The histological existence and evaluation of polycyclic aromatic hydrocarbons (PAHs) in milk fish (*Chanos chanos*) and hard clam (*Meretrix* spp.) in the coastal waters of Tarakan City, North Kalimantan, Indonesia

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Abstract. Polycyclic aromatic hydrocarbons (PAHs) are mutagenic and carcinogenic compounds whose presence in the coastal waters of Tarakan City can affect the life of biota, especially milkfish (*Chanos chanos*) and hard clam (*Meretrix* spp.). The purpose of this study was to determine the potential of bioaccumulation and risk for *Meretrix* spp.and *C. chanos* exposed to PAHs. Samples of *C. chanos* and *Meretrix* spp., extracted from selected locations, were analyzed using GC-MS type Thermo Trace 1310 Single Quadrupole Mass Spectrometer in order to obtain 14 types of priority PAHs (USEPA). The concentration in the *C. chanos* body ranged between 0.05-9.91 ng g^{-1} and *Meretrix* spp. 0.04-14.14 ng g^{-1}. Cyclic 2-4 aromatic hydrocarbons dominate the exposure of the biota bodies, with a concentration level corresponding to a moderate contamination. Histology tests describe disorganization and fusion of lamellae, edema and necrosis, regressing tubules and an irregular meshwork of meat.

Key Words: contamination, brackish waters, biomarker, bioaccumulation, risk.

Introduction. Concentrations of polycyclic aromatic hydrocarbons (PAHs) at certain levels in seawater and sediments can be toxic to benthic and pelagic marine organisms (Arias et al 2009). PAHs enter the fish's body through gills and food chains. Bivalvia, as a filter feeder, has the potential to accumulate various types of pollutants in its tissue (Moore et al 2015), including PAHs. Concentrations of PAHs of 1-50 ppb in the environment can cause sub-lethal responses in some sensitive organisms (Neff 1979). Concentrations of 108 ppm of benzene, 28 ppm of toluene and 3.8 ppm of naphthalene in *Metacarcinus magister* (Dungeness crab) 22.80 ppm of toluene, 16.69 ppm of xilinx and 12.52 ppm of 1.3.5-trimethyl benzene in *Gambara affinis* (mosquitofish) and *Carassius auratus* (goldfish) have toxic effects on organisms of these species (Brenniman et al 1975; Caldwell et al 1977). Small concentrations of phenanthrene and benzo[b]fluoranthene can cause genotoxic effects on *Ruditapes decussatus* (Bivalvia, Veneridae) (Martins et al 2013). *Mytilus galloprovincialis* specimens, when exposed to cyclopenta (cd) pyrene for 30 days, showed mutagenic activity in their bodies (Fabbri et al 2006).

Various social and economic activities occurring in the City of Tarakan, along watersheds and coastal areas, can raise the entry of pollutants, including PAHs, into the environment. PAH inputs in aquatic ecosystems are often correlated with the level of urbanization (Dunbar et al 2001; Van der Oost et al 2003). Besides, it can originate from port loading and unloading activities, atmospheric deposition (Fabbri et al 2006) and combustion of fuel oil (Yan et al 2016).

Tarakan has the potential for sustainable fish resources, especially shrimp farming and fish ponds. However, there is a downward trend. For example, shrimp, fish, and crab exports in 2010 were 11,000 tons, 4 tons and 199 kg, respectively, against only 9,000 tons, 923,000 kg and 65,000 kg, respectively, in 2013 (BPS 2011; BPS 2014). This decrease is thought to be caused by an increase in anthropogenic waste discharge, including PAH. One type of fish cultivated in Tarakan City is the milkfish (*Chanos chanos*). *C. chanos* is one commodity of traditional ponds whose production has decreased. This decrease is thought to be caused by pollutants, including PAHs that enter and accumulate. The presence of PAH will potentially influence another species, the hard clam (*Meretrix* spp.). The mussels are a favorite food, especially when visiting Amal Beach in the east of the town of Tarakan. The sediment filter feeders make this shell very susceptible to contamination. *Meretrix* spp. mussels and *C. chanos* are part of the human food chain, so their PAHs exposure presents a danger to the human health, due to the high mutagenicity and carcinogenicity of these compounds.

Information on PAH accumulation in fish resources in Indonesia is minimal, in particular in the northern regions of Indonesia, such as the coastal city of Tarakan, where oil and gas industrial activities exist since 1896. Previous research has revealed the presence of PAH contaminants in the coastal waters, accumulating in nomei fish (*Harpadon nehereus*) in concentrations of 27 to 422 ng g^{-1} (Achyani et al 2015). Information about the extent to which the polycyclic aromatic hydrocarbons (PAH) accumulate in marine food sources, in particular in *Meretrix* spp. and *C. chanos*, is necessary. The purpose of this study was to determine the potential for bioaccumulation and risk in *Meretrix* spp. and *C. chanos* exposed to PAHs in the coastal waters of North Borneo Tarakan City.

Material and Method

Description of the study site. The sampling was carried out at the pond fish farming, while clam samples were taken from locations enlargement in Tarakan Amal Beach East (Figure 1). Fish and clam samples were collected according to the standard amount for PAH analysis needs. Organs to be analyzed are the gills, liver and meat, which were collected using a dissecting kit, then stored in a freezer until analyzed.

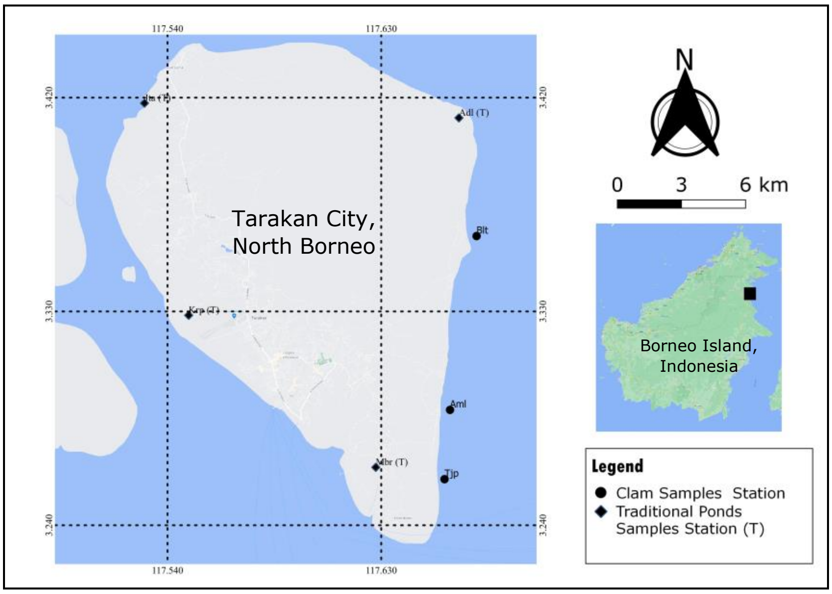

Figure 1. Sampling of *Chanos chanos* at the traditional pond fish farming and clam enlargement locations in Tarakan Amal Beach, East Tarakan City, North Borneo, Indonesia.

Sample analysis. Fourteen priority PAHs by USEPA were used in this study, namely: naphthalene (Nap), acenaphthene (Ace), acenaphthylene (Acy), fluorene (Flu), phenanthrene

(Phe), fluoranthene (Fla), pyrene (Pyr), benzo[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), dibenzo[a, h]anthracene (DbA), and Benzo[g, h, i]pyrelene (BgP). PAH extraction in water and sediment samples was performed using the soxhlet method (Achyani et al 2015). The analysis of PAHs for all sample types used a GC-MS Type Thermo Trace 1310 single quadrupole mass spectrometer, with a DB5 MS GC-MS fused silica column of 30 m length and a diameter of 0.32 mm inline. GC temperature was set at 40°C for 1 minute and programmed to raise up to 300°C at a pace of 6°C min^{-1}, then maintained for 20 minutes at 300°C.

The status of PAH contamination in fish and shellfish refers to the criteria presented in Table 1.

Table 1
Classification of PAH contaminated fish body and bivalves status (Gomes et al 2010)

Classification	*PAH concentration (ng g^{-1})*
Not contaminated	<10
Small contamination value	99-100
Medium contamination value	100-1,000
High contamination value	>1,000

The PAHs distributions, based on the characteristics of the aquatic environment, were analyzed using the principal component analysis (PCA) (Bengen 2000). Histological tests were performed to determine the extent of the impact of PAH on the exposed organs, using histological preparations from gills, liver and meat. Histology testing is generally performed following the steps: organs measurements, fixations, cutting organs, washing, dehydration, cleaning, paraffin infiltration, incision, staining and microscopic observation.

Results and Discussion

Presence of PAHs. Fourteen types of PAH were found in fish biota and *Meretrix* spp. (Table 2), which illustrates that both organisms have been exposed to PAH.

Table 2
The type and presence of PAH identified in the gills, liver and flesh of *Chanos chanos* and *Meretrix* spp.

PAHs	*Chanos chanos*			*Meretrix spp.*		
	Gill	*Liver*	*Meat*	*Gill*	*Liver*	*Meat*
Naphthalene	+	+	+	+	+	+
Acenaphthylene	+	−	+	−	−	+
Acenaphthene	+	+	+	−	+	+
Fluorene	+	+	+	−	+	+
Phenanthrene	+	−	−	−	−	+
Fluoranthene	+	+	+	+	+	+
Pyrene	+	+	+	+	+	+
Chrysene	+	+	+	+	+	+
Benzo (a) anthracene	+	+	+	+	+	+
Benzo (b) fluoranthene	+	+	+	+	+	+
Benzo (k) fluoranthene	+	+	+	+	+	+
Benzo (a) pyrene	+	+	+	−	+	+
Dibenzo (a, h) anthracene	+	+	+	+	+	+
Benzo (g, h, i) perylene	+	+	+	−	+	+

(+) detected; (-) not detected.

Cyclic type 2-4 PAHs seem to predominate in specific organs. PCA analysis showed the dominant PAH distribution in by organ: cyclic hydrocarbons of types 2 and 4 were found in all clam organs, also in fish meat and liver, whereas cyclic hydrocarbon type 3 was only found in fish gills (Figure 2). Fish and clam can be exposed to PAHs through contaminated sediments and water, breathing, food, detritus and skin absorption (fish) (Logan 2007). Compounds like Acy, Ace, Flu and Phe were not identified. This difference may be due to the level of contaminants in the environment as well as to the different levels of intake and excretion (metabolic pathways) by the organisms.

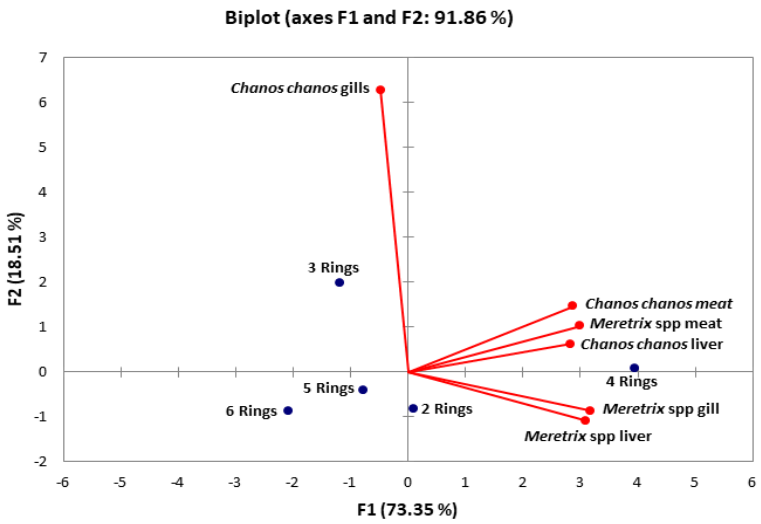

Figure 2. Distribution of the presence of PAHs is based on the number of chains of carbon atoms in each organ biota.

The PCA analysis results show two main components, respectively contributing 73.35 and 18.51% of the variability of the data with a total variability of 91.86%. The dominant PAH distribution was cyclic hydrocarbons of types 2 and 4.

The concentration of PAHs based on the type of organ in *C. chanos* and *Meretrix* spp. varies (Table 3).

Table 3

Concentrations of total and individual PAHs in *Chanos chanos* and *Meretrix* spp. in each organ (ng g^{-1})

PAHs	*Chanos chanos*			*Meretrix spp.*		
	Gill	*Liver*	*Meat*	*Gill*	*Liver*	*Meat*
Naphthalene	10.92	8.76	8.19	8.24	5.89	3.01
Acenaphthylene	0.34	nd	0.21	nd	nd	0.20
Acenaphthene	42.12	7.90	5.76	1.06	nd	2.34
Fluorene	0.47	1.34	0.08	0.28	nd	10.23
Phenanthrene	3.17	nd	nd	nd	nd	4.97
Fluoranthene	0.31	7.46	1.29	3.72	0.47	59.50
Pyrene	1.53	6.51	0.95	2.92	0.66	55.90
Chrysene	10.39	10.39	4.42	3.99	14.14	8.05
Benzo (a) anthracene	5.10	9.94	7.79	3.54	1.06	6.99
Benzo (b) fluoranthene	4.85	9.91	0.22	0.97	1.08	1.34
Benzo (k) fluoranthene	8.20	9.91	0.16	0.96	0.90	1.39
Benzo (a) pyrene	3.89	0.42	0.05	1.17	nd	0.69
Dibenzo (a, h) anthracene	nd	0.16	0.07	0.99	0.46	0.04
Benzo (g, h, i) perylene	10.57	0.05	0.03	0.16	nd	0.04
Low molecular weight	54.62	17.99	14.24	9.57	5.89	20.74
High molecular weight	44.85	54.73	14.97	18.45	18.78	133.94
∑14PAHs	99.47	72.72	29.21	28.02	24.67	154.68
Total		201.40			207.37	

nd - not identified.

ΣPAHs concentration level in *C. chanos* was 201.40 ng g^{-1}, distributed as following: 99.47 ng g^{-1} in the gill, 72.72 ng g^{-1} in the liver and 29.21 ng g^{-1} in the meat. The highest concentrations were observed for the BgP (10.57 ng g^{-1}) and Chr (10.39 ng g^{-1}), in the gill organ, and the lowest concentration for the BgP (0.03 ng g^{-1}) in the meat. PAH with an octanol-water partition coefficient (Kow) 2 to 6.5 are mostly absorbed through the gills (Baussant et al 2001), which explains why the concentration of Chr (Log Kow 5.30) and BgP (Log Kow 6.20) in the gills are higher. Due to its lipophilic nature, PAH tends to accumulate in tissues with a higher lipid content, such as the gill, followed by the liver and muscles (Frapiccini et al 2018). Gills are the first and most important organ that accumulates pollutants in waters, due to a direct contact with the water and dissolved sediments exposed to PAH.

Concentrations of PAHs in the liver showed a decrease compared to the gills as the first entry route. This decrease indicates the efficiency of the biotransformation capacity of the liver in fish (Carrasco et al 2012; Zhao et al 2014). The liver contains the primary detoxifying tissues and high levels of detoxification enzymes, such as the cytochrome P450. In this organ, PAHs go through biotransformation by detoxifying enzymes and become hydrophilic and easier to be excreted by organisms (Frapiccini et al 2018). Concentrations in fish meat are smaller than in the liver. The elimination of PAHs from the fish body depends on the passive diffusion and active biotransformation in the liver, followed by the excretion of bile. The passive diffusion occurs in the gills and skin, are characteristic especially for the PAHs with a low molecular weight. Biotransformation and excretion are specific to the PAHs with a high molecular weight. PAHs transformed into hydroxy-PAHs are excreted in the bile (Jonsson et al 2004).

The concentration of ΣPAHs in *Meretrix* spp. is 207.37 ng g^{-1}, with an accumulation distribution as follows: 154.68 ng g^{-1} in the meat, 28.02 ng g^{-1} in the gills and 24.67 ng g^{-1} in the liver. The highest concentration was observed for the Fla (59.50 ng g^{-1}) and the lowest for the DbA and BgP (0.04 ng g^{-1}), in the meat. High concentrations in meat show the different levels of metabolization in the bivalve tissue (Wang et al 2020), the total lipid content's physiological role in the PAH metabolism (Wang et al 2005) rendering the detoxification in gills and liver suboptimal or disrupted. The gills are an early entry of these contaminants, due to direct contact with water and surrounding environment, resulting in larger and faster accumulation. These organs are more susceptible to the oxidative stress so that the antioxidant mechanisms should be faster and more efficient than in any other tissue (Nicholson & Lam 2005).

The concentration in milkfish, based on the type of organ, decreases in the order gills>liver>meat, while in *Meretrix* spp. organs it decreases in the order meat>gill>liver, illustrating the different metabolic processes of each biota. PAHs are more easily metabolized by invertebrates and are generally more efficiently metabolized in vertebrates such as fish. In fish, the metabolism of residues in tissues is typically slow and the resulting metabolites can be found in bile (Hylland 2006).

The concentrations of PAHs in *Meretrix* spp. are higher than in the milkfish. The levels of LMW and HMW PAHs differ between milkfish and *Meretrix* spp. Concentrations of LMW in *Meretrix* spp. are smaller than concentrations of HMW and conversely in the milkfish. Different routes of entry and a high exposure of each organism is assumed to be the cause. For example, *C. chanos* has a greater exposure to dissolved PAHs, while the *Meretrix* spp. is exposed to both the dissolved and particulate contaminants present in the water column. Hydrocarbons with a lower molecular weight are most soluble, whereas compounds with higher molecular weight are more easily absorbed by smaller resuspended particles, becoming a source of significant pollution for the *Meretrix* spp. (Baumard et al 1998). PAH concentrations decreased sequentially with the wet weight in biota groups, as follows: isopods>oligochaetes>water plants>anodonta>molluscs and chironomids>fish (Douben 2003). ∑PAHs for milkfish were 201.40 ng g^{-1} and for *Meretrix* spp. shells they were 207.37 ng g^{-1}. Gomes et al (2010) classified ΣPAHs concentration levels of 100-1,000 ng g^{-1} as moderate. The presence of PAHs in fish and shellfish body is of medium level and compared to other locations, the concentrations were small (Table 4).

Table 4
Comparison of the concentration level of PAHs in the body of biota, at the coastal town of Tarakan with other locations in the world (ng g^{-1})

Location	*Biota*	*∑PAHs*	*Concentration*	*Level*	*References*
Tarakan, North Borneo	*Chanos chanos*	16	201.40 ww	Moderate	Present study
	Meretrix spp.		207.37 ww	Moderate	
Tarakan, North Borneo	*Horpodon neherus* meat	16	605-1067 dw	Moderate-high	Achyani et al 2015
	Horpodon neherus liver		977-1679 dw	Moderate-high	
Hainan Island, China	*Pinctada martensii*	16	597.1-2332 dw	Moderate-high	Wang et al 2020
	Perna viridis		818.5-2153 dw	Moderate-high	
Niger Delta	*Littorina littorea*	16	7.150-30.100 ww	High	Nwaichi & Ntorgbo 2016
	Crassostrea virginica		3.670-105.000 ww	High	
	Periophthalmus koeleuteri		45.900-171.900 ww	High	
Turkish straits systems					
Istanbul Strait	*Mytilus galloprovincialis*	16	1.2-589 dw	Non-moderate	Balcioglu et al 2014
Marmara Sea	*Mytilus galloprovincialis*		0.94-36.4 dw	Non	
Canakkale Strait	*Mytilus galloprovincialis*		0.4-47.9 dw	Non-low	
Bays of Pozzuoli and Naples, Tyrrhenian Sea, Italy	*Mytilus galloprovincialis* (wild)	15	4.47-905.66	Non-moderate	Mercogliano et al 2016
	Mytilus galloprovincialis (farm)		0.71-1314.45	Non-high	
Bizerte Lagoon, Tunisia	*Mytilus galloprovincialis*	15	107.4-430.7 dw	Moderate-high	Barhoumi et al 2016
	Anguilla anguilla		114.5–133.7 dw	Moderate	
Mediterranean Sea	*Donax trunculus*	16	212.7-282.2 ww	Moderate	Ferrante et al 2018
	Sardina pilchardus		28.67-78.52 ww	Non	
	Solea solea		20.67-37.46 ww	Non	
Mundau Lagoon, Brazil	Bivalvia (*Mytella charruana*)	17	41.4-52.5 ww	Non	Maioli et al 2010
Southern Nigeria	Bivalves (*Arca senilis*)	16	3000-16.000 ww	High	Moslen et al 2019
Northern British Columbia, Canada	Mussels (lipid normalized)	37	0.51–13.08	Non	Thompson et al 2017
	Clams		0.86–17.55	Non	
	Cockles		0.74–9.73	Non	
	Mussels (Non-lipid normalized)		0.009-0.116	Non	
	Clams		0.012–0.239	Non	
	Cockles		0.008–0.095	Non	

ww - wet weight; dw - dry weight; non - not contaminated.

Histological tests on the gills, liver and meat of milk fish and *Meretrix* spp. showed a change in the structure of the biota organs due to the exposure to PAHs. In the *Meretrix* spp. gills there were no changes in the frontal cilia, lateral cilia and gill epithelium (Figure 3).

The liver tubule lumen changed, with regressing tubules (Figure 4), while in the meat there were found irregular mesh works which lead to necrosis (Figure 5). Exposure to PAHs in the gills causes necrosis, inflammation and edema of branchial lamellae and ciliated epithelium (Maisano et al 2016; Al-hashem 2017), while in the liver it causes tubular atrophy (Zacchi et al 2019).

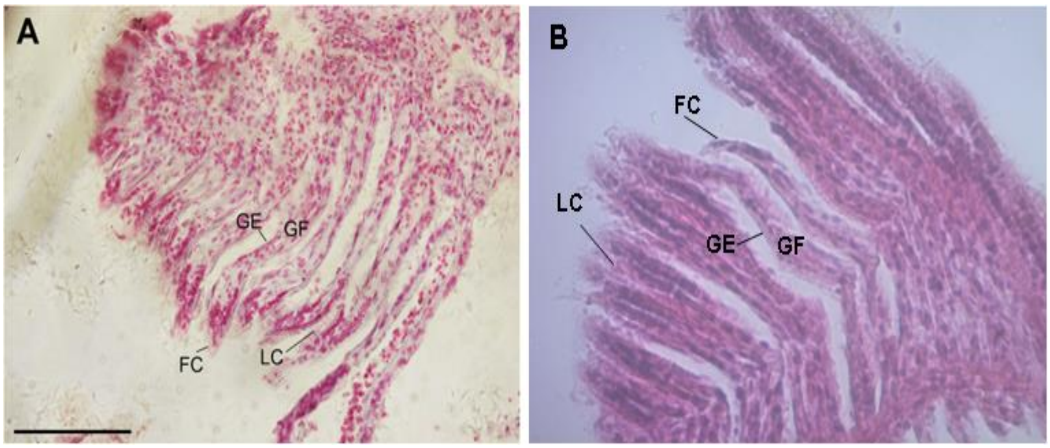

Figure 3. *Meretrix* spp. gills (B) don't show any deformities in frontal cilia, lateral cilia and gill epithelium compared to normal (A). (A) Normal gills clam (Wang et al 2020); (B) *Meretrix* spp. gill. Gill filaments (GF); frontal cilia (FC); lateral cilia (LC); gill epithelium (GE).

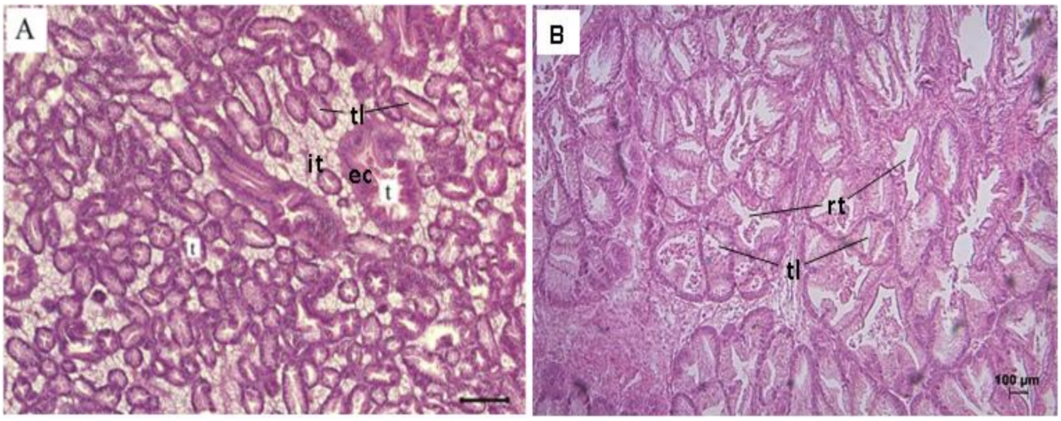

Figure 4. Liver *Meretrix* spp. show changes in the lumen tubules, compared to normal liver. (A) Normal *Meretrix* spp. liver (Zacchi et al 2019); (B) *Meretrix* spp. Liver. Lumen tubule (tl); digestive tubules (t); epithelial cells (ec); interstitial tissue (it); regressing tubules (rt).

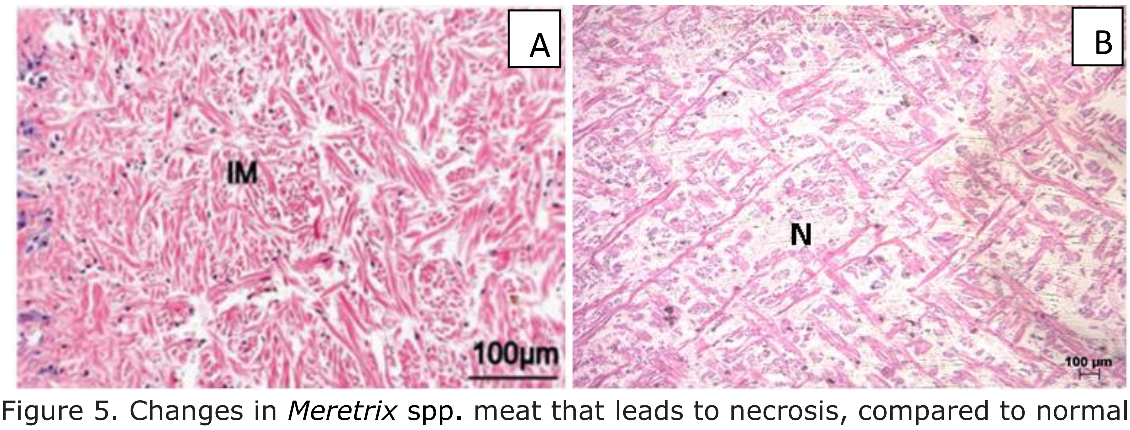

Figure 5. Changes in *Meretrix* spp. meat that leads to necrosis, compared to normal meat. (A) Normal *Meretrix* spp. meat (McElwain & Bullard 2014); (B) *Meretrix* spp. meat. Irregular meshwork (IM); Necrosis (N).

Histological tests on milkfish also showed that there were structural changes in the gills, liver and meat organs, likely due to exposure to PAHs. The milkfish gills at the Andulung location illustrate the lamellae disorganization. At Mamburungan, Karang Anyar Pantai, the same conditions were recorded, with the presence of fusion of lamellae, edema and necrosis (Figure 6). Gill changes due to the exposure to PAHs can cause interlamellar epithelial, lamellar and mucous cells hyperplasia, light dilation of blood vessels and congestion (Martins et al 2016; Akinsanya et al 2018). Besides, it can cause hypertrophy and fusion of the secondary lamellae, adhesions, edema and epidermal hyperplasia (Akinsanya et al 2018; Filfilan & Othman 2019).

Liver histology illustrates a suspicion of inflammation and alleged necrosis of the milkfish liver from Mamburungan and Karang Anyar Pantai (Figure 7). The meat shows a form of deformation (Figure 8). Exposure of the fish body to PAHs can cause hepatic necrosis, renal tissue mineralization, lipofuscin, blood accumulation, leukocytosis, an increase in macrophage numbers and inflammation, while exposure of meat can increase pigmented macrophage centers (Bentivegna et al 2015; Filfilan & Othman 2019; Mai et al 2019).

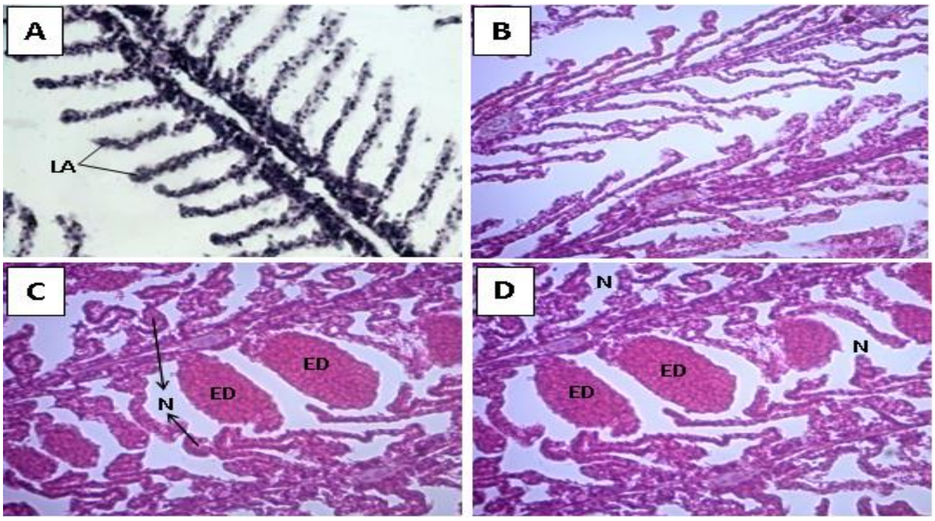

Figure 6. Changes in the gills of *Chanos chanos* in the form of changes in the shape of lamella, edema and necrosis, compared with normal gills. (A) Normal milkfish gills (Hidayati et al 2010); (B) Andulung location; (C) Mamburungan; (D) Karang Anyar Pantai. Lamellae (LA); edema (ED); necrosis (N).

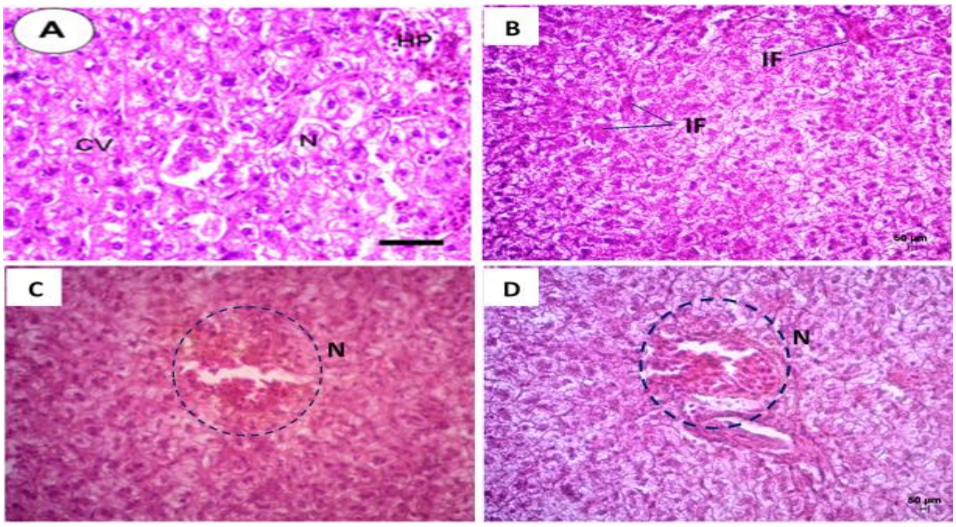

Figure 7. Changes in *Chanos chanos* liver in the form of inflammation and necrosis, compared to normal liver. (A) Normal milkfish liver; (B) Location of Andulung; (C) Mamburungan; (D) Karang Anyar Pantai. Central vein (CV); hepatic plate (HP); nuclei (N); inflammation (IF); necrosis (N).

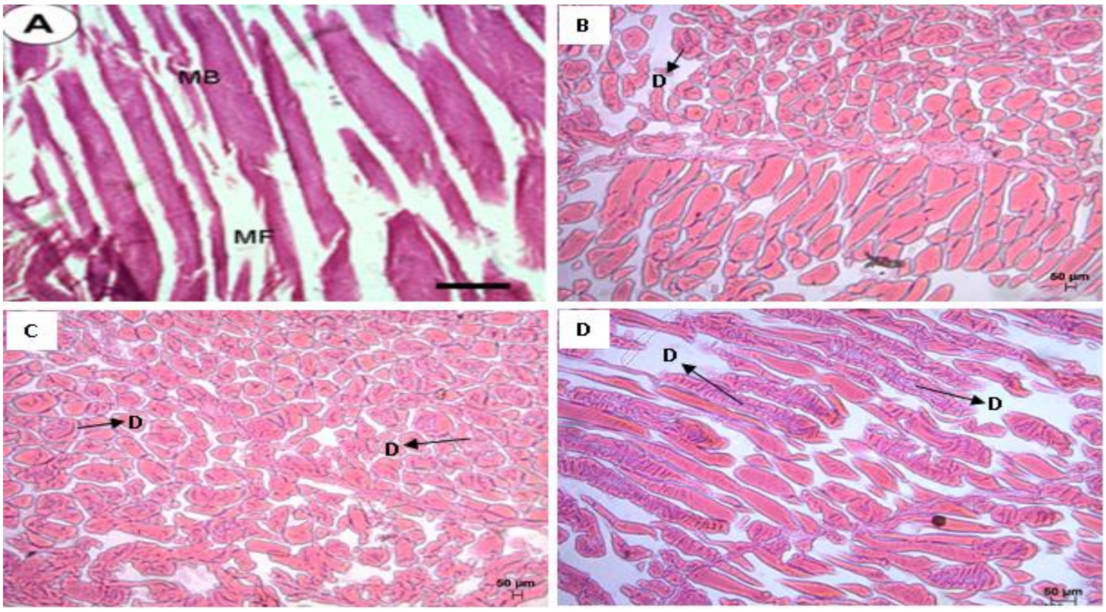

Figure 8. Changes in *Chanos chanos* in the form of deformation, compared to normal gills. (A) Normal milkfish meat; (B) Location of Andulung; (C) Mamburungan; Karang (D) Anyar Pantai. Deformation (D).

Conclusions. A significant exposure of aquatic biota to PAH at the Tarakan City coastal area was found in *Meretrix* spp. and *C. chanos*, which is indicated by the presence of 14 PAHs accumulated in organs. The level of PAH concentration in *Meretrix* spp. is higher than in milkfish. The highest concentration is in the *Meretrix* spp. shells' liver and gills in milkfish. The level of PAH contamination in *Meretrix* spp. and milk fish is moderate, but it still has an impact in terms of histological changes in gills, liver and biota meat.

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Conflict of interests. None reported.

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