International Journal of Fauna and Biological Studies
Available online at www.faunajournal.com

IJFBS International Journal of Fauna And Biological Studies

E-ISSN 2347-2677
P-ISSN 2394-0522
www.faunajournal.com
IJFBS 2022; 9(3): 41-47
Received: 05-03-2022
Accepted: 17-04-2022

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Daily variations in the diet of the freshwater oyster *Etheria elliptica* (Lamarck, 1807) in the Pendjari River (Benin)

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DOI: https://doi.org/10.22271/23940522.2022.v9.i3a.903

Abstract
Variations in the diet of the oyster *Etheria elliptica* were studied over a daily cycle at the Pendjari River. Stomach content items were identified. Diet composition was analyzed using the point alloted method. The oyster fed mainly of phytoplankton (99.55%) and zooplankton (0.41%). The Diatoms *Coscinodiscus rudolfii* (31.73%) and *Eunotia bilunaris* (23.93%) constitute the preferential foods, while the Cyanophytes (*Microcoleus lacustris*, 14.15%) and the Chlorophytes *Stigeoclonium aestivale* (10.64%) and *Gonatozygon monotaenium* (9.13%) are the secondary foods. The other are additional food items. On the daily cycle, the trophic activity is intense almost all day with a preference for the night hours between 6 p.m -12 a.m. The diet compositions showed significant variations between the hours of the day (9 a.m. to 6 p.m.) and night (9 p.m. to 6 a.m.). *Etheria elliptica* is a phytophagous with a varied trophic activity, feeding mainly on diatoms. The trophic activity intensifies during the night hours.

Keywords: *Etheria elliptica*, Diet, phytoplankton, zooplankton, Pendjari River

1. Introduction
In general, molluscs and more specifically bivalves (oysters, mussels, clams, etc.) play a significant role in the economy of many countries around the world [1-2].
In Benin, oysters are fishery resources exploited by inland fishing. In the south, the mangrove oyster *Crassostrea gasar* is exploited in Lake Nokoué, the lagoons of Porto Novo and the coastal lagoon mainly by women and generates important sources of income [3-4]. In the north of the country, this exploitation takes place in the freshwaters of the Pendjari River with the unique species *Etheria elliptica* [5]; the only species of freshwater oyster currently known in West Africa. To sustainably guarantee the oyster needs of local populations and preserve the survival of the species, one of the proposed solutions is its introduction into culture. To do this, scientific information is necessary on the ecology and biology of the species, in particular on its diet. In addition to studies on the feeding habits of certain oyster species such as *Crassostrea madrasensis* in India [6], on *Crassostrea gasar* at Lake Nokoué [7], work on the diet of tropical oysters is rather rare.The freshwater oyster *Etheria elliptica* (Mollusca: Bivalvia) belongs to the Etheridae family. The family Etheriidae (Unionoida) consists of four genera: *Etheria*, *Bartlettia*, *Acostaea* and *Pseudomulleria*. All of them dwell in river waters, almost entirely in the tropics in South America, Africa and India. *Etheria elliptica* (Lamarck, 1807), found in the Niger, Congo and Nile basins and also in Madagascar, was studied by Jinja in Lake Victoria. It is cemented indifferently by one of its valves becoming bilaterally asymmetrical to a sedimented support in water. It is also very irregular where attached to an uneven surface. The oyster is a filter feeder. To feed, it opens its valves to let in water that it expels after taking its food (plankton). Food particles are filtered and oxygen absorbed through gills that are covered in microscopic cilia that push water inward.
Currently, with the exception of some data on *Etheria elliptica* reproduction [8-9] and ecology [5-10], there are no data on oyster diet. It is to fill this knowledge gap that this study on the diet of the oyster Etheria elliptica (Lamarck, 1807) was undertaken in the Pendjari River. The specific objectives of this study are to: 1) determine the general daily food composition of the species, 2) identify the preferential foods, secondary foods and accessory foods and 3) analyze the variations in the diet according to the hours of day and night.

2. Material and methods

2.1. Study area

This study is carried out in the Pendjari River, located about 550 km from Cotonou in the Pendjari Biosphere Reserve (RBP) in the extreme northwest of the Republic of Benin (Figure 1). Its geographical limits are between 10 degrees 30' and 11 degrees 30' North latitude, 0 degree 50' and 2 degrees 00' East longitude [11].

The choice of this river for this study is explained by the exploitation freshwater oysters by local populations, on the one hand; and by the framework of this study which is the conservation of the resource by its introduction into breeding, on the other hand.

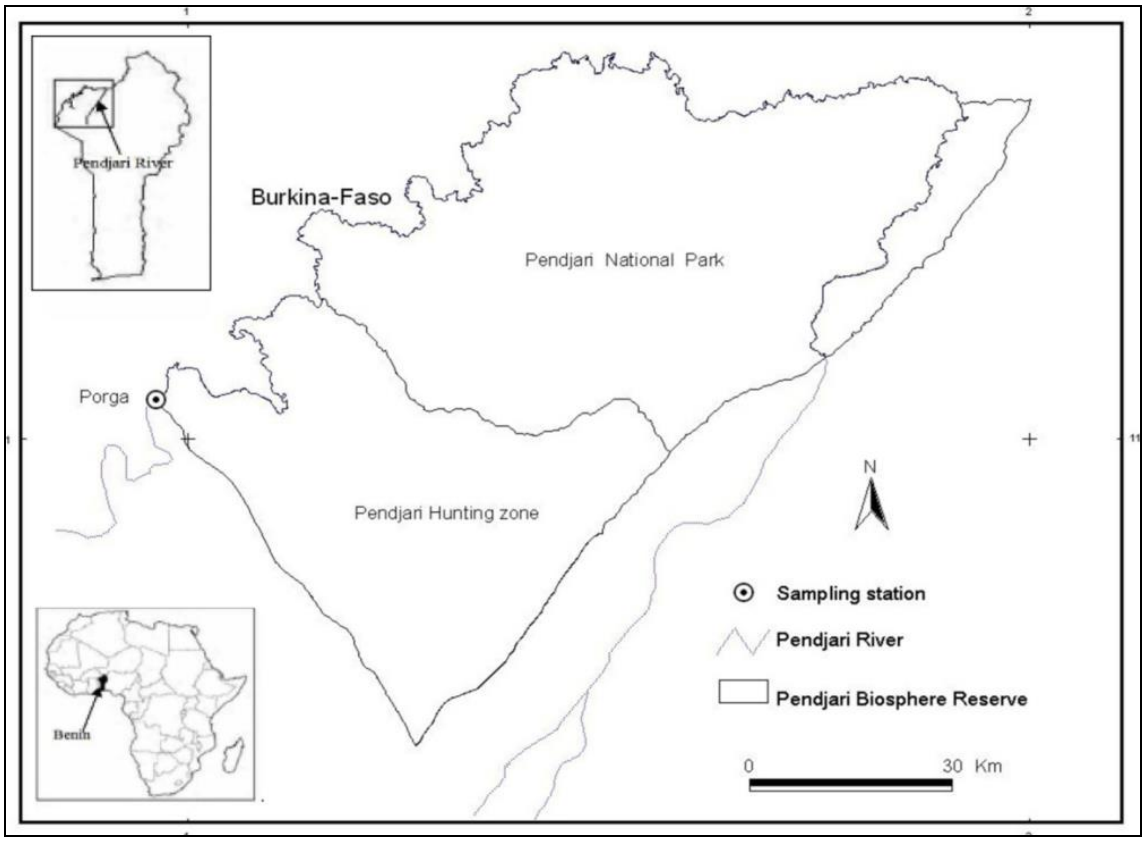

Fig 1: The Pendjari River with the Porga sampling station (Benin)

2.2. Sampling

The oyster samples were collected at the Porga station, in the Pendjari River in Benin (Figure 1). A total of 30 specimens of all sizes were collected at regular 3-hours intervals over a 24-hours cycle (3 a.m, 6 a.m, 9 a.m, 12 p.m, 3 p.m, 6 p.m, 9 p.m, 12 a.m) and stored automatically in 5% formalin to fix stomach contents and stop digestion. The samples were then transported to the laboratory for the determination of the stomach contents of the oysters.

2.3. Diet Study Methods

The study of the diet of the sampled oyster individuals was carried out in the laboratory. Like fish, the method used is the analysis of stomach contents. The common method of data analysis is the volumetric method used for fish. Thus, the contents of the stomach of each specimen is introduced into a graduated tube containing water, the volume or weight of each food item or of all the foods of each fish is estimated using the observed drop. However, this method can only be applied to macroorganisms because this level difference is difficult or even impossible to observe in the stomach of microphages such as oysters. Thus, the volumetric method is not suitable for this study.

Accordingly, the points method. Each oyster specimen is stripped of its valves with a knife and then the stomach is dissected with a scalpel. After the dissection, a total of points (0 to 20) is assigned to the stomach according to its level of filling (empty, nearly empty, 1/4, 1/2, 2/3 or completely full) and according to the size of the individual. The total points can reach 30 depending on the case [12]. The contents of the stomach are then diluted in a volume of water of 10 ml. When the density of items is too high, a new dilution is carried out (x10, x20 etc.) in order to facilitate the identification and counting of prey. The stomach contents collected in pillboxes are observed in small volumes of 1 ml under a light microscope (Magnification x100). The different taxa of the foods consumed were determined using available studies [14-15-16].

The number of individuals per identified species is estimated by the observer. Indeed, this number is not counted to the nearest unit; but estimated according to the method of Hynes [12]. The possible biases resulting from the imprecision of the count disappear after the treatment of several samples by the same observer. Moreover, the count is exhaustive if the volume of dilution is 10 ml. But when it exceeds 10ml, for example 30ml, 10ml is taken from the total volume for the treatment for a final extrapolation. The relative importance of items in the stomach is determined using the point method. Indeed, the identified species are classified in their different taxonomic groups (Item) then points are attributed to each item. These points (1; 2; 4; 8; 16) are awarded according to the volume of each group and the size of the animal while

taking into account the score for the stomach: the cumulative points are equal to the score of the stomach.
The methodology used in the data processing is based on the calculation of the Preponderance Index (PI) [17]. It is calculated by taking into account the percentages of occurrence and volume

$$PI = \frac{ViOi}{\sum ViOi} \times 100$$

Where, Oi =occurrence of volumeet Vi =indice volume
At the end of all this preliminary work carried out, correlation tests of the ranks of Spearman then those of x^2 were carried out using the Statistica 6 software in order to categorize the diet of the oysters and to compare its variation. depending on the different time slots.

3. Results

3.1. General daily food composition

The general profile of the diet of the oyster *Etheria elliptica* as well as its composition according to the photoperiodism were analyzed. A total of 223 oyster stomachs, between 20-80mm in size, were examined, all of which are almost full. The diet of the 223 stomachs observed presents 61 types of food with a predominance of phytoplankton reaching 96% of the preponderance index (PI). Among phytoplankton, diatoms are the most abundant (58.43%). Then come the Cyanophytes (20.78%) then the Chlorophytes (19.94%) and finally the Euglenophytes (0.38%) (Table 1).

Table 1: Diel variations of gut content of the oyster *Etheria elliptica* %IP = Index of Preponderance index (%) ; n = number of stomachs examined.

Food item		Food species	Global (n=223) %IP	Day (n=113)%IP	Night (n=110)%IP
Phytoplankton	Chlorophyceae	*Gonatozygon monotaenium*	9.13	14.87	4.44
		Audouinella violacea	0.02	0.03	0.01
		Cladophora sp	0.00	0.00	0.01
		Closterium aciculare	0.11	0.12	0.09
		Cosmarium candianum	0.01	0.00	0.02
		Stigeoclonium aestivale	10.64	13.16	7.86
		Ulotrix zonata	0.03	0.01	0.05
	Cyanophyceae	*Artrospira sp*	1.10	0.97	1.22
		Chroococcus sp	0.24	0.08	0.48
		Coelosphaerium sp	0.56	0.37	0.78
		Lyngbya martensiana	4.26	2.25	6.84
		Merismopedia elegans	0.05	0.06	0.05
		Microcoleus lacustris	14.15	14.40	13.45
		Microcystis aeruginosa	0.40	0.32	0.48
		Synechosystis aquatilis	0.01	0.00	0.03
	Diatomophyceae	*Aulacosira ambigua*	0.18	0.13	0.25
		Navicula cuspidata	1.10	0.77	1.46
		Navicula placentula	0.01	0.00	0.03
		Amphora inflexa	0.25	0.25	0.25
		Coscinodiscus rudolfii	31.73	27.98	34.35
		Cyclotella meneghiniana	0.16	0.07	0.27
		Cymbella ventricosa	0.07	0.07	0.08
		Diatoma mesodon	0.00	0.00	0.01
		Diatoma sp	0.30	0.16	0.47
		Eunotia bilunaris	23.93	21.80	25.40
		Gyrosigma hippocampus	0.66	1.46	0.15
		Melosira granulata	0.04	0.00	0.11
		Surirella sp	0.00	0.00	0.01
	Euglénophyceae	*Euglena viridis*	0.09	0.05	0.14
		Phacus longicauda	0.29	0.10	0.57
Zooplankton		*Asplanchna girodi*	0.24	0.12	0.40
		Cephalodella gibba	0.00	0.00	0.01
		Dicranophorus prionacis	0.05	0.03	0.09
		Keratella tropica	0.01	0.03	0.00
		Epiphanes brachionus	0.00	0.00	0.01
		Hepadella patella	0.00	0.00	0.00
		Pompholys sulcata	0.02	0.00	0.05
		Notommata pseudocerberusta	0.08	0.25	0.00
		Trichocerca chattoni	0.00	0.00	0.02
Other items	Plants	*Meridion circulare*	0.00	0.01	0.00
		Plant matter (detritus)	0.00	0.01	0.00
	Animals	*Animal matter (detritus)*	0.00	0.01	0.00
		Insect undetermined	0.00	0.01	0.00
		Tintinnidae	0.04	0.05	0.04
		Total	100.00	100.00	100.00

The analysis of the composition of the diet of the specimens of the oyster *Etheria elliptica* revealed that the species *Coscinodiscus rudolfii* (Diatoms) is classified in the category of preferential food. Secondary foods are *Eunotia bilunaris* (Diatoms), *Microcoleus lacustris* (Cyanophytes), *Stigeoclonium aestivale* (Chlorophytes) and *Gonatozygon monotaenium* (Chlorophytes). The remaining foods (*Lyngbya martensiana, Artrospira sp, Navicula cuspidata* and other foods) are classified as accessory foods.

3.2. Nycthemeral feeding rhythm of the *Etheria elliptica* oyster

The cumulative points allocated revealed the variations in the trophic activity of the oysters over the daily cycle. They reach their maximum values at 6 p.m. (524 points) at dusk and at night between 9 p.m. (496 points) and midnight (501 points). The trophic activity is minimal in the morning at 9 a.m. (395 pts) (Figure 2).

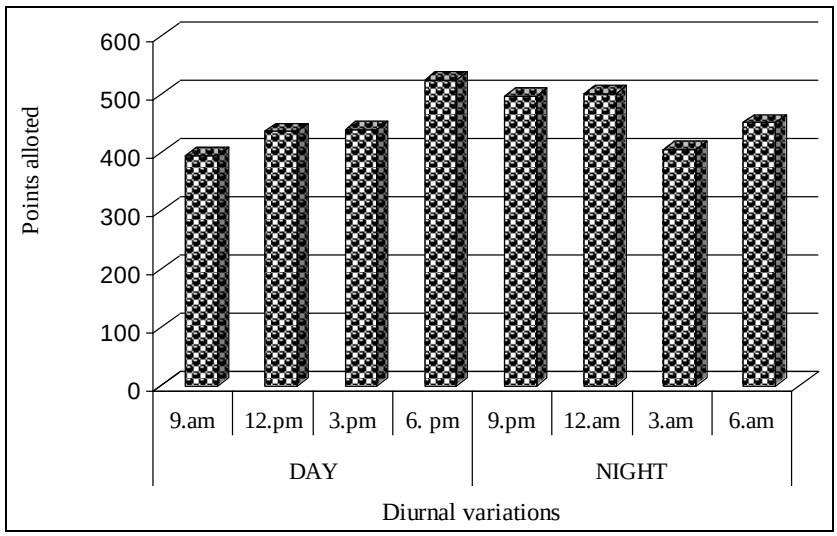

Fig 2: Feeding periods of the oyster *Etheria elliptica* over a daily cycle in the Pendjari River.

3.3. Diurnal variations in diet composition

Times of Day

During daylight hours (9 a.m. to 6 p.m.), Diatoms come first with a predominance of the *Coscinodiscus rudolfii* species. Nevertheless, the values of the diatom preponderance index gradually decrease during the day (70.22% at 9 a.m. 53.84% at 12 p.m.; 50.54% at 3 p.m. and 36.08% at 6 p.m.). The consumption of Chlorophytes increases gradually during the day (Preponderance Index: 11.38% at 9 a.m. 31.67% at 12 p.m.; 31.55% at 3 p.m. and 33.79% at 6 p.m.). The Cyanophytes are found in the respective proportions of 17.54% IP; 14.11% IP; 16.21% PI and 28.23% PI (Figure 3). The diatoms *Coscinodiscus rudolfii* and *Eunotia bulinaris* are the most consumed food items followed by the Chlorophytes *Gonatozygon monitaenium* and *Stigeoclonium aestivale* (Figure 4).

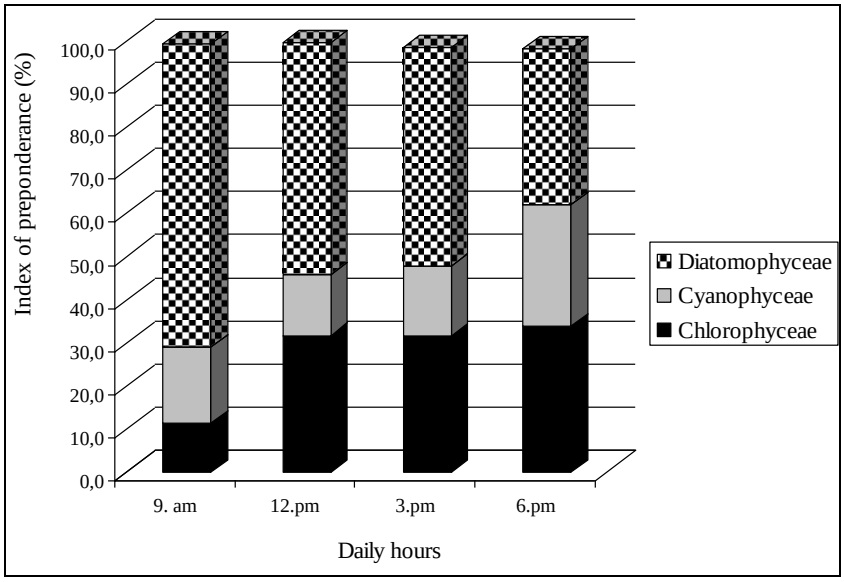

Fig 3: Variations in diet of the oyster *Etheria elliptica* during the hours of the day.

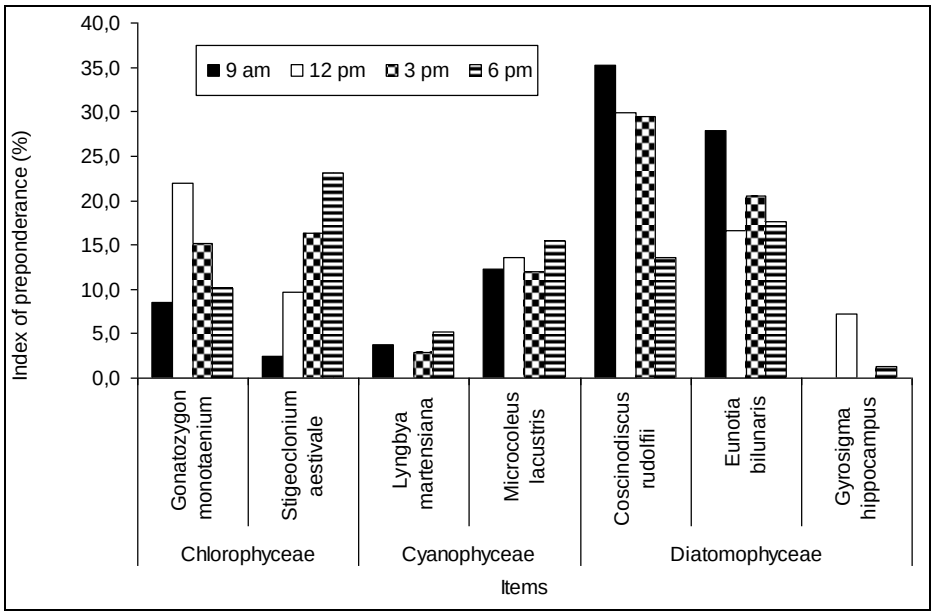

Fig 4: Variation in diet composition of the oyster *Etheria elliptica* during the day in relation to Phyla and species.

Night hours

During the hours of the night (9 p.m. to 6 a.m.), Diatoms regardless of the time slot considered are abundant with a preponderance of the species *Coscinodiscus rudolfii* and *Eunotia bulinaris* (Figure 5 and Figure 6). In addition, the Chlorophyte preponderance index decreases gradually from one time slot to another during the night (24.08% at 9 p.m.; 11.74% at midnight; 8.96% at 3 a.m. and 6.82% at 6 a.m.). Diatoms vary overnight with the percentages of the Preponderance Index (PI) of 48.85% at 9 p.m.; 67.41% at 24 hours; 68.50% at 3 a.m. and 61.26% at 6 a.m. while Cyanophytes have respective percentages of 24.64%; 19.36%; 21.01% and 30.85% (Figure 6).

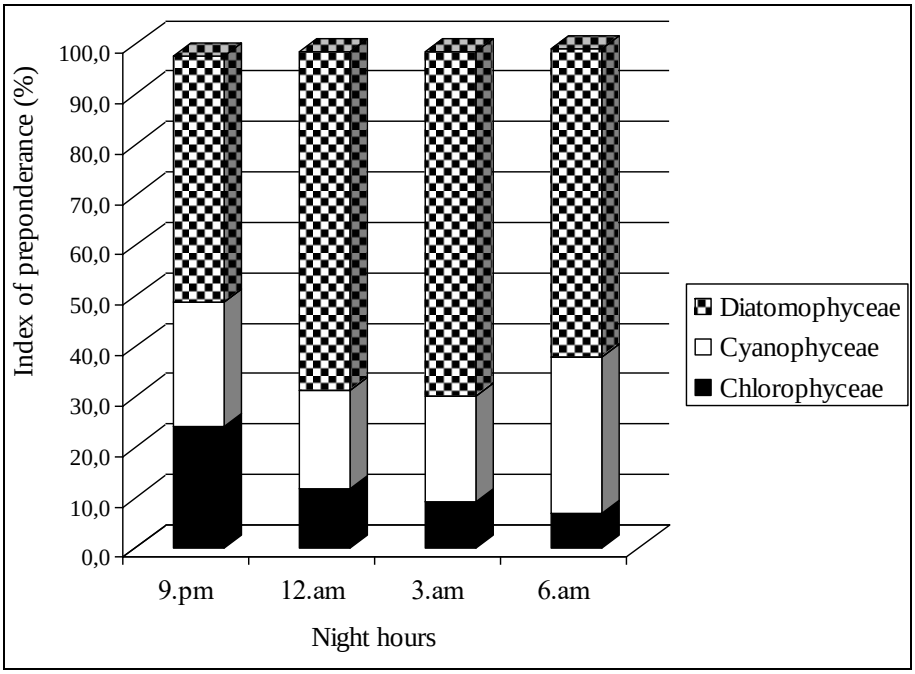

Fig 5: Variation in the diet of the oyster *Etheria elliptica* during the night according to the abundant Phyla

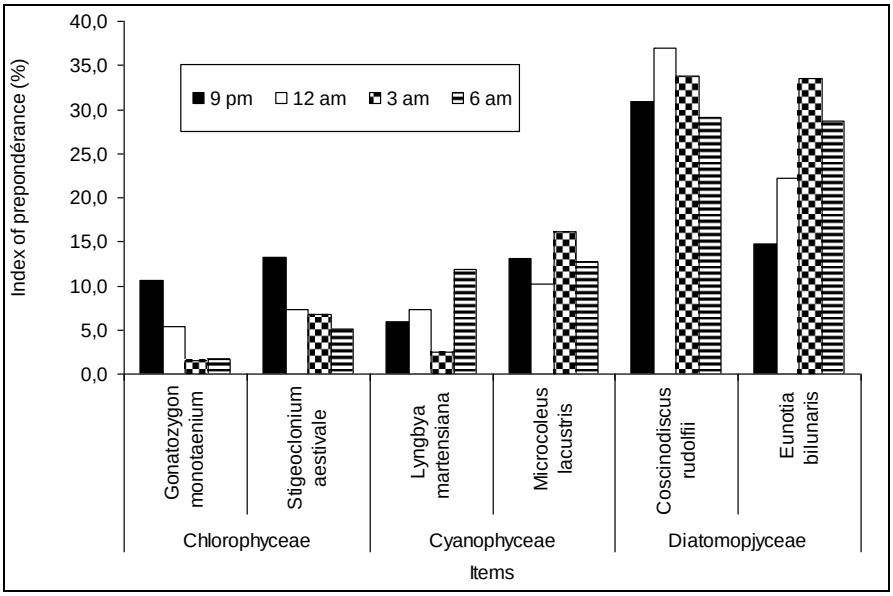

Fig. 6 : Variation in the diet of the oyster *Etheria elliptica* during the night according to the abundant Phyla and species.

4. Discussion

The daily profile of the diet of the oyster *Etheria elliptica* confirms that this species is a phytophagous that feeds mainly on phytoplankton. Similar results are previously reported by Thangavelu [6] as well as Zabi and Le Loeuff [18], who indicated that oysters are phytophagous. *Etheria elliptica* has a marked preference for diatoms (58.43%); cyanophytes (20.78%), chlorophytes (19.94%) and finally euglenophytes (0.38%). These results are almost similar to those obtained by Thangavelu [6] for the species *Crassostrea madrasensis* in Lake Pulicat (South India). Indeed, the diet of this species is essentially composed of diatoms (52.8%).

Previously, Yonge [19] reported that *E. elliptica* fed on small-sized phytoplankton species in running waters. Bogan [20] noted that freshwater bivalve molluscs, such as *Etheria elliptica*, filter large amounts of diatoms, blue-green algae, bacteria as well as fine organic particles. Thus, the Pacific oyster (*Crassostrea gigas*) has an omnivorous diet mainly oriented towards benthic diatoms (70% of the abundance) [21]. Similarly, the mangrove oyster *Crassostrea gasar* feeds mainly on phytoplankton (diatoms and peridinians) [18]. In southern India, the natural diet of the edible oyster *Crassostrea madrasensis* is dominated by diatoms (52.8%)[6].

Bacillariophycae taxa predominate and constitute more than 50% of phytoplankton taxa in the fresh and brackish waters of the Bonny River in Nigeria. In freshwaters, the Bacillariophycae are the most important with 38 taxa out of 82 [22].

The high consumption of phytoplankton species confirms the trend that diatoms constitute the class of planktonic species of the algal population of West African water bodies and rivers [23].

This would explain the predominance of diatoms in the diet. The diatoms *Coscinodiscus spp* and *Eunotia spp*. The abundance of diatom species in the river would explain their abundant consumption by specimens of the *E. elliptica* oyster. However, Thanghavelu[6] analyzed the correlation between the abundance of diatoms in the stomach of the oyster Crassostrea madrasensis and their proportion in Lake Pulicat in India. The results showed a preference of the *Crassostrea madrasensis* oyster for benthic diatoms even when they are in low quantity in the natural environment.

The diatom *Coscinodiscus* is a preferential food of suspension-eating bivalves. It is consumed abundantly by the oyster *Crassostrea madrasensis* [6], the bivalve *Pecten maximus* [24]. Beninger and Decottignies [24] showed that *Coscinodiscus* capsules are attractive to suspension-eating bivalves. This diatom is one of the preferred phyoplanktons of *Etheria elliptica*.

The analysis of the circadian rhythms of the oyster *Etheria elliptica* was carried out. The day (9 a.m., 12 p.m., 3 p.m., 6 p.m.) and night (9 p.m., midnight, 3 a.m., 6 a.m.) periods were chosen in consideration of the work of Isumbisho and Kaningini [25] on the time of feeding and diet of *Limnothrissa miodon* larvae in the southern part of Lake Kivu (Bukavu Basin) which considered the day from 8 a.m. to 6 p.m. and the night from 8 p.m. to 6 a.m. Thus, the peak of tropic activity observed between 6 p.m. and midnight indicates that the *Etheria elliptica* oyster feeds on the daily cycle with intense trophic activity at the beginning of the night (9 p.m.). It therefore appears that the feeding activity of the oyster is exclusively diurnal and extends from 9 a.m. to 6 p.m., with intense feeding activity between 6 p.m. and midnight.

5. Conclusion

The study of the daily variations in the diet of the oyster *Etheria elliptica* has provided scientific information on its diet, hitherto little studied. The species of diatoms *Coscinodiscus rudolfii* and *Eunotia bilunaris* constitute the preferential food of the species while *Microcoleus lacustris* (Cyanophyte), *Stigeoclonium aestivale* (Chlorophyte) and *Gonatozygon monotaenium* (Chlorophyte) are secondary foods. The other foods are additional items. The different variations observed at the level of the time slots show that the trophic activity of the oyster is intense at dusk. *Etheria elliptica* is a phytophagous with a trophic activity which would intensify during the night.

6. Acknowledgements

We thank the fishermen of the Pendjari River for their active contribution to the collection of oyster samples especially during the cold hours of the night.

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