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Mudskipper Fish as a Bio-indicator for Heavy Metals Pollution in a Coastal Wetland

Heri B. Santoso^{1,2*}, Eko Suhartono³, Rizmi Yunita⁴, Danang Biyatmoko⁵

¹-Postgraduate Program, Doctoral Department of Agricultural Science, Lambung Mangkurat University. Banjarbaru, South Kalimantan 70714, Indonesia

²-Department of Biology, Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University. Banjarbaru, South Kalimantan, Indonesia

³-Department of Medical Chemistry/Biochemistry, Faculty of Medicine, Lambung Mangkurat University. Banjarbaru, South Kalimantan, Indonesia

⁴-Department of Aquatic Resources Management, Faculty of Fisheries and Marine Sciences, Lambung Mangkurat University. Banjarbaru, South Kalimantan, Indonesia

⁵-Department of Animal Science, Faculty of Agriculture, Lambung Mangkurat University. Banjarbaru, South Kalimantan, Indonesia

* Corresponding Author: heribudisantoso@ulm.ac.id

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ABSTRACT

Mudskippers are important for ecotoxicological studies of coastal wetlands. They are recognized as a potential bio-indicator in estuarine water biomonitoring programs due to their natural abundance, high resistance to polluted environmental conditions, benthic habitat, high trophic levels in the aquatic food chain, and sensitivity to environmental changes. Mudskippers have the ability to accumulate heavy metals in their tissues and also have a strong propensity for determining the biological impact of pollutants on coastal wetlands. Hence, Mudskippers have the ability to serve as ideal sentinel organisms for the biomonitoring of heavy metal pollution. The application of biomarkers is very important for biomonitoring, as it is a direct indicator of all the toxic effects of heavy metals on organisms and is less expensive to use compared to chemical indicators, especially for heavy metal pollutants with very low concentrations. Biomarkers responses such as genotoxicity and immunotoxicity have not been applied as diagnostic tools to monitor the effect of heavy metals on mudskippers, while oxidative stress biomarker is widely used to diagnose and evaluate the adverse effects of heavy metals on mudskippers. So, these biomarkers need to be developed for more comprehensive information on the heavy metal toxicity in fishes. Thus, oxidative stress, genotoxicity, and immunotoxicity biomarkers can be incorporated into standard biomonitoring programs as new studies and approaches to resolve the global issue of heavy metal pollution in estuaries and the degradation of coastal wetland ecosystems.

INTRODUCTION

Heavy metal pollution is considered as a serious threat to the biological preservation of the estuary ecosystem with negative impacts on the health of organisms and the

ecosystem functions. The main effects of long-term heavy metal pollution include biodiversity and habitat degradation. These pollutants can disrupt living organisms at the molecular, cellular, and physiological levels, resulting in adverse effects at the population and community levels (Lionetto *et al.*, 2019). Heavy metal pollution mostly comes from anthropogenic practices as a result of industrialization, farming operation, and urban growth (AnvariFar *et al.*, 2018). Heavy metals that often pollute estuaries are Cu, Zn, Fe, Pb, Cd, Hg, and Ag (Marques *et al.*, 2019). However, society often overlooks the health effects of heavy metals and our overall knowledge about the biological effects of heavy metals on estuary ecosystems is minimal. Accordingly, establishing a biomonitoring program using biomarkers as an early identification tool is mandatory for preventing the harmful effects of heavy metal pollutants on the estuary ecosystem.

The purpose of the biomonitoring program is to measure the environmental quality through the use of living organisms on a regular and systematic basis or through their normal responses as markers in the environment (Seriani *et al.*, 2015; Sweidan *et al.*, 2015; Ghisi *et al.*, 2017; de Oliveira *et al.*, 2019; Decou *et al.*, 2019). The biomarker approach, as an early warning signal for ecosystem degradation, is based on the identification and quantification of molecular, biochemical, physiological, genetic, and cellular modifications in bio-indicator organisms (Ballesteros *et al.*, 2017; Bertrand *et al.*, 2018). The response to these modifications was studied depending on the bioavailability and bioaccumulation of heavy metals in the organism's body either due to single or multi-pollutant exposure (Calado *et al.*, 2020). The use of various complementary biomarker responses in bio-indicator species has now been widely used and validated in laboratory and field studies as biomonitoring tools for estuary and marine environments (Beg *et al.*, 2015).

The effectiveness of the biomonitoring program for heavy metal pollution in waters depends on the selection of the correct and appropriate bio-indicator species. A good bioindicator should have certain characters such as (i) high abundance; (ii) easy to handle, easy to manage in the laboratory, easy to locate, and readily identifiable; (iii) it can absorb numerous amounts of pollutants without fatal effects, making it suitable for ecotoxicological assessment; (iv) the sessile way of life (animals attached to inanimate objects or living animals), thereby representing local pollution; (v) the population is quite large and broadly dispersed, such that it is convenient to replicate and compare; (vi) the long life span, enabling comparisons between various ages; (vii) have target cells, tissues and organs suitable for further research at the cellular and molecular level; (viii) adaptable and tolerant of various conditions; (ix) occupy an important role in the food chain; (x) and react rapidly to early exposure to various xenobiotics. The ideal bio-indicator is certainly difficult to identify, but the identification of possible multi-character bio-indicator species could be applied according to the objectives of the biomonitoring program (Zhou *et al.*, 2008; Bertrand *et al.*, 2016; Javed *et al.*, 2017; Nimet *et al.*, 2020). These characteristics are sufficiently addressed by fish species, which makes fish an effective model as a biomonitoring tool for heavy metal pollutants in estuaries. Fish responds very sensitively and quickly to the quality of the aquatic environment. Thus, it can serve as a bio-indicator of environmental pollution (Farombi *et al.*, 2007; Rautenberg *et al.*, 2015; Kim *et al.*, 2017; Rajeshkumar and Li, 2018; Salgado *et al.*, 2019; Sinha *et al.*, 2020; Adegun *et al.*, 2020).

Mudskippers are often used for biomonitoring the estuary water quality, especially in coastal wetland ecosystems. Coastal wetlands are tidal wetlands that are a part of the estuary ecosystem. Mudskipper fish has a distinct biological uniqueness among other fish species. In general, they can not only breathe on land and in water like amphibian, but also has a broad tolerance to environmental stressors, benthic life, and resistance to organic and inorganic contaminants. Besides, mudskippers are widely distributed, plentiful in natural abundance, they are at the top of the food chain, and are suitable bioindicators for the long-term toxic effects of pollutants (Hidayaturrahmah *et al.*, 2019). Mudskippers dwell in mudflats of the intertidal coastal zones and mangrove forest floors of tropical and subtropical areas. These areas are known as the most degraded habitat types on the earth since they are continuously exposed to heavy metal pollution either from anthropogenic practices (Ferreira *et al.*, 2019; Marques *et al.*, 2019) or from naturally occurring pollution due to sedimentation and flooding (Barbee *et al.*, 2014; Zhang *et al.*, 2019).

Mudskippers can absorb heavy metals via various types of exposures, such as direct contact, ingestion, drinking, and inhalation (Barbee *et al.*, 2014). Heavy metal hazardous in biological systems could stimulate excessive production of reactive oxygen species (ROS), leading to abnormalities in cellular metabolic processes resulting in oxidative stress (Basirun *et al.*, 2019; Gavrić *et al.*, 2019). As a result of this kind of stress, an antioxidant defense mechanism is developed and could be used as a biomarker in biomonitoring programs for pollution risk assessment (van der Oost *et al.*, 2003). Heavy metals could also cause imbalances in cellular redox reactions, leading to lipid and protein lysis, DNA damage, as well as carcinogenic and immunosuppressive effects (Gao *et al.*, 2019; Lee *et al.*, 2019; Sinha *et al.*, 2020).

Intensive studies were conducted to develop biomarkers on mudskippers for biomonitoring heavy metal pollution in coastal wetland ecosystems. Oxidative stress, genotoxicity, and immunotoxicity biomarkers could be used as early warning systems in the biomonitoring programs (Radwan *et al.*, 2020). Biomarker diagnostic techniques have already been developed in aqua-toxicological studies, for the early identification of heavy metal pollution in fish, because they are reliable, precise, eco-friendly, and cost-effective. This study aims to provide a comprehensive review on the potential of using mudskipper as a bio-indicator species by assessing the response of biomarkers to heavy metal pollutants. Hence, to evaluate pollution in the coastal wetland environment.

MATERIALS AND METHODS

The review of mudskippers as a bio-indicator of heavy metal contamination in the coastal wetlands was performed by reviewing relevant indexed Scopus and Google Scholar articles until mid-2020. The keywords used were "mudskippers", "biomonitoring", "biomarkers", "bioindicator", "bioaccumulation", "estuarine pollution", "coastal wetland pollution", and "heavy metals". We focused on the following mudskipper species; "*Periophthalmus*", "*Periophthalmodon*", "*Boleophthalmus*", "*Pseudapocryptes*", "*Apocryptes*", "*Scartelaos*", and "*Zappa*".

About 150 articles from several countries, in Africa and Asia, were discovered through Scopus and Google Scholar. Abstracts of these articles were used for the initial screening. We excluded the articles with not relevant abstracts or too broad discussions. Of the 150 articles, 105 were referred to be used for the review material. Twenty relevant

articles, the core of this review, were summarized in a table including data about the bioaccumulation of heavy metals in mudskippers living in coastal wetlands and the responses of mudskippers to heavy metal exposures in the estuary.

From the 105 relevant articles used for compiling this review, 20 articles were on mudskippers (*Periophthalmus* (7), *Periophthalmodon* (4), *Boleophthalmus* (3), *Gobius* (2), *Neogobius*(2), *Pseudapocryptes* (1), and *Zosterisessor* (1)), 25 articles were on the biological aspects and the uniqueness of mudskippers, and 60 articles on the response of biomarkers, bioindicators, and bioaccumulation of heavy metals in other estuarine fish species.

RESULTS AND DISCUSSION

The biology uniqueness of mudskippers (Teleostei: Gobiidae: Oxudercinae)

Mudskipper is a unique fish with an amphibian-like lifestyle (Figure 1). The amphibian lifestyle is defined by its ability to remain entirely terrestrial as part of their normal daily life cycle (Al-behbehani & Ebrahim, 2010; Dabruzzi *et al.*, 2019). According to Ansari *et al.* (2014), 34 species of mudskippers were identified in 7 genera, namely *Periophthalmodon*, *Periophthalmus*, *Boleophthalmus*, *Scartelaos*, *Pseudapocryptes*, *Zappa*, and *Apocryptes*. The *Periophthalmus* genus includes 18 species (Dabruzzi *et al.*, 2019). At present, five genera namely *Boleophthalmus*, *Periophthalmodon*, *Periophthalmus*, *Scartelaos*, and *Zappa* (the slender mudskipper) with 32 species exist mainly in the soft-bottom mudflat zones, mangal habitats, river deltas, and muddy coastlines throughout the Indo-Pacific to southern Japan and west to the Atlantic coast of Africa (Dabruzzi *et al.*, 2019). Their terrestrial activity varies according to each species. *Periophthalmus* and *Periophthalmodon* spend the majority of their time out of the water, although their terrestrial lifestyle differs among species of these genera. On the other hand, *Scartelaos* is primarily aquatic, followed by less water-dependent *Boleophthalmus* (Polgar and Lim, 2015; Lorente-Martínez *et al.*, 2018).

The giant mudskipper, *P. schlosseri*, is one of the major species of Oxudercine gobies that can be found in tropical and subtropical countries such as Malaysia, Indonesia, Australia, India, and Africa (Looi *et al.*, 2016). Studies on mudskipper's mitogenomics, molecular instrument for the protection of biodiversity (Yi *et al.*, 2016), are currently ongoing to explore mudskippers genes to find new genera and species that may not have been identified. The unique living environment allows the Mudskippers to undergo unusual physiological and ecological adaptations, as well as genetic modifications.

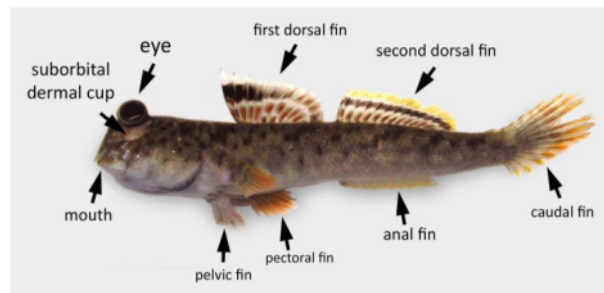


Figure 1. External morphology of a mudskipper, *Periophthalmus variabilis*. © Gianluca Polgar (Polgar, 2010)

Mudskippers like to “skip” on land, particularly in muddy areas or shallow waters near mangroves in the estuarine region when the water recedes, so it is called a mudskipper. The shape of its face is very distinctive with two eyes protruding above the head like frogs’ eyes and a nicely shaped dorsal fin. Its body is elliptical, like a torpedo, with a rounded tail-end. The length of the body ranges from a few centimeter to nearly 30 cm. We can measure the age and growth of the mudskipper by calculating the growth rings of the second pectoral radial bone (Nanami and Takegaki, 2005). This species is characterized by spending 90% of its time living on land, climbing up the roots of mangrove trees, jumping long distances, and walking on mud. These characteristics are supported by the existence of pectoral fins that have been evolved into sturdy legs and can be used to walk in mud (Al-behbehani and Ebrahim, 2010). The base of the pectoral fins is sturdy enough that can be bent and serve as arms for creeping, crawling, and leaping (Wicaksono *et al.*, 2016). According to Wicaksono *et al.* (2020), *Periophthalmus variabilis* has unusual climbing and jumping ability, it can cross the water surface by leaping and can change its direction while on the water surface. The Mudskipper terrestrial movement is characterized by a constant wave motion on land and has a temporary ballistic-like leap motion on the water surface. Terrestrial movement is influenced by the size of the fish. The medium-sized species make more leaps on water surface and fewer leaps out of the water than the smaller or larger similar species (Magellan, 2016). Further studies are required on other environmental factors related to the mudskipper’s behavior in terrestrial areas. This ability to survive on land is assisted by its ability to breathe through the skin surface and the mucous membranes in its mouth and throat (Ansari *et al.*, 2014; Zaccone *et al.*, 2017).

The two most terrestrial genera, *Periophthalmodon* and *Periophthalmus* spend most of their time out of water. These genera have a special modification to their gill structure and hyper-vascularization of epithelial tissues that allow aerial respiration while they are out of water.

Mudskippers are known as air-breathing fish because they use air directly through their skin (Looi *et al.*, 2016). Their epidermis contains characteristic structure and numerous capillaries for cutaneous respiration. The mudskippers’ air phase pattern in their burrows has been reviewed. Most of the mudskippers create their burrows in anoxic mud in the littoral zone. At high tide, the burrows are flooded with water and the fish is enclosed there (for up to 10 hours) to escape from predators, such as piscivorous fishes.

Mudskippers perform a sequence of the air-deposition acts to establish an air phase in their burrows, which is a rapidly repeated sequence of activities. These activities include inflating the buccal chamber (part of the air-breathing organ) with air on the mudflat surface, transporting it to the burrow, releasing it, and immediately returning to the surface with a deflated buccal chamber (Lee *et al.* 2005). However, since they possess aquatic larvae and cannot tolerate desiccation, they still depend on water. Thus, mudskippers roll-on mud, immerse in shallow pools that persist at low tides or often shift from land to burrows that were built in the mud and submerged at high tides. These burrows are also used as shelters for reproduction and hiding from enemies (Lorente-Martínez *et al.*, 2018). The oxygen in the mudskipper's burrow air phase therefore be necessary for intraburrow aerial respiration. The skin of mudskippers is the main interface between the body and the environment and plays a key role in host defense (Lauriano *et al.*, 2018).

The tidal flats created by river estuaries and the floor of the mangrove forest ecosystem are the natural habitats of mudskippers (Al-behbehani and Ebrahim, 2010; Perumal, 2011; Shirani *et al.*, 2012b). In Iran, mudskippers are also found in freshwater (Sharifian *et al.*, 2018). They are commonly distributed in Africa, Madagascar, India, Southeast Asia, northern Australia, South China, southern Japan, Samoa, the Tonga Islands, Saudi Arabia, the Gulf of Kuwait, Polynesia, and Indonesia (Polgar, 2012; Polgar and Lim, 2015). The coastal regions of Southeast Asia, Australia, and Papua have the largest species diversity (Ansari *et al.*, 2014). Mudskippers are found to be active in mud with very sticky particles. Mudskippers also form mixed colonies with digging crabs (Fiddler crabs-Caidae) (Al-behbehani and Ebrahim, 2010). The species is carnivorous feeding primarily on insects (diptera) and tiny crabs (such as the fiddler crabs) (Sharifian *et al.*, 2018). Adult fish are strongly terrestrial and have not been observed to venture into the water. Courtship activity was observed and fertilized eggs were retrieved from burrows in both the brackish and freshwater habitats (Mai *et al.*, 2019). In intertidal habitats, mudskippers can achieve densities of >4 individuals/m² (Polgar, 2012).

Mudskippers are closely correlated with coastal wetlands and tidal mudflats and will probably follow the fate of these critically endangered ecosystems (Polgar, 2012). The natural habitat of the mudskipper can be preserved by reforestation of the intertidal zone with the planting of mangroves. This operation is feasible to boost the coastal ecosystem and promote marine biodiversity. The reforestation of mangrove forests also protects the coastline from strong currents and promotes the deposition of sediment and organic matter in the intertidal zone, thereby enhancing the consistency of the mud and increasing the survival and growth of intertidal zone fauna. Efforts to control trash, untreated wastewater, and pollutants directly into estuarine waters would definitely help protect mudskippers. Law policies and regulations on the preservation of the aquatic ecosystem must be addressed in order to ensure the preservation of fauna biodiversity in the estuarine environment.

According to Takiyama *et al.* (2016), the adaptive mechanism of mudskipper species to travel and forage on land is designed to enhance air vision. *Periophthalmus modestus* forages in the mudflat at low tide by capturing polychaetes and crustaceans. However, during high tide, it rises and remains on the wood and does not actively feed. The sensory and motoric capabilities of *P. modestus* are well suited to the terrestrial lifestyle, i.e. its eyes are fitted with thick corneas and flat lenses which are designed to

enhance vision during terrestrial activities. A characteristic trait of *P. modestus* orientation behavior is to target the target using a specialized retinal area by rolling the eyes and lifting the head before moving to strike targets positioned above eye level. Another species, *P. argentilineatus*, can travel on the mudflat at low tide and leap on its pectoral and tail fins. This species can sense the presence of objects from a considerable distance whenever it comes out of the water. This behavior is an adaptation for foraging and terrestrial activities where buoyancy is negligible.

Under favorable conditions, mudskippers emerge during the low tide in search of food, locate mates, and protect their territories. Some species are known to keep emerging for hours, or even days, waiting for the tide to return. The long emersion time is supported by various key adaptations, including (i) expanding the surface of the respiratory exchange; (ii) special biochemical and physiological modifications to prevent ammonia toxicity (Ip and Chew, 2010); (iii) and strong skin resistance to evaporative water loss (Dabruzzi et al., 2019). However, in summer, mudskippers can experience a rise in body temperature that endangers their life, as well as intense dryness/dehydration when switching from water to air. Even so, field studies have confirmed that several mudskippers of the genus *Periophthalmus* and *Boleophthalmus* have been able to survive and be active in temperatures between 38 and 40°C, which is an extreme temperature that can kill fish in general. Most mudskippers avoid the most extreme low temperatures by settling in their nests where the temperature can be several degrees warmer than the air. Mudskippers will reappear into the air after the temperature increase. Several species of mudskippers have been observed spending the winter in their burrows. Exposure to air temperature of 1°C is rarely lethal to the mudskippers. However, *Boleophthalmus pectinirostris* is an exception, which can survive in mud habitats at temperatures of 0.8°C. The temperature range for the mudskipper's behavior activity and survival was between 11.4°C - 37.0°C (Dabruzzi et al., 2019). Mudskippers are typical amphibious fish that have diverse methods to ameliorate ammonia toxicity during the exposure to environmental ammonia (You et al., 2018). The mudskippers are euryhaline and can tolerate a sudden and dramatic shift in salinity (Soltanian and Fereidouni, 2019). They can tolerate a very wide range of fluctuations in salinity and temperature (Chen et al., 2015). Mudskippers that are traveling through the land between aquatic habitats are likely to face sudden changes in a variety of environmental factors, including salinity (Sutton et al., 2018).

The locals also believed that mudskippers have significant medicinal importance (Ikram et al., 2010). They could also be a reliable or decorative fish for aquarium (Song et al., 2020). The species has little commercial value in Iran, but it is the main food source for intertidal birds and may be used as indicators for anthropogenic practices on coastal ecosystems (Sharifian et al., 2018). Due to its high-quality protein with well-balanced amino acids, *Pseudapocryptes elongatus* is desirable for human consumption in India (Mahadevan et al., 2019).

Mudskippers as potential biomonitoring tool for heavy metals pollution in the coastal wetland

Heavy metal pollution in water causes biological disturbance to organisms, where it can be identified and assessed by specific biological tests on relevant bioindicators such as mudskippers. Early identification of biomarker responses in potential bioindicators

plays an important role in the success of biomonitoring before the effects of heavy metal pollution adversely affect the entire population or community (Georgieva *et al.*, 2016; de Almeida Duarte *et al.*, 2017; Bouzahouane *et al.*, 2018). The biomonitoring approach is more accurate and reliable than chemical water quality monitoring. Water chemical analysis offers direct information about the identity, nature, and volume of harmful chemical material in aquatic biota, water sources, and sediments¹². However, it cannot provide details about bioavailability and the role of its toxicity effects on aquatic biota (Seriani *et al.*, 2015; Sweidan *et al.*, 2015). It also cannot explicitly determine the direct effects on organisms, populations, and communities (Montenegro *et al.*, 2020). The shortcomings of this monitoring will now be strengthened by the use of biomonitoring methods strategy.

Mudskippers are used as bio-indicators of pollution in coastal wetland habitats, from the 1980s to the present, since they have distinctive morphological and ecological structure, have amphibians-like lifestyle, could accumulate different pollutants, exposed directly to various pollutants, and are euryhaline organisms (Ansari *et al.*, 2013; Bertrand *et al.*, 2018). They are sentinel organisms (guard organisms) that are ideal for detecting the effects of pollutants on water and sediments. A sentinel organism must have some special characteristics such as a wide geographical range, high sensitivity to environmental pollutants, and the dominant species in their habitat (Shirani *et al.*, 2012a; 2012b). Researches on the potential of mudskippers as a bio-indicator for heavy metal pollution in several countries began with an analysis of heavy metal bioaccumulation in various body tissues and establishing an analysis of behavioral and biomarker responses (Table 1).

Table 1. Summary of variations in the response of biomarkers on mudskippers to heavy metal pollution

Mudskipper species	Heavy metals	Exposure route	Organ	Responses	Reference
<i>Zosterisessor ophiocephalus</i>	Hg, Se	Mining sites	Soft tissues	Bioaccumulation	Acquavita and Bettoso (2018)
<i>Gobius boddarti</i>	Fe, Cu, Zn, Cd, Pb	Polluted sites	Soft tissues	Bioaccumulation	Ahmed <i>et al.</i> (2011)
<i>Periophthalmodon schlosseri</i>	Cu, Zn, Pb, Cd, Ni	Polluted sites	Scale, muscle, bone, gills, operculum, intestine, liver, cartilage	Bioaccumulation	Buhari and Ismail (2016)
<i>Periophthalmus waltoni</i>	Zn, Cu, Cd, Fe	Polluted sites	Muscle, gills, liver	Bioaccumulation	Bu-Olayan and Thomas (2008)
<i>Periophthalmus dipses</i>	Cr	Experimental	Liver, brain, muscle	Inhibitors enzyme ATPase in the liver, brain, muscular tissues	Chhaya <i>et al.</i> (1997)
<i>Periophthalmus koelreuteri</i>	Cu, Zn, Pb	Polluted sites	Muscle, gills, liver	Bioaccumulation	Eboh <i>et al.</i> (2006)
<i>Boleophthalmus pectinirostris</i>	Cd	Experimental	Testes	The testis presents abnormal morphology and MT mRNA expression	Han <i>et al.</i> (2015)

Mudskipper species	Heavy metals	Exposure route	Organ	Responses	Reference
<i>Periophthalmodon schlosseri</i>	Zn, Cu, Cd, Pb	Polluted sites	Scales, muscles, bones, gills, liver	Bioaccumulation in different tissues	Ikrum et al. (2010)
<i>Neogobius melanostomus</i>	Pb, Cd, Cu	Polluted sites	Gonad, liver	An increase in the antioxidant enzyme activities and the level of oxidized serum proteins. A decrease in the size and weight gonad & liver	Kovyrshina and Rudneva (2018)
<i>Neogobius melanostomus</i>	Pb, Cd, Cu	Polluted sites	Blood	The activities of antioxidant enzymes (CAT, SOD, PER, GR, NADP, GT)	Kovyrshina and Rudneva (2016)
<i>Periophthalmus argentilineatus</i>	Al, Zn, Fe, Pb, Cd, Cu, Co, Cr, Ni	Polluted sites	Muscle tissues	Bioaccumulation	Kruitwagen et al. (2008)
<i>Periophthalmus modestus</i>	As, Cd, Cr, Cu, Hg, Ni, Pb, Zn	Polluted sites	Muscle	Bioaccumulation in muscle	Liu et al. (2019)
<i>Periophthalmodon schlosseri</i>	Hg, MeHg, Se	Polluted sites	Muscle, liver, gill, gastro-intestinal tract	Bioaccumulation in the multiple tissues	Looi et al. (2016)
<i>Boleophthalmus pectinirostris</i>	Pb	Experimental	Gill, liver	Antioxidant enzyme activity (SOD, CAT, GPX, GSH), MDA content, expression of SOD, GST, HSP-70, and HSP-90 genes in the gill and liver	Jing et al. (2017)
<i>Gobius niger</i>	Various toxic compounds such as heavy metals	Impacted stations	Gonad, liver, muscles	The ethoxyresorufin-O-deethylase (EROD) activity, GST activity, total glutathione concentrations, the formation of TBARS	Louiz et al. (2016)
<i>Periophthalmus sp</i>	Cr, Ni, Cu, Pb, Ag, Cd	Polluted sites	Muscle	Bioaccumulation	Moslen and Miebaka (2016)
<i>Boleophthalmus boddaerti</i>	Zn, Mn, Cu, Fe, Co, Ni, Cd, Cr, Sr	Polluted sites	Muscle	Bioaccumulation	Patel et al. (1985)
<i>Periophthalmodon schlosseri</i>	Ag, As, Cd, Cr, Cu, Hg, Pb, Zn	Experimental	Brain	Inhibition of acetylcholinesterase activity	Sabullah et al. (2015)
<i>Periophthalmus waltoni</i>	Pb, Zn, Ni, V	Polluted site	Blood	Alkaline phosphatase and alanine aminotransferase were higher in fish	Sarhadizadeh et al. (2014)
<i>Pseudapocryptes lanceolatus</i>	Fe, Cu, Ag	Polluted site	The olfactory apparatus	Bioaccumulation and neurodegenerative disorders in ciliated olfactory sensory receptor neuron	Sarkar and De (2016)

Recently, the use of biomarkers has gained a significant attention as a responsive parameter to estimate the exposure or effects of heavy metal pollution and polycyclic aromatic hydrocarbons. Successful biomarkers, in routine biomonitoring programs, must be applicable in laboratory and field conditions. Biomarkers serve as a screening tool to monitor the effect of pollutants. Several biomarkers have been used to detect and understand the adverse effects of heavy metal pollutants (such as, oxidative stress, genotoxicity, and immunotoxicity). These biomarkers could provide information on early identification and quantification of impacts of the pollutant during the initial exposure, hence, permitting prevention and/or restorative attempts to the affected ecosystem as soon as possible. The analysis of the oxidative stress biomarker response in mudskippers is more advanced than the immunotoxicity and genotoxicity responses, thereby opening up possibilities for potential studies.

The studies of mudskippers as a bio-indicator of heavy metal pollution were not conducted in the coastal wetlands of Indonesia, but were mainly in Peninsular Malaysia and the Persian Gulf. Mudskippers were used as a biomonitoring tool for heavy metal pollution in Peninsular Malaysia with acetylcholinesterase inhibition approach (Sabullah *et al.*, 2015), while in the Persian Gulf, it was used as a bio-indicator for polycyclic aromatic hydrocarbons (Sinaei *et al.*, 2012; Sinaei and Rahmanpour, 2013; Sinaei and Mashinchian, 2014; Ansari *et al.*, 2014). Ikram *et al.* (2010) added that the mudskippers could be the future biomonitoring organisms for the contamination of coastal mud in the intertidal zone of Peninsular Malaysia with Cd and Pb.

Jing *et al.* (2017) stated that, the exposure of mudskippers to Pb resulted in an excessive production of reactive oxygen species (ROS). Oxidative stress is thought to be the key path to the initiation of heavy metal toxicity in fish. Oxidative stress is a mismatch between the production of ROS and the response of cellular antioxidants (such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione (GSH), and glutathione s-transferase (GST)), which allows the usage of the antioxidant response as the oxidative damage as a reliable and sensitive biomarkers for the evaluation of oxidative stress in fish subjected to heavy metals (Beg *et al.*, 2015; Lee *et al.*, 2019; Adeogun *et al.*, 2020). To mitigate the harmful effects of ROS, fish built an efficient antioxidant defense mechanism, including enzymatic antioxidants (such as CAT, GPx, glutathione reductase (GR), glucose-6-phosphate dehydrogenase (G6PDH), and GST), as well as non-enzymatic antioxidants (such as GSH and metallothionein) (Livingstone, 2001; Ferreira *et al.*, 2019; Sinha *et al.*, 2020). This mechanism protects the cells from lipid peroxidation, protein oxidation, and DNA/RNA damage through eliminating the production of ROS and free radicals (Do *et al.*, 2019).

Thiobarbituric acid reactive substances (TBARS) have been used to calculate lipid peroxidation (Ponton *et al.*, 2016). Lipid peroxidation induces oxidative stress and disrupts cell activities (Li *et al.*, 2010; Kim *et al.*, 2017). Lee *et al.* (2019) reported that Pb affects the mechanism of lipid peroxidation (LPO) in animals and humans. Also, the increase in TBARS due to Pb exposure resulted in oxidative stress in fish. Oxidative stress by Pb toxicity causes lipid peroxidation (LPO) in biological membranes. TBARS with malondialdehyde (MDA) is a sensitive indicator of lipid peroxides detection as a membrane lipid peroxidation substance (Do *et al.*, 2019). Lee *et al.* (2019) suggested that lipid peroxidation and TBARS levels in fish subjected to oxidative stress due to heavy metals exposure and other environmental toxins are used to demonstrate the level of lipid

1eroxidation. Excessive ROS production due to heavy metal toxicity can cause an increase in TBARS and oxidative stress to the gills, intestines, brain, and fish muscles resulting in cell damages (Kaya and Akbulut, 2015). TBARS has the potential as an oxidative stress biomarker in fish because fish contains a lot of highly unsaturated fatty acids (HUFA) (Copat *et al.*, 2019). This statement is consistent with the findings of Louiz *et al.* (2016) who used GST and TBARS levels as biomarkers of oxidative stress in *Gobius niger* (Gobiidae) exposed to heavy metals in the Byzerta lagoon, Tunisia. Thus Pb toxicity has a detrimental effect on fish by inducing oxidative stress and ROS production, as well as antioxidant responses such as SOD, CAT, GSH, GST, and TBARS. The emergence of antioxidants and TBARS responses are major biomarkers of oxidative stress in fish exposed to Pb (Lee *et al.*, 2019).

In addition to inducing oxidative stress, heavy metal pollution in estuaries also results in genotoxicity in fish. The origin of the nucleate or DNA is changed due to the unnecessary induction of ROS. Since DNA is very important to cells, a relevant method has been developed to evaluate DNA modification induced by ROS. The HPLC method and immunological techniques were used to calculate the formation of oxidized bases in fish, particularly 8-oxoguanine (8-OG). Meanwhile, the Comet test is used to diagnose differences in DNA damages and is used as a genotoxicity biomarker in fish exposed to heavy metals (Lushchak, 2011; Ben Ameer *et al.*, 2012; Ghisi *et al.*, 2017). However, the studies on genotoxicity biomarkers due to heavy metals exposure to Mudskippers have not been reported, and thus further studies for biomonitoring are required.

Exposure to heavy metals in the estuary environment often disrupts the fish's immune responses. For example, exposure to Pb has resulted in changes in fish's immune system and has also resulted in physiological, biochemical, and neurological disorders (Paul *et al.*, 2014). Dunier (1996) indicated that exposure to Pb in fish induces the decrease of hematopoietic activity in the spleen, phagocytic activity, and decreased antibody production. Adeyemo *et al.* (2010) stated that the immune system stimulation and tissue damage in fish exposed to Pb may lead to an increase in lymphocytes. However, long-term contact will trigger a decline in lymphocytes and white blood cells due to immune system damages. The number of white blood cells and lymphocytes in fish exposed to Pb will be reduced. This is triggered by the secretion of cortisol as a stress response due to Pb exposure, which decreases the life of lymphocytes or improves their apoptosis. Heavy metal toxicity significantly reduced fish antibody titres. Lee *et al.*, (2019) stated that Pb exposure affected the immune response to factors such as plasma immunoglobulin M (IgM) and lysozyme production. Pb is believed to affect the immune response by regulating the expression of cytokines (Yin *et al.*, 2018). Cytokines including interleukins (ILs) and tumor necrosis factor (TNF) are proteins that control the signal in different cells that trigger the immune response and play an important role in the regulation of immune mechanisms. Interleukin 10 (IL-10) is anti-inflammatory cytokine, and tumor necrosis factor- α (TNF- α) is involved in inflammation and apoptosis (Dai *et al.*, 2018). Expression of IL-10 and TNF- α mRNA was changed in crucian carp exposed to Pb, causing a significant tissue damage due to enhancing the inflammatory immune response (Dai *et al.*, 2018).

Heavy metals such as Pb enhance the expression of Hsp70 in fish (Yin *et al.*, 2018). Hsp70 has the essential functions of intracellular transport and chaperoning, and tumor-specific antigen production by stimulating the immune system of T cells and NK cells. Hsp70 has an important function in intracellular transport, and the production of tumor-specific antigens by activating the immune function of T cells and NK cells. Additionally, Hsp70 stimulates the release of cytokines such as ILs and TNFs (Radons and Multhoff, 2005).

Many studies have shown that heavy metals such as Pb adversely affect immune responses such as lymphocytes, leukocytes, inflammation, and apoptosis in fish. Pb not only prevents the function of various biomolecules, but also acts as immunotoxicity by preventing intracellular signal transduction. Therefore, changes in fish immune response have been used as an important indicator to assess the toxic effects of heavy metal exposure (Lee *et al.*, 2019). Thus, heavy metals accumulation in fish induces oxidative stress by generating ROS, and the antioxidant responses such as SOD, CAT, GST, GSH and TBARS in fish are activated to protect them from oxidative stress. Heavy metals exposure also causes genotoxicity and immunosuppression. Unfortunately, however, studies on the response of immunotoxicity biomarkers related to heavy metal exposure on Mudskippers are minimal, even though this species has the potential as a sentinel organism and as bioindicators for monitoring the effects of heavy metals in the biomonitoring program of coastal wetland ecosystems.

Future ecotoxicological studies can significantly broaden their scope by concentrating on the biological peculiarities and diversity of oxudercine species. Future studies could investigate the effect of anthropogenic influences on mudskippers populations and their current conservation status. Future mudskippers studies could be applied not only to investigate the biological significance and complexity of its genus, but also to study its ecotoxicology. Future studies may determine the impact of anthropogenic effects on the biomarker response, population structure, and conservation status. It is hoped that in the future, mudskippers may have the potential to become a well-known flagship species to support estuaries environmental conservation in the Indo-Pacific West and the Atlantic coast of Africa.

CONCLUSION

Mudskippers are gobioid teleosts, euryhaline, amphibious and air-breathing fish. They are broadly dispersed along the West African coast and the Indo-Pacific region. The fish lives in mangrove and tidal ecosystems at the soft bottom peri-tidal environment in the estuarine region, especially in coastal wetlands. The estuary is the most degraded habitat since it is often exposed to heavy metal pollution due to commercial, agricultural, and domestic activities. Some species are amongst the best-adapted fishes for an amphibious lifestyle. Mudskippers are benthic burrowers in anoxic sediments, and since tidal mudflats are effective sediment traps and sinks for nutrients and other chemical compounds, they are continuously in contact with various forms of pollutants generated by commercial, agricultural, and domestic activities. Thanks to their natural abundance, tremendous resilience to heavily polluted environments, and benthic habits, mudskippers

are often used as model organisms in aquatic ecotoxicological studies to assess heavy metals in coastal areas and to study their effects on intertidal inhabitants. Mudskippers could bioaccumulate metals in their body tissues and bio-magnify them to higher trophic levels, so that they can be used as a biomonitoring agents that help scientists to preserve coastal wetland habitats to become more sustainable and productive. It has also been suggested as a possible bio-indicator for heavy metal pollution in the tropical intertidal coastal mudflat. Mudskippers can be used as biomonitors for the differential habitat degradation of tropical coastal wetlands occurring along the intertidal zone. Biomarker responses such as oxidative stress, genotoxicity, and immunotoxicity were useful for early identification of heavy metals toxicity and offer complimentary knowledge in the biomonitoring program. Thus, these biomarker responses could develop guidelines for effective and efficient coastal wetland habitat management. So, it can be said that biomarkers are required to monitor environmental changes earlier by assessing the effect of xenobiotic compounds, including heavy metals on the mudskippers.

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