Prospects for the development of oyster cultivation in Tarakan, North Kalimantan in the perspective of a comparative study in Sabah, Malaysia

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Abstract. Oysters are commercial shellfish that have high sales and nutritional value. This study aimed to identify the potential for oyster cultivation in Amal Beach, Tarakan City, through a comparative study from the perspective of oyster cultivation in Tuaran, Sabah, Malaysia. Research has been carried out in Tuaran Oyster Farm, Sabah Malaysia (May 2019) and the coastal waters of Amal Beach (May 2020). Physical parameters were carried out in situ (temperature, surface current velocity, brightness, salinity, dissolved oxygen, and pH), while chemical parameters (ammonia, nitrate, nitrite, and phosphate) were analyzed in the laboratory. The preservation of samples for morphological character data collection was carried out using the wet-keeping method. Tarakan oyster DNA sequencing samples were analyzed by performing multiple sequence alignment using the Mega X program. Outgroup DNA sequencing samples were obtained from GeneBank access codes EU660790.1 *Crassostrea gigas*, AJ5539132 *Crassostrea iredalei*, and JF9155221.1 *Saccostrea* sp. The results showed that all water quality parameters in the study were within the normal range appropriate for oyster life. Tarakan oyster samples were identified by nucleotide BLAST analysis, the specimen with 100% similarity with the GeneBank access code KM460878.1 was *Crassostrea ariakensis*. Thus, the Tarakan wild oyster is *C. ariakensis*, which can be developed for aquaculture.
Key Words: *C. iredalei*, Suminoe oyster, *C. ariakensis*, Amal Beach, DNA sequencing.

Introduction. Tarakan Island in North Kalimantan is an archipelago with considerable potential for fisheries resources. Tarakan City has an area of ±657.53 km^2 consisting of an ocean area of 406.53 km^2 and a land area of 250.80 km^2, which also comprises marine fisheries and coastal fisheries in the form of aquaculture ponds and brackish water (BPS 2016). The Government of Tarakan City, through the Department of Marine Affairs and Fisheries, has determined ten leading commodities in the aquaculture sector to be developed and stimulated in production, including tiger prawns, milkfish, seaweed, catfish, pangasius, tilapia, gourami, carp, grouper, and Soka crab but the list is still open (DP3K 2019). Opportunities exist for developing the potential of other aquaculture commodities such as Shellfish. North Kalimantan is an area that borders Malaysia, namely the Sabah Region. Geographically, Tarakan and Sabah are both located in the Equator and are influenced by the waters of the Sulu Coral Triangle. Based on the similarity of geographical conditions, there are similarities in fisheries potential between Sabah (Malaysia) and Tarakan City (Indonesia). The seafood menu is a culinary favorite for Southeast Asian people, especially Indonesia and Malaysia. Tarakan people's consumption of shellfish protein is only limited to Kapah and Anadara in several culinary centers such as the Amal Beach shop, which sells Kapah and Tarakan food street seafood or which provides Kapah shellfish menu. At the same time, the oyster has not become a commercial commodity that is traded, which is very different from Sabah Malaysia, which has long sold the oyster menu in its culinary choices. Sabah contributed with 98% to the total oyster production and 97% to the total Malaysian wholesale value. Sabah also has the most oyster culturists (344) compared to the number of culturists in Peninsular Malaysia (DOF 2013).

Bivalve aquaculture is an essential source of affordable animal protein for coastal communities. Its sustainability is highly influenced by the suitability of the environment in which it is carried out (Soon & Ransangan 2016). The oysters (Ostreidae) are one of the economically essential bivalves for the community in Malaysia (Izwandy 2006). Pacific Oysters are known by several common names, such as Giant Oysters, Pacific Oysters, Gigas, Portuguese Oysters, Immigrant Oysters (Wang et al 2007). Oysters are included in Kingdom Animalia, Molluscs phylum, Bivalve classes, Ostreoidea orders, Ostreidae family, genera *Crassostrea* and *Ostrea*, species including *Crassostrea gigas*, *Crassostrea iridescent*, *Crassostrea angulate*, *Crassostrea virginica*, and *Ostrea edulis*. Pacific oysters have features such as a rough shell surface shape, such as layers and irregularities, the size of the shell is not the same between the top and bottom, the bottom cover is convex. In contrast, the top cover is flat. The cover is generally white with purplish spots starting from the umbo and an asymmetrical body shape. Inside the shell, a dark indentation is attached to the anterior adductor muscle, never black or purple (Suneja 2014). Oysters like warm and protected waters, from sloping waters with mud substrate, sand or gravel, and rocks (Silulu et al 2013). According to Wang et al (2007), oysters that live in the fine substrate have a relatively smooth or slightly layered shell surface shape compared to oysters that live on coarse substrates. Oysters have the potential to be nutritious and high-protein foods.

Oyster farming requires low technology, which almost all fishermen can apply. The oysters feeding only rely on the natural phytoplankton, therefore they can be easily aquacultured by the local communities and they can adapt to the selected areas. Oyster farming is an ideal choice for alternative livelihood for local coastal communities since it is considered green aquaculture, where no feed or antibiotics are required for the farming. The main objective of this study was to identify the potential for oyster cultivation in Amal Beach, Tarakan City, through a comparative study perspective on oyster cultivation in Tuaran, Sabah, Malaysia.

Material and Method

Study time and place. This research will be conducted in Tuaran Oyster Farm, Sabah Malaysia (May 2019) and Amal Beach water coastal areas (May 2020). Sampling was carried out from three different stations (farm oyster Malaysia owned by Pacik YB, farm oyster Malaysia owned by Pacik Lakin, and wild oyster from the Amal Beach) with three replications using the stratified random sampling method. Stations are distinguished with reference to the Tuaran Oyster Farm’s GPS position and geographic situation, considering the water access, the location of mangroves, the coastal areas large distance from the estuary. Analysis of physical parameters, by YSI Pro30 (606082A), was carried out in situ (temperature, surface current velocity, brightness, salinity, DO, and pH), but for the chemical parameters (ammonia, nitrate, nitrite, phosphate) it was carried out in the laboratory. The process of observing the morphology of all samples of Sabah vs Tarakan oyster (n=6) was carried out by measuring the shell height, Anterior-Posterior length (AP), Dorsal-Valve length (DV) and thickness (T) using a digital dial caliper (cm), and by weighing the total weight of meat and gonads using a digital scale (g).

Preservation of samples. *C. iredalei* samples were analyzed and measured in the laboratory for morphometric parameters (length, width and thickness/height of shell, in mm) (Winder 2011), their body weight (g) and histology analysis. Histology preparations were carried out in the chemical laboratory of the Borneo Marine Research Institute, the University of Malaysia Sabah, using the paraffin method. The oyster sample was fixed with Bouin's solution, then hydrated with ethanol at 70, 80, 90, and 95%, then clarified with xylol and sliced thinly with a microtome. Preservation of body oysters is done with 10% NBF (Neutral Buffer Formalin) fluid and identification of Ostreidae using a mollusk identification manual book. Samples obtained in the field are stored and labeled by stations of origin and replication number and cleaned first of the substrate and dirt.

Sample preservation for morphological character data retrieval is carried out by the wet-keeping method. In the first instance, the sample was photographed for documentation using a digital camera. The sample was put in a container, treated with NBF 10%, and labeled.

Molecular identification. Molecular identification of *C. ariakensis* was carried out in the MST laboratory of the Aquaculture Department, IPB University Bogor.

DNA amplification. Isolation of *C. ariakensis* DNA used the Wizard Genomic DNA Purification Kit (Promega). Amplification using 18S rRNA primer Forwards 5′-CAGCAGCCGCGGTAATTCC-3′ and Reverse 5′-CCCGTGTTGAGTCAAATTAAGC-3′ with the following PCR program's parameters: initial denaturation at 95°C for 2 min, followed by 30 cycles of each 95°C for 1 min, primer annealing at 52°C for 1 min and final primer extension at 72°C for 5 min (Magare et al 2015). Amplified gene products were visualized on 1% agarose gel under UV-Vis. The 40 µL amplicon is then sent to the 1st Base Laboratory SDN. Bhd. Malaysia for sequencing. A sequence homology analysis was performed by comparing Oyster Tarakan nucleotides using the BLAST-N (Basic Local Alignment Search Tool for Nucleotide) program in the GenBank database (NCBI 2022).

Genetic diversity. Genetic diversity was determined based on phylogenetic analysis based on neighbors joining trees (NJ) (Saitou & Nei 1987) and maximum-parsimony (MP) method with 1,000 replicates bootstrap analysis to evaluate the internal node of the tree. Analysis of the *C. ariakensis* DNA sequencing sample was initiated by performing multiple sequence alignment using the Mega X program. Outgroup DNA sequencing samples were obtained from the GeneBank access codes EU660790.1 *C. gigas*, AJ553913.2 *C. iredalei*, and JF9155221.1 *Saccostrea* sp. Genetic distances within and between populations were calculated based on the Kimura 2-parameter model.

Statistical analysis. Analysis of waters physics and chemistry are performed in situ and ex situ (laboratory). Oyster species were confirmed in Tarakan through morphometric observations and PCR analysis methods followed by sequencing DNA using 18S rRNA primer pairs to ascertain the type of oyster found in Amal Beach with species data in the GeneBank (BLAST-N 2022).

Results and Discussion. This comparative study gathers information about oysters cultivated in Sabah, its environmental characteristics and the types of oysters found in the Amal Beach location and commonly consumed by local people, of a great value for oyster cultivators in Tarakan City (Figure 1).

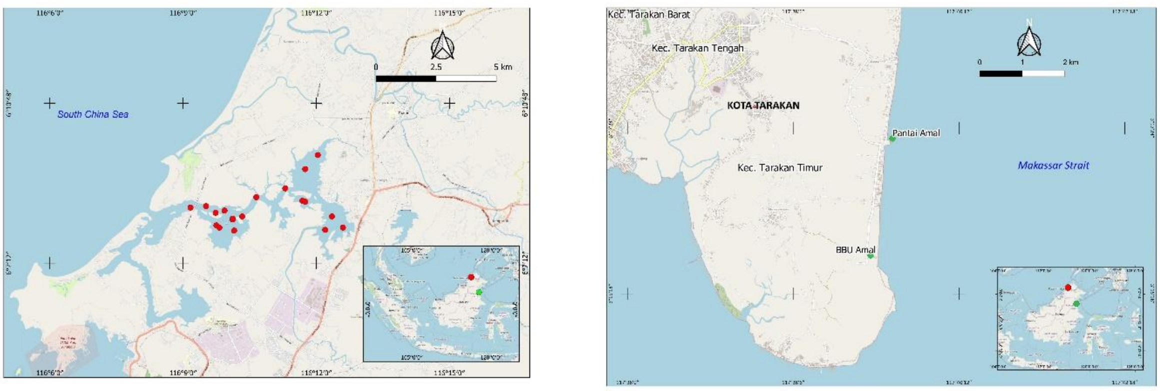


Figure 1. Location of sampling site.

Oyster farming in Sabah, Malaysia, is an activity that has been carried out by coastal communities for a long time. It is very easy to find oyster commodities that are sold in the Malaysian market, but unlike North Kalimantan, especially Tarakan, there has never been a location for oyster cultivation that is managed in a sustainable manner. The people of Tarakan only produce in natural conditions for consumption and not for sale. The opportunity offered by the oysters' selling value and by a long history of oyster cultivation development in Sabah, where the weather conditions are not radically different.

The oyster shell will be opened again after getting the appropriate water. Waters that are relatively calm, but still have movements, with a salinity of 15-30 ppt are better candidates for oyster habitats (Galtsoff 1964). Only certain types of oysters live up to 45 ppt at 33°C (Bardach et al 1972). Adult oysters can survive several days in lower salinity water, but oyster seeds will die if they experience sudden changes in salinity, such as in the rainy season. The summary of water parameters from Tuaran and Tarakan are shown in Table 1.

Table 1

Water quality parameter

Location	*Temp. (°C)*	*DO (mg L^{-1})*	*SPC (mS/cm)*	*Salinity (ppt)*	*pH*	*Depth (m)*	*Sechi disc (m)*
Farm Pacik YB	31.1-32.6	4.30-4.59	45.8-47.2	29.50-30.47	7.35-7.48	2.0-2.1	0.4-0.5
Kg. Berunggis	31.9-32.9	3.80-5.50	46.1-47.3	29.70-30.50	7.37-7.55	1.2-1.3	0.3-0.4
Petronas	31.0-32.7	4.95-5.38	46.2-47.8	29.78-30.87	7.50-7.66	1.4-4.9	0.5-1.0
Near Mangroves	30.9-31.6	5.30-5.77	47.7-47.8	30.87-30.92	8.03-8.20	1.7-5.2	0.9-1.5
Estuary	31.2-31.4	5.90-6.09	47.00-47.7	30.86-30.89	8.16-8.21	1.4-4.5	1.4-1.5
Farm Pacik Lakin	32.0-32.2	5.07-5.85	47.8-47.9	30.95-31.01	7.86-8.04	1.7-1.9	1.3-1.5
Under the Bridge	31.1-31.5	5.72	47.8	30.91-30.92	7.99-8.11	1.2-5.9	1.2-1.5
Amal Beach	29.5-32.1	4.9-6.0	Not measured	30.00-31.00	7.50-8.60	2.0-4.0	0.7-1.2
BBU Amal	30.0-31.5	4.9-6.6	Not measured	30.00-31.00	7.23-8.50	1.8-5.0	0.6-0.8

The measurements of water quality parameter values (in situ using YSI Pro30 606082A), namely temperature, DO, specific conductivity (SpC), salinity, pH, depth, and water transparency (using a Secchi disk), were within the normal range for oysters. Likewise, ammonia, phosphate, nitrate, and nitrite measurements in the laboratory for both water samples in Sabah and Tarakan have typical values for oyster life. Chemical parameters of water quality at the checking point station in Tuaran, Sabah, Malaysia had the following values: ammonia 0.026-0.470 mg L^{-1}, phosphate 0.018-0.046 mg L^{-1}, nitrate 0.002-0.008 mg L^{-1} and nitrite 0-0.099 mg L^{-1}, while at the Amal Beach, Tarakan, North Kalimantan checking point station, the values were: ammonia 0.014-0.100 mg L^{-1}, phosphate 0.004-0.123 mg L^{-1}, nitrate 0.016-0.426 mg L^{-1} and nitrite 0-0.060 mg L^{-1}.

Crassostrea oysters are a group of shellfish that live in intertidal areas (the part of the coast that is submerged in water below the highest tide limit). Oysters can be found everywhere in the world on sheltered beaches, salt lakes and lagoons. Some types of oysters have adapted to environmental conditions that vary according to the changing seasons. However, the appropriate salinity and temperature are environmental factors that are indispensable for the life of oysters, namely: a salinity of 30-36 ppt and a temperature of 30-33°C. The specialty of the oyster lies in its ability to stay alive at low tide, it can even survive up to 49 hours without water. If the oyster is at an inappropriate salinity such as in freshwater, the shell will close tightly.

C. iredalei (Figure 2a) have a maximum shell height (AP) of 15 cm, generally up to 8 cm. In the measurement of this study (n=6), the mean and standard deviation of the Tuaran oyster samples were respectively AP 6.35±1.10 cm, DV 4.15±0.89 cm, T 2.40±0.63 cm, gonad weight 0.98±0.41 g, and whole oyster weight 26.37±12.31 g. It attaches to hard objects or grows in bunches, on various soft bottoms, especially in bays and estuaries with slightly reduced salinity. It is an important commercial species in Malaysia, produced from both wild and cultivated stocks. *C. iredalei* has a dirty white outer shell, often reddened with pale greyish brown. The right valve is often with several darker purplish-grey radial bands in the early stages of growth. The inside of the valve is white and shiny, often with an irregular area of chalky white, dark purple-brown in the posterior adductor scar. *C. iredalei* cultivated commercially, namely black scar oyster (Mustaffa et al 2009).

(a) (b)

Figure 2. (a) Tuaran cultivated oyster, *Crassostrea iredalei*; (b) Tarakan wild oyster, *Crassostrea ariakensis.*

C. ariakensis (Figure 2b) is size maximum shell height (AP) 45 cm, commonly to 15 cm. In the measurement of this study (n=6), the mean and standard deviation of the Tarakan oyster samples were respectively AP 8.10±0.31 cm, DV 5.48±0.86 cm, T 2.86±0.60 cm, gonad weight 0.40±0.19 g, meat weight 6.25±1.67 g, and whole oyster weight 59.71.37±17.53 g. *C. ariakensis* is a major commercial species introduced in aquaculture in many countries. They were primarily restricted to the temperate and subtropical western Pacific. *C. ariakensis* is closely related to *C. nippona* and *C. hongkongensis* (Hong et al 2014). *C. ariakensis* are a native estuaries species in Southern and Northern China. *C. ariakensis* has a yellow flesh color, with a high economic value, and suitable for higher salinity environments (Qin et al 2020).

The purity of the Tarakan oyster DNA genome was measured using a GeneQuant pro UV/Vis Spectrophotometer, by determining the protein absorbance at 260/280 nm, and the ratio value was 1.86 g L^{-1}. Electrophoresis of the Tarakan oyster PCR product with agarose gel (1.5% w/v) obtained the following amplification of 650 bp DNA fragment (Figure 3). BLAST analysis of Tarakan oyster nucleotides resulted in a genetic identification 100% similar to the GeneBank record with the accession number KM460878.1 *C. ariakensis*. Thus, the wild oyster of Tarakan is the Suminoe oyster of *C. ariakensis*.

The evolutionary history was inferred using the Neighbor-Joining method (Saitou et al 1987). The optimal tree is shown in Figure 4. The percentage of replicate trees where the related taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method (Kimura 1980) and are expressed (units) in number of base substitutions per site. This analysis involved 4 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 312 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al 2018).

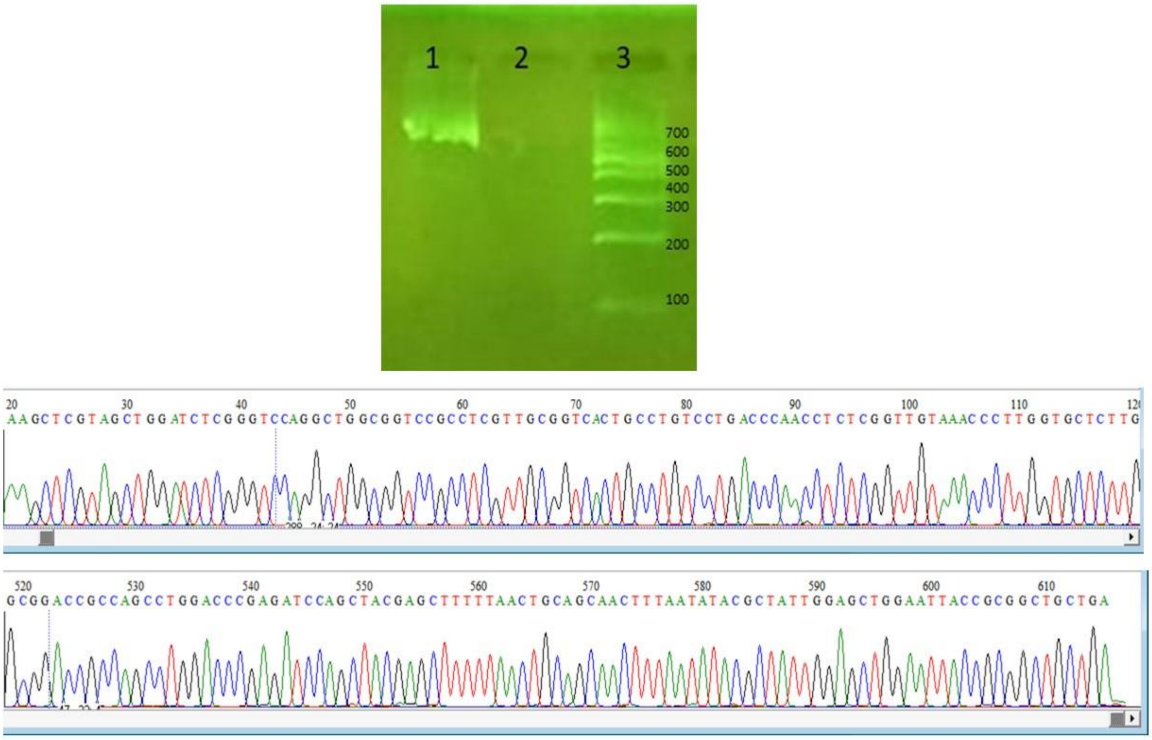


Figure 3. Electrophoresis agarose UV-Vis amplicon sample Tarakan oyster, forward and reverse sequencing DNA from amplicon Tarakan oyster. (1: Amplicon, 2: Negative control ddH2O, 3: 100bp "Geneaid" Ladder marker).

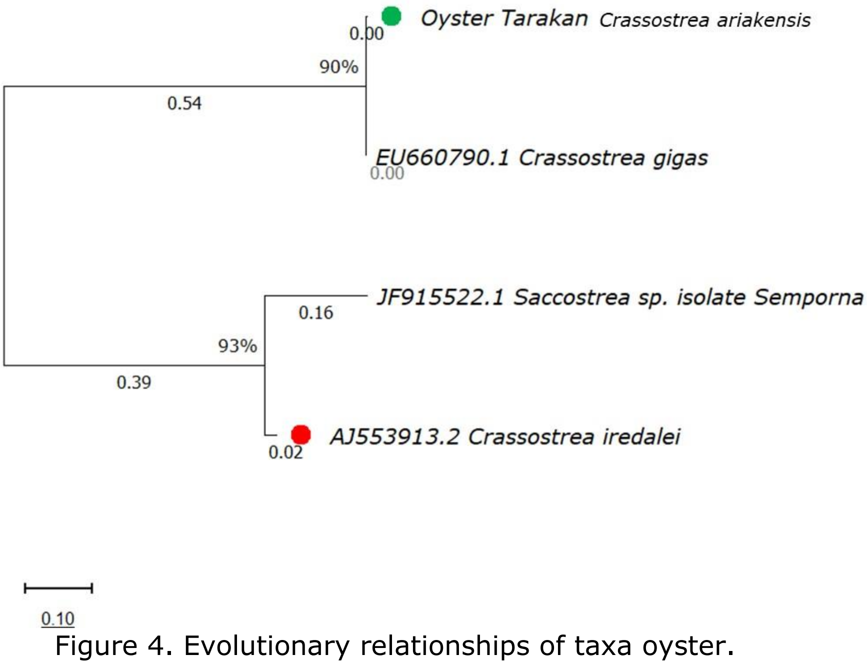
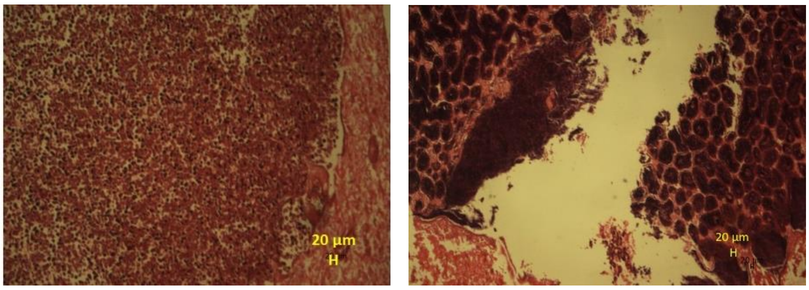


Figure 4. Evolutionary relationships of taxa oyster.

The phylogenetic tree, showing the results of the reconstruction of commercial oyster samples, formed two clusters. The first cluster of *C. ariakensis* had 90% similarity with *C. giggas*. The second cluster of *C. iredalei* has 93% similarity to *Saccostrea* sp. The boostrap value is indicated by the numbers located on the branches of the phylogenetic tree. This study shows a bootstrap value ≥90 where the level of species' similarity is high with the GenBank database records, so that the phylogenetic tree reconstruction can be trusted for its accuracy.

According to Galtsoff (1964), based on sexual habits, oysters are divided into two categories: (1) first, non-incubating or oviparous species that release their eggs into the water so that fertilization occurs outside the organism, for example *Crassostrea* sp. and (2) incubation species in which fertilization occurs in the gill cavity and the larvae are incubated and released after reaching a larviparous stage, for example *Ostrea* sp. According to Guo et al (1996), the gonadal species *Crassostrea* sp. is very dependent on age, so the older group tends to be dominated by females (hermaphroditic protandric). According to Batista (2007) gametogenesis in bivalves is controlled by internal factors (hormones) and exogenous (temperature, salinity, and feed).

Oysters can reach sexual maturity at the size of 50 to 80 mm (Rosell 1990). Fertilization occurs externally, with sperm being released continuously as dense white streams and eggs are released aided by contraction of adductor muscles (Figure 5a). A single female can release millions of eggs (Figure 5b). In Malaysia, the peaks of oysters' spawning season are between April to June and October to December (Devakie et al 2009).



(a) (b)

Figure 5. (a) Histology photo spermatogenic cell of the male *Crassostrea iredalei*; (b) Histology photo oocytes of the female *Crassostrea iredalei.*

It is necessary to cultivate oysters to maintain their availability in nature. Oyster cultivation strategy benefits from its tendency to stick to the substrate and to live in groups. Many developed countries have carried out oyster cultivation activities and have succeeded. Spat oysters will look for a place to attach, grow, develop, and reproduce. Gangnery et al (2004) proposed a method of oyster cultivation with a media in the form of a polyethylene rope to which dead clam shells were tied for larvae attachment. The rope is wrapped around wooden slats that are still submerged at high and low tide until the larvae or spats reach the size of an adult oyster for harvesting. A high level of knowledge and awareness is needed to understand the bioecology of shellfish, life cycle, reproduction, behaviour and habitat conditions for the development of oyster aquaculture.

The collection of spat oysters from nature is a basic principle of shellfish cultivation. Spat oysters are collected with collectors that have a variety of shapes, materials and ways of operation. A good location for spat oyster collection is a stable bottom with no substrate movement (sand and mud movement), with low but continuous current velocity and a place where eddy currents occur.

Conclusions. Based on the study results, the geographical conditions of Sabah and Tarakan Island are not very different. The water quality parameter values in the estuary and along the coastline, at the Tuaran and Tarakan wild oyster farm locations, supported the growth and life of the commercial oysters observed. Sabah has succeeded in developing a *C. iredalei* oyster cultivation business and to manage it in a sustainable industrial manner. At the same time, Tarakan only takes oysters from nature but does not have a cultivation industry and has not been managed commercially in a sustainable way. Tarakan has the potential and opportunity to commercially develop aquaculture of *C. ariakensis* at Amal Beach or *C. iredalei* oysters at Tuaran Sabah Malaysia. A reconstruction genetic confirmation through the phylogenetic tree with Mega X application and database GeneBank showed a boostrap value ≥90% between Tarakan oyster *C. ariakensis*, oyster code access EU660790.1 *C. gigas*, oyster code access AJ553913.2 *C. iredalei*, and oyster code access JF9155221.1 *Saccostrea* sp. (Semporna islands), so that the genetic tree reconstruction in this study can be trusted for its accuracy.

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Conflict of interest. The authors declare no conflict of interest.

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