

Morphological and genetic characteristics (mitochondrial DNA) as a basis for determining the direction of giant prawn (*Macrobrachium rosenbergii*) resources management in Riau Province, Indonesia

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Abstract. Giant prawns (*Macrobrachium rosenbergii*) are one of the essential freshwater commodities that has high economic value. The distribution of giant prawns in Riau Province is extensive, almost found in all inland waters, especially in large rivers such as Indragiri, Kampar, Siak, and Rokan. In the early 80s, changes in environmental conditions began to occur in the giant prawn habitat. This study aimed to determine the distribution, phenetic, and genetics of giant prawns to determine the direction of giant prawns resource management in Riau Province. Methods The research used survey methods and experimental methods (in vitro). The research stages include sample collection, species identification, morphometric and mtDNA of giant prawns, and water quality. Then data analysis was conducted: distribution analysis, phenetic analysis, and sequence data analysis. The results showed a relatively large genetic distance between the population of Okura giant prawns and the Tapung strain, which was 0.00443. Populations that have a lower genetic distance value indicate that the population has a closer kinship relationship, and the higher the genetic distance, the more distant kinship.

Key Words: aquatic resources management, in vitro, mtDNA, phenetic, river, sequencing.

Introduction. The giant prawn is one of the most economically important species for fisheries in Indonesia. In Southeast Asia, giant prawns are harvested wildly from nature. Global giant prawns production even reached 216,856 tons in 2014 (Ma et al 2020). The spread of giant prawns in nature ranges from southern Vietnam to Pakistan in South Asia, northern Australia, and islands in the Pacific Ocean (Chand et al 2005; Hurwood et al 2014; Kumar et al 2017).

The development of modern cultivation techniques made giant prawns to spread widely to the American continent (Fujimura & Okamoto 1972; Kueh et al 2017). This spread extends to mainland China (Yang et al 2012; Thanh et al 2015; Du et al 2021; Jiang et al 2020; Nguyen Thanh et al 2015). Indonesia has a relatively wide spread of giant prawns (Hurwood et al 2014; Megawati et al 2021; Sopian et al 2017).

Riau is one of the provinces that makes of the giant prawns a vital commodity (Fauzi et al 2021). Giant prawns are found in all inland waters of the Riau Province, mainly in large rivers like the Indragiri River, Kampar River, Siak River, and Rokan River. Alterations to the habitats of giant prawns started to occur at the beginning of the 80s. These changes happened because in the period of 1982-1983 land clearing began throughout Indonesia, covering an area of 36 million ha, including Riau Province. Although giant prawns are still the main catch of fishermen, the catch has decreased.

Besides deforestation along the watershed, the decline in water quality and habitat also has a terrible impact on the life of giant prawns. Giant prawns grow and develop (maturation of the gonads and mating) in fresh waters in both upstream rivers, flooded swamps, and middle rivers and migrate to brackish waters to incubate their eggs

and develop to the post-larval stage. In the development of life, giant prawns are very dependent on the quality of the waters that become the medium of life, especially in the process of growing by molting (Utomo 2004; Du et al 2021; Sopian et al 2017).

Understanding genetic diversity is essential in developing conservation strategies. The application of genetic markers allows rapid development in parent investigation, genetic diversity evaluation, inbreeding determination, and species identification (Liu & Cordes 2004; Elsheikh et al 2015; Kueh et al 2017; Ma et al 2020). Several studies of giant prawn related to genetics have been done before. For examples are the use of allozymes (Hedgecock et al 1979), mtDNA (de Bruyn et al 2005), the use of microsatellites (Charoentawee et al 2006; Thanh et al 2015), and the use of single nucleotide polymorphisms (Jung et al 2009; Agarwal et al 2016; Alam et al 2017).

In the present study, we aimed to identify genetic diversity in wild species of giant prawns to support the future management and conservation of giant prawns.

Material and Method. This research was conducted from April to September 2021. Sample collection was carried out in three major rivers, including the Siak River, Kampar River, and Rokan River. Identification and morphometric analysis were carried out at the Laboratory of Aquatic Ecology and Marine Microbiology, Faculty of Fisheries and Marine Affairs, University of Riau. This study uses survey methods and experimental methods (in vitro). Sample identification refers to Weber and Beufort (1913) and Kottelat et al (1993).

Meanwhile, morphometric analysis refers to Costa et al (2003), Strauss and Bookstein (1982), Caillet et al (1996), and Tan and Ng (2000). The collection of fish samples in the field is carried out using various fishing gear (trawls and hooks) according to the conditions of the collection location. All samples obtained were then recorded for specific characteristics such as body color, fin color, and other characteristics that are thought to be lost or changed if preserved. Then some samples were photographed with a digital camera. Each sample is labeled in the form of a sample code. Large prawns were injected with 10% formalin to avoid sample damage due to fungi and bacteria. All giant prawn samples were then put into a plastic box filled with 4% formalin, stretched in such a way as to position the body and fins, and then brought to the laboratory. The procedure for treating this specimen refers to Caillet et al (1996). The laboratory samples were identified using identification keys from Weber and de Beaufort (1916) and Kottelat et al (1993). Color characters were identified based on sample photos, while morphometric and meristic characters were identified using a stereo microscope and digital calipers. All identification descriptions are recorded and used as evidence of the validity of the species identification. Sources of cells used for DNA extraction were pieces of giant prawn organs, namely swimming legs with a weight of 5-10 mg. The method used is standard phenol-chloroform (Elsheikh et al 2015; Jiang et al 2020).

Results. Measurement of water quality aims to determine the value of water quality regarding its physics and chemistry. Water quality has a significant influence on the survival of aquatic organisms such as giant prawns. Water quality measurement was carried out three times at four stations where giant prawns were caught. The results of water quality measurements can be seen in Table 1.

Table 1
Results of water quality measurement during research

No.	Parameter	Sampling Location			
		I Siak (Okura)	II (Rokan)	III (Tapung)	IV (Kampar)
1.	Temperature (°C)	28-29	29-30	29-30	29-31
2.	Depth (m)	2.06	3.58	2.72	2.65
3.	Brightness (cm)	19.67-20.23	19.43-22.42	19.23-23.27	20.78-23.43
4.	Current velocity (m/second)	0.28-0.35	0.42-0.49	0.38-0.45	0.36-0,50

5.	DO (ppm)	3.54-3,88	3.83-4,8	4.11-4,41	3.89-4.12
6.	CO ²	6.12-7.36	7.43-8.25	7.73-8.88	7.32-8.45
7.	Salinity (‰)	0	0	0	0
8.	pH	5.67-6.34	6.26-6.93	6.61-6.92	6.63-6.98
9.	Alkalinity (ppm)	72-86	98-101	108-111	106-113
10.	Hardness (mg/L CaCO ₃)	67-73	76-84	72-90	74-96

The water temperature of the rivers in Riau is still within tolerance limits to support the life and growth of giant prawns. The water temperature measurements at four stations show a range of values of 28°C-31°C (Table 1). The highest average water temperature was found at station 4 with a minimum value of 29°C and a maximum of 31°C, and the lowest was at station 1 with a minimum value of 28°C and a maximum of 29°C. The low water temperature at station 1 is due to this area being located in a large area of peat water. This follows the opinion of Kusbiyanto (2009), who states that the maximum temperature of peat waters is 29°C.

Water clarity is a measure of the clarity of the water. The higher the brightness of the water, the higher the light penetrates the water column. The results of measurements of water brightness at four points on rivers in Riau show a range of values between 19.23 cm – 23.43 cm (Table 1), with the lowest value found at Kampar III station at 19.23 cm and the highest at Rokan station IV is 23.43 cm. This condition describes the rivers in Riau are relatively murky. This condition is thought to be due to organic matter and soil particles that enter it. This brightness is also influenced by sunlight entering the waters. Following the facts on the ground, this is that there are many domestic activities along the rivers in Riau.

The brightness values obtained from four stations in rivers in Riau during the study were classified as unsuitable for giant prawn life. This follows the opinion of Hurwood et al (2014), which states that the brightness of the waters that are good for crustacean life (giant prawn, crab, and lobster) is in the range of 30-90 cm.

Current velocity is one factor that has an essential role in river waters. This relates to the distribution of organisms in these waters, dissolved gases, and minerals contained in the water (Jyväsjärvi et al 2009; Kuguru et al 2019; Suresh et al 2015). The classification of current speed consists of 4 categories, namely the category of slow currents with speeds in the range of 0 – 0.25 m/sec and medium currents with speeds in the range of 0.25 – 0.50 m/sec. Second, the category of fast currents with speeds in the range of 0.5 – 1 m/s and the category of swift currents with speeds above one m/s. The classification of water flow velocity at several research stations in rivers in Riau in this study is included in the current medium category, namely with a value of 0.31 m/sec at station 1, then 0.45 m/sec at station 2, at station 3 with an average 0.41 m/s and 0.43 m/s.

The current speed, which is classified as slow to moderate, carries many food particles originating from upstream areas and insects and leaves that fall from trees that are held for a long time in water bodies that are a source of food for aquatic life organisms. In line with that, rivers with slow to moderate currents are ideal habitats for aquatic organisms that do not have particular adaptations against heavy water currents. The current speed is caused by the nature of the water flowing from upstream to downstream. The upstream part of the river is faster than the downstream due to the higher slope level and relatively narrower water body than the downstream area. Medium to fast currents is found in the Riau rivers.

Dissolved oxygen in waters is needed for the respiration process and is one of the main components for the metabolism of aquatic biota, including giant prawns. Dissolved oxygen in waters can be sourced from oxygen diffusion in the atmosphere and the photosynthetic activity of phytoplankton (Carvalho et al 2021). The measurements of dissolved oxygen content at each station (Table 1) show a value that varies between 3.54 ppm - 4.80 ppm, with the lowest value found at station 1 and the highest at station 3. These results indicate that the dissolved oxygen condition at the sampling station can still support the life of giant prawns. This condition follows the quality standard limits

required by Indonesian Government Law No. 82 of 2001 (Class II) that the optimal DO for waters is 4.

According to Li et al (2021) and Wei et al (2019), a good dissolved oxygen level for fish is at least three mg/L, while according to Hurwood et al (2014), the optimum range of dissolved oxygen for giant prawns is 3-7 mg/L with a lethal limit of less than 1 mg/L. Dissolved oxygen in the waters can be sourced from the diffusion of oxygen in the atmosphere and the photosynthetic activity of phytoplankton. Dissolved oxygen levels in these three rivers tends to be low. The low dissolved oxygen is thought to be high in the canopy cover around the river, which is generally dominated by pandanus water, so the formation of oxygen through photosynthesis to water bodies is not going well.

DNA isolation is the first step in DNA-based molecular analysis. Genomic DNA checking on giant prawns from four varieties was performed using a gel electrophoresis process. The resulting giant prawn genome DNA is more than 10,000 bp in size (Figure 1). The average size of giant prawn mitochondrial DNA exceeds 10,000 bp, as in *Litopenaeus vannamei* and *Fenneropenaeus chinensis*, which are 15,989 bp and 15,004 bp (Li et al 2012; Song et al 2020) and *Penaeus monodon* is 15,984 bp (Prasanna Kumar et al 2012; Srivastava et al 2017). The quality of the resulting genomic DNA will form a pattern of bands with different thickness levels. This condition is related to the concentration of DNA obtained. The higher the concentration obtained, the thicker and brighter the band formed (Li et al 2012). The genome obtained from the extraction was amplified using mtDNA COI primers (LCO1490 and HCO2198).

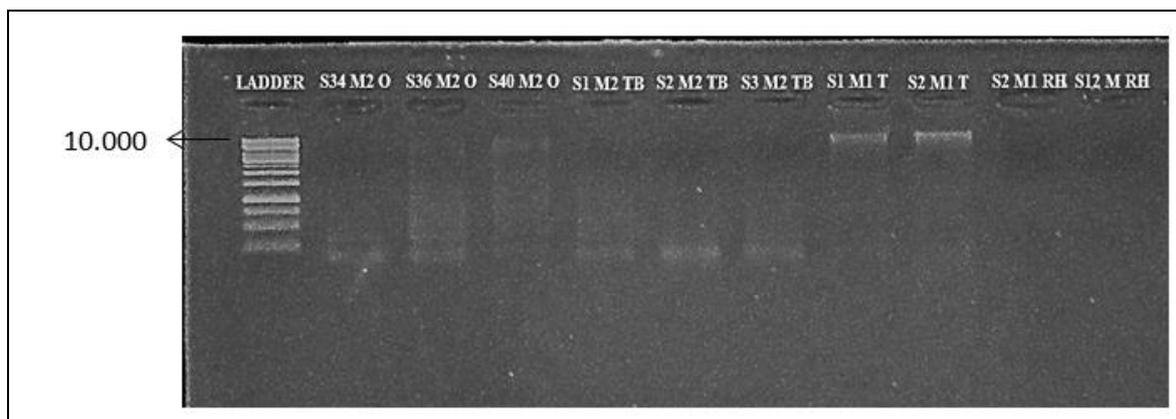


Figure 1. Visualization of the giant prawn mtDNA (COI) genome. Code SMO (Sample Okura), SMTB (Sample Teratak Reed), SMT (Sample Tapung), SMRH (Sample Rokan Hilir), Ladder 1 Kb (Thermoscientific).

The genetic distance value is presented in the form of a matrix, as shown in Table 1, while the dendrogram illustration is shown in Figure 2. The genetic distance between the Okura giant prawn population and the Tapung strain is quite large, namely 0.00443. The genetic distance between the two and other populations can be seen in Table 1. Populations with a lower genetic distance value indicate that the population has closer kinship, and the higher the genetic distance, the more distant kinship (Bala et al 2017; Ma et al 2020).

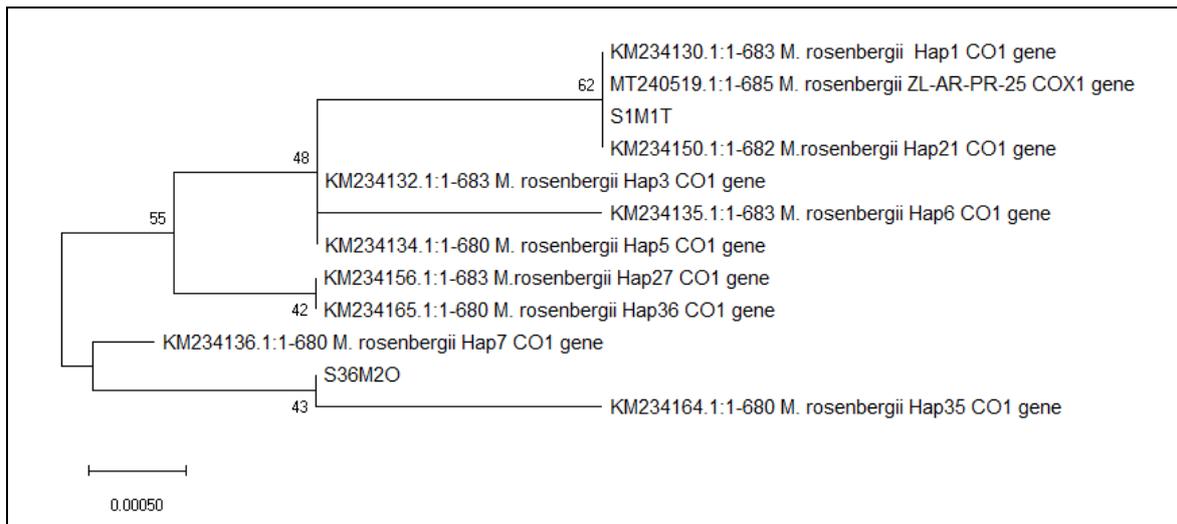


Figure 2. Dendrogram of genetic distance of 2 giant prawn varieties (Okuran and Tapung) with other strains found in GenBank data based on mtDNA (COI) sequences (Sayers et al 2022).

The genetic distance information can be used to determine which individuals or populations will be selected as crosses. The farther (higher) the genetic distance of a population, the higher the heterosis effect when crossed. The dendrogram illustration provides information on the kinship of the two giant prawn populations with other haploid strains contained in the NCBI data (Sayers et al 2022). The analysis results show that there is a genetic structure as a description of the population separation between the Okura and Tapung populations. The genetic distance data mapped using the pairwise distance program (MEGA X) shows that the Okura strain and the Tapung population are in different clusters (Figure 2). This follows the location of the giant prawns' habitat, which is fragmented by region. The genetic distance dendrogram showed that the Okura strain had a closer relationship with the Hap7, Hap27, Hap35, and Hap36 strains, which was 0.0014.

The Tapung strain has 100% the same kinship with Hap1, Hap21, and VLARPR vouchers. The kinship pattern of a population is thought to occur due to the spread and migration process (gene flow). Gene flow or migration is a process of gene transfer between populations (Chang et al 2013; Elsheikh et al 2015). This interpopulation gene transfer can be caused by the movement of individuals or populations from one habitat to another or vice versa due to human intervention or naturally due to the location of habitats between populations. Analysis of the relationship between length and weight aims to determine the growth characteristics of giant prawns. The relationship between the length and weight of *M. rosenbergii* giant prawn during the study is presented in Figure 3, Figure 4 and Figure 5.

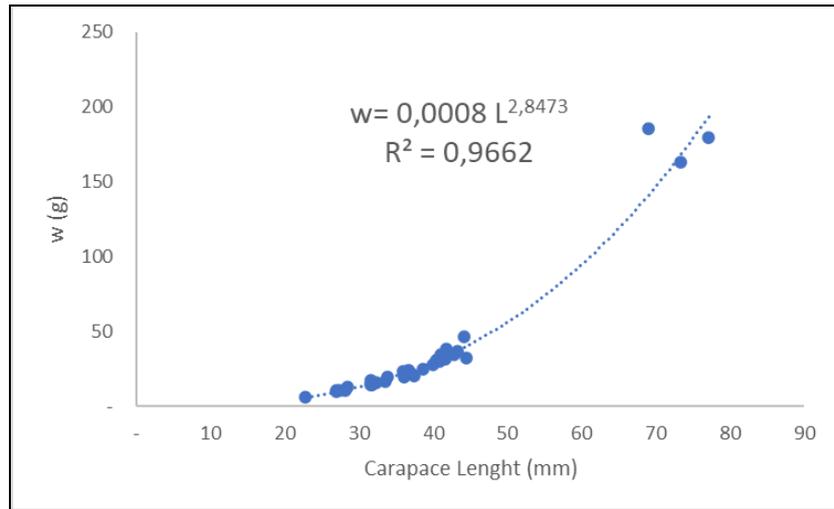


Figure 3. Correlation between carapace length and weight of giant prawns Okura in Siak River.

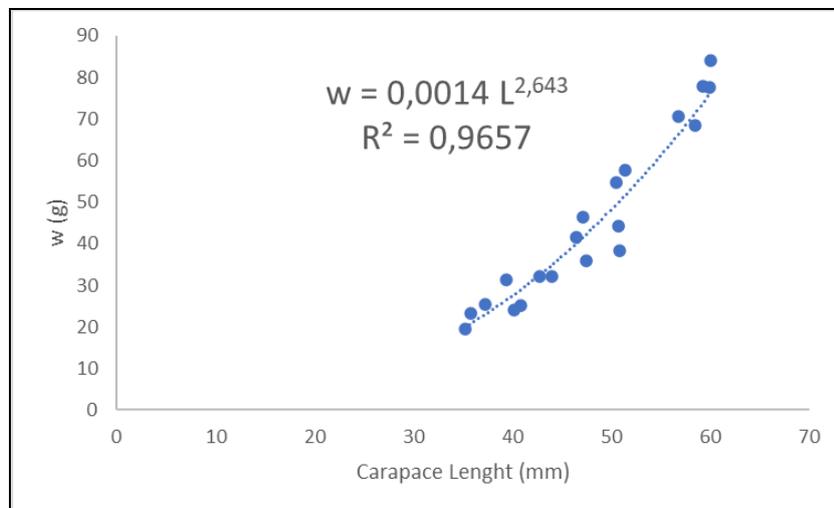


Figure 4. Correlation between carapace length and weight of giant prawns in the Tapung River.

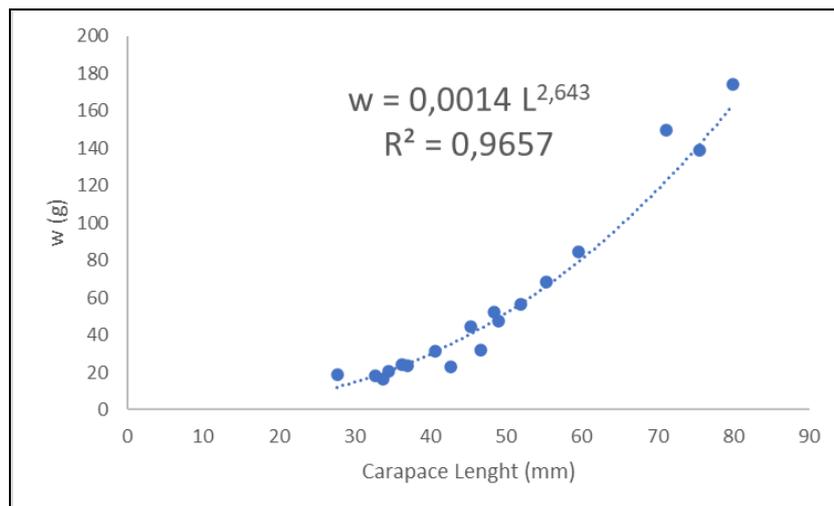


Figure 5. Correlation between carapace length and weight of giant prawns in Kampar River.

Data on the relationship between the length and weight of giant prawns is one of the parameters that can be used to analyze the growth pattern of a group of giant prawns that is useful in fisheries management activities (Hossain et al 2017; Kumar et al 2017; Suresh et al 2015). Based on the test on the value of b, for giant prawns, the results of

the growth properties of giant prawns differed at each station; at station 1 and station 4, the growth of giant prawns was negative allometric, where the allometric growth was negative, namely the increase in length of the giant prawn was faster than the increase in weight (lean), presumably due to environmental conditions of the waters and food that does not support the life of giant prawns in these waters. While at stations 2 and 3, the growth of giant prawns was positive allometric, where the growth of prawns increased in length was slower than weight gain (plump). This was presumably due to the sex of giant prawns, which was dominated by females carrying their eggs. For the giant prawn *Acetes intermedius*, the b values for male and female giant prawns were 2.979 and 3.249 (Musel et al 2019).

According to Ricker (1975), the functional value of b regression represents body shape. It is directly related to weight, which is influenced by ecological factors such as temperature, food supply, spawning conditions as well as gender, age, fishing time, area, and fishing vessel. According to Du et al (2021) and Jiang et al (2020), the characteristic of the length-weight relationship in fish and invertebrates is that the value of the exponent (b) is 3 when the weight growth is isometric (without changing shape). If the value of b is different from 3, the weight growth is said to be allometric (changes in giant prawn shape as it grows bigger). Allometric growth may be negative ($b < 3$) or positive ($b > 3$).

Conclusions. The water quality of rivers in Riau is characterised by temperature ranging from 28°C – 31°C, DO ranging from 3.54 ppm to 4.8 ppm, pH ranging from 5.67 – 6.98, alkalinity ranging from 72 mg/L -113 mg/L, and hardness ranging from 67-96 mg/L CaCO₃. Giant prawns (*Macrobrachium rosenbergii*) in rivers of the Riau Province have a negative allometric growth pattern where the increase in length of giant prawns is faster than the increase in weight (lean). In other words, the increase in body weight is not as fast as the increase in carapace length. There is a relatively large genetic distance between the Okura prawn population and the Tapung strain, 0.00443. Populations with a lower genetic distance value indicate that the population has a closer kinship relationship, and the higher the genetic distance, the more distant kinship.

Conflict of interest. The authors declare that there is no conflict of interest.

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