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Comparative study of reproduction cycle of mangrove oyster (*Crassostrea gasar*) of the lagoons Ebrie and Aby (Côte d'Ivoire)

Jean Noel Yapi[*1], Mélecony Célestin Ble[2], Assoi Olivier Etchian[1], Vincent Kadjo[1], Kouakou Yao[1]

[1]*Formation Unit and Research of Nature Sciences, University Nangui Abrogoua, Abidjan, Côte d'Ivoire*

[2]*Aquaculture Department, Centre of Oceanology Research, Abidjan, Côte d'Ivoire*

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Abstract

This study aims to give the bases of an efficient management of oyster stocks of the lagoons of the Ivory Coast and further bases of oyster-farming. It characterizes the reproduction cycle of mangrove oyster *Crassostrea gasar* in two lagoons of Côte d'Ivoire. The Ebrié lagoon; on the level of Grand-Bassam and Azito and the Aby lagoon; on the level of Assinie. Thus, a monthly sampling of 30 oysters per site for 12 months (October 2015 at September 2016) has been made. For each oyster, the linear and ponderal parameters were determined. In addition, the gonad of each oyster was extracted and subjected to a histological study. The results showed that *C. gasar* has a cycle of reproduction made up of five (5) stages sexual maturity. The sex-ratio is in favour of the females with respectively the ratio (male/female) of 1:2.9 ratios in Assinie, 1:4 in Grand-Bassam and 1:2.6 in Azito. It was noted that *C. gasar* has a continuous reproduction. However, stages III and IV were more present during the small rainy season (SRS) and the small dry season (SDS) at the level of the sites of Assinie and Grand-Bassam. It is during these two seasons that the gonadosomatic index and the condition factor had its greater values. As for the site of Azito, it recorded constancy in the evolution of gonadosomatic index and the condition factor. At the end of this study, it would be interesting to consider the culture of the mangrove oysters (*C. gasar*) in an environment controlled to ensure the perennity.

***Corresponding Author:** Jean Noel Yapi ✉ yapinoel83@gmail.com

Introduction

The mangrove oyster (*Crassostrea gasar*) is an endemic bivalve mollusc of the coastal zones West-African (Niekles, 1995; Von Cosel, 1995) and East South-American.

Localized on the level of Ivory Coast littoral lagoon in particular, the Ebrié lagoon and Aby, this oyster is the subject of an intense exploitation by the coastal populations (Yapi *et al*., 2016) and records a negative allometry on the level of its growth (Yapi *et al*., 2017). Indeed, according to these authors, this food product constitutes an important source of protein and income for these populations. However, according to Christensen and Pauly,1997, the intensity of fishing can modify the capacity of reception or "carrying capacity" of an ecosystem by deteriorating the structure of trophic flows and their potential productivity. In addition, according toMagali, 2009, anthropic pressure causes a continual degradation of the ecosystems of mangrove, which could upset their functioning and to cause a reduction of the organisms which are present.

Facing this situation, it becomes imperative to find solutions which could ensure the perennity of oysters of our lagoons while protecting their habitat. A plausible solution to arrive there is to cultivate these oysters. That necessarily passes by knowledge of the reproduction parameters. Thus, the aim of this work is to determine, the different stages of sexual maturity, the sexual cycle, the gonadosomatic index, the sexual maturity index and the condition factor of oysters of the lagoons Ebrié and Aby.

Materials and methods

Study area

This study was carried out on the level of the coastal zone of the lagoons Ebrié and Aby, from October 2015 to September 2016. The sampling of this work was carried out in two coastal lagoons of Côte d'Ivoire. In particular, the Ebrié lagoon and the Aby lagoon. Concerning the Ebrié lagoon, two localities were retained: that of Grand-Bassam and Azito. While Assinie was the only locality retained for the Aby lagoon. The Fig. 1 gives a glance of the study zone.

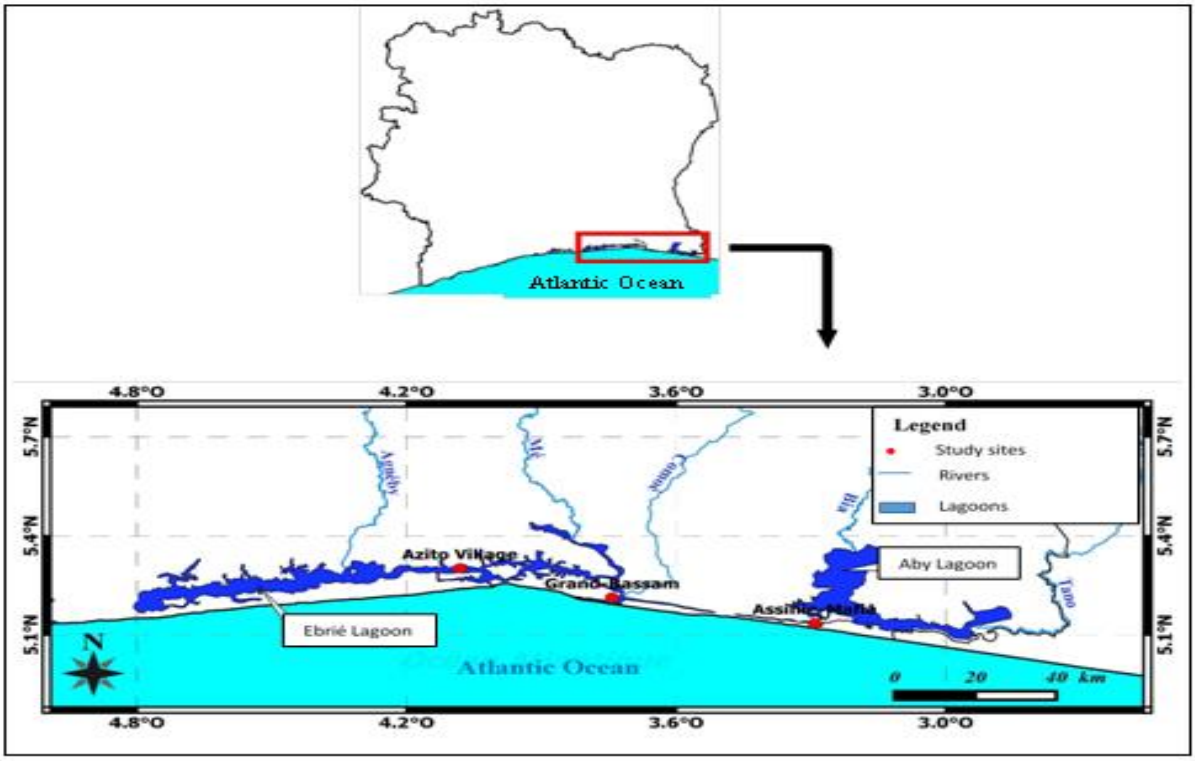

Fig. 1. Geographical situation of the study sites (Assinie, Grand-Bassam and Azito) on the level of lagoons Ebrie and Aby.

Biological materials

The study was carried out on a sample of 1080 oysters because of 360 oysters per site. The size of the individuals varied from 53 to 109 mm for Grand-Bassam; from 41 to 112 mm for Assinie and from 44 to 101 mm for Azito. As for the weights, it varied from 17.65 to 105.22g in Grand-Bassam, 26.11 to 155.25g in Assinie and 17.81 to 129.8g in Azito.

Data sampling and processing

A monthly sampling of thirty (30) oysters was carried out for 12 months. In particular, from October 2015 to September 2016.Thus, for each oyster, it was determined:

Total length: distance between the anterior edge and the posterior edge; Body weight: whole weight of the individual without the foreign bodies on the shell; Weight of the fresh flesh: fresh visceral weight, drained during at least 30 minutes on filter paper and Weight of the gonadic portion.

Study of sexual maturation

The study of sexual maturation started with the fixing of the gonad portions with for mol (10%) during 48h.After this phase of fixing, each sample of gonad was put in cassette and was subjected to the different histological processes, until inclusion in the paraffin. Then, cuts of 5 µm thickness was carried out and mounted on the slides.

These various slides were subjected thereafter to a coloring in hematoxylin eosin. After the coloring phase, the plates were assembled with Eukitt adhesive on the slides. The preparations were observed under an optical microscope and the different stages of sexual maturity were determined according to criteria of classification of Normand *et al.,* 2008 and Gomes *et al.*, 2014.

Sex-ratio

The sex-ratio is the numerical report of two sexes in a population. It is expressed either by the rate of masculinity (ratio of the number of males by the number of females) or by the rate of femininity (ratio of the number of females by the number of males) (Kartas and Quignard, 1984). In this present work, the formula used is in favour of the rate of masculinity and the ratio determined was made according to the terrestrial seasons according to the following formula.

$$\text{Sex} - \text{ratio} = \frac{\text{number of males}}{\text{number of females}} \times 100$$

Gonadosomatic index (GI)

The gonadosomatic index is the report of the gonad weight on the flesh weight of oyster without its shell. It's determined starting from the following formula:

$$GI = \frac{\text{Gonad weight}}{\text{Flesh weight}} \times 100$$

Maturity index (MI)

The maturity index informs about the maturity condition of the individuals in a population at a precise period. It is determined according to the following formula:

$$MI = \sum(\%\ \text{of each stage of maturity} \times \text{maturity Factor})$$

Maturity factors are: 0 = undetermined; I = start of development; II = development; III = mature and IV = Emission

Condition index

The condition index was determined according to seasons

$K = \frac{W}{Lt^3}$ x 100 (Lawal-Are and Owolabi, 2012)

W: Body weight

Lt: Total length

Statistical analysis

The software used for the various statistical analyses is R version 2.12.1. Thus, the different values of sex-ratio were submitted to G-test (Kh^2) with this software in order to appreciate if the difference is significant.

Results

Gonadic development

The gonad histological study made it possible to note that the sexual cycle of *Crassostrea gasar* is characterized by five stages of maturity. However, the stage 0 is common to both sexes (Fig. 2), the four other stages being specific for each sex (Fig. 3 and Fig. 4). In addition, the gonad of the same individual can present several cells at various stages of maturity.

Table 1. Seasonal evolution of the sex-ratio.

Terrestrial seasons	Sex-ratio			X^2	$P > X^2$
	Assinie	Bassam	Azito		
SRS	1:4.2	1:6.3	1:3.5	1.16	0.56
GDS	1:1.9	1:3.1	1:2.7	0.46	0.80
GRS	1:3.7	1:3.3	1:1.6	1.12	0.57
SDS	1:2.9	1:5.3	1:3.8	0.97	0.62

Seasonal evolution of the sex-ratio

It arises from the analysis of this Table 1 that the sex-ratio is in favour of the females on the level of each site. In addition, although apparently this ratio is different from one locality to another, statistical analyses plead of a similarity of the sex-ratio between the localities for a same season ($P > 0.05$).

The average during the small rain season (SRS) for the three sites being of 1:4.7.This average pass to 1:2.6 during the great dry season (GDS), to 1:2.9 during the great rain season (GRS) and finally to 1:4 during the small dry season (SDS).

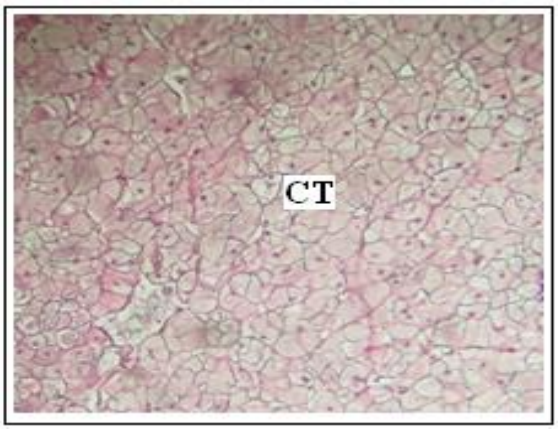

Fig. 2. Stage 0 (resting stage)or stage indeterminate of gonadic maturity of mangrove oyster, *Crassostrea gasar*: Bar: 100 µm. 400X.Stage common to both sexes (male and female). CT. connective tissue.

Seasonal variation of the gonadic development

Within sight of Fig. 5, it arises that large majority of the individuals on the level of Assinie (52%) and Grand-Bassam (48.67%) are at the stage 0 (unspecified stage) during the GDS and the SRS. The four other stages are present at all the seasons but with more or less variable proportions. The site of Azito records the presence of the five stages at every season of the year.

Gonadosomatic index (GI)

The Fig.6 presents the evolution of the gonadosomatic index female of the three sites. According to the figure, the indices gonadosomatic of the sites of Assinie and Grand-Bassam evolve in the same way. Indeed, on the level of these two localities, the highest values of the gonadosomatic index are recorded during July to November. The variation of this index during this time is 0.07 to 0.09 for Assinie and 0.06 to 0.08 for Grand-Bassam. The small values of index for these sites are recorded during the period of December to June with an average of 0.03.As for the site of Azito, the gonadosomatic index remained almost constant during all the study period with very weak variations (0.05 to 0.07) and an average of 0.06.On the level of the males (Fig. 7), the evolution of gonadosomatic index is similar to that of the females for the sites of Assinie and Grand-Bassam. Just like in the case of females of these two sites, the highest values of the index gonadosomatic of males are recorded from July to November and the low values from December to June.

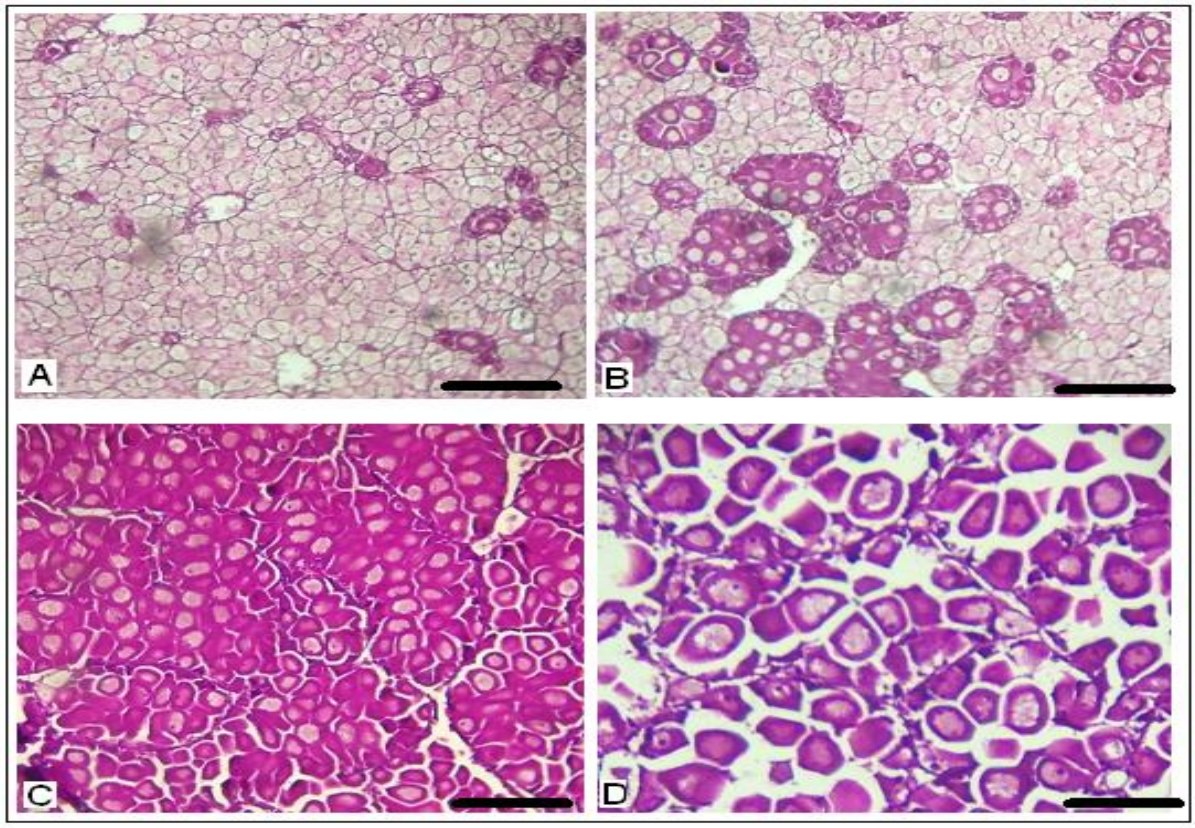

Fig. 3. Stages of sexual maturity of the female oyster *Crassostrea gasar*: (A): stage I (Gametogenesis); (B): stage II(Repletion = Pre-spawning); (C): stage III (Partial spawning) and (D): stage IV(Complete spawning). Bar: 100 µm. 400X.

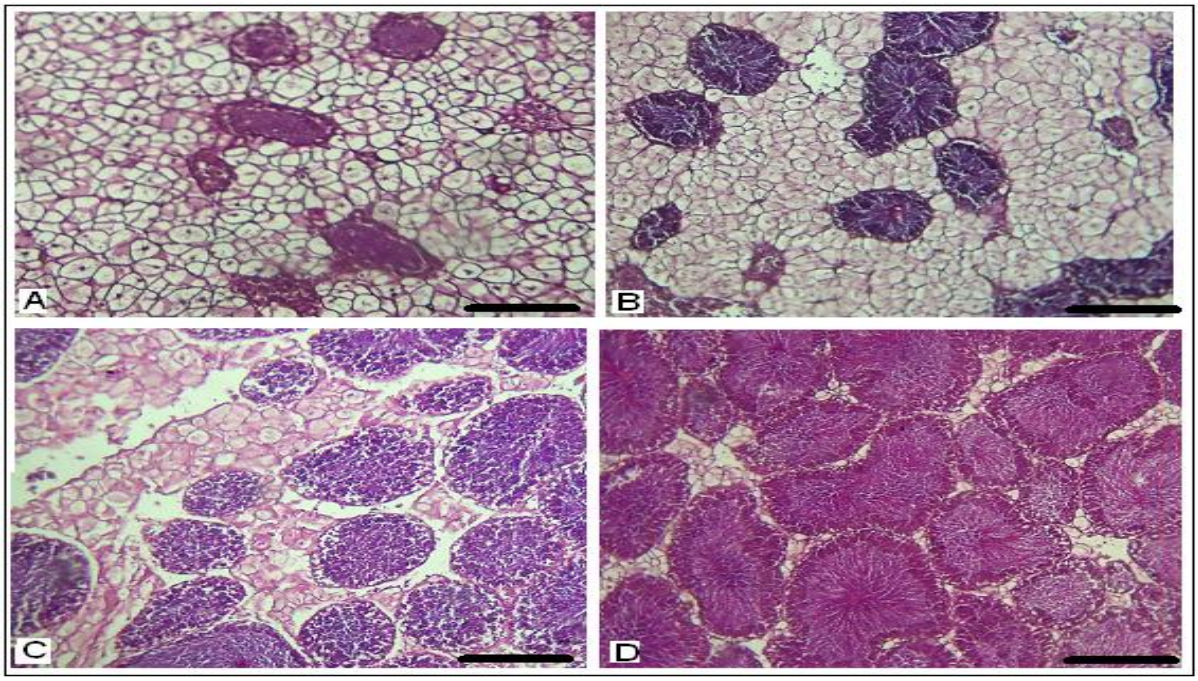

Fig. 4. Stages of sexual maturity of the male oyster *Crassostrea gasar***:** (A): stage I (Gametogenesis); (B): stage II (Repletion = Pre-spawning); (C): stage III (Partial spawning) and (D): stage IV (Complete spawning). Bar: 100 µm. 400X.

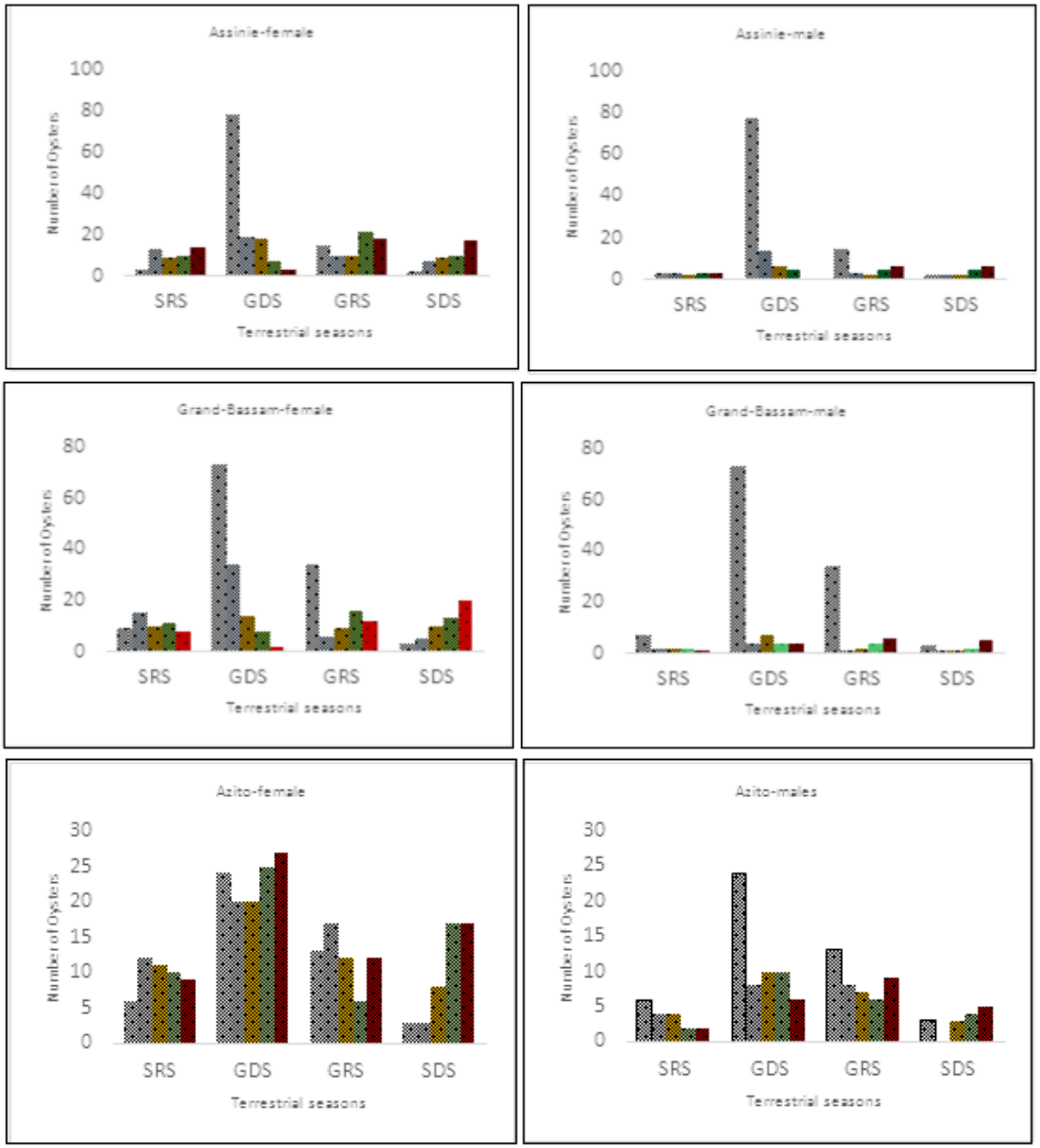

Fig. 5. Seasonal variation of the stages of sexual maturities of oysters of the localities of Assinie; Grand-Bassam and Azito

On the level of the site of Azito, just like in the case of females of this site, the gonadosomatic index evolved regularly as a whole. Although a fall of this index was recorded during April.

Gonadosomatic index and maturity index

In the localities of Assinie and Grand-Bassam, two peaks of GI are recorded for the males and the females.

The first peak is recorded during the small rain season (SRS) and the second during the small dry season (SDS) (Fig.8).As for the maturity index (MI), it records a similar evolution within the same sex for these two sites. However, on the level of Azito, the GI records more or less a constant evolution as well for the females and for the males. It is the same for the maturity index on the level of the two sexes.

Condition factor

The Fig. 9 shows the evolution of condition factor of the female oysters and the males on the level of the three localities. On the level of the females, the variation of condition factor is notable. Indeed, the highest values are recorded during the SRS and the SDS. While the GDS and the GRS are characterized by low values of this factor. However, on the level of the males, the analysis of the figure shows that the condition factor significantly did not vary during these different seasons. Indeed, this factor remained more or less constant within each locality even if the comparison between sites is in favour of the site of Azito.

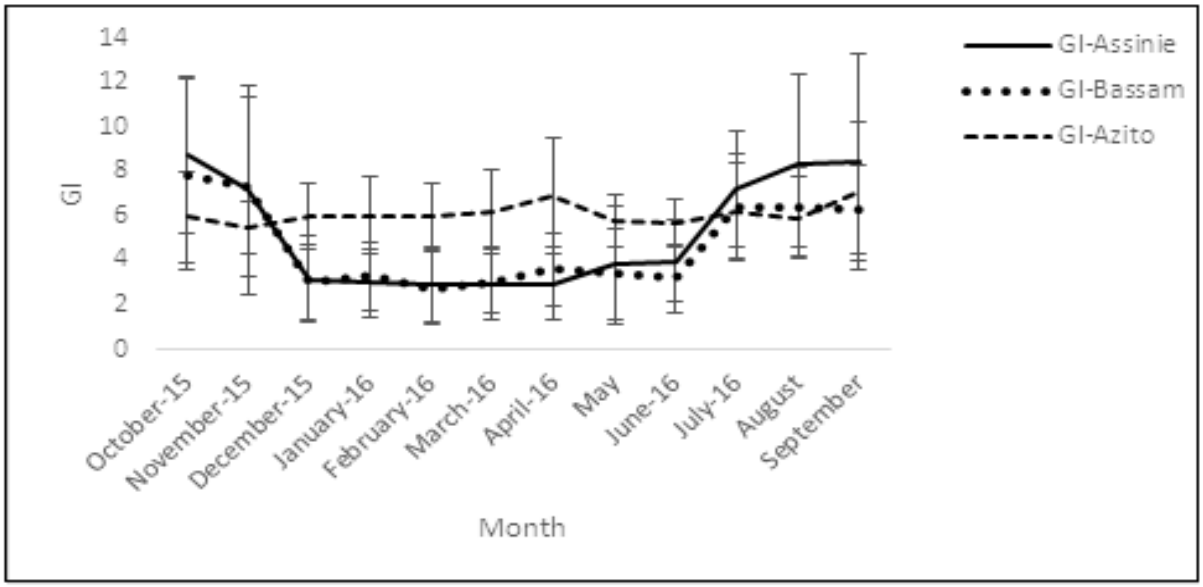

Fig. 6. Evolution of gonadosomatic index of female oysters of Assinie, Grand-Bassam and Azito (period from October 2015 to September 2016).

Discussion

The histological study of tissue gonadic of *Crassostrea gasar* revealed five stages of maturation of the sexual cells for the females and also for the males. Similar results were already obtained by Gomes *et al.,* 2014. Indeed, these authors undertook a study of the kind in the northern part of the Bay of Santa Catarina in Island on this species and they are agreed at the same number of stage of development of the gonadic cells.

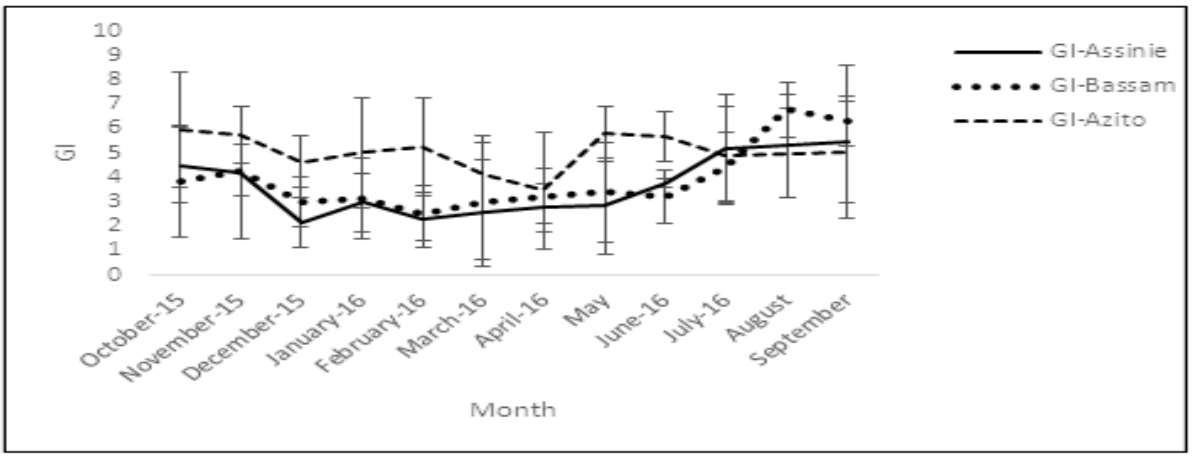

Fig. 7. Evolution of gonadosomatic index of male oysters of Assinie, Grand-Bassam and Azito (period from October 2015 to September 2016).

In addition, the works of Ramos *et al.*, 2013 showed conclusions identical as to the number of stages and the structural organization of the sexual cells.

The sex-ratio is a parameter difficult to estimate. Because it varies according to several parameters among which, the season, distribution, the age, environment and the migratory behavior.

The oyster is an alternate protandric hermaphrodite, (Guo *et al.*, 1998; Lango-Reynoso, 1999), the evaluation of this parameter becomes again more difficult. Indeed, for certain authors, the orientation of his sexual genus would depend either on environmental factors, or of genetic factors (Guo *et al.*, 1998), or again of a simultaneous action of these two determinisms (Yusa, 2007).

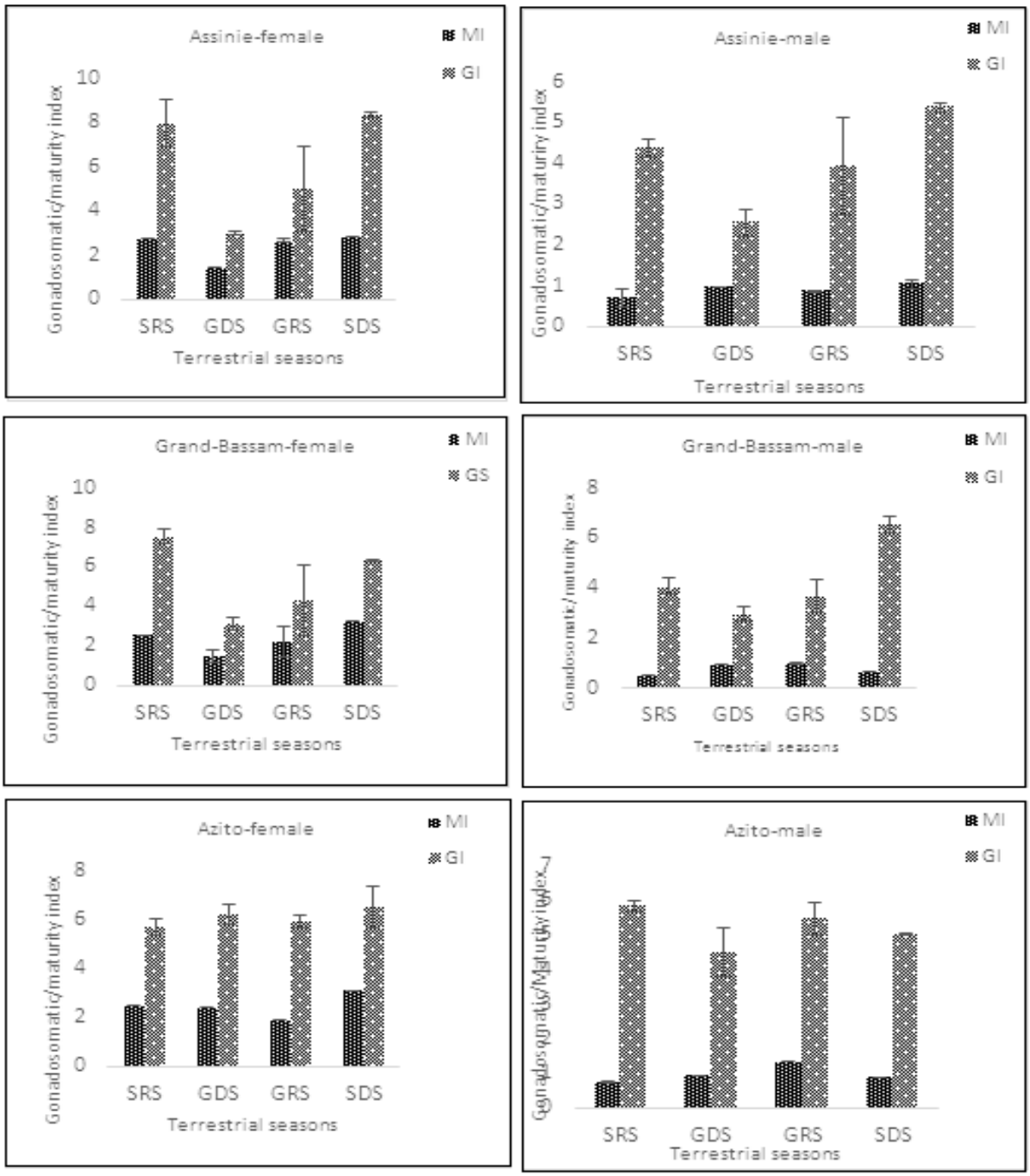

Fig. 8. Seasonal variation of the gonadosomatic index and the of maturity index of *Crassostrea gasar* (A and B) Assinie; (C and D) Grand-Bassam and (E and F) Azito for the period from October 2015 to September 2016.

In this work, the sex-ratio is in favour of the females on all the sites in any season. To believe these authors (Yusa, 2007; Guo *et al.*, 1998), the environmental conditions observed in this study would be in favour of female sex. However, the genetic factor would seem more plausible. In spite of higher salinity on the level of the site of Azito, the sex-ratio remains in favour of the females. Moreover, the works made by Dioh on the same species showed similar results (Dioh, 1976). The results of this work also show that *C. gasar* has a gonadic maturation related to the environmental conditions in particular, the salinity.

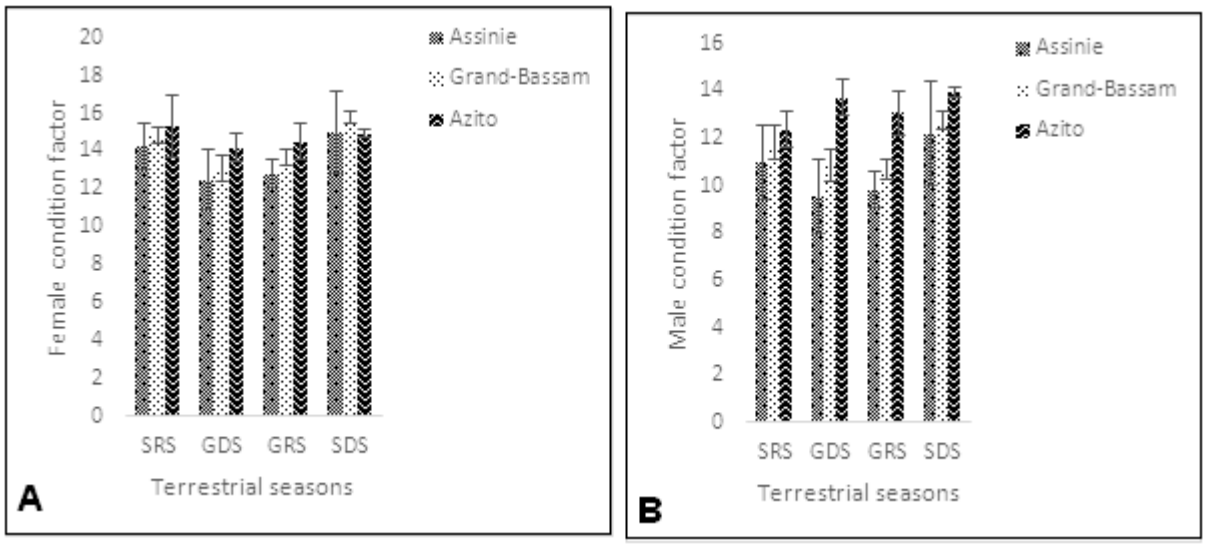

Fig. 9. Seasonal evolution of the condition factor of oysters of the three sites: (A) females and (B) males.

Indeed, the gonadic development evolves with the increase in the rate of salinity. Thus, the undetermined stage appears of November to May, when one records the weak values of salinity. Moreover, the different stages appear with the increase in salinity. The stage IV, last stage of the development gonadic, appears with the higher values of salinity. This narrow correlation between the development gonadic and the salinity would be confirmed by the results of development gonadic of Azito. Indeed, on this site which presents the higher values of salinities at any season, compared with those of the sites of Assinie and Grand-Bassam, the presence of all the stages of development gonadic is recorded at all seasons. Similar results were already observed in 2000 by Galvao and collaborators during their work on the same species (Galvao *et al.,* 2000). These authors had recorded the presence of all the stages of the development gonadic over all the period of their study. In addition, several authors have showed the influence of the environmental parameters (temperature, salinity and the availability of food) on the development gonadic of the bivalves (Loosanoff and Davis, 1951; Utting, 1993; Utting and Millican, 1997). In this work, the similarity of the temperature on the level of the three sites would carry to say the gametogenesis of this oyster is on the influence of salinity. It is in this sense that, the authors like Siqueira, 2008 and Castilho-Westphal, (2012) stipulated during their work on *C. gasar* that salinity was the factor more determining in their distribution. Moreover, according to these same authors, the change of salinity level can modify the cycle of reproduction of this species. Indeed, during their work, these authors had noted that the oysters remained with the two closed valves when they were maintained in fresh waters. What would limit the food and thus the quantity of energy necessary for a harmonious realization of the gametogenesis. In addition, of the studies undertaken on *Crassostrea virginica*, neighbor species to *C. gasar*, by Butler in 1949 had shown that the salinity has direct incidence on its physiology and its reproductive capacity. More recently, in 2011 (Lenz and Boehs, 2011) at the time of study on the reproductive biology of *C. rhizophorae* in two different environments noted that these

oysters were in various stages of the reproduction cycle throughout the year. They had allotted this fact to the environmental parameters, in particular salinity.

The evolution of the gonadosomatic indices (GI) of Assinie and Grand-Bassam is similar as well in the females as in the males. The greatest values of these indices recorded between October and November (SRS) and between July and September (ending GRS - beginning SDS) would indicate the period of reproduction and would confirm the thesis according to which the gametogenisis cycle is related to the salinity of the environment. Indeed, these same periods correspond to the periods of higher salinities for these two sites.

Moreover, evolution of GI of the site of Azito (as well in the females as in the males) which is higher than that of the two others sites would indicate than the oysters and in particular those of this site have a continuous reproduction. The values of salinity of this site (Yapi *et al.*, 2017) would carry to affirm that this parameter is an element which would strongly contribute to a continuity of the reproduction at this species. The values of condition index (K) obtained at *C. gasar* are relatively low on the level of each site as well in the females as in the males. However, the higher values are recorded during the SRS and the SDS for the two sexes as regards the sites of Assinie and Grand-Bassam. This situation could be due to the weight of the individuals which reaches its maximum because of the gonads which reach their optimum development. Indeed, these two seasons correspond to the period of the most accentuated reproduction where one finds the greatest number of individuals at the mature stage on the level of these sites. The weak values of K recorded would thus correspond to the periods of sexual rest of the species. The weak variation of K observed on the level of the site of Azito would be explained by the presence at every moment on this site of individuals of all the stages.

Conclusion

The study of the sexual cycle of *Crassostrea gasar* made it possible to identify five stages of maturation of the sexual cells with a sex-ratio in favour of the females on the level of the three sites. That is to say respectively the sex-ratio (male/female) of 1:2.9 ratios in Assinie, 1:4 in Grand-Bassam and 1:2.6 in Azito. During this study, it was noted that the oyster has a continuous reproduction and that the continuity of this reproduction was closely related to the physico-chemical conditions of the environment in particular, the salinity. Indeed, the presence of the five stages of maturity was recorded during all seasons. However, stages III and IV were more present during the small rainy season (SRS) and the small dry season (SDS) at the level of the sites of Assinie and Grand-Bassam.

It is during these two seasons that salinity has had its greater values. It is during these two seasons that salinity has had its greater values, like the gonadosomatic index and the condition factor. As for the site of Azito, it recorded constancy in the evolution of gonadosomatic index and the condition factor.

On the level of this site, the five stages of gonadic maturity were observed at very close frequencies during the four seasons. At the end of this study, it would be interesting to consider the culture of the mangrove oysters (*C. gasar*) in an environment controlled to ensure the perennity of the species while placing an oyster of quality at the disposal of the population of the Ivory Coast and why not another populations.

Acknowledgment

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