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Polydora index and histopathological lesions in the mangrove oyster *Crassostrea gasar* from the Aby and Ebrié lagoon systems

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ABSTRACT

The mangrove oyster *Crassostrea gasar* plays a vital ecological and socio-economic role in the Ébrié and Aby lagoons of Côte d'Ivoire. However, it is threatened by infestations of boring worms from the *Polydora* genus, which cause shell damage that can reduce both its commercial value and physiological health. This study aims to quantify the *Polydora* Index (PI) and the prevalence of tissue lesions in *C. gasar* at three sites, Azito and Grand-Bassam on Ébrié Lagoon and Assinie on Aby Lagoon, based on a sample of 1,080 oysters. Infestation levels were classified into five severity classes and histopathological lesions were assessed in the mantle, gills, and gonads. Results show that Assinie had the lowest PI (0.16) and only exhibited gill necrosis (21.1%). Grand-Bassam and Azito had higher PI (0.24 and 0.33, respectively) and a greater prevalence of lesions: necrosis (32.5% to 51.4%), abscesses (12.2% to 41.9%), and neoplasia (14.2% to 28.6%). A clear correlation was observed between shell infestation levels and lesion prevalence, especially in Azito, where up to 85% of infested oysters showed necrosis. The study highlights the need to monitor such infestations as indicators of environmental stress and potential threats to oyster farming.

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INTRODUCTION

The mangrove oyster *Crassostrea gasar* (Adanson, 1757) is a species of major ecological and socio-economic importance in the Ebrié and Aby lagoons of Côte d'Ivoire (Yapi *et al.*, 2016). It contributes not only to the food security of local communities but also plays a role in water filtration, oyster bed structuring, and the overall productivity of lagoon ecosystems (da Silva *et al.*, 2014 ; Queiroga *et al.*, 2013).

However, several threats compromise its health, among which is infestation by boring worms of the genus *Polydora*, responsible for shell lesions (mud blisters, burrows, cracks) that can impair both its commercial value and biological functions (Catherine *et al.*, 1990; Chambon *et al.*, 2007). Classical studies on *Crassostrea gigas* have shown that *Polydora* infestation can lead not only to physical alterations of the shell but also to significant physiological impacts.

Catherine *et al.* (1990) described *Polydora* -related shell anomalies associated with antifouling paints along the French coast. Additionally, (Chambon *et al.*, 2007) studied the effect of *Polydora* on respiratory behavior and oxidative stress in *C. gigas*. Recent work, such as that of (Sato-Okoshi *et al.*, 2023), has examined oyster shell-boring infestations by Polydoria species (Annelida : Spionidae) in cultivated oysters in Normandy. Zhang *et al.* (2020) also reported histological changes in the mantle region of *Patinopecten yessoensis* in response to *Polydora*. Regarding *Crassostrea gasar*, studies have highlighted its vulnerability to parasites and environmental stressors (Queiroga *et al.*, 2015). These authors examined parasites infecting *C. gasar* in northeastern Brazil, notably *Perkinsus* spp. and *Nematopsis* sp., and reported high prevalence rates.(Costa *et al.*, 2023) assessed the biological responses of *C. gasar* exposed to different concentrations of suspended matter in a biofloc system, showing effects on growth and physiology. (Yapi *et al.*, 2017) studied population dynamics of *C. gasar* in the Ebrié (Grand-Bassam, Azito) and Aby (Assinie) lagoons, but without focusing on *Polydora* -related lesions.

There is thus a gap in the literature characterized by a lack of studies quantifying the *Polydora* index (a measure of infestation) and the prevalence of shell lesions in *C. gasar* from the Ebrié and Aby lagoons. This is a critical issue, as these lesions can reduce market value, increase susceptibility to other pathogens or stressors, and serve as indicators of the environmental health of lagoon ecosystems. The present study addresses this gap, with the objectives of determining the *Polydora* index and lesion prevalence in *C. gasar* populations from Grand-Bassam and Azito (Ebrié Lagoon) and Assinie (Aby Lagoon).

MATERIALS AND METHODS

This study was conducted on two (2) lagoon systems in Côte d'Ivoire, namely the Aby Lagoon at Assinie and the Ebrié Lagoon at Grand-Bassam and Azito (Fig. 1).

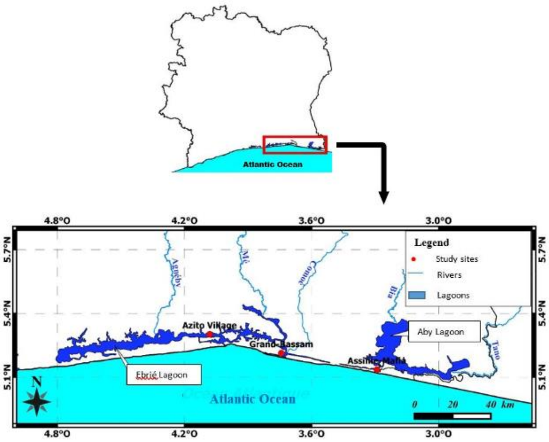

Fig. 1. Geographical location of the study sites in the Aby and Ébrié lagoon systems (Côte d'Ivoire)

It involved 1080 oysters, with 360 oysters per site, and aimed to determine the level of shell infestation and histopathological lesions of the mantle, gill, and gonad of the mangrove oyster *Crassostrea gasar*. The shell infestation study was carried out by assessing the boring levels on the oyster shells. For this purpose, both valves of each oyster were examined visually to determine the level of infestation by *Polydora* worms, based on five oyster classes as defined by Ifremer (Catherine *et al.*, 1996). These classes are : Class 0 (no worm traces), Class 1 (few galleries with limited extension, no chambers), Class 2 (no more than 2 chambers and infested surface less than 10%), Class 3 (more than 2 chambers or infested surface between 10% and 25%), and Class 4 (infested surface greater than 25%). From the infestation level, the *Polydora* Index used within the REMORA network (Fleury *et al.*, 2001) was calculated using formula 1.

$$IP = 0.00 \times p0 + 0.25 \times p1 + 0.5 \times p2 + 0.75 \times p3 + 1.00 \times p4 \quad (1)$$

In this formula, p0, p1, p2, p3, and p4 represent the frequencies of oysters in classes 0, 1, 2, 3, and 4, respectively (Fleury *et al.*, 2001). According to these authors, the *Polydora* Index also accounts for structures created by all polychaetes, not only annelids of the genus *Polydora*. The histopathological study was based on the classical histology technique described by (Martoja and Martoja-Pierson, 1967). Thus, in order to identify potential lesions present in the mantle, gills, and gonads of oysters from the two lagoon systems at Assinie, Grand-Bassam, and Azito, histological sections were performed. After analyzing the obtained sections, the prevalence of observed lesions was determined using formula 2.

Lesion prevalence= {(number of cases for a lesion)/(total number of oysters)} × 100

All collected data were subjected to statistical analysis using R software version 3.1.3 with a significance level of 5%. A global comparison of counts or proportions was performed using the Chi-square test. Furthermore, when the Chi-square test was significant, post-hoc tests for two proportions were conducted.

RESULTS

Chambering status of oysters from Assinie (Aby Lagoon) and from Azito and Grand-Bassam (Ébrié Lagoon)

Table 1 shows the chambering levels of oysters from the different study sites. It appears that, at each site, individuals in the first class (class 0) are significantly more numerous in Assinie compared to Grand-Bassam and Azito, by 1.14 and 1.43 times respectively ($p < 0.05$).

Among the three sites, Assinie is the only one with no individuals in the highest chambering class (class 4). For this class, oysters from Azito are significantly more represented ($p < 0.05$). In fact, Azito is the most affected site, with 60% of oysters exhibiting shell-boring (chambering), while Grand-Bassam and Assinie record 50% and 43% of chambered individuals, respectively.

Table 1. Oyster classes of boring-shell from Assinie in the Aby lagoon and from Azito and Grand-Bassam in the Ebrié lagoon

Sites	Number	Number of boring-shell oyster				
		Class 0	Class 1	Class 2	Class 3	Class 4
Azito	360	143^{c} (0.40)	86^{c} (0.24)	47^{a} (0.13)	37^{a} (0.10)	47^{a} (0.13)
Grand-Bassam	360	179^{b} (0.50)	101^{a} (0.28)	36^{b} (0.10)	9^{c} (0.03)	35^{b} (0.10)
Assinie	360	205^{a} (0.57)	92^{b} (0.26)	47^{a} (0.13)	16^{b} (0.04)	0^{c} (0.00)
p-value		0.001	0.001	0.001	0.001	0.001

The letters "a," "b," and "c" indicate, within the same category, a significant difference in the degree of shell-boring infestation among oysters between the three sites. The values in parentheses represent the proportions of infestation for each category. The statistical test used is the G-test from the R software.

Table 2. *Polydora* Index (PI) of the different study sites (Azito, Grand-Bassam, and Assinie)

Lagoon	Localities	*Polydora* index
Aby	Assinie	0.16^{c}
Ebrie	Azito	0.33^{a}
	Grand-Bassam	0.24^{b}
p-value		0.001

The letters "a", "b", and "c" indicate significant differences between the *Polydora* indices of the three localities. The statistical test used is the Chi-squared test (Chi^2) performed with R software version 3.1.3.

***Polydora* index**

Table 2 shows *Polydora* index (PI) ranging from 0.16 to 0.33 depending on the lagoon system and location. The site with the lowest index is Assinie. The index value at this site is significantly lower than those of the two other sites. Indeed, the PI value at Assinie is 1.52 and 2.06 times lower than those at Grand-Bassam and Azito, respectively. In this study, Azito appears as the site with the highest PI value.

Lesion prevalence in the mantle, gills, and gonads of oysters from the Aby and Ebrié Lagoons

Table 3 presents the lesion prevalence observed in oysters from the three study sites. According to the Table 3, the gills exhibited only one type of lesion, namely necrosis. Thus, 32.5% of oysters showed branchial necrosis at Grand-Bassam, compared to 21.11% in Assinie and 51.39% in Azito. In the gonads and mantle, three types of lesions were identified. However, these lesions were only observed in individuals from the Grand-Bassam and Azito sites. These include pustules or abscesses, neoplasia, and necrosis. Among the 360 oysters examined at each of these two sites, 14.17% and 23.33% of oysters presented neoplasia in the gonads and mantle, respectively, at Grand-Bassam, compared to 4.72% and 28.61% at Azito.

As for pustules, 12.22% and 18.61% of oysters exhibited them in the gonads and mantle, respectively, at Grand-Bassam, while Azito recorded 41.94% and 28.33% in the same order. Finally, the proportion of oysters with necrosis in the gonads and mantle was 8.06% and 11.94% at Grand-Bassam, compared to 16.94% and 37.50% at Azito, respectively.

Table 3. Lesional prevalence of the mantle, gills, and gonads of oysters from Aby (Assinie) and Ebrié (Grand-Bassam and Azito) lagoon systems

Tissues	Sites	Number	Prevalence (%)	X^2	*p*-value
Necrosis					
Gill	Assinie	360	21.11^c	15.42	<0.001
	Grand-Bassam	360	32.50^b		
	Azito	360	51.39^a		
Neoplasia					
Gonad	Assinie	360	00.00^c	20.65	<0.001
	Grand-Bassam	360	14.17^a		
	Azito	360	04.72^b		
Neoplasia					
Mantle	Assinie	360	00.00^c	43.70	<0.001
	Grand-Bassam	360	23.33^b		
	Azito	360	28.61^a		
Abscess					
Gonad	Assinie	360	00.00^c	62.25	<0.001
	Grand-Bassam	360	12.22^b		
	Azito	360	41.94^a		
Abscess					
Mantle	Assinie	360	00.00^c	41.04	<0.001
	Grand-Bassam	360	18.61^b		
	Azito	360	28.33^a		

The test used for the comparison of prevalence is the Chi-square test. The letters 'a', 'b', and 'c' indicate, for the same tissue, a significant difference in lesion prevalence between the three sites.

Prevalence of different lesions observed across study sites

Fig. 2 shows the prevalence of the different lesions observed at each site. According to the Fig. 2, only the two sites located in the Ebrié Lagoon (Grand-Bassam and Azito) exhibited all three types of lesions: necrosis, abscesses, and neoplasia. The Assinie site, which is the only site located in the Aby Lagoon, recorded only necrosis, with a prevalence of 21.1% which is 1.54 times and 2.44 times lower than those recorded in Grand-Bassam and Azito, respectively. Azito was the most affected site, with a necrosis prevalence of 51.4%, neoplasia at 28.6%, and abscesses at 41.9%. These prevalence rates were, respectively, 1.58 times, 1.23 times, and 3.43 times higher than those observed at Grand-Bassam.

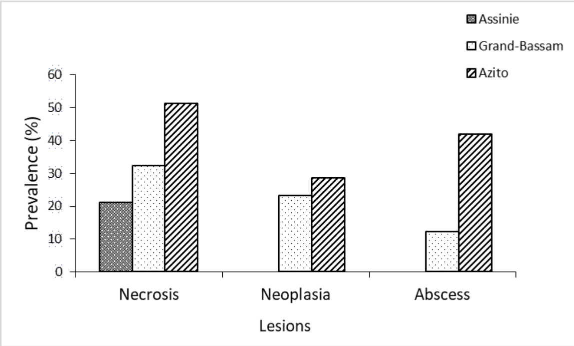

Fig. 2. Prevalence of different lesions in the three study sites

Prevalence of tissue lesions in burrowed oysters

Table 4 presents the prevalence of tissue lesions in oysters with infested shells. It is noteworthy that the prevalence of lesions increases with the number of oysters affected by shell damage. Indeed, the Assinie site, which recorded 155 oysters with damaged shells, showed only one type of lesion—necrosis. In contrast, a higher prevalence and diversity of lesions were recorded at the Grand-Bassam and Azito sites, which had 181 and 217 affected oysters, respectively. Moreover, the prevalence of necrosis was significantly higher in individuals from Azito (85.25%) compared to those from Grand-Bassam (64.64%) and Assinie (49.03%). Regarding neoplasia, Azito again ranked highest with a prevalence of 47.47%, slightly ahead of Grand-Bassam (46.41%). As for abscesses, the prevalence at the Azito site (69.59%) was more than 2.86 times higher than that recorded at Grand-Bassam.

Within each site, the prevalence of lesions varied. The Assinie site recorded only one type of lesion (necrosis) with a prevalence of 49.03%. At Azito, necrosis prevalence (85.25%) was significantly 1.23 times and 1.80 times higher than that of abscesses and neoplasia, respectively. At the Grand-Bassam site, the highest prevalence was also observed for necrosis (64.64%), followed by neoplasia (46.41%), and finally abscesses (24.31%).

Table 4. Prevalence of gonadal, mantle, and gill tissues in oysters with infested shells

Lagoons	Sites	Oyster shell-boring infestation	Lesions prevalences			*p*-value
			Necrosis (%)	Neoplasia (%)	Abscess (%)	
Aby	Assinie	155	49.03^{cA}	0.00^{bB}	0.00^{cB}	0.00
Ebrié	Grand-Bassam	181	64.64^{bA}	46.41^{aB}	24.31^{bC}	0.00
	Azito	217	85.25^{aA}	47.47^{aC}	69.59^{aB}	0.00
p-value		0.00	0.00	0.00	0.00	

Within the same column, the lowercase letters a, b and c indicate a significant difference, while within the same row, the uppercase letters A, B and C indicate a significant difference.

DISCUSSION

This study aimed to evaluate the *Polydora* index and histopathological lesions in the mantle, gill, and gonad tissues of the mangrove oyster Crassostrea gasar across three sites belonging to two lagoon systems in Côte d'Ivoire. These include the Assinie site in the Aby Lagoon system and the Grand-Bassam and Azito sites in the Ébrié Lagoon system. Based on the results, Assinie exhibited not only the lowest *Polydora* index but also only one type of lesion.

In contrast, Azito and Grand-Bassam recorded the highest numbers of burrowed (chambered) oysters, as well as the highest numbers and diversity of lesions. Furthermore, these two sites shared similar lesion types. The reduced number of burrowed oysters and lesions observed at Assinie suggests that oyster habitats at this site are less infested by *Polydora* worms and potentially less polluted compared to Grand-Bassam and Azito. The similarity in lesion types at Azito and Grand-Bassam may be explained by the comparable physicochemical parameters of the environment and the possible presence of similar pollutants, given that both sites belong to the same lagoon system. Indeed, being part of the same lagoon, these sites are likely exposed to the same types of physical or chemical stressors, although the intensity may differ between locations. Chambon *et al.*, 2007, demonstrated that environmental heterogeneity can significantly alter larval development and survival capacities of bentho-pelagic organisms, and that infestation by *Polydora* sp. modifies respiratory behavior and induces oxidative stress in *Crassostrea gigas*. Moreover, these authors reported that harmful algal blooms also have detrimental effects on bivalves, causing behavioral changes, tissue lesions, and altered immune responses-often associated with immunosuppression or activation of antioxidant defenses. In addition, histopathological studies by (Haberkorn *et al.*, 2010) on bivalve mollusks from polluted aquatic environments revealed an increased incidence of necrosis, tissue lesions, and vacuolization of the digestive epithelium. According to the same source, several histopathological studies also documented the presence of neoplasms (cancerous tumors) in the gonadal tissues of these mollusks. A macroscopic histopathological study conducted on tissues of the mangrove oyster *Crassostrea rhizophorae*, native to coastal areas of eastern South America, revealed tissue lesions caused by protozoan and metazoan parasites of the genera *Nematopsis* and *Tylocephalum*, respectively (Hégaret and Wikfors, 2005; Hégaret *et al.*, 2007, 2011). Similarly, a study on *Crassostrea gasar* from the Mamanguape River estuary (Brazil) by (Queiroga *et al.*, 2013) identified the presence of *Perkinsus* sp., *Nematopsis* sp., *Tylocephalum* sp., and other parasites, with associated tissue damage in infected organs. Another study by (Zhang *et al.*, 2020) on the scallop *Patinopecten yessoensis* infested by *Polydora* showed histological changes in the mantle, particularly an increase in mucous cells secreting acid mucopolysaccharides and alterations in mantle folds, suggesting a defensive response akin to the formation of repair layers in the shell. These structural changes may be analogous to the mantle lesions observed in the present study on mangrove oysters. (Joshy *et al.*, 2022) examined pathological changes in various bivalve species in relation to pollution, finding vacuolization, hemocyte infiltration, parasitic infestations, lamellar disorganization, and prokaryotic inclusions in gills and digestive glands. These types of lesions closely resemble those reported in this study when comparing sites.

Finally, in a similar context, (Cole *et al.*, 2020) investigated the spatial and seasonal patterns of infestation by *Polydora* websteri in farmed oysters from the Gulf of Mexico. They found that infestation intensity varied significantly depending on location, stocking density, and local pollution levels, supporting the idea that more polluted or disturbed sites tend to exhibit higher *Polydora* indices and more severe lesions.

CONCLUSION

This study provides the first quantification of the *Polydora* index and associated histopathological lesions in *Crassostrea gasar* from the Ébrié and Aby lagoons in Côte d'Ivoire. It revealed the specific characteristics of each site. The Assinie site (Aby Lagoon) showed better oyster health, with a low *Polydora* index and only one type of lesion, the gill necrosis, indicating a less stressful environment. In contrast, the Grand-Bassam and Azito sites (Ébrié Lagoon) had oysters in poorer health, with higher *Polydora* indices and a greater diversity of lesions (necrosis, abscesses, neoplasia), reflecting a more polluted environment.

A strong correlation was observed between *Polydora* infestations and internal tissue lesions, indicating a direct impact on oyster health, likely worsened by environmental stressors such as pollution. The similarity in lesion profiles between Azito and Grand-Bassam further suggests the influence of shared environmental conditions within the same lagoon system. These findings highlight the need for regular health monitoring of oysters, which serve as effective bioindicators, and for management strategies aimed at reducing environmental stress, particularly in the heavily impacted Ébrié Lagoon. Further studies should incorporate analyses of environmental parameters and assess the long-term impacts of infestations on oyster reproduction and survival.

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