

The potential use of *Octolasmis* spp. parasites in mud crabs *Scylla* spp. as a bioindicator for mercury pollution

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Abstract. Nur I, Aris EA, Yusnaini Y, Beavis S. 2021. The potential use of *Octolasmis* spp. parasites in mud crabs *Scylla* spp. as a bioindicator for mercury pollution. *Biodiversitas* 22: 3764-3772. Artisanal small-scale gold mining and the use of mercury is widespread across Indonesia, often characterized by relatively short-lived gold rushes. In the late and post-mining phases, mercury stored in mine tailings and river beds was transported down catchment, posing risks to ecosystems and human health over much longer time scales. These risks can be under-rated when mercury concentrations in water, sediments, and aquatic species are low enough to meet relevant guideline thresholds. In this study in Southeast Sulawesi, Indonesia, we have investigated the Costraca barnacle, *Octolasmis*, as a bioindicator of mercury contamination. The presence of *Octolasmis* parasites in mud crabs, *Scylla* spp., and the accumulation of mercury (Hg) in the hosts, parasites, and the ambient environment, were analyzed across both dry and wet seasons. Severe infestation of *Octolasmis* was assessed using prevalence, abundance, and intensity. Hg concentrations were significantly higher in the *Octolasmis* parasites than in the host tissues, water and sediments. The mean bioconcentration factor (BCF) of Hg equaled 7938.21 from water to parasites, and 28.91 from the host's gill tissue to the parasites. The results suggest that *Octolasmis* spp. can be used effectively as a bioindicator in coastal catchments impacted by mercury contamination, even when concentrations of mercury are low in water and river sediments. The study provides the first report of *Octolasmis* spp. parasitized to mud crabs as a reliable bioindicator of Hg contamination and pollution.

Keywords: Artisanal small scale gold mining, bioindicator, mangrove, mud crab, parasite, pollution

INTRODUCTION

Environmental stress caused by contamination from anthropogenic activities, is an increasing concern. Specifically, environmental pollution by heavy metals, and associated toxicity, negatively impact living systems over the medium and long term due to bioaccumulation and biomagnification (Singh et al. 2011; Pandey and Madhury 2014).

In these settings, the uptake and absorption of metals in major organs and muscle tissue of biota (Singh et al. 2011; Copat et al. 2013; Armah et al. 2014; Bawuro et al. 2018; Thompson and Darwish 2019), results in higher concentrations within an organism than the surrounding environment (Sivakumar and Li 2018). Aquatic biota can be particularly vulnerable due to their prolonged exposure and uptake from various sources, including water, sediments and biota lower in the food chain (US. EPA 2000; Kahlon et al. 2018). Furthermore, the degree of metal accumulation is not only determined by environmental conditions and the trophic position of the organism, but also by species characteristics (Jakimska et al. 2011; Sow et al. 2019).

National and international water quality standards can be applied to assessments of water bodies with reference to a wide range of physical and chemical parameters,

including heavy metals. However, in the last few decades, bioindicators have also been increasingly used to identify pollutant loads and environmental risks at, and downstream of contaminated sites. Bioindicators are species or communities whose presence/absence or condition can be used to qualitatively assess environmental health and biogeographic change (Parmar et al. 2016). Many studies on bioindicators in aquatic environments have focussed on contaminants within fish tissues (Joeharnani and Trijuno 2015; Bawuro et al. 2018; Junaidi et al. 2019; Sow et al. 2019; Andini et al. 2019; Emiyarti et al. 2020). However, the use of certain fish endoparasites have also been identified as particularly useful bioindicators of heavy metal pollution (Al-Hasawi 2019; Mehana et al. 2020) due to their capacity for bioconcentration (Brázová et al. 2012). For example, an early study of *acanthocephalan* parasites ('thorny-headed worms') indicated significantly greater concentrations of heavy metals than in the host body, providing the role parasites could play as environmental quality indicators (Sures et al. 2017; Mehana et al. 2020; Molbert et al. 2020). Tellez and Merchant (2015) also found that trematode parasites in the intestine of American alligator, *Alligator mississippiensis*, are preferable magnifiers of heavy metal, while Hassan et al. (2016) found similar in nematode helminths in the muscles of kosher fish, *Epinephelus summana*. Ectoparasites, which

live on the external surfaces of the host, are of special interest, as they are in direct contact with both the host and the surrounding environment. A number of studies have explored environmental factors that affect monogenean parasites (flatworms very commonly found on fish gills, skin and fins) also demonstrating that they are reliable indicators of water quality due to contamination by heavy metals (Modu et al. 2012). Notably, most studies have focussed on fish parasites with little information on the accumulation of heavy metals in crustacea such as crabs, and their ectoparasites. This is an important gap, as crabs are common coastal species that are particularly vulnerable to the effects of heavy metal uptake and bioaccumulation due to their direct contact with both water and sediments, and their limited capacity to move away from contaminated sites.

In Indonesia, mud crabs (*Scylla* spp.) are an important intertidal species in terms of both mangrove ecosystem structure and fishery production, although little is known about their cultivation, diseases and conservation (Furukawa et al. 2014). Nevertheless, several studies have been carried out on their parasitic diseases, especially in crabs living in areas polluted with heavy metals (Olgunoğlu and Olgunoğlu 2016; Aris et al. 2018; Hasni et al. 2020). The pedunculate barnacle *Octolasmis* is a common crustacean that has a stalk or main stem attached to crustacean (Blomsterberg et al. 2004). This organism is found in mud crabs (Ihwan et al. 2014), spiny lobsters (Nur and Yusnaini 2018) and portunid crabs (Heirina et al. 2021). They can bring negative health effects on their host, such as decreased oxygen intake, shortened molting intervals, less movement and buoyancy, and making the host more vulnerable to predators. (Li et al. 2015; Hasan et al. 2019). Moreover, infected hosts with a high-intensity infection get less food and influence the ingestion of hosts (Xue and Wu 2002; Hasan et al. 2019). The attachment of enormous amount of pedunculate barnacles causes the red sternum syndrome, mud crabs become weakened and afflicted with other pathogens (Lerssutthichawal and Penprapai 2013). The red sternum syndrome in mud crabs is characterized by low levels of protein and urea nitrogen in the hemolymph, which then lead to a serious effect on protein metabolism (Kankamol and Salaenoi 2018). Infestation becomes permanent after the final or terminal molt by the host has taken place, with the parasites colonizing the surfaces of the carapace and appendages, or residing within the host's branchial chambers. Earlier studies have suggested that higher parasitic infestations occur on females - as a consequence of the long period between their molts (Shields 1992; Hudson and Lester 1994) - and on larger crabs, with size possibly being a proxy for age (Hudson and Lester 1994) and gender.

The premise of this paper is that the examination of these mud crabs, and their ectoparasites, can provide useful insights for biomonitoring heavy metal contamination in coastal catchments. Along the coast of Bombana Regency in Southeast Sulawesi, Indonesia, mud crabs are abundant within the intertidal zone. A number of catchments in the Regency, whose rivers flow into these coastal habitats,

have seen rapid and extensive development of artisanal small-scale gold mining (ASGM), with associated broadscale use of mercury in the extraction of gold. Downstream waters including the habitats of mud crabs might be contaminated with mercury (Beavis and McWilliam 2018). Mercury is a highly toxic heavy metal that bioaccumulates and biomagnifies in aquatic food webs (Monroy et al. 2014). Its impacts on the environment and human health in ASGM areas have been extensively documented (World Health Organization 2016).

The aim of this study was to determine bioaccumulation of mercury (Hg) in host mud crabs *Scylla* spp. and associated *Octolasmis* spp. parasites in an estuarine intertidal zone, located downstream of a recent gold mining area. A further aim was to establish whether the *Octolasmis* spp. parasites can be used as an 'accumulation' bioindicator to monitor environmental changes associated with heavy metal pollution, even when this is occurring at low ambient concentrations. The data may be used to provide information for future biomonitoring studies of Hg polluted coastal waters.

MATERIALS AND METHODS

The background of study area

The study site (-4.627934, 122.009633) is located near Anugrah Village, Lantari Jaya District, Bombana Regency, Southeast Sulawesi Province, Indonesia, within a mangrove forest on an estuarine waterway where ASGM has operated ~6km+ upstream (Figure 1). A gold rush occurred in the catchment in 2008, with very rapid development drawing in tens of thousands of miners into the area, who employed the use of mercury in gold separation processes (Jaelani et al. 2018). Nevertheless, mining activity had decreased significantly by 2015, there are only several small mining concessions operating currently. There is still significant storage of mercury in extensive mine tailings and river bed sediments up-catchment. At the study site itself, aquaculture ponds extend laterally out from the waterway and mangrove forests.

Among several mangrove plant species, red (*Rhizophora mucronata*) and white (*Sonneratia alba*) mangroves are the most dominant ones. Many species of crabs are also found on the research site. However, in particular, crabs from the genus *Scylla*, of the family Portunidae, have commercial value in which the most commonly caught species include *Scylla tranquebarica*, *S. serrata* and *S. olivacea*.

The study area has a humid tropical climate with fairly constant temperatures and distinct dry and wet seasons. The daily dry season temperature during the sampling period was 25.9-26.2 °C and precipitation was 0.6-46.2 mm. By contrast, during the wet season, daily temperatures were 25.2-25.3 °C and precipitation 138.6-162.2 mm (Indonesian Agency for Meteorology, Climatology and Geophysics 2017-2018).

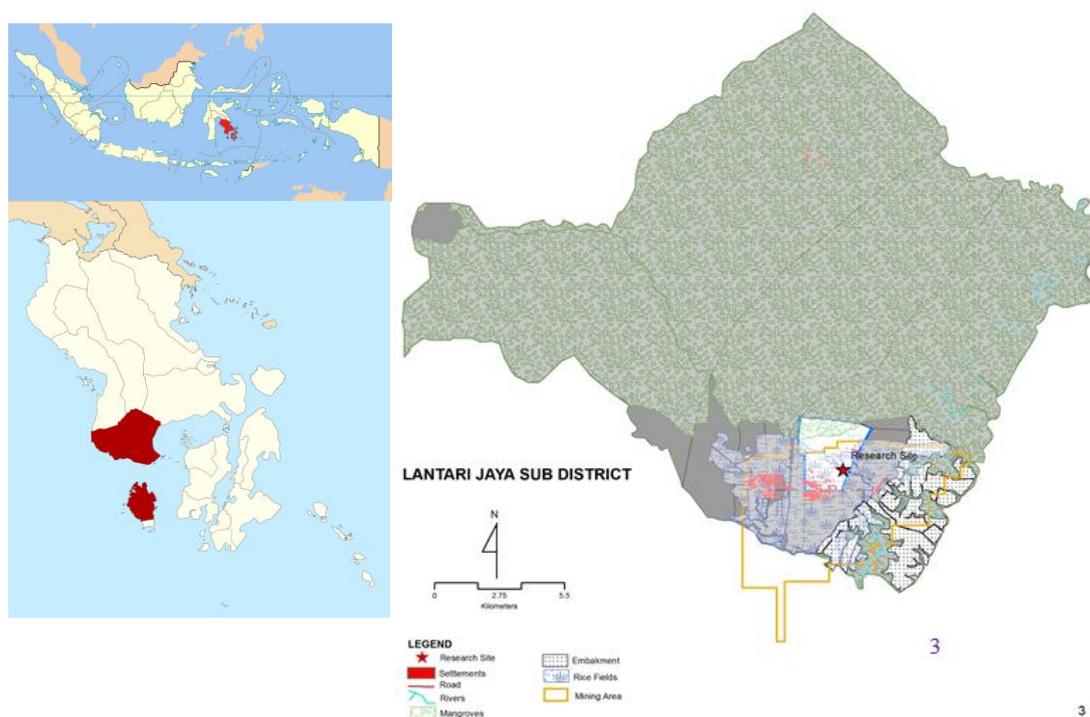


Figure 1. Research location (red star). Samples were collected from mangrove area in Lantari Jaya Sub-District, Bombana Regency, SE-Sulawesi Province, Indonesia

Research methods

This study has applied a descriptive exploratory research method that aims to describe the phenomenon of certain parasites in polluted waters having the ability to absorb and bio-accumulate heavy metals at higher concentrations than the surrounding environment. This then lays the foundations to explore the potential of parasites being used as biological markers for changes in water quality due to Hg contamination, even when those ambient heavy metal concentrations are low. The study focuses on the effects of pollution on the activity of parasitism, specifically in relation to the accumulation of Hg in dominant ectoparasites, in addition to the ability of parasites to absorb heavy metal Hg compared to the infected hosts.

We first analyzed the dynamics of crustacean parasitic infestations in crab *Scylla* spp. based on season and gender of hosts, based on the assumption that the infestation level of parasites will vary in certain seasons and according to host gender. The level of pollution in the environment was assessed monthly by analyzing Hg in water and sediments. Furthermore, we analyzed the level of absorption of heavy metal Hg in crabs and their external ectoparasite, *Octolasmis* spp. The bioconcentration factor (BCF) of the parasite relative to the host body tissue and surrounding environment has been used to establish the potential of the parasite as a bioindicator in polluted coastal environments.

This study was undertaken across two distinct dry and wet seasons. Sampling of water, sediment, crabs, and parasites was carried out monthly during the dry season between October and mid-December 2017, while sampling in the wet season was completed between late-December

2017 and February 2018. The flood tide between October and mid-December ranged between 52 to 89 cm, while the ebb tide was -84 to -130 cm. During the wet season, the flood tide between late December and February was ~77 to 84 cm, while the ebb tide was -76 to -125 cm (data from Research Agency of Indonesian Maritime Affairs and Fisheries 2017-2018).

Three replicates of water samples were collected during ebb tide at a depth of ~0.75m, using 250ml glass bottles. Nitric acid (HNO₃) was added to decrease pH to <2 to minimize adsorptive losses and microbial activity prior to analysis, then placed on ice in a styrofoam container and transported to the laboratory where they were stored at 4°C prior to analysis. (APHA 2005). Measurement of water physicochemical parameters was carried out in the field just below the water surface using a Hanna Instruments Type HI 98107 pHep multimeter for pH and temperature, and an ATAGO Master S10 .2471 hand refractometer for salinity. Duplicate samples were analyzed for dissolved oxygen (DO), nitrate (NO₃), and ammonia (NH₃) in laboratories of the Faculty of Fisheries and Marine Sciences at Halu Oleo University. These analyses included: (i) the Azide Modification of the Winkler Method for DO; (ii) the Brucin method (SNI 06-2480-1991) for nitrate) and (ii) spectrophotometry for ammonia. The results of water quality measurements are shown in Table 1. In addition, analysis of water for total Hg used cold vapor atomic absorption spectrophotometry (SNI 6989.78-2011/ U.S EPA method 245.1).

Sediments were extracted to a depth of ±20 cm from the sampling site using polyvinyl chloride (PVC) pipe. The sediment samples were sealed, transported, and stored at 4°C immediately before Hg analysis, also based on the cold vapor atomic absorption spectrophotometry method (U.S. EPA method 245.1).

Table 1. Mean values of water quality measurement results during the sampling period

Parameter	Unit	n	Measurement	
			Dry season	Wet season
pH	-	15	6.5	6.5
Temperature	°C	15	27.5	29
Salinity	ppt	15	28	26
Alkalinity	mg/L	10	130.5	90.08
DO	mg/L	10	4.76	5.2
Nitrate	mg/L	10	0.0163	0.0132
Ammonia	mg/L	10	0.1223	0.0315

A total of 159 mud crabs (*Scylla* sp.) were collected, with an average size of 271.97±51.08 g. All crabs were collected by professional fishermen using net-bamboo trap pots. The crabs were transported alive to the Fish Health Laboratory at Halu Oleo University, SE-Sulawesi Indonesia, they were washed with distilled water to remove the sand and sediments found adhering to their body, and their weight and gender were recorded. Crabs were killed humanely by piercing of the ganglia in the brain and ventral-nerve mass using an awl.

The parasites were then removed alive from the gills. Examination of crabs for ectoparasites *Octolasmis* spp. were characterized their morphology referencing previous relevant studies (Ihwan et al. 2014; Rasheed and Mustaqim 2017).

Crabs were examined for *Octolasmis* infestation, all *Octolasmis* were removed and counted. Muscle and gills from each crab were dissected and prepared for further mercury determination. Dried samples (each weighing 2 g) were oven-dried for 40–48 hours at 60 °C and then were transferred to glass beaker to prepare for Hg analysis through acid digestion (tissues were digested with 5 mL of nitric acid (65%). The samples were cooled to room temperature following complete digestion and diluted to 25 mL with double distilled water. All the digested samples were analyzed three times for Hg based on the AOAC (2012) method using an Atomic Absorption Spectrophotometer (Agilent, USA) and the instrument was calibrated with standard solutions prepared from commercially available chemicals (Merck, Germany). The results were expressed as total Hg mg/kg dry mass. A parallel review of the quality assurance was provided by the Certified Reference Material (ICP multi-element standard solution IX, Merck, Germany) for each series of analyses. During the validation cycle, the detection and recovery limits were determined. Detection limits were at least three times the standard deviation of the blank measurement. Measurement of heavy metal Hg was conducted at Integrated Chemical Laboratory, IPB University.

The proportion of parasite-infected host individuals (parasite prevalence), the number of parasites per infected host (parasite intensity), and, the number of parasites per host (parasite abundance) are all useful metrics for quantifying parasite infection in a host population. In this study, the total number of parasites from the carapace and gills from each crab were counted, and the prevalence,

mean intensity, and abundance data were calculated according to the formulae of Margolis et al. (1982) and Bush et al. (1997):

$$Prevalence = \frac{\text{Number of infected hosts}}{\text{Number of hosts examined}} \quad (1)$$

$$Intensity = \frac{\text{Number of individuals of a particular parasite species}}{\text{Number of infected hosts in a sample}} \quad (2)$$

$$Abundance = \frac{\text{Number of individuals of a particular parasite species}}{\text{Number of hosts examined}} \quad (3)$$

Hg bioaccumulation analysis in the parasites and crabs from exposure to mercury in the brackish water and sediments utilized bioconcentration factors (BCF). Bioconcentration factor analysis was based on the content of Hg in the biota divided by Hg concentrations in the brackish water or sediment at the study site. Bioconcentration factors were calculated using the following formula (Wang 2016; Azeez 2021):

$$BCF = CB/CW \quad (4)$$

Where, BCF is a bioconcentration factor; CB is the concentration of heavy metals in biota (parasite or crab); CW is the concentration of heavy metals in ambient medium (crab, water or sediment).

Data analysis

Data of parasite infestation based on gender of the host and season were analyzed for equality of variances using a homogeneity test (Levene's Test). The T-test was applied for normally distributed data to determine if a statistically significant difference is present in the means of the two variables. Meanwhile, analysis of bioaccumulation data established whether the data distribution was non-gaussian, and if so, then a non-parametric test (Kruskal-Wallis) was applied. Differences of influence between categories were analyzed using the Mann-Whitney U test. Statistical analysis using SPSS version 25.0.

RESULTS AND DISCUSSION

Species of ectoparasites

In total, 159 individual mud crabs with a bodyweight ranging from 169.5 g to 385.3 g were examined for parasites and pedunculate barnacles, *Octolasmis* spp. were found.

Octolasmis infestation was verified in the branchial chambers of the mud crabs (Figure 2), where the respiratory current provides food and oxygen (Li et al. 2014). The distribution pattern of *Octolasmis* which was found in this study was concentrated in the middle part of the gill surface, however, Hasan et al. (2019) concluded that the site was determined by water current. The cyprids of *Octolasmis* were also found in the branchial chamber of portunid crab within the attachment disc surrounded by a cuticular skirt (Blomsterberg et al. 2004). Although in some literature, it is stated that *Octolasmis* reflects the

specialized epibiotic lifestyle, some evidence suggest that the association between epibiont and rhizocephalan-parasitized crabs particularly in long-term parasitized hosts, can pose a significant burden and increase host mortality (Li et al. 2015). Moreover, in our recent studies, it is explained that they cause damage to the morphology and gill tissues. Histological examination revealed that the tissue had deteriorated into hemorrhage, necrosis, and severe vacuolization (unpublished data). This condition had effects in reducing the efficiency of gas exchange and required a large amount of energy in respiration. In this case, *Octolasmis* is referred to as a parasite because of all the damage and disruption which is caused by it toward the host.

Prevalence, abundance and intensity of parasitic infestation

The data collected in this study suggest high prevalence, abundance, and mean intensity values of *Octolasmis*. Examination of the hosts found higher prevalence, abundance and intensity in the female hosts than the male (Figure 3), and this was statistically significant for abundance and intensity (P-value of <0.05). The prevalence in both sexes occurs at very high values of 97.26% and 90.31% in females and males, respectively. Parasitic dynamics across the dry and wet indicate that prevalence, abundance, and mean intensity of parasites are not affected by seasonal changes (Figure 4).

Mercury (Hg) accumulation in mud crabs and the surrounding environment

The mean concentrations of Hg in the tissues (muscles and gills) of mud crabs and the ambient environment of the study site, such as soil sediment samples and water, are

presented in Table 2. Hg concentration varied as follows: water<crab gills<crab muscle<sediment<parasite with concentrations in the parasites being one order of magnitude greater than the host, and several orders of magnitude greater than in the surrounding water. The difference in mean Hg concentration between the parasites and the surrounding environment (brackish water and sediment) and host tissues (gill and muscle) was statistically significant (Kruskal Wallis test, $P=0.046<0.05$).

The low mercury content in the crab samples analyzed in this study has been attributed to the low mercury concentrations in the surrounding environment, despite upstream artisanal gold mining in the recent past. Mean concentrations of mercury in the water were 0.0003 mg/L and 0.200 mg/kg in the sediment (Table 2). The value for water meets the Indonesian national guideline threshold of 0.001 mg/L (State Minister of Environment Decree No.51/2004).

Mercury (Hg) accumulation in parasites and crab hosts

The Hg concentration in the parasites relative to host tissues and their surroundings was expressed in terms of the bioconcentration factor (BCF). The results of this study indicate that *Octolasmis* accumulates mercury present in the environment, particularly where it contaminates ambient water (Table 3).

Analysis of mercury in crab muscle and the parasites indicates an order of magnitude greater concentrations in *Octolasmis* relative to its host (Table 4). There is still some evidence to suggest seasonal variation in the concentration of mercury in the parasites, with higher values during the wet season.



Figure 2. Colonization of *Octolasmis* attached on the gill chamber of a mud crab

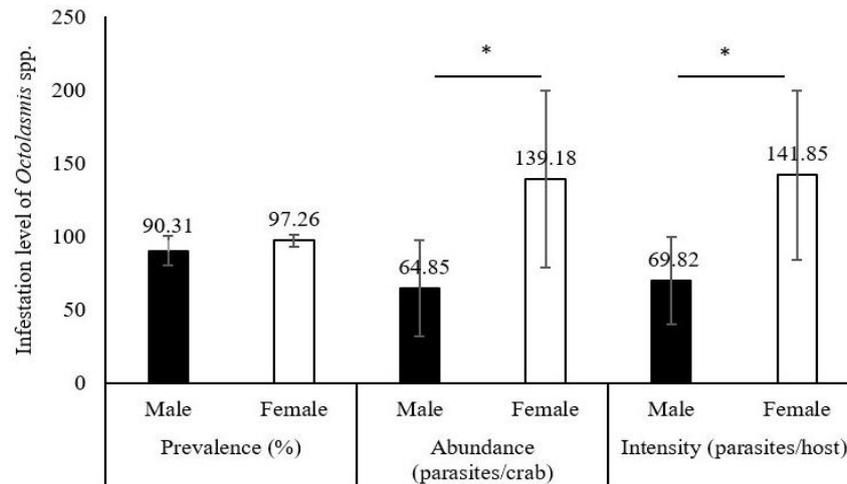


Figure 3. Host gender variation in the infestation level of mud crabs by *Octolasmis* spp. The size of crabs ranges from 247.68±42.65 g for males and 265.42±58.09 g for females. The total number of crab samples is 85 males and 74 females. The data are expressed as mean values with standard deviation (SD) error bars. Superscript (*) indicates P-value significant at 0.05 level

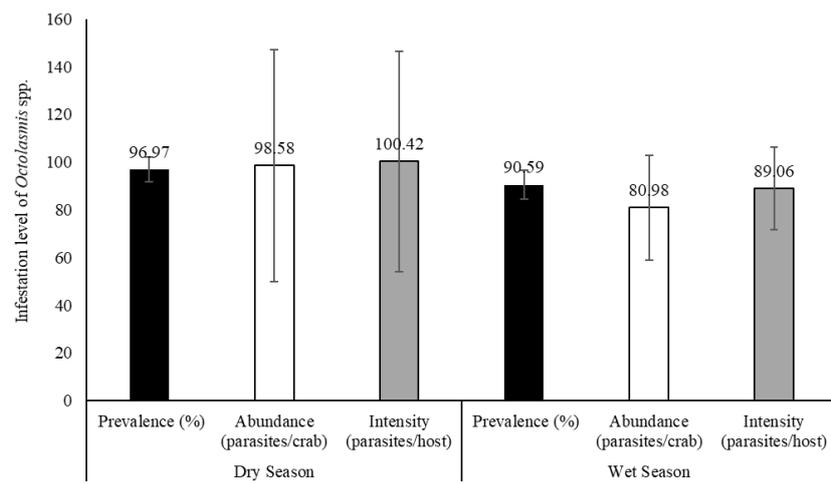


Figure 4. Seasonal variation in the infestation level of mud crabs by *Octolasmis* spp, in which no statistically significant difference is found between dry season and wet season. The data are expressed as mean values with standard deviation (SD) error bars. Note: number of *Octolasmis* = 8.199 in the dry season and 6,977 in the wet season

Table 2. Concentration of mercury (Hg) in the parasites, water, sediment, and crab muscle and gills

Hg concentration (mg/kg) or (mg/L)	Parasites	Water	Sediment	Muscle crabs	Gill crabs
	5.875±0.784 ^a	0.0003 ^b	0.20±0.064 ^b	0.126±0.094 ^b	0.082±0.003 ^b

Note: The data is mean and standard deviation (SD). Superscript indicates a statistically significant among samples (p<0.05). Total number of parasites = 15,176 individual

Table 3. The bioconcentration factor (BCF) of Hg of parasites (in relation to habitat and in relation to host)

	Parasites-water	Parasites-sediment	Parasite-gill host	Muscle host-water
BCF	7,938.21	14.83	28.91	996.09

Table 4. The concentration of mercury (Hg) in both the crab muscle tissue and parasites during sampling period

Sampling time	Hg concentration (mg/ml)	
	Crab muscle	Parasite <i>Octolasmis</i> sp.
Dry Season	0.0825±0.113 ^a	0.068±0.045 ^a
Wet Season	0.6566±0.645 ^a	5.875±0.784 ^b

Note: The data is mean and standard deviation (SD). Superscript indicates a statistically significant among samples (p<0.05).

The low Hg concentrations in the host can be attributed to heavy metal absorption by the parasites at the site of the infection, which may help reduce the concentration and favors the survival of the host. This is supported by data shown in Table 3, where the parasite's ability to accumulate Hg in water was higher (Mean BCF=7938.21) than its host (BCF=996.09).

Very high numbers of infections (prevalence, mean abundance and mean intensity) in this study indicate that the parasites, *Octolasmis*, are successful in finding an appropriate host, invading it, surviving and then reproducing. This is assumed to be a function of optimal environmental conditions, such as water temperature, salinity and nutrient availability all of which satisfied national and international water quality guidelines for marine waters across both wet and dry seasons.

In accordance with the criteria specified by Williams and Bunkley-Williams (1996), prevalence at 90-98% is classified as severe or constant infection and the results presented here indicate severe infection in males and very severe infection in females. The higher prevalence, abundance and intensity in female hosts (Figure 3) confirms earlier findings of Shields (1992) and it is assumed to be a function of their larger size. In this study, sample found the average weight of females is greater than that of males, this is in contrast to that obtained by Pudiawati and Patria (2017) on *S. oceanica* in mangrove area in Tanjung Lesung, Banten, Indonesia. However, Santos and Bueno (2002); Silva-Inácio et al. (2016) stated that there was no correlation found between the prevalence of *O. lowei* infestation and the carapace width of males or females in blue crabs and portunid crab.

Notably, this severity of infection occurred across both wet and dry seasons, contrary to expectations based on the previous work of Khidr et al. (2012); Saha and Bandyopadhyay (2017) where seasonality of prevalence was attributed to changes in environmental condition, host specificity, hosts' habits and body condition. This level of infestation, relatively constant over time, provided ideal conditions for assessing these parasites for their capacity to bioaccumulate mercury and to establish their suitability as a bioindicator of environmental contamination.

Previous research has demonstrated that uptake of contaminants by aquatic animals can encourage increased parasitism (Tellez and Merchant 2015). There is evidence that parasites are significantly more common in poor water quality conditions (Gilbert and Avenant-Oldewage 2017). It is possible, that at the study site the presence of mercury in water and sediments, originating from upstream gold mining activities, could be a causal factor in the prevalence of the *Octolasmis* parasites. However, this requires further investigation.

A comparison of Hg concentration in water and sediments, the vital organs of the mud crabs and *Octolasmis* has provided the basis for quantifying bioaccumulation in both the host and parasites. The concentration of mercury in the water and sediments is relatively low, and meets national and international water quality guidelines for aquatic ecosystems. For example, the ANZECC and ARMCANZ (2000) guidelines for fresh and

marine water quality provide Hg trigger values of 0.4 µg/L for protection of 95% species, to 0.1µg/L in moderately disturbed environments (for protection of 99% of species). Uptake of Hg by mud crabs *Scylla* spp. is evident in the concentration in their gills and muscle tissue (Table 2), although the concentrations are still low. The mean concentration of mercury lies below the standard threshold for most countries of 0.5 mg/kg (for example, Commission Regulation 1881/2006/EC; US EPA 2000). This is also the case in Indonesia, where the national standard for the maximum limit of mercury contamination in crustaceans is 1.0 mg/kg (BSN 2009). Despite this, the presence of Hg in the tissues of crabs should be of concern particularly with reference to its entry into the food chain via predation, and potential consumption by humans (Nur et al. 2020).

Contrarily, concentration of Hg in the parasites is much higher than its environmental surroundings and its host (Tables 2, 3, and 4). This confirms earlier studies using parasites as bioindicators, that have also reported higher heavy metal concentrations in the parasite relative to the host. This can be assumed to (i) the influence of the parasites on the host's capacity to detoxify pollutants (Gismondi et al. 2012; Molbert et al. 2020), or heavy metal load (Sures et al. 2017), (ii) the effect of pollutants on the prevalence and intensity of parasites (Ugokwe and Awobode 2015; Borkovcova et al. 2020; Nur et al. 2020); and (iii) the adult life stage of *Octolasmis* and associated exposure to Hg.

Regarding seasonal variation, concentrations of Hg in the host muscle are slightly higher in wet season than in dry season (Table 4). The same happens also to parasites. However, the opposite result was reported by Al-Abbawy et al. (2021) that the level of heavy metals in aquatic plant tissues throughout the summer season was higher than in the various seasons. It is uncertain whether the concentration of Hg in the host would be absorbed by barnacles as it is common with endoparasites in fish, but it was found that high level of Hg in parasites might be related with the reduction of host energy. It is assumed that high infestation of *Octolasmis* could cause heavy physiological load on its crab host, then it could reduce the host's ability to allocate resources for heavy metal clearance of its body tissues. Dominant barnacles are suspension feeders (Nishizaki and Carrington 2014), however, it was unclear whether high level of Hg was related to feeding habits of this barnacle species. It may differ from endoparasites in fish which absorb nutrients from intestine, but through the gills in which *Octolasmis* colonizes. The barnacle's exposure with Hg contained in sediment is a strong reason why higher Hg levels were found, while their simpler body did not easily neutralize the pollutant. In this case, more studies are required to be done.

The significance of the parasite having higher heavy metal concentrations, and bioaccumulation, is that even under low ambient levels of heavy metal contamination, the presence of this toxicant in an ecosystem is quantifiable. This has implications for identifying the continuing risks associated with legacy mines. In these settings, despite the cessation of major decrease in mining operations, episodic mobilization and transport of heavy

metals stored within mine tailings and river sediments continue to deliver contaminated waters downstream affecting land, freshwater, and marine ecosystems. In this case study, the monitoring of mercury in water, sediment, and mud crabs would suggest that relevant guidelines for aquatic ecosystems and human food consumption are being met. However, the Hg concentrations within *Octolasmis* is evidence for continuing risks to those ecosystems and human health due to exposure and uptake at a lower trophic level.

In conclusion, this study has demonstrated that the Hg concentrations in parasites were significantly higher than the concentrations observed in the host crab tissues and the surrounding habitat. This finding suggests that *Octolasmis* spp. could potentially be used as a bioindicator, not only for the risks occurring within a contaminated area, but also sensitive to environmental changes.

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