

## **Genetic variability and performance of Asian swamp eel *Monopterus albus* (Zuiew, 1793) from West Java cultured in saline water medium**

### **Keragaman genetik dan keragaan belut sawah *Monopterus albus* (Zuiew, 1793) asal Jawa Barat yang dibudidaya dalam air bersalinitas**

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#### **ABSTRACT**

Asian swamp eel *Monopterus albus* (Zuiew, 1793) is freshwater fish species which is prospective for domestic and export markets. The production is limited depend on the catches of natural population. The cultivation of eel has been carried out to increase the production for sustainability. This study was conducted to evaluate the genetic variability and performance of Asian swamp eel from West Java and its potential cultivation in water based media with salinity. Three populations from West Java were collected of different altitudes from Sukabumi (673 m asl), Cianjur (429 m asl), Karawang (51 m asl) sized 19–26.5 cm and weighed 4.95–11.4 g. The cultivation was performed during 30 days in water media without substrate at salinity 6 g/L with density of 1 kg/m<sup>2</sup> and maintenance at container 50×30×30 cm completed with shelter pipe of ¾ inches diameter and 20 cm length, height of water 10 cm and water exchange 100% every day, fed at satiation using *Tubificidae* once a day. Genetically, all of the populations showed low heterozygosity at 1.19–1.23% and genetic distance 0.01–0.04. Asian swamp eel adapted better at water salinity 6 g/L which indicated by low mesure of osmotic gradient and blood glucose. Asian swamp eel from Karawang showed superior at survival rate (90%) and daily growth rate (1.42/day).

Keywords: Asian swamp eel, *Monopterus albus*, cultivation, genetic variability, salinity

#### **ABSTRAK**

Belut sawah *Monopterus albus* (Zuiew, 1793) merupakan komoditas ikan air tawar potensial di pasar domestik maupun ekspor, namun produksinya masih mengandalkan hasil tangkapan dari alam karena budidaya belum berkembang. Penelitian ini bertujuan untuk mengevaluasi keragaman genetik dan keragaan belut sawah asal Jawa Barat dan potensinya untuk dikembangkan dengan teknik budidaya di air bersalinitas tanpa lumpur. Tiga populasi belut sawah dikoleksi dari lokasi di Jawa Barat dengan ketinggian berbeda yaitu Sukabumi (673 m dpl), Cianjur (429 m dpl), Karawang (51 m dpl). Sumber genetik belut berukuran 19–26,5 cm dan bobot berkisar 4,95–11,4 g dipelihara selama 30 hari dalam media air tanpa substrat bersalinitas 6 g/L. Wadah pemeliharaan berukuran 50×30×30 cm dilengkapi shelter pipa paralon diameter ¾ inci dan panjang 20 cm serta ketinggian air 10 cm. Padat penebaran ikan 1 kg/m<sup>2</sup> (20 ekor/wadah), serta pergantian air 100% dilakukan setiap hari dan pemberian pakan berupa *Tubificidae* secara at satiation satu kali sehari. Secara genetik ketiga populasi menunjukkan tingkat heterosigositas yang rendah yaitu berkisar 0,19–0,23 % dan jarak genetik 0,01–0,04. Belut sawah menunjukkan respons adaptasi yang baik dalam media air tanpa substrat pada salinitas 6 g/L berdasarkan indikator beban osmotik dan kadar glukosa yang rendah. Belut sawah asal Karawang unggul pada laju pertumbuhan harian (1,42/hari) dan kelangsungan hidup (90 %).

Kata kunci: belut sawah *Monopterus albus*, budidaya, keragaman genetik, salinitas

## INTRODUCTION

Asian swamp eel *Monopterus albus* (Zuiew, 1793) is a potential freshwater species in domestic and international markets. The distribution areas of Asian swamp eel population include India, China, Japan, Malaysia, Indonesia, Bangladesh, Thailand, and Vietnam. Its habitat is in an area with an elevation range of 0–1,000 m asl, a temperature range of 25–32 °C and a pH range of 5–9, and it also has diverse morphological characterizations. In Indonesia, Asian swamp eel is spread in Java, Sumatra, Sulawesi, Kalimantan, and West Nusa Tenggara. The exports of Asian swamp eel were addressed to China, Hong Kong, Japan, Singapore, Chinese Taipei, Korea, and Thailand (Khanh & Ngan, 2010).

Asian swamp eel belongs in facultative air breather species that has an additional breathing apparatus in the form of a thin mucus skin on its oral cavity and takes oxygen directly from the air (Iversen, 2013). Asian swamp eel is cannibal, but the culture system with a high stocking density can reduce its cannibalism (Khanh & Ngan, 2010). A substrate has functions as an infiltration, a nutrient screen and an aeration for the nutrient requirement. Spawning system of Asian swamp eel is polygamy in which a male eel can fertilize several female eels that choose it with a female quantity of 2–3 individuals (Matsumoto *et al.*, 2011).

The production of Asian swamp eel becomes a serious challenge, because the population of eel in nature decreases caused by the large use of pesticides and over exploitation (Lei *et al.*, 2012). According to Nugroho *et al.* (2012), the aquaculture development based on local species has to be conducted to maintain biodiversity through species conservation on farmer level (on farm conservation) for food security. The intensive culture technique of Asian swamp eel in water medium without mud is prospective to be studied for a sustainable production fulfillment to stop the capture of eels from nature. Domestication and culture process by increasing medium salinity were reported to be able to reduce physiological stress and increased production performance (Karim, 2007; Langkosono, 2007).

The physiological ability of an organism in responding changes in its living environment is influenced by its genetical component (Piferrer *et al.*, 2012). In general a population that has a higher genetic variability shows its ability to respond a better environment in its adaptation process to feed

sufficiency, water quality, and disease resistance (Gjedrem, 2010). Domestication process of a species allows an organism to survive, grow, and breed in an artificial environment. Genetic variability of population closely relates to growth performance and economical properties such as survival and feed efficiency. According to Dunham (2004), genetic variability is important for long-term sustainability of a species or population so allowing it to adapt to environmental changes.

Studies about genetic variability of Asian swamp eel has still been little studied (Matsumoto *et al.*, 2010; Li *et al.*, 2013). Moreover, environmental optimization that supports adaptation process and production performance of Asian swamp eel through environmental engineering with saline water medium has still not widely been studied. This study aimed to evaluate genetic variability and performance of Asian swamp eels from West Java and its adaptation response on the culture in saline water medium without mud.

## MATERIALS AND METHODS

### The collecting of experimental animals

Asian swamp eels from West Java were obtained from three paddy fields with different elevations, including Cianjur, Sukabumi, and Karawang (Table 1). Acclimatization and culture experiment of eels were performed in Experimental Pond Faculty of Fisheries and Marine Science Bogor Agricultural University

### Genetic variability analysis

Genetic variability analysis was performed by observing DNA variability through Random Amplified Polymorphic DNA (RAPD) method (Wei *et al.*, 2006). Genetic variability analysis was done at Laboratory of Research and Development Center of Freshwater Aquaculture (RDCFA) Bogor. The DNA extraction using five individuals from each population was performed using phenolchloroform isoamylalcohol (25:24:1) followed by PCR (Polymerase Chain Reaction) process using three universal primers including OPA-09 primer (5'-GGGTAACGCC-3'), OPC-02 primer (5'-GATAGGCGTC-3'), OPC-05 primer (5'-GATGACCGCC-3'). Polymerase chain reaction (PCR) process was started with initial denaturation at a temperature of 97 °C for 10 min, then followed by 40 subsequent cycles at a temperature of 94 °C for 1 min, annealing at a temperature of 36 °C for 1 min, elongation at a

Table 1. Characteristics of locations for collecting Asian swamp eels from West Java and water quality in Cianjur, Sukabumi, and Karawang

Characteristic of location	Population origin		
	Cianjur	Sukabumi	Karawang
Area elevation (m asl)	429	673	51
Temperature (°C)	24.22–30.94 (20–31)*	23.49–24.93 (17–30)*	31.59–34.70 (22–34)*
Conductivity (ms/cm)	0.19–0.32	0.12–0.19	0.00–0.40
Turbidity (ntu)	15–104	0–275	0–1000
TDS (g/L)	0.12–0.21	0.08–0.12	0.00–0.26
DO (mg/L)	5.91–13.67	1.41–7.68	4.5–13.55
pH	6.64–7.48	7.44–9.36	7.23–7.79
Salinity (g/L)	0.10–0.20	0.0–0.10	0.0–0.20

\*Source: Meteorological, Climatological, and Geophysical Agency (2015).

temperature of 72 °C for 1.5 min, final elongation at a temperature of 72 °C for 5 min, and stabilizing at a temperature of 4 °C for 3 min. PCR products were electrophoresed using agarose gel 2%.

### The culture of Asian swamp eels

The rearing system of Asian swamp eels was performed in a water with a salinity of 6 g/L, a height of 10 cm and without mud filled in opaque plastic containers, blue colored tank (50×30×30 cm), equipped with shelters in the form of pipe pieces with a length of 20 cm, and a diameter of ¾ inch. Asian swamp eels sizing 19.0–126.5 cm with a weight of 4.95–11.4 g were stocked at a density of 1 kg/m<sup>2</sup> (Khanh & Ngan, 2010) for each population replicate. The culture acclimatization was conducted for seven days by fasting experimental eels and the subsequent rearing was conducted for 30 days with water replacement conducted every day by replacing 80% water of total water volume in the medium with a new water. Feed given to experimental eels was silk worm Tubificidae once a day to apparent satiation.

### Observations of parameters

Production performance was observed every ten days including survival, length growth, absolute weight, daily growth rate, and biomass (Goddard, 1996). Blood glucose level and osmotic gradient were measured at the end of the study with GLUCODR® kit dan osmometer according to a method described by Karim (2007). Water quality parameters during the culture of Asian swamp eels in recirculation system in a water

with a salinity of 6 g/L were on controlled ranges: temperature at 26–28 °C, pH at 7–8, and dissolved oxygen at 6.1–6.5 mg/L.

### Statistical analysis

Genetic polymorphism level and heterozygosity were analyzed using TFPGA (Tools for Population Genetic Analysis) program. Interpopulation genetic relationship was analyzed based on genetic distance and dendrogram using UPGMA (unweighted pair group method with arithmetic mean). Production performance was analyzed through analysis of variance at a confidence level of 95% and was further analyzed through Duncan's test, while osmotic rate and blood glucose level were analyzed through descriptive statistic.

## RESULTS AND DISCUSSION

### Genetic variability

The result of the amplification of DNA samples of Asian swamp eels from Sukabumi, Cianjur and Karawang using three primers including OPA-09 primer, OPC-02 primer and OPC-05 primer is presented in Figure 1. Fragments sized 300–3000 bp with a fragment number range of 9–14 (Cianjur), 9–13 (Karawang), and the lowest was 6–12 (Sukabumi).

Genetic variability level of Asian swamp eels from West Java ranged 76.19–83.33% with a heterozygosity of 0.1947–0.2372 (Table 2). Asian swamp eel populations from West Java were thought to be geographically isolated but their reproduction rates were not limited in the

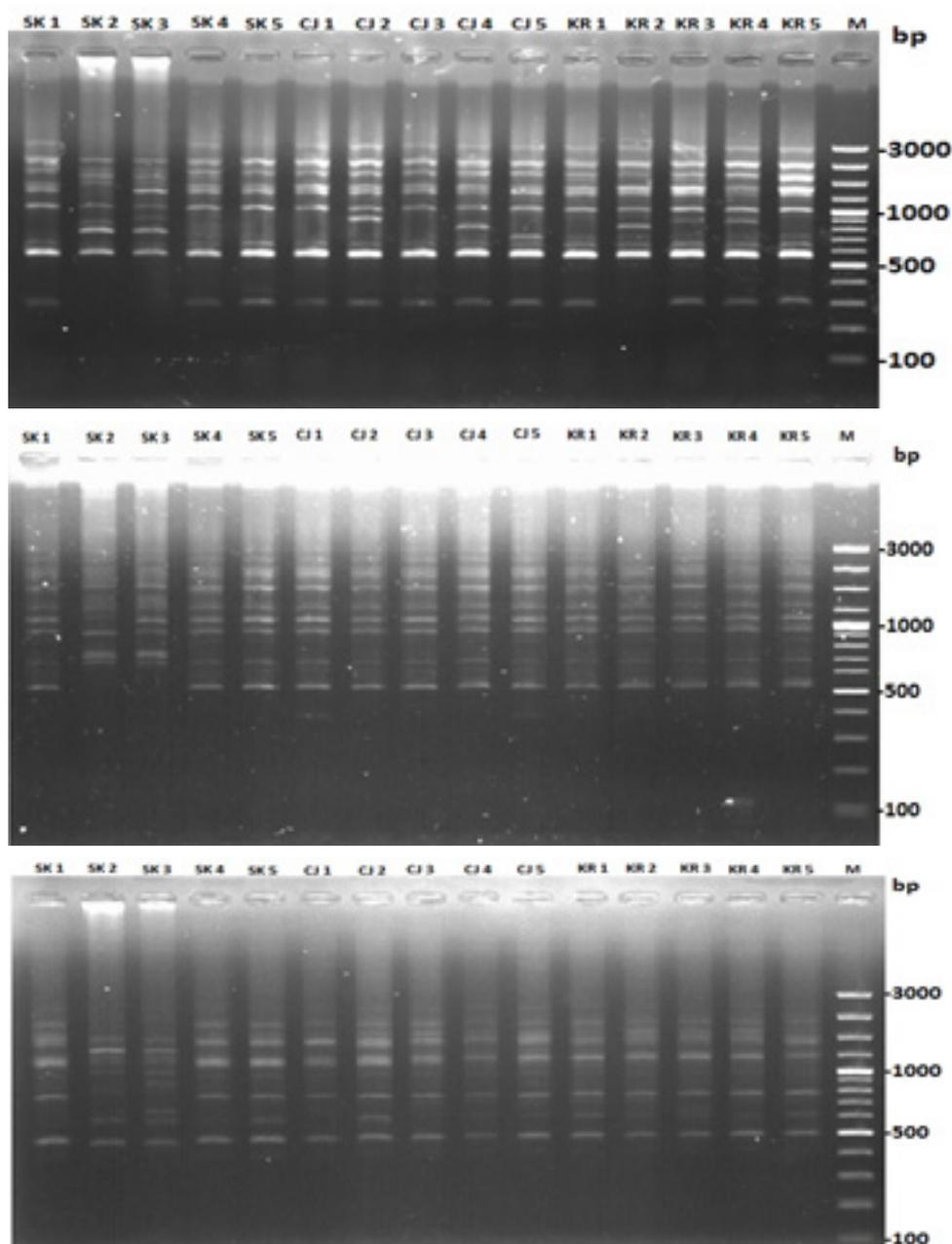


Figure 1. The amplification of Asian swamp eel DNA using OPA-09 primer, OPC-02 primer, OPC-05 primer. Note: SK 1–5 = Sukabumi population, CJ 1–5 = Cianjur population, KR 1–5 = Karawang population, and marker leader (M).

populations so their heterozygosity was low but their genetic variability was high. Populations that are isolated and are limited in genetic source for the continuity of generations allow to experience gene washout, but their genetic variabilities provide success chances to adapt to environmental changes to survive in a long-term period (Wei *et al.*, 2006).

Asian swamp eels from Cianjur, Karawang, and Sukabumi showed a close relationship with a genetic variability difference of 2–5%. Genetic variability difference can increase genetic distance among populations influenced by conditions of

native habitats of Asian swamp eel populations (Kusmini *et al.*, 2011; Lorenzen *et al.*, 2012). According to Setijaningsih *et al.* (2007), the high or the low value of similarity index is influenced by forming genetic resources, the higher the similarity index indicates an exclusivity or suspected to be a closed population.

#### **Performance of Asian swamp eels in the culture system without any substrate**

The adaptation response of Asian swamp eels to the culture system without any substrate with a salinity of 6 g/L demonstrated a decrease pattern

on osmotic load to the water medium compared to a salinity of 0 g/L (Figure 3). Osmotic rate of Asian swamp eel from Karawang was the lowest in the water with and without salinity compared to eels from Cianjur and Sukabumi. High osmotic rate in the rearing system without any substrate with a salinity of 0 g/L causes an organism becoming stress so required an energy to overcome stress through the breakdown of glucose in the blood. Stress is a physiological response to maintain homeostasis through cellular metabolism regulation causing a decrease of blood volume, liver glycogen reserves, and the increasing of blood glucose (Affandi & Tang, 2000).

In general blood glucose decreased starting from week-2 of the rearing period (Figure 4).

The decreasing trend of blood glucose levels on those three Asian swamp eel populations was a good adaptation response indicator to stress in the culture system without any substrate. In the population of eels from Sukabumi blood glucose level increased on week two then decreasing on week three. A failure in responding unusual environmental conditions could cause eels becoming stress so required an energy to maintain homeostasis through cellular metabolism regulation that causes a decrease in liver glycogen and an increase in blood glucose (Hadiroseyani *et al.*, 2016). According to Purchase *et al.* (2009) stress is an adaptation to physiological changes which was resulted from various environmental stressors.

Table 2. Genetic variability based on the number and the size of amplified DNA fragments from three Asian swamp eel populations from West Java

Population origin	The number of DNA fragments	The size of DNA fragments (bp)	Polymorphism (%)	Heterozygosity
Cianjur	9–14	300–3,000	83.33	0.2291
Sukabumi	6–12	300–3,000	76.19	0.1947
Karawang	9–13	300–3,000	83.33	0.2372

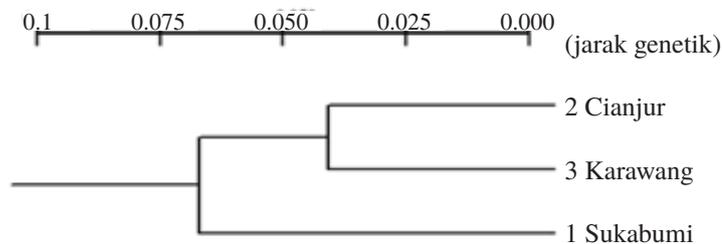


Figure 2. Genetic relationship dendrogram of Asian swamp eels from West Java (Cianjur, Sukabumi, and Karawang).

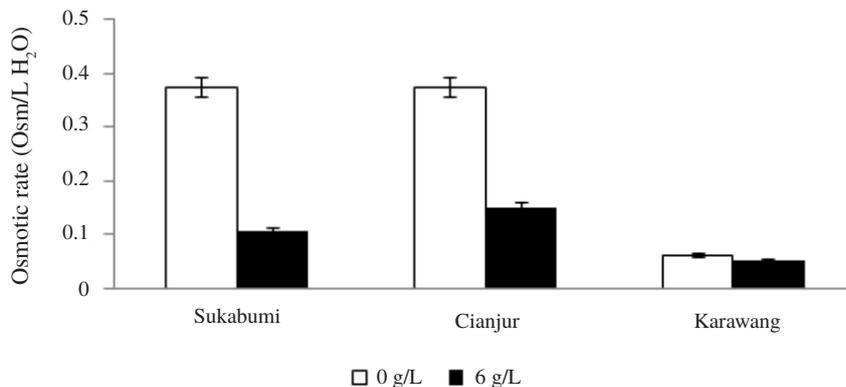


Figure 3. Osmotic rate of Asian swamp eels from Sukabumi, Cianjur, and Karawang with the culture system without any substrate in a water with a salinity of 6 g/L after a 30-day-rearing.

Physiological response of an organism for balancing ion contents in its body fluid with ion contents in the environment influences production phenotype and survival of an organism. Asian swamp eel from Karawang was superior on daily growth rate (1.42%) on a 30-day rearing in a water without any substrate with a salinity of 6 g/L ( $P < 0.05$ ), while the highest biomass was generated in eel from Sukabumi, but its absolute length gain was the lowest, while survivals were not significantly different among those three populations ranging 76.67–90% (Table 3).

Genetic variability is a key parameter of the fitness of a population that ensures its sustainability and ability to passively respond natural or artificial selection (Lorenzen *et al.*, 2012). Asian swamp eel populations from Sukabumi, Cianjur, and Karawang had genetic variability over 75% but their heterozygosity was low that was 0.2 (Table 3). Genetic variability commonly positively correlates with a success chance to adapt to changes in environmental conditions, but in Asian swamp eel from Sukabumi there was a significant difference in temperature at its habitat (23–24 °C) with the environmental condition in the culture system (26–28 °C).

The high polymorphism level in a population shows the effectiveness of an individual on the reproduction process in its habitat (Kristanto & Kusriani, 2007; Kusmini *et al.*, 2011). Low genetic variability is thought to relate to limited gene exchange or narrow population size so inbreeding increases and impacts on a slow growth and a low adaptation level. If a genetic potential is supported by an appropriate environment so it will be expressed optimally and will produce maximum phenotype too (Ath-thar *et al.*, 2014). A population with a high genetic variability has a higher surviving chance, because many alternative genes or gene combinations are available to respond changes in encountered environmental conditions (Dunham, 2004).

Li *et al.* (2013) declared the presence of differences in genetic variability between eels that live in nature and cultured eels. Eels that live in nature have a higher genetic variability compared to cultured eels. Liu *et al.* (2009) also stated that the genetic variabilities of cultured populations are lower compared to wild populations. The low genetic variability will lead to degradation in ability to adapt to new environments.

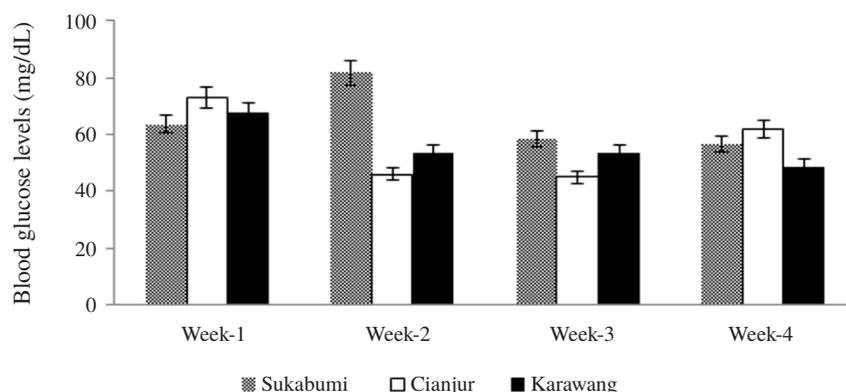


Figure 4. Blood glucose levels of Asian swamp eels from Sukabumi, Cianjur, and Karawang on week 1–4 in the rearing system with the culture system without any substrate and a salinity of 6 g/L

Table 3. Phenotype performance of Asian swamp eels from Sukabumi, Cianjur, and Karawang population reared for 30 days in the culture system with a water medium without any substrate at a salinity of 6 g/L

Phenotype performance	Population origin		
	Cianjur	Sukabumi	Karawang
Daily growth rate (%/day)	0.69±0.09a	0.58±0.12a	1.42±0.20b
Absolute length growth (cm/day)	1.42±0.23b	2.31±0.27a	1.88±0.11a
Survival (%)	87.50±7.50a	76.67±2.89a	90.00±5.00a
Biomass (g)	27.69±0.29b	17.74±0.00a	19.43±7.79ab

## CONCLUSION

Genetic variability level of Asian swamp eels from West Java ranged 76–83% with a heterozygosity of 0.19–0.23 and had a genetic distance of 0.01–0.04. The decreasing of osmotic rate and blood glucose of Asian swamp eels cultured in a water medium at a salinity of 6 g/L indicated a good adaptation response to stress. Asian swamp eel from Karawang was superior on daily growth rate by resulting a value of 1.42%/day, an absolute length growth of 1,88 cm/day, and a survival of 90%.

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