ISSN: 2277-9655
Scientific Journal Impact Factor: 3.449
(ISRA), Impact Factor: 1.852

INTERNATIONAL JOURNAL OF ENGINEERING SCIENCES & RESEARCH TECHNOLOGY

Comparative Study of the Lipid Content and the Fatty Acid Composition in the Parasite (*Mothocya Belonae*) and in the Muscle of its host (*Belone Belone*), (Teleost, Belonidae) Collected in the Bay of Monastir (Central Mediterranean)

Mohamed Ali Ben Smida [1*], Alĕs Bolje[2], Hassen Mejri M'hamed El Cafsi[1] and Rafika Fehri-Bedoui[1]

[1]Université de Tunis El Manar, Faculté des Sciences de Tunis, 05/UR/09-05 Eco-physiologie et Environnement Aquatique, 2092 Tunis, Tunisie

[2] Fisheries Research Institute of Slovenia, S1-1211, Ljubjana-Šmartmo, Sp. Gameljne, 61a Slovenia

ali.bensmida@Laposte.net

Abstract

The fatty acid composition of the parasite, *Mothocya belonae*, and the muscle of its host, *Belone belone* (Garfish), were compared. The saturated, monounsaturated and polyunsaturated fatty acids in parasite and the host are respectively 49% to 52.9%; 25.12% to 28.8% and 25.87% to18.2 % of total fatty acids.

The parasite is characterized by palmitic (C16: 0), oleic (C18: 1n-9), arachidonic acid (C20: 4n-6) and EPA (C20: 5n-3) with respective percentages of 29.1%, 17.6%, 3.9% and 7.7% of total fatty acids. Parasite tissues are distinguished by their high EPA + DHA with a rate of 19.4% of total fatty acids.

Keywords: Total fatty acids (TFA), Host muscle, Parasite, *Belone belone*, *Mothocya belonae*.

Introduction

The pathogens of marine fish and freshwater fish such as viruses, bacteria, protozoa and metazoa are very diverse (Cassia et al., 2004). In the wild, parasitism contributes to increase the natural mortality in fish populations and may affect their migratory behavior. This finding was observed in eel infested by the worm Anguillicola crassus that resulted in a decrease in its catches (Audenaert et al, 2003 ; Lambert et al, 2003). However, in rearing conditions, parasitized fish with a disgusting aspect would lead to decrease the production which causes real financial losses. Thus, research on parasitism of reared organisms provides valuable data on the origin of the parasites, their location on the fish and their life cycle (Sommerville, 1986).

The main purpose of these studies is to control the environment, the impact of the parasite on the fish (Stewart et al, 2004.), the research of a prophylaxis and the implementation of an adequate prevention (Sarusic, 1986; Brisinelli, 1896).

In contrast, in the natural environment, studies on parasitism are essentially descriptive, limited to the specific identification of the parasite (Neifar et al, 2004 ; Amine et al, 2006 Blahoua et al., 2009; Vinoth, 2010). Some studies have focused on the environmental aspects related to research the different stages of the parasite and genetic characterization in relation to its habitat (Blasco-Costa et al., 2012).

In the case of an ectoparasitic infestation in particular by copepods and isopods, the parasite is constrained to take advantage of its host. For this, it needs to strengthen its attachment to the host in order to ensure its biological functions of nutrition, growth and reproduction necessary for its own spread. Some authors report the harmless effect of the copepod (*Perodermo cylindncum*) on the physiology of sardine (Ktari et al., 1980). In contrast, in the same species, El Gharbi et al. (1983) identified serious alterations in the points of impact of the parasite. Faced with these conflicting interpretations and given that the only source of nutrition of the parasite is its own host, we undertook a study based on comparative analysis of fatty acids present in the muscle of infected fish specimens, *Belone belone* (garfish), and fatty acids present in the parasite, *Mothocya belonae* , attached to the gills of the host.

Materials and methods

Samples of garfish parasitized (n = 6) were collected from fishermen in the Bay of Monastir in July 2013 (Figure 1). The parasite, *M. belonae*, is attached to the fish gills. The weight of the parasite vary between 0.8g and 1.10g. Weights and the total

ISSN: 2277-9655
Scientific Journal Impact Factor: 3.449
(ISRA), Impact Factor: 1.852

lengths of the parasitized fish are shown in Table 1. Samples of 0.5g of parasite were grouped to reach a fresh weight of 3 g. Samples of 1 g of parasitized fish were grouped to reach a fresh weight of 6 g. The total lipid extraction was performed according to the method of Folch et al. (1957) in a chloroform-methanol (2:1, v / v). The total lipids obtained were stored in chloroform-methanol- butylated-hydroxytoluene (BHT) at -28°C. For further analysis, the fatty acids were transformed into methyl esters, according to the Cecchi et al. (1985). The quantification of the fatty acids is based on an internal standard not present in our samples, methyl nonadecanoate or C19:0 (Sigma Aldrich, Corporate Headquarters, St Louis, MO). Methyl esters of TFAs were separated, identified, and quantitated by gas chromatography using a HP 6890 gas chromatograph with a split/splitless injector with electronic pressure control and a flame ionisation detector was used for the analysis. Separation was performed with a 30 m HP Innowax capillary column with an internal diameter of 250 micrometers and a 0.25 micron film thickness, the stationary polar phase of the column being polyethylene glycol. We identified the different fatty acids contained in garfish by comparing the retention times of the fatty acids under focus with those of a mixture of methyl esters SUPELCO (PUFA-3). To compare the quantity of each fatty acid contained in the different sections, we analysed the variance (ANOVA) with an interval of reliability of 95% ($p < 0.05$). The comparison of the mean values is based on the Duncan test using the SPSS 13 program. Data are presented as mean ± SE and submitted to the Student test to determine significant differences between means.

Results and discussion

Table 2 shows the contents of the total fatty acids of the host (*B. belone*) and the parasite (*M. belonae*). These contents showed a significant differences in the parasite and its host. The fatty acid composition of the host muscle and the parasite is shown in table 3. A total of 23 fatty acids have been identified. The fatty acid composition of the parasite is characterized by 49% saturated fatty acid SFA dominated by palmitic (C16: 0, 29.12%), 25.12% monounsaturated fatty acid MUFA dominated by oleic acid (C18: 1n -9, 29.12%), a percentage of 25.87% of polyunsaturated fatty acids PUFA n-3 represented by the complex EPA + DHA (C20: 5n-3 + C22: 6n-3, 19.40%) and 4.56% PUFA dominated by n-6 arachidonic acid (C20: 4n-6, 3.90%).

SFA and MUFA, didn't show a significant variations between the host muscle and the parasite (Table 3). Parasite's PUFAs differ significantly from those of the host . This variation is explained by the percentage of arachidonic acid and EPA which vary very significantly between the two individuals (Table 3).A lipid content of 0.99g/100g of fresh weight parasite promotes rapid growth. This growth requires an increase in all cellular membranes where lipids are stored. These reserves play a fundamental in the osmotic pressure that allows the parasite to survive during both difficult and reproductive periods (Schoffeniels, 1976; Tocher et al., 2010).

Comparative analysis of fatty acids in the host muscle and the parasite (Table 3) allows the detection of transfer between the host and the parasite. The presence of miristic acid (C12: 0, 2%) in the parasite would be a consequence of a very high percentage of this fatty acid in the host (> 18%). Similarly, higher percentages of arachidonic acid (C20: 4n-6, 3.90%), DHA (C22: 6n-3, 11.67%) and Hexadecadienoic acid (C15: 1 0.15%) in the parasite may result from relatively high proportions of these three fatty acids (2.16%, 10.79% and 0.55%) in the host. Lloyd and Barrett (1981) reported that *Fasciola hepatica* absorbed by diffusion the short chain fatty acids (acetate, propionate, butyrate). Therefore, it is suggested that *Mothocya belonae* could follow these strategies for absorption C12: 0, C15: 1, C20: 4n-6 and C22: 6n-3 present at different percentages. It is suggested that Mothocya belonae would be able to transform the C12: 0 in C16: 0 by chain elongation. This phenomenon has been reported by Furlong (1991) in the trematode *Schistosoma mensoni* living in the vascular system of the mammalian host. This mechanism has been shown in *Spirometra mansonoides* worm parasite of terrestrial mammals and the aquatic organisms including fish, respectively, in the adult and larval stages for the C16: 0, C18: 0, C18: 1, C18: 2 and C18 : 3 which are transformed into C20 and C22 (Meyer et al., 1966).

Palmitic acid would act as precursor for the synthesis of saturated and unsaturated fatty acids in *M. belonae*. (Barrett (1981) suggested that in most organisms, the cycle of fatty acid synthesis stops at the palmitate which acted as precursor for both long chain fatty acids saturated or unsaturated.

The fatty acid groups (SFA MUFA and PUFA) are common to the host and parasite. However they differ in their respective percentages. The presence of certain acids such as C14: 0, C16: 0, C18: 0, C18: 1n-9, C20: 5n-3 and C22: 6n-3 in significant quantities reflects the physiological importance of these fatty acids in both the host and the parasite. It has been reported that the palmitic and oleic acids are incorporated into the neutral lipid *P. microbothrium*

ISSN: 2277-9655
Scientific Journal Impact Factor: 3.449
(ISRA), Impact Factor: 1.852

(Awharitoma et al., 1990). Therefore, we can assume that the presence of oleic acid and palmitic with respective percentages of 17.67% and 29.12% for *M. belonae* could explain their incorporation in the lipid reserves.

Probably, *Mothocya belonae* is able to change the fatty acids obtained from the host. For the same amount of TFA, the parasite has low percentages of C18: 2n-6 (0.66%) and C18: 3n-3 (0.02%) and relatively high percentages of arachidonic acid (3.90%), EPA (7.72 %) and DHA (11.67%). Tocher (2003) assumed that polyunsaturated long chain fatty acids present in the ectoparasite of salmonids, *Lepeophtheirus salmonis*, may come either from a phenomenon of elongation and desaturation of short chain fatty acids or they are generated by selective oxidation of C18: 2n-6 and C18: 3n-3 and selective retention of arachidonic acid, EPA and DHA. Thus, the result of the relationship *Belone belone-Mothocya belonae* would result in a fatty acid absorption by the parasite. Consequently, the energetic potential of the parasite would be mainly set for the benefit of its reproduction.

The biochemical composition of fish is greatly influenced by their diet composition (Orban *et al.*, 2007). *M. belonae* constraint to get its food from its own host, any variation in the diet of the fish is reflected in the fatty acid composition of the parasite (Tocher, 2003). However, the fatty acid composition of *M. belonae* showed a difference from that of the garfish particularly in terms of percentage of C15: 1. Fehri-Bedoui et al., (2013) suggested that the C15: 1 is not generated from an endogenous synthesis, but rather from the garfish alimentation. It seems that this fatty acid does not present an important role for the parasite

In conclusion, it appears that the muscle of the host (*B. belone*) and its parasite (*M. belonae*) present, qualitatively, similar lipid profiles. Quantitatively, these profiles present significant differences ; the parasite seems to be able to modify the fatty acids by elongation of their chains. This strategy allows the parasite to provide nutritionnal added value to the fatty acids extracted from the host to ensure its own survival

References

1. *Cassier, P., Brugerolle, G., Combes, C., Grain, J. and Raibaut, A., **1998.** Le parasitisme. Un équilibre dynamique. Ed. Masson, Paris, 366 p.*
2. *Audenaert, V., Huyse, T., Goemans, G., Belpaire, C. and Volckaert, F.A.M., **2003**. Spatio-temporal dynamics of the parasitic nematode Anguillicola crassus in Flanders, Belgium, Dis. Aquat. Org., 56: 223–233.*
3. *Lambert, P., Feunteun, E. and Crivelli, A.J., **2003**. L'anguille, un défi pour les scientifiques compte rendu des journées anguilles du CRISAM la Tour du Valat, 26-29 mars 2001, Bull. Fr. Pêche Piscic., 368 : 1-8.*
4. *Sommerville, C., **1986** - Maladies parasitaires chez les poissons d'élevage. In Pathologie des espèces élevées en aquaculture marine en Méditerranée. Mediterranean regional aquaculture project PRM/DA. Doc. FAO, p. 247.* http://www.fao.org/docrep/005/AC910F/AC910F00.htm
5. *Stewart, C., Johnson Treasurer, S., Bravo, K., Nagasawa, K. and Kabata, Z., 2004. A review of the impact of parasitic copepods on marine aquaculture. Zool. Stud., 43: 229-243.*
6. *Sarusic, G., 1986 - Contrôle et traitements du poisson. In Pathologie des espèces élevées en aquaculture marine en Méditerranée. Mediterranean regional aquaculture project PRM/DA. Doc. FAO, p. 247.* http://www.fao.org/docrep/005/AC910F/AC910F00.htm
7. *Brisinelli, W., **1896.** Traitements et contrôles : les vaccins : In Pathologie des espèces élevées en aquaculture marine en Méditerranée. Mediterranean regional aquaculture project PRM/DA. Doc. FAO, p. 247.* http://www.fao.org/docrep/005/AC910F/AC910F00.htm
8. *Neifar, L., Euzet, L. and Oliver, G. 2004. Lamellodiscus (Plathelminthes, Monogenea, Diplectanidae) nouveaux parasites branchiaux des poissons marins du genre Pagrus (Teleostei, Sparidae). Zoosystema 26 (3), 365-376.*
9. *Amine, F., Neifar, L. and Euzet, L., **2006**. Lamellodiscus sanfilippoi n. sp. (Monogenea, Diplectanidae) parasite from the gills of Diplodus sargus (Teleostei, Sparidae) in Mediterranean sea Parasite, 13, (1): 45–49* http://dx.doi.org/10.1051/parasite/2006131045
10. *Blahoua, K.G. Pariselle, A.V., N'douba, T. Kon and Kouassi, N.J., **2009.** Description of three new monogenean gill parasites from Mormyrus rume (Valenciennes, 1846) (Teleostei: Mormyridae) in Ivory Coast*

ISSN: 2277-9655
Scientific Journal Impact Factor: 3.449
(ISRA), Impact Factor: 1.852

Parasite, 16, 51–56 http://dx.doi.org/10.1051/parasite/2009161051

11. *Vinoth, R., Kumar, T.T.A., Ravichandran, S., Gopi, M. and Rameshkumar, G., **2010**. Infestation of copepod parasites in food fishes of Vellar estuary, Southeast coast of India. Acta Parasitologica Globalis 1 (1): 01-05.*
12. *Blasco-Costa, I., Waters, J.M. and Poulin, R., **2012**. Swimming against the current: genetic structure, host mobility and the drift paradox in trematode parasites.Molecular Ecology, 21, 207–217.* doi: 10.1111/j.1365-294X.2011.05374.x
13. *Ktari, M.H. and Abdelmoula, A., **1980**. - Note sur la présence et les effets du Copépode (Heller, 1868) parasite de la sardine Sardina pilchardus(Walbaum, 1792) des côtes tunisiennes. Bull. Inst, nat. Sci. Tech. Océanogr. Pêches. Salammbô 7, pp. 103-112.*
14. *El Gharbi S., Rousset, V. and Raibaut, A., **1985.** Biologie du copépode Lernaeenicus sprattae (sowerby, 1806) et ses actions pathogènes sur les populations de sardines des côtes du Languedoc-Roussillon. Rev. Trav. Inst. Pêches marit., 47 (3 et 4) : 191-201.*
15. *Folch, J., Lees, M. and Sloane-Stanley, G.A., 1957. A simple method for the isolation and purification of total lipids from animal tissues, Journal of Biological Chemistry, Vol. 226, 1, pp. 497-509.*
16. *Cecchi G., Basini, S. and Castano, C., **1985.** Méthanolyse rapide des huiles en solvant, Revue française des corps gras, 32 (4), pp. 163-164.*
17. *Schoffeniels, E, 1976. Biochemical approaches to osmoregulatory process I, Crustacea. In Perspectives In Experimental Biology I (ed. Spencer Davies), pp. 107-124. Oxford, Pergamon.*
18. *Tocher, J.A., Dick, J.R., Bron, J. E., Shinn, A.P. and D. R. 2010. Lipid and fatty acid composition of parasitic caligid copepods belonging to the genus Lepeophtheirus*
19. *Lloyd, G.M. and Barrett, J., 1983.* Fasciola hepatica: Carbohydrate metabolism of the adult. *Exp Parasitol. 56(1):81-8.*
20. *Furlong, ST., 1991. Unique roles for lipids in Schistosoma mansoni. Parasitol. Today. 7, pp. 59-62.*
21. *Meyer F, Kimura S and Mueller JF. 1966. Lipid metabolism in the larval and adult forms of the tapeworm Spirometra mansonoides. J. Biol.Chem. 241, pp. 4224-4232*
22. *Barrett, J., **1981.** Biochemistry of parasitic helminths. Baltimore: University Park Press.*
23. *Awharitoma,* A.O, Opute, F.I, Ali, S.N. and Obiamiwe, B.A., 1990. Lipid biosynthesis in Paramphistomum microbothrium (Trematoda). Angew Parasitol. 31(1) :51-3.
24. *Tocher, D.R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Rev. Fisheries Sci. 11, 107-184.*
25. *Orban, E. Nevigato, T., Masci, M., Di Lina, G., Castani, I., Caproni, R., et al., 2007. Nutritional quality of European perch (Perca fluviatilis) from lakes of Central Italy. Food Chemistry, 100, 482-490. O'Sullivan, G. (2006). Fish INFOnetwork Market Report. Pangasius 7/06. FAO.*
26. *Fehri-Bedoui, R., Ben Smida, M.A., Mejri, H., and EL Cafsi, M., **2013.** Impact of a blood-sucking parasite on the chemical composition of fatty acids in the white muscle of Garfish (Belone belone, Belonidae) from Tunisian coasts (Central Mediterranean) ; Afr. J. of Biotechnology, 12 (44), pp. 6335-6339.*

ISSN: 2277-9655
Scientific Journal Impact Factor: 3.449
(ISRA), Impact Factor: 1.852

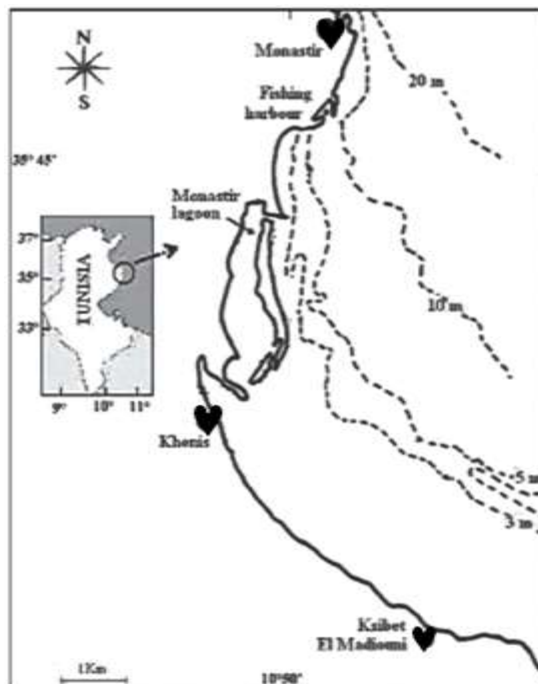

Figure 1. Location of the Bay of Monastir (Central Mediterranean) ♥ Sampling sites.

Table 1. Size and weight of the studied specimens collected in the Bay of Monastir (Host: Belone belone; Parasite: Mothocya belonae).

	Parasitized *fish : Belone belone*		Parasite : *Mothocya belonae*
Specimen	Total length (cm)	Total weight (g)	Total weight (g)
E1	25	50.40	0,8
E2	29	70.00	0,95
E3	32.1	90.20	1,05
E4	28,5	70,07	0,63
E5	20	30.85	1,10
E6	40.1	100.20	0,92

Table 2. Total fatty acids in the host muscle and the parasite.

	Host muscle *(Belone belone)*	Parasite *(Mothocya belonae)*	*P*
TFA g/100g PF	0.81±0.06	0.99±0.22	**

*P≤0.05 ; **P≤0.01 ; ***P≤0.001 ; ns >0.05

ISSN: 2277-9655
Scientific Journal Impact Factor: 3.449
(ISRA), Impact Factor: 1.852

Table 3. Fatty acids composition in the host (parasitized garfish) and the parasite M. belonae from the Bay of Monastir (Central Mediterranean).

Fatty acids	Host muscle	Parasite	*p*
C12 :0	18.20±0.91	2.01±0.57	***
C14 :0	7.02±2.75	8.05±2.19	ns
C15 :0	1.63±0.34	0.38±0.05	***
C16 :0	18.33±2.69	29.12±1.92	***
C17 :0	1.10±0.10	2.10±0.50	ns
C18 :0	6.87±1.21	7.31±1.40	ns
C15 :1	20.22±4.23	0.04±0.00	***
C16 :1n-9	1.95±0.27	2.02±0.29	ns
C16 :1n-7	0.97±0.14	1.01±0.14	ns
C18 :1n-9	3.27±0.81	17.67±1.54	***
C18 :1n-7	1.57±0.13	3.48±0.19	***
C20 :1	0.35±0.10	0.28±0.17	ns
C22 :1	0.45±0.04	0.60±0.17	ns
C16 :2n-4	0.51±0.11	0.15±0.00	***
C18 :2n-6	0.22±0.05	0.66±0.09	***
C20 :2n-6	0.16±0.02	0.07±0.02	*
C18 :3n-3	0.06 ±0.02	0.02±0.00	ns
C18 :4n-3	0.87±0.45	0.32±0.11	ns
C20 :4n-6	2.16±0.20	3.90±0.23	***
C20 :4n-3	0.27±0.07	0.13±0.02	ns
C20 :5n-3	1.24±0.06	7.72±0.92	***
C22 :5n-3	1.92±0.29	1.20±0.16	ns
C22 :6n-3	10.79±1.27	11.67±1.13	ns
$C_{12:0}+C_{14:0}+C_{16:0}$	43.38±6.17	39.19±3.58	ns
SFA	52.98±5.61	49.00±2.97	ns
MUFA	28.81±4.32	25.12±1.78	ns
PUFA	18.21±1.54	25.87±1.80	***
UFA	47.02±5.61	50.99±2.97	ns
n-3	15.16±1.43	21.08±1.56	*
n-6	2.54±0.24	4.56±0.28	***
n-7	2.55±0.05	4.49±0.29	***
n-9	6.02±0.91	20.58±0.58	***
EPA+DHA	12.03±1.31	19.40±1.51	*
n-3/n-6	6.02±0.36	4.55±0.19	**
UFA/SFA	0.99±0.20	1.07±0.10	ns
PUFA/MUFA	0.68±0.07	1.04±0.08	**
PUFA/SFA	0.37±0.06	0.54±0.06	ns

*P≤0.05 ; **P≤0.01 ; ***P≤0.001 ; ns >0.05