Effect of Seasonality on the Diet and Biochemical Composition of the Stomach Contents Tilapia *Sarotherodon melanotheron melanotheron* (Rüppell, 1852) in three Aquatic Ecosystems of Côte d'Ivoire (Sector VI of Ebrié lagoon, Ayamé Lake 1 and Aby-Nord lagoon).

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ABSTRACT---- ***Culture of Sarotherodon melanotheron presents major difficulty, which is the valorization of artificial food by this species. Thus, this study aims to identify preferential prey of this species in three aquatic ecosystems (sector VI of Ebrié lagoon, Ayamé Lake 1 and Aby-Nord lagoon), with a view to developing natural food resources in farming environments. The sampling was carried out from March 2017 to February 2018 using 80 to 100 mm gillnets. Stomachs of 343 specimens were analyzed by microscopic observation, preys were identified, feed index (AI) was calculated and nutritional quality of food bolus has been determined. IA shows a preference of specimens from sector VI for Coscinodiscus sp. at dry season (IA=58.63%), rainy season (IA=65.97%) and flood season (IA=21.06%). Coscinodiscus sp. remains an essential prey in Aby lagoon at high rainy season (IA=45.66%), a dominant prey at small dry season (IA=76.06%) and small rainy season (IA=92.29%). On the other hand, in Ayamé Lake 1, Aulacoseira sp. is important prey (IA =19.03%) in high dry season, essential prey (IA=48.19%) at high rainy season and dominant prey (IA=61.21%) at small dry season. At alimentary bolus, highest contents of proteins (22.44%) and lipids (2.36%) were obtained at high dry season in the Aby lagoon. The contents of hydrolysable organic matter (4499.50 to 781.3 mg.g-1) and minerals (161.3 to 406.2 mg.g-1) remain high in three aquatic ecosystem regardless of the season. In conclusion, species has a preference for genera Coscinodiscus sp. and Aulacoseira sp and quality of food bolus is superior in lagoon.***

Keywords---- *Sarotherodon melanotheron melanotheron*, *Coscinodiscus* sp., *Aulacoseira* sp., diet

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1. INTRODUCTION

Increasing aquaculture production in Cote d'Ivoire necessarily involves diversifying and popularizing other species of fish other than the tilapia *Oreochromis niloticus*. This species represents nearly 96% of the species farmed[1] but it has difficulty growing in brackish water[2]. Thus the black-chinned tilapia, *Sarotherodon melanotheron*, recognized for its high euryhalinity and its ability to resist heavy pollution[3] appears to be an ideal species to popularize in brackish water environments[4.] Unfortunately, studies have shown that *S. melanotheron* shows poor growth with artificial foods, thus reducing its culture[5]. Interesting results were demonstrated on the growth of the species in systems with intermediate environmental conditions between intensive farming environment and natural environment such as the enclosure-acadja system[5, 6]. It would be beneficial in the present study to explore the diet of the subspecies in the environments where its presence is reported in order to improve the food formulation of the species in the breeding environment.

Sarotherodon melanotheron represents 59% of fishing catches of Ivorian lagoon[7]and about 29% in Ayamé Lake 1[8]. These environments thus appear as natural habitats for this species.

The study of the diet of the tilapia *Sarotherodon melanotheron* in these aquatic ecosystems will consider preferential prey, seasonal variation and nutritional quality of these prey in different environmental conditions. The knowledge of these parameters could be beneficial in the food reformulation of the species in intensive and extensive farming.

2. MATERIALS AND METHODS

2.1. Study Area

The study was conducted during the period from March 2017 to February 2018 in 03 aquatic ecosystems of Cote d'Ivoire among which sector VI of the Ebrié lagoon (3°40' and 4°50' West longitude and 5°2' and 5°10' North latitude) and the Aby-Nord lagoon (3°40' and 4°50' West longitude and 5°2' and 5°10' North latitude) are brackish water and Ayamé Lake 1 (5°30' North latitude, 3° West longitude) which is freshwater environment (Figure 1). These study areas were chosen for ease of access and the presence of a natural stock of Sarotherodon melanotheron.

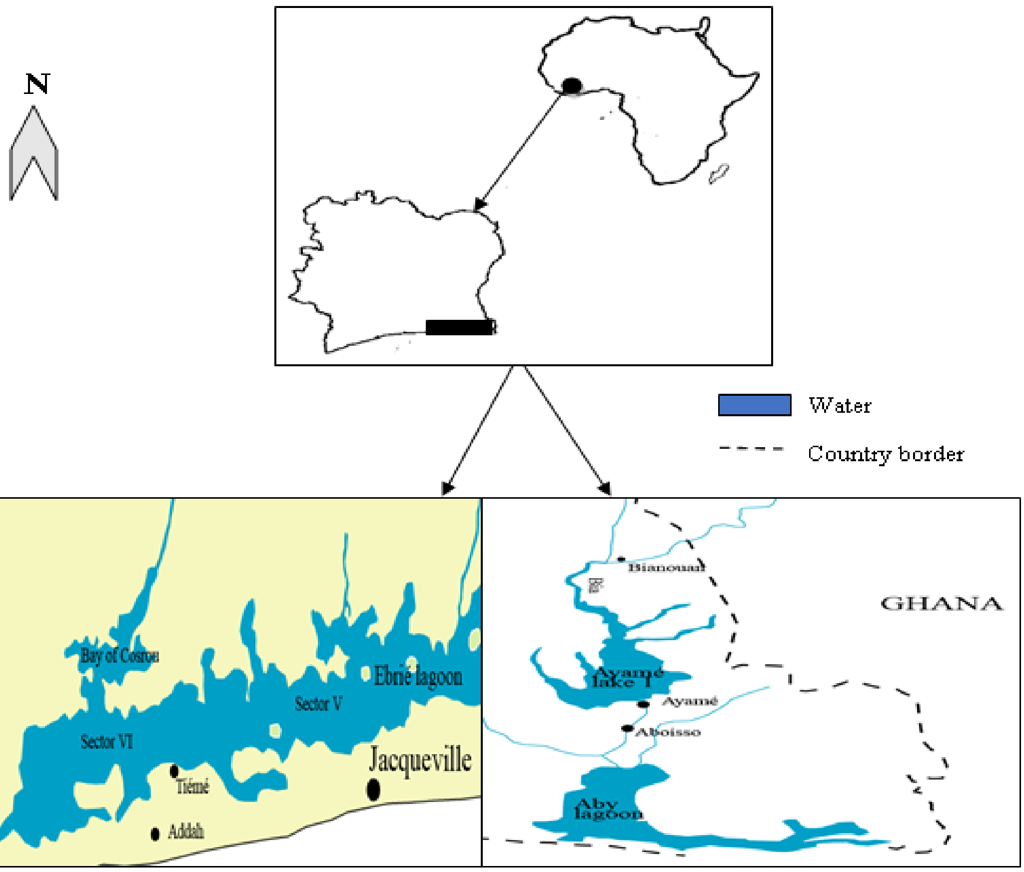

Figure 1: Map showing the sector VI of Ebrie lagoon, Ayame Lake 1 and northern area of Aby lagoon.

2.2. Fish Sampling

Monthly fish samples were obtained using carp gillnets of 80 to 100 mm mesh size. The fish were kept chilled in an ice chest immediately after capture and sent to the laboratory. At the laboratory, fish were identified, dissected using stainless dissection materials and the digestive system removed. The stomach was then taken out by cutting the alimentary tract between the end of esophagus and the pylorus. Empty stomachs were counted and the Vacuity index (Vi) was calculated.

*Vacuity index:*The Vacuity index (Vi) was calculated using the Equation[9]:

$Vi = 100\ (N_{es}/\ N_{st})$[9], where N_{es} is the number of empty stomachs and N_{ts} is the total number of stomachs examined.

Part of the sampled full stomachs is kept in a 5 % formalin solution for microscopic observation and the other part is used for biochemical analysis.

2.3. Identification of Prey

Identification of prey was determined by method of Utermöhl[10] modified by Laplace-Treyture et al.[11]. The stomach contents of fish were then homogenized by manual agitation. Observation of preys was carried out with an inverted microscope after complete sedimentation of the cells in a tank. Identification of phytoplankton prey was carried out using keys of Ouattara et al. [12], Prygiel and Coste[13]. For zooplankton prey, keys of Dumont et al. [14] and Coulter[15] were used. Observation data made it possible to détermine parameters such as frequency of occurrence (Fo), volumetric index (VI) and feed index (IA).

Frequency of occurrence (Fo) : The frequency of occurrence of each food item was calculated using the formula: $F0(\%) = 100\ X\ Ns/Nt$ [16], where Ns is the number of stomach containing a given prey and Nt is the number of non-empty stomach.

Volumetric percentage (V) **:** The volumetric percentage of each food item was calculated using the formula: $V(\%) = Vi\ X\ 100/Vt$[17]**,** where Vi is the total volume of each prey category and Vt the total volume of all prey.

Feed index (IA) **:** The Feed Index of each prey category was calculated using the formula: $IA(\%) = Fo\ X\ V/100$ [18], where Fo is Frequency of occurrence each prey category and V the volumetric percentage each prey category. Lauzanne[18] was used to indicate the importance of each food category (>50: dominant, 25-50: essential, 10-25: important, <10: secondary)

2.4. Biochemical Analysis

Moisture content and the lipid content were determined by AOAC[19] method, the protein content by Kjeldahl method. The contents of Total Organic Matter (TOM), Hydrolysable Organic Matter (HOM), Organic Matter Resistant to Hydrolysis (OMRH) and Minerals (Min) were determined according to the method of Buddington[20], modified by Bowen et al. [21].

2.5. Statistical Analysis

Kruskal-wallis test was performed to check if there is a significant difference between seasonal data (vacuity index and biochemical parameters). For significant difference cases, the U Mann-Whitney a posteriori test is performed to compare these data two by two. These tests were performed at the 5% threshold with Statistica 7.1 software. A statistical analysis of the similarity (ANOSIM) between profiles was performed with primer 7 software. R of ANOSIM indicates the degree of similarity of groups. These are considered different for $p<0.05$ and $R>0.25$ ($R>0.75$, very separated groups; $0.50 < R < 0.75$: separated groups with overlaps; $0.25 < R < 0.5$: separated groups with very strong overlaps.

3. RESULTS

3.1. Vacuity index

Variation of the vacuity index in the three environments is shown in Figure 2. In sector VI, the coefficient (22.5%) was significantly high during the flood season. This index was high during the rainy season for fish from the Aby-Nord lagoon (65.00%) and during the same season for fish from Ayamé Lake 1 (35.00%).

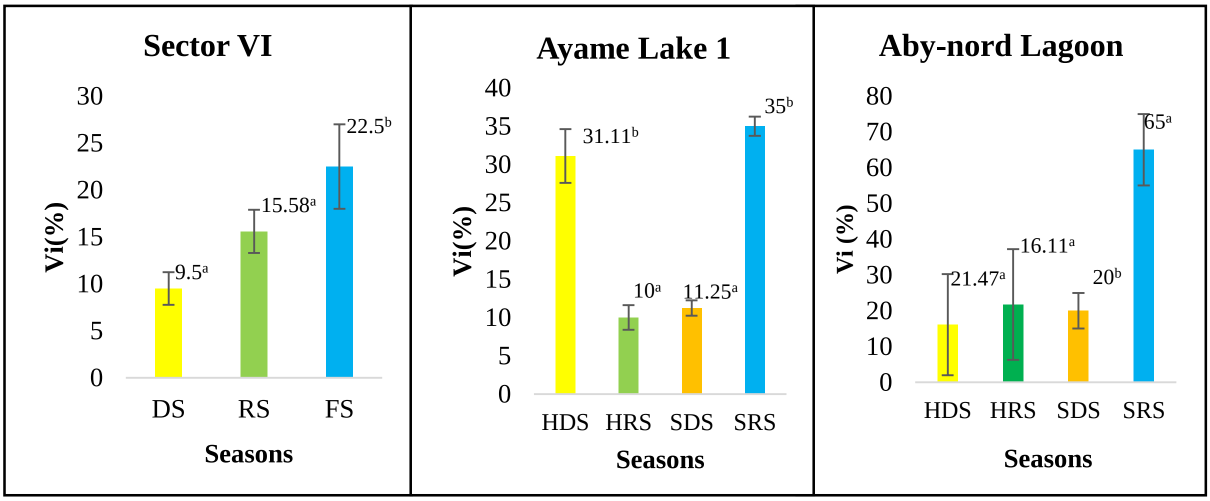

Figure 2: Seasonal variations of Vacuity index *S. m. melanotheron* fished in sector VI of Ebrie lagoon, Ayame Lake 1 and Aby nord lagoon

HDS : High dry season ; HRS : High rainy season; SDS : Small dry season ; SRS : Small rainy season; DS : Dry season ; RS : Rainy season ; FS : Flood season., (a, b): numbers with the same letters on the same line are not significantly different (P>0.05)

3.2. Inventoried prey in fish sampled in the three aquatic ecosystem

The prey inventoried in the fish sampled in Ebrié lagoon, Aby-Nord lagoon and man-made Ayame Lake 1 are shown in Figure 3. The prey identified is phytoplanktons, zooplanktons, debris and oocytes. Phytoplankton represented more than 60% of the prey inventoried in the three environments. These phytoplankton prey are divided into several class including diatoms, Chlorophyceae, Conjugatophyceae, Cyanophyceae, Dinophyceae, Chryptophyceae Dinophyceae and Xanthophyceae.

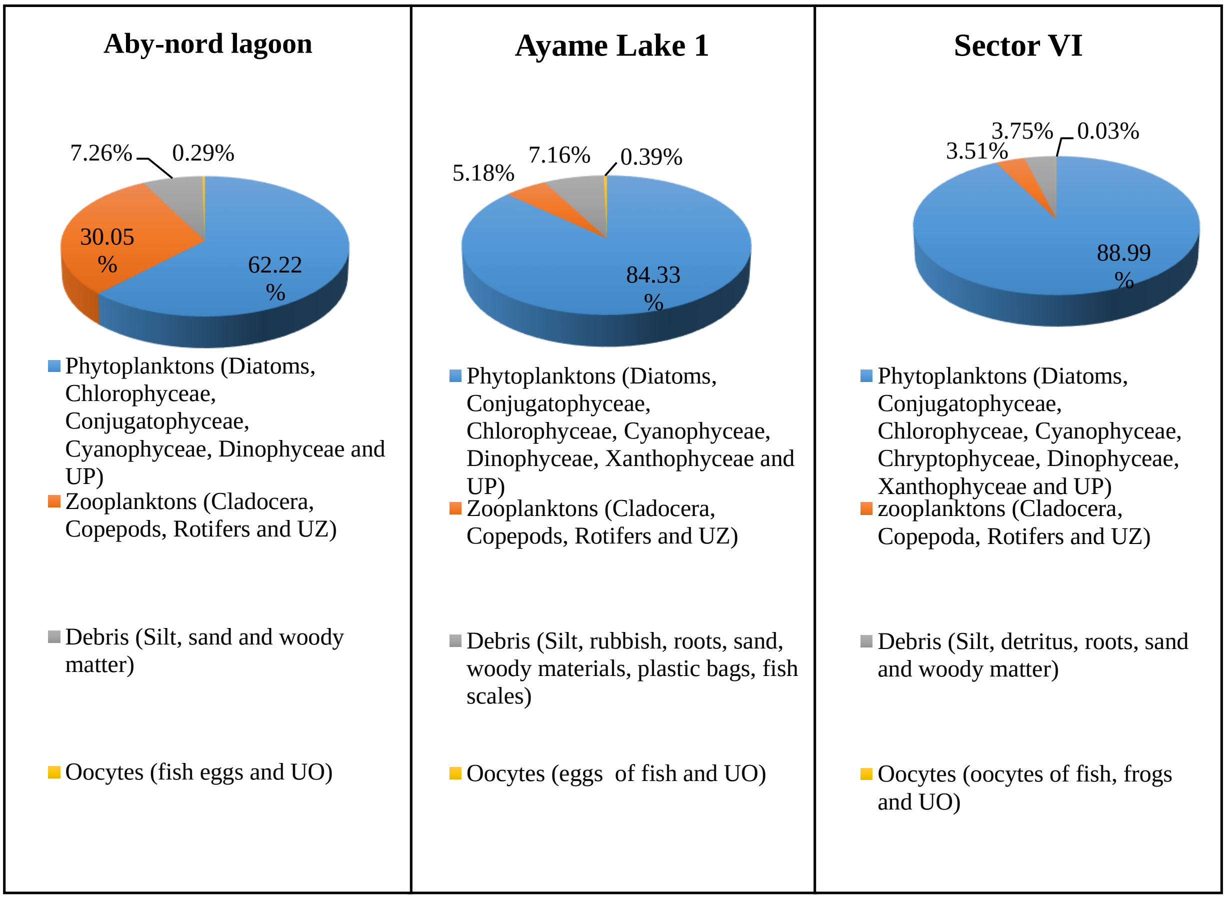

Figure 3**:** Inventoried prey in *S. m. melanotheron* fished in sector VI of Ebrié lagoon, Ayame Lake 1 and Aby nord lagoon.

UP: Unidentified phytoplanktons, UZ : Unidentified zooplanktons UO : unidentified oocytes

3.3. Feed index of inventoried prey

Tables I, II and III present the different feed index in the three aquatic ecosystems. Food bolus in the three environments consisted essentially of phytoplankton (dominant prey), except in high dry season in Aby-Nord lagoon where zooplankton (IA=77%) was the dominant prey. Zooplankton is essentially composed of the genus *Brachionus* sp. (IA=42.66%). In this lagoon, *Coscinodiscus* sp. was the essential prey (IA=45.66%) in high rainy season and the dominant prey (IA=76.06%) in small dry season and small rainy season (IA= 92.29%). In Ebrié lagoon, *Coscinodiscus* sp. was the dominant prey during rainy season (IA =58.63%) and dry season (IA=65.97%). At Ayame lake 1, the genus *Aulacoseira* appeared as the important prey in high dry season (IA = 19.03), the essential prey in high rainy season (48.19%) and the dominant prey in small dry season (IA=61.21%).

Table I: Frequency of occurrence (Fo), Volumetric percentage (V) and Feed Index (IA) of important, essential and dominant prey of *S. m. melanotheron* specimens caught in sector VI of the Ebrie lagoon.

Taxa	*Seasons*								
	Dry season			Rainy season			Flood season		
	Fo(%)	**V(%)**	**IA(%)**	**Fo(%)**	**V(%)**	**IA(%)**	**Fo(%)**	**V(%)**	**IA(%)**
Phytoplanktons	100	95.54	95.54	100	92.97	92.97	100	78.45	78.45
Diatoms	100	62.16	62.16	100	70.25	70.25	100	33.82	33.82
Coscinodiscus sp.	100	58.63	58.63	100	65.97	65.97	83.33	25.28	21.06
Cyanophyceae	66.67	18.81	12.54			+	100	25.79	25.79
Chlorophyceae	83.33	12.52	10.43	87.5	11.86	10.38	100	16.04	16.04

+ : secondary prey

Table II: Frequency of occurrence (Fo), Volumetric percentage (V) and Feed Index (IA) of important, essential and dominant prey of *S. m. melanotheron* specimens caught in Ayame lake 1

Taxa	Seasons											
	High dry season			High rainy season			Small dry season			Small rainy season		
	Fo(%)	**V(%)**	**IA(%)**	**Fo(%)**	**V(%)**	**IA(%)**	**Fo(%)**	**V(%)**	**IA(%)**	**Fo(%)**	**V(%)**	**IA(%)**
Phytoplanktons	100	81.05	81.05	100	91.62	91.62	100	92.27	92.27	100	72.36	72.36
Diatoms	100	36.16	36.16	100	52.77	52.77	100	64.94	64.94	100	45.62	45.62
Aulacoseira sp.	100	19.03	19.03	100	48.19	48.19	100	61.21	61.21	100	16.8	16.8
Guinardia sp.			+	-	-	-	-	-	-	100	10.14	10.14
Conjugatophyceae	100	17.91	17.91	100	31.3	31.3	100	15.18	15.18	80	1.77	+
Staurastrum sp.	100	12.58	12.58	100	23.02	23.02	100	14.04	14.04	80	1.05	+
Chlorophyceae			+			+			+	100	10.66	10.66
Debris			+			+			+	100	22.72	22.72

-: absent prey; +: secondary prey

Table III: Frequency of occurrence (Fo), Volumetric percentage (V) and Feed Index (IA) of important, essential and dominant prey of *S. m. melanotheron* specimens caught in sector VI of Ebrie lagoon

Taxa	Seasons											
	High dry season			High rainy season			Small dry season			Small rainy season		
	Fo(%)	**V(%)**	**IA(%)**	**Fo(%)**	**V(%)**	**IA(%)**	**Fo(%)**	**V(%)**	**IA(%)**	**Fo(%)**	**V(%)**	**IA(%)**
Phytoplanktons	100	20.64	20.64	100	49.69	49.69	100	79.29	79.29	100	99.28	99.28
Diatoms	80	17.06	16 .65	100	49.57	49.57	100	78.2	78.2	100	98.62	98.62
Coscinodiscus sp	60	10.28	6.17	100	45.66	45.66	100	76.06	76.06	100	92.29	92.29
Zooplanktons	100	77	77	100	22.74	22.74	100	20.26	20.26	40	0.53	0.21
Rotifers	100	52.82	52.82	100	21.62	21.62	100	11.96	11.96			+
Brachionus sp	100	42.66	42.66	100	17.23	17.23			+			+
Debris			+	100	27.43	27.43			+			+

+ : secondary prey

3.4. Seasonal variation in diet

Seasonal variations in diet of tilapia *S. m. melanotheron* in the 3 aquatic ecosystems are represented in Table IV. In sector VI, the prey of flood season was significantly different from that of rainy season (R=0.57). In Ayamé lake 1, prey of small rainy season (flood season) was significantly different from that of high rainy season (R=0.88), also from that of small dry season with little overlap (R =0.644). In Aby-Nord lagoon, diet of small rainy season (flood season) was separated (R = 1) from that of high dry season, also from that of high rainy season (R = 0.56) and that of small dry season (R=0.69). There appeared to be a clear difference between prey of high dry season and small rainy season (R=0.856).

Table IV: R-statistic values between seasonal prey groups of *S. m. melanotheron* fished in Ayame Lake1, Aby-Nord lagoon and sector VI of the Ebrie lagoon

Study areas					
Sector VI		**Ayame Lake 1**		***Aby-Nord lagoon***	
Groups	**R-Statistic**	**Groups**	**R-Statistic**	**Groups**	**R-Statistic**
DS, RS	0.065	HDS, HRS	0.276	HDS, HRS	0.438
DS, FS	0.254	HDS, SDS	0.193	HDS, SDS	0.856
RS, FS	0.567	HDS, SRS	0.333	HDS, SRS	1.
		HRS, SDS	0.144	HRS, SDS	0.
		HRS, SRS	0.88	HRS, SRS	0.556
		SDS, SRS	0.644	SDS, SRS	0.692

HDS : High dry season ; HRS : High rainy season; SDS : Small dry season ; SRS : Small rainy season; DS : Dry season ; RS : Rainy season ; FS : Flood season.

3.5. Biochemical composition of food bolus

Table V shows the biochemical composition of stomach contents of fish caught in the 03 aquatic ecosystems. Protein contents had varied from 6.78 to 15.57% and 19.74 to 22.44% respectively in Ayamé Lake 1 and Aby-Nord lagoon. The Kruskal-wallis test shows that the protein content varied significantly between the different seasons in both environments. On other hand, no significant difference ($p > 0.05$) was observed in fish from sector VI in terms of protein content (17.99 to 20.71%). In terms of hydrolysable organic matter (HOM) content, high values were obtained during the small dry season (781.3 mg.g-1) in Ayamé 1 Lake. In sector VI and Aby-Nord lagoon, no significant difference was recorded at the content of hydrolysable matter. Moreover, significantly low levels ($p<0.05$) of HROM were observed in Ebrie lagoon (32.3±17.25 to 64.5±18.30 mg.g-1), Aby lagoon (6.25±1.98-48.39±4.25 mg.g-1) and in Lake Ayamé during high rainy season (31.7±0.49 mg.g-1) and small dry season (31.20±4.1 mg.g-1).

Table V: Seasonal variations in the content of dry matter, lipids, and proteins, MOT, HROM, HOM and Min of the food bolus of *S. m. melanotheron* fished in sector VI, Ayame Lake 1 and Aby-nord lagoon

Study areas	Seasons	Biochemical parameters						
		Dry matter (%)	**Lipids (%)**	**Proteins (%)**	**TOM $(mg.g^{-1})$**	**HROM $(mg.g^{1})$**	**HOM $(mg.g^{-1})$**	**Min $(mg.g^{-1})$**
Sector VI	DS	8.85±0.23^{a}	0.97±0.05^{a}	19.47±2.93^{a}	713.21±96.23^{a}	63.0±43.48^{a}	650.2±52.7^{a}	286.8±96.2^{a}
	RS	9.77±2.04^{a}	1.02±0.17^{a}	20.71±2.31^{a}	806.5±56.99^{a}	32.3±17.25^{a}	774.2±39.74^{a}	193.5±56.99^{a}
	FS	14.51±2.38^{b}	1.32±0.03^{b}	17.99±2.21^{a}	838.7±63.95^{a}	64.5±18.30^{a}	774.2±45.75^{a}	161.3±63.95^{a}
Ayame Lake1	HDS	13.58±1.35^{b}	0.74±0.21^{a}	10.38±0.83^{ab}	651.7±57.98^{a}	94.2±1.06^{b}	499.50±47.73^{a}	348.29±57.98^{a}
	HRS	18.41±1.36^{c}	1.51±0.33^{a}	13.23±2.2^{bc}	593.7±48.79^{a}	31.7±0.49^{a}	620.00±15.06^{a}	406.3±48.79^{a}
	SDS	10.11±1.03^{a}	1.31±0.31^{a}	15.57±2.42^{c}	812.5±72.22^{a}	31.20±4.1^{a}	781.3±27.11^{b}	187.5±72.22^{a}
	SRS	17.49±0.9^{c}	1.08±0.25^{a}	6.78±1.25^{a}	718.7±51.09^{a}	125.00±16.69^{b}	593.7±34.41^{a}	281.29±51.09^{a}
Aby-nord lagoon	HDS	14.55±2.9^{a}	2.36±0.32^{b}	22.44±1.36^{b}	661.29±22.81^{a}	48.39±4.25^{c}	612.9± 45.62^{a}	338.71±22.81^{a}
	HRS	23.4±14.28^{a}	1.40±0.34^{a}	21.42±0.91^{ab}	593.8±26.30^{a}	31.30±0.42 ^{b}	562.49±17.39^{a}	406.2±26.30^{a}
	SDS	27.7±20.79^{a}	1.46±0.15^{a}	19.74±1.17^{a}	662.5±30.69^{a}	6.25±1.98^{a}	656.25±25.88^{a}	337.5±30.69^{a}
	SRS	19.25±10.25^{a}	1.26±0.24^{a}	19.58±1.04^{a}	625.00±31.82^{a}	31.25 ±3.15^{b}	593.74±21.06^{a}	375.00±31.82^{a}

HDS : High dry season ; HRS : High rainy season; SDS : Small dry season ; SRS : Small rainy season; DS : Dry season ; RS : Rainy season ; FS : Flood season; TOM: Total Organic Matter ; HOM: Hydrolysable Organic Matter ; HROM: Hydrolysis-Resistant Organic Matter; (a, b, c): numbers with the same letters on the same line are not significantly different (P>0.05)

4. DISCUSSION

High values of vacuity index at flood season in sector VI and small rainy season in Ayame Lake 1 and Aby-Nord lagoon would be related to the heavy rainfall resulting in high turbidity. High turbidities can have an unfavorable effect on the gills of filter-feeding fish and could therefore limit their trophic activity [22]. The results show a diet composed mainly of phytoplankton in the three environments. During flood season and small rainy season, identified phytoplankton families varied significantly. This is decrease in the Lauzanne feed index of *Coscinodiscus* sp. to detriment of prey from the

Cyanophyceae class in sector VI. At the Aby-Nord lagoon, feed index of Rotifers decreases when that of *Coscinodiscus* sp. increases. In lake, presence of *Guinardia* sp and debris in the stomachs is significant while feed index of *Staurastrum* sp. decreases. These variations can be explained by dilution of phytoplankton due to floods which leads to trophic disturbances[23, 24]**.** In addition, a zooplanktonophagous diet was observed during high dry season in Aby-Nord lagoon during this study. The almost phytoplankton-eating diet of fish during floods and rainy season could be attributed to heavy rainfall and flooding, which promote the supply of new nutrients and stir up native nutrients present in different strata of the aquatic ecosystems in favor of the phytoplankton production[25]. This strong primary production observed favors zooplankton expansion, which would thus explain zooplankton diet in dry season. But according to Ouattara[26], the tilapia *Sarotherodon melanotheron* exploits zooplankton resources during periods of scarcity. The changing diet according to the available resources of environment reveals plasticity of trophic spectrum of species[27].

Prey class inventoried in *Sarotherodon melanotheron* during our study are similar to those of Kide *et al*. [28] in the Banc of Arguin National Park (Mauritania) for the diet of *Sarotherodon melanotheron*. These authors identified mainly phytoplanktonic prey (Diatoms, Bacillariophyceae, Chlorophyceae, Conjugatophyceae, Dinophyceae, Cyanophyceae and Chrysophyceae) but also zooplanktonic prey and debris in the species.

As the gut is the main site of nutrient absorption in fish[29], composition of stomach contents reveals the quality of the food ingested. The composition of food bolus shows a low protein content during the study. These values could be related to the phytophagous diet of the fish, mainly diatoms which are low in protein estimated at 20%[30]**.** This value is in line with the different protein contents obtained in the high dry season and the high rainy season of the Aby-nord lagoon but higher than the protein contents of the other aquatic ecosystems. The low content (6.78%) in the small rainy season in Man-made Ayame1 Lake is linked to high feed value (22.72%) of debris. In the Aby lagoon, the high protein content (22.44%) in dry season is due to the presence of zooplankton (IA= 77%) in diet.

Lipid content remains very low in the food bowl in all seasons, this content is below 2%. These low values could be related to phytoplankton diet of *Sarotherodon melanotheron*. According to Dejoye[31] lipid content of microalgae is very low under normal conditions and can vary from 1.23-3.3% depending on extraction solvent.

High content of HOM (minimum value: 499.50±47.73 mg.g-1) is related to its food bolus composition, dominated by zooplankton and phytoplankton. Diatoms are known to be an important source of easily digestible food due to the structure of their cell walls[32]. At the same time, zooplankton also have a very high digestibility, sometimes limiting the time of their observation[33].

Significantly low levels of HROM observed in Ebrie lagoon (32.3±17.25 to 64.5±18.30 mg.g-1), Aby lagoon (6.25±1.98-48.39±4.25 mg.g-1) and Ayame 1 Lake during high rainy season (31.7±0.49 mg.g-1) and the small dry season (31.20±4.1 mg.g-1) would be correlated with a high contribution to diatom diet, essentially composed of *Coscinodiscus* sp. and *Aulacoseira* sp. would be correlated with a strong contribution to diatom diet, essentially composed of prey of *Coscinodiscus* sp. and *Aulacoseira* sp. In the latter aquatic ecosystem, during high dry season and the small rainy season the OMRH contents; respectively 94.2±1.06 mg.g-1 and 125.00±16.69 mg.g-1 were relatively high. Latter value coincided with period of high presence of debris in the food bowl (AI=22.72%), essentially composed of sand, pieces of bag, sand and scales. These constituents, whose biological value would be marginal, would contribute to the high HROM content. Thus Kennel[34] had maintained that when phytophagous fish ingest a lot of silt and sand, the organic matter is diluted in the mineral matter, thus decreasing the efficiency of the digestive enzymes.

Furthermore, high mineral content of the food bolus (minimum content: 161.3±63.95 mg.g-1 and maximum content: 406.3±48.79 mg.g-1) could have two possible explanations: it would either come from frustile, which is very rich in silica[35]**,** or from bioaccumulation. According to Ben Amor[36], microalgae have a high capacity for heavy metal accumulation. An alga such as Chlorella vulgaris is capable of absorbing several heavy metals, including chromium[37]. In aquaculture, bioaccumulative capacity of algae can be exploited as a means of nutritional enrichment.

5. CONCLUSION

Aquatic ecosystems studied offer a wide food spectrum to the tilapia *Sarotherodon melanotheron*, resulting in high feeding activity. The feeding of this species is preferentially oriented towards phytoplankton, particularly Diatoms. Specifically, the genus *Aulacoseira* sp is more consumed in Ayame 1 Lake and the genus *Coscinodiscus* sp. is in both lagoon ecosystems. In terms of biochemical composition of the food bolus, the protein and lipid contents are low, respectively 20 and 2%, regardless of the season. The proportion of hydrolysable organic matter in the stomach contents is very high in the total organic matter.

6. REFERENCES

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