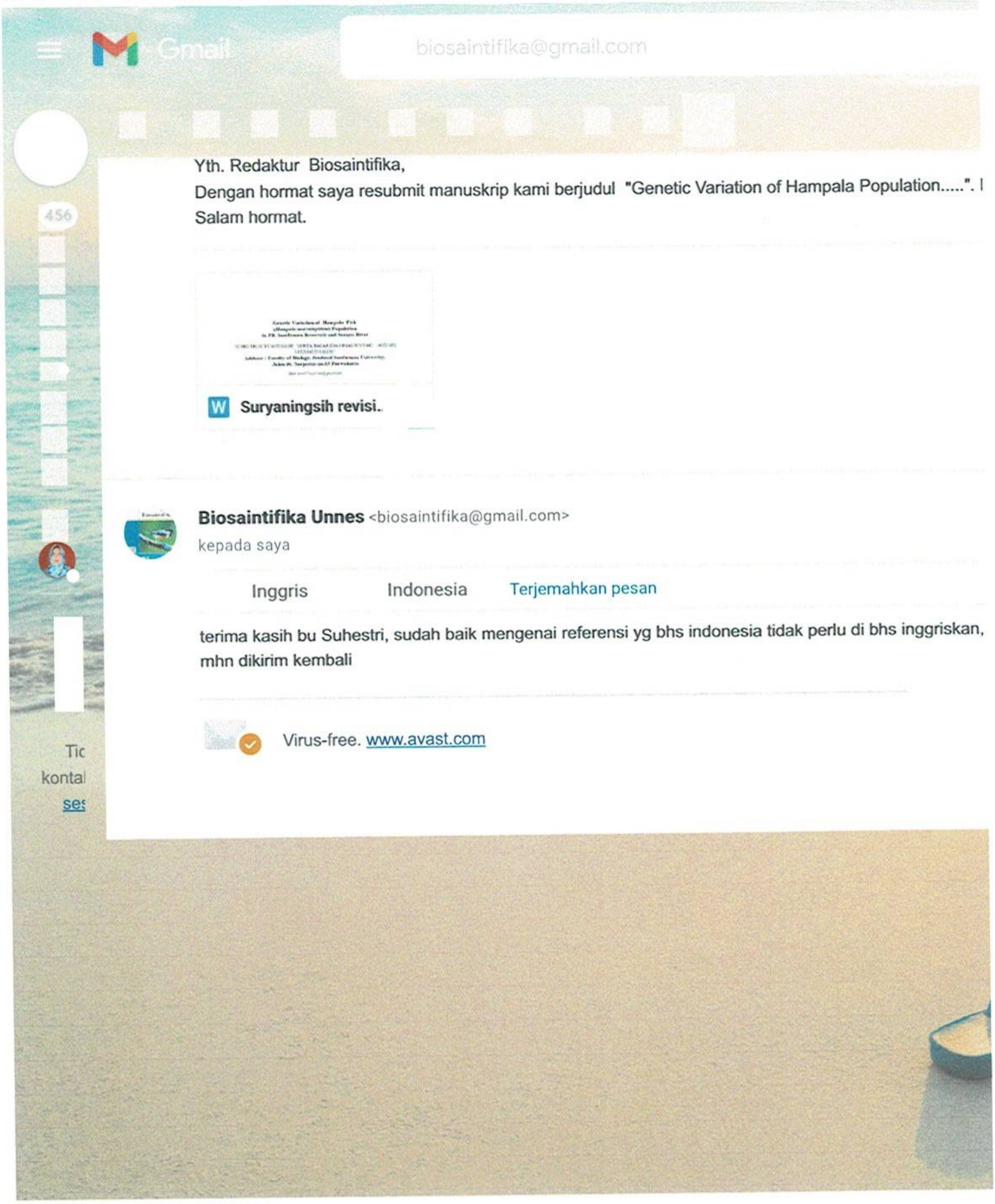

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