SUSTAIN-AQUA: 22-23 October

How do the distances to fish farms affects the fatty acids profiles and stable carbon and nitrogen isomers in different hard-bottom organisms

A translocation study

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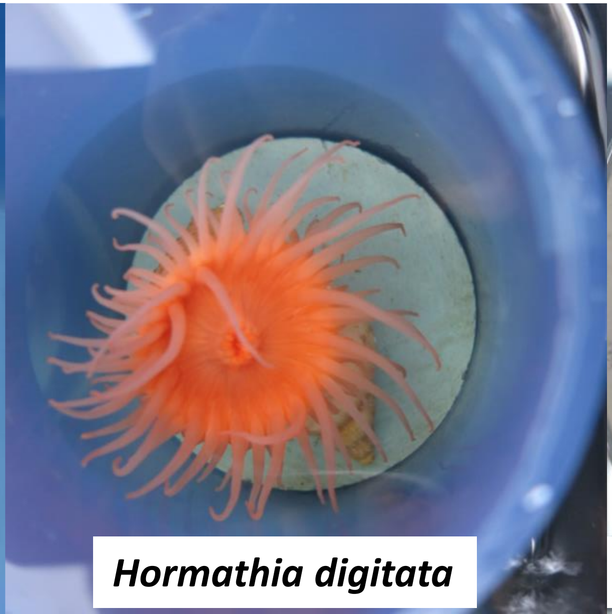
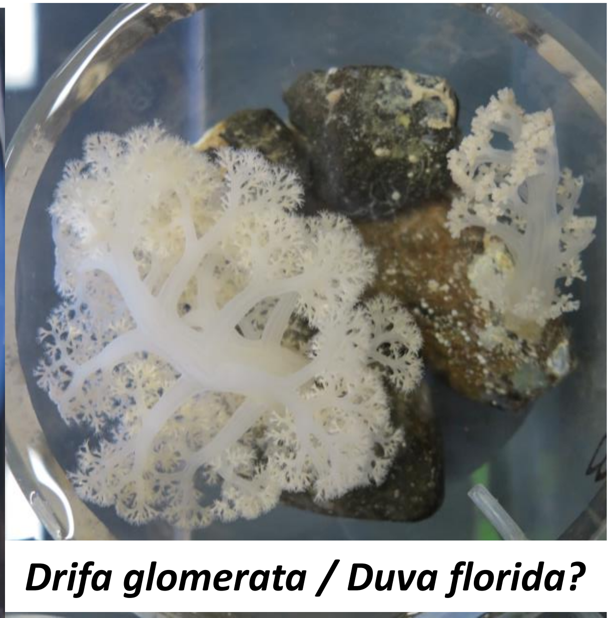
Polymastia sp.

Craniella zetlandica



Hormathia digitata

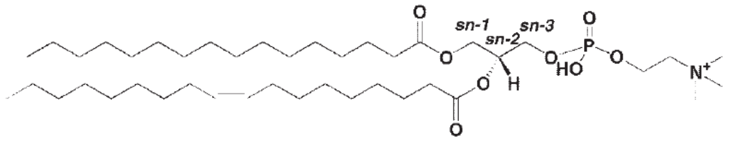


Drifa glomerata / Duva florida?

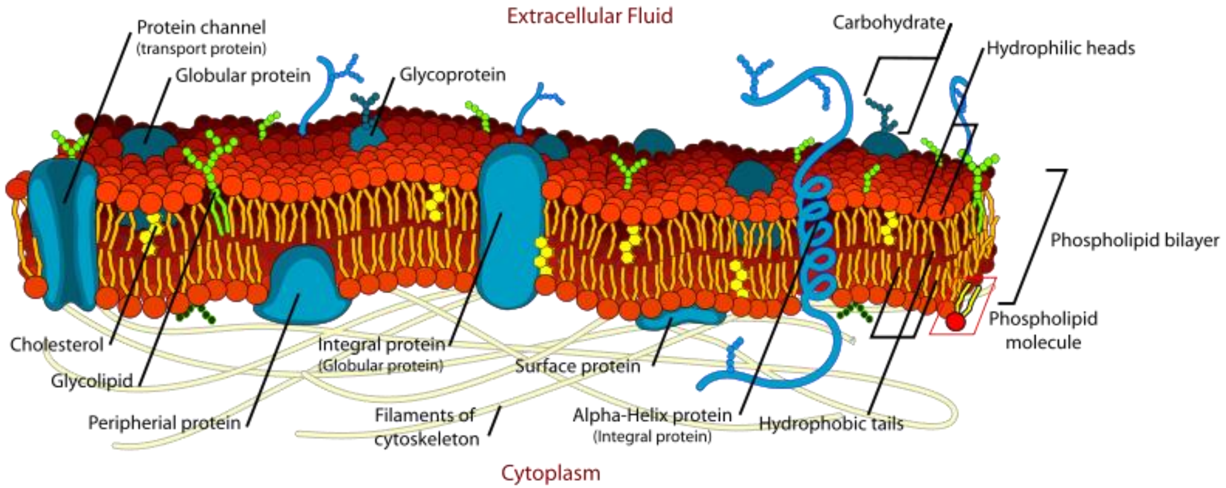
Outline

1. Super short introduction to lipids
2. Fatty acids trophic markers as geochemical tracers of organic waste from fish farms
3. Analytical methods
4. Results
5. Conclusion

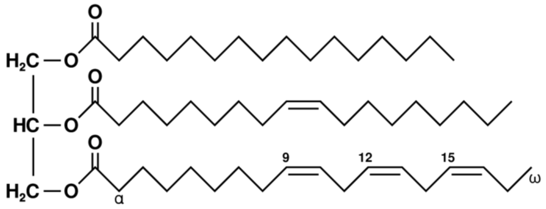
Amphipathic lipids.
Phospholipids; Structural lipids in cell membrane

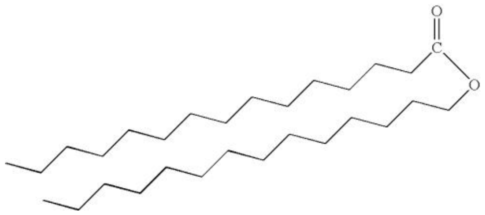


PC



Hydrophobic lipids
Energy storages:triacylglycerol, waxesters





SFA

(a) Palmitic acid (16:0)

MUFA

(b) Oleic acid (18:1 n-9)

PUFA

(c) Linoleic aic (18:2 n-6)

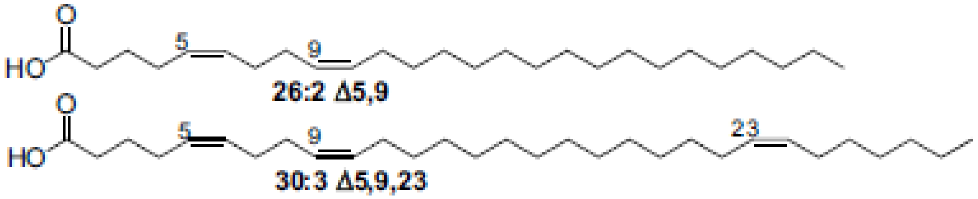
(d) α-linolenic acid (18:3 n-3)

(e) Arachidonic acid (20:4 n-6)

(f) EPA (20:5 n-3)

Figure 2. Structures of some important fatty acids

NMI-PUFA



Non-methyl-interupted (NMI)-PUFA

Vol. 7: 1–13, 2015
doi: 10.3354/aei00132

AQUACULTURE ENVIRONMENT INTERACTIONS
Aquacult Environ Interact

Published online May 18

Use of fatty acid profiles to monitor the escape history of farmed Atlantic salmon

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rtment of Biology, Norwegian University of Science and Technology, 7489 Trondheim, Norway

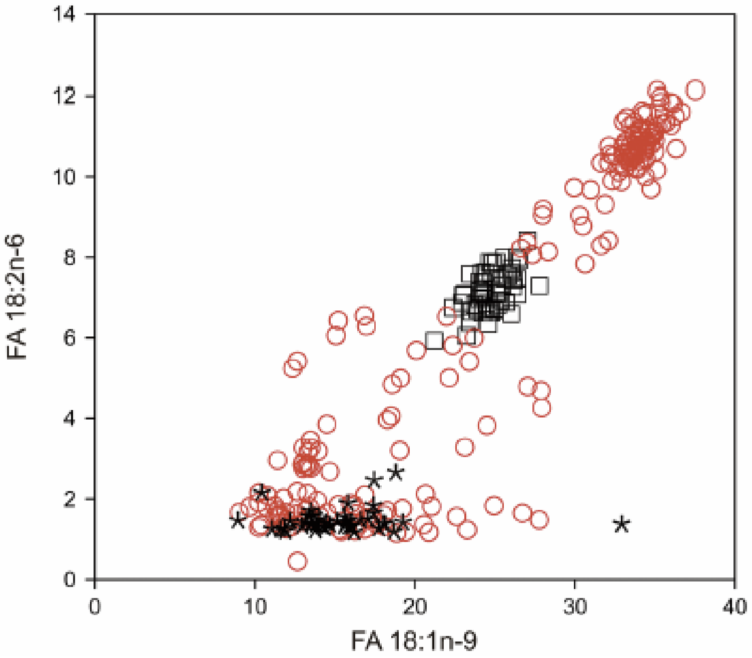


Fig. 5. *Salmo salar*. Level of fatty acids (FAs, in %) 18:2n-6 against 18:1n-9 for escaped farmed salmon (red circles), presumably escaped brood fish (black squares) and wild salmon (asterisks)

Table 6. Content of selected fatty acids (FAs) (>4 %) in salmon food pellets obtained from fish farms between 2011and 2013. All FAs are presented in Table S2 in the Supplement at www.int-res.com/articles/suppl/q007p001_supp.pdf

Producer	Recommended use	16:0	18:1n-9	20:1n-9	22:1n-11	18:2n-6	18:3n-3	20:5n-3	22:6n-3
Bio Mar Salmon group	Summer, fish >0.5 kg	14.13	18.11	5.01	6.00	8.40	3.18	8.50	7.14
Bio Mar Salmon group	All year, large salmon	10.71	26.78	6.65	9.06	10.78	3.68	4.59	4.07
Skretting	All year, fish >0.5 kg	11.28	28.90	4.76	5.87	11.46	4.19	5.61	4.15
EWOS Florø	All year, fish >0.5 kg	9.18	34.24	5.43	6.78	12.87	5.82	3.64	3.51
EWOS	Summer, smolts	18.06	8.74	1.43	1.06	4.02	1.05	14.46	8.73
EWOS	Summer, large salmon	11.87	34.14	1.43	0.55	12.12	4.45	7.01	4.27
Bio Mar Salmon group	Winter, large salmon	9.97	37.76	2.38	1.54	15.11	5.95	4.35	4.15
EWOS	Winter, small salmon	12.04	35.99	1.90	1.51	13.05	5.30	5.03	4.17
EWOS	Winter, large salmon	10.12	36.61	4.37	5.85	13.48	5.67	3.16	3.58
EWOS	Winter, large salmon	10.10	37.01	4.26	5.58	13.60	5.81	3.13	3.52
EWOS	Winter, large salmon	10.72	36.50	3.70	4.12	14.22	5.49	3.03	3.25
Skretting	Winter, large salmon	10.77	33.34	2.85	3.73	12.41	5.28	5.15	5.21
Skretting	Winter, small salmon	10.77	23.29	6.96	13.48	9.23	3.47	4.56	5.15
Skretting Vitalis SA	Broodstock feed	15.67	10.37	5.84	9.31	3.98	1.20	10.63	9.07

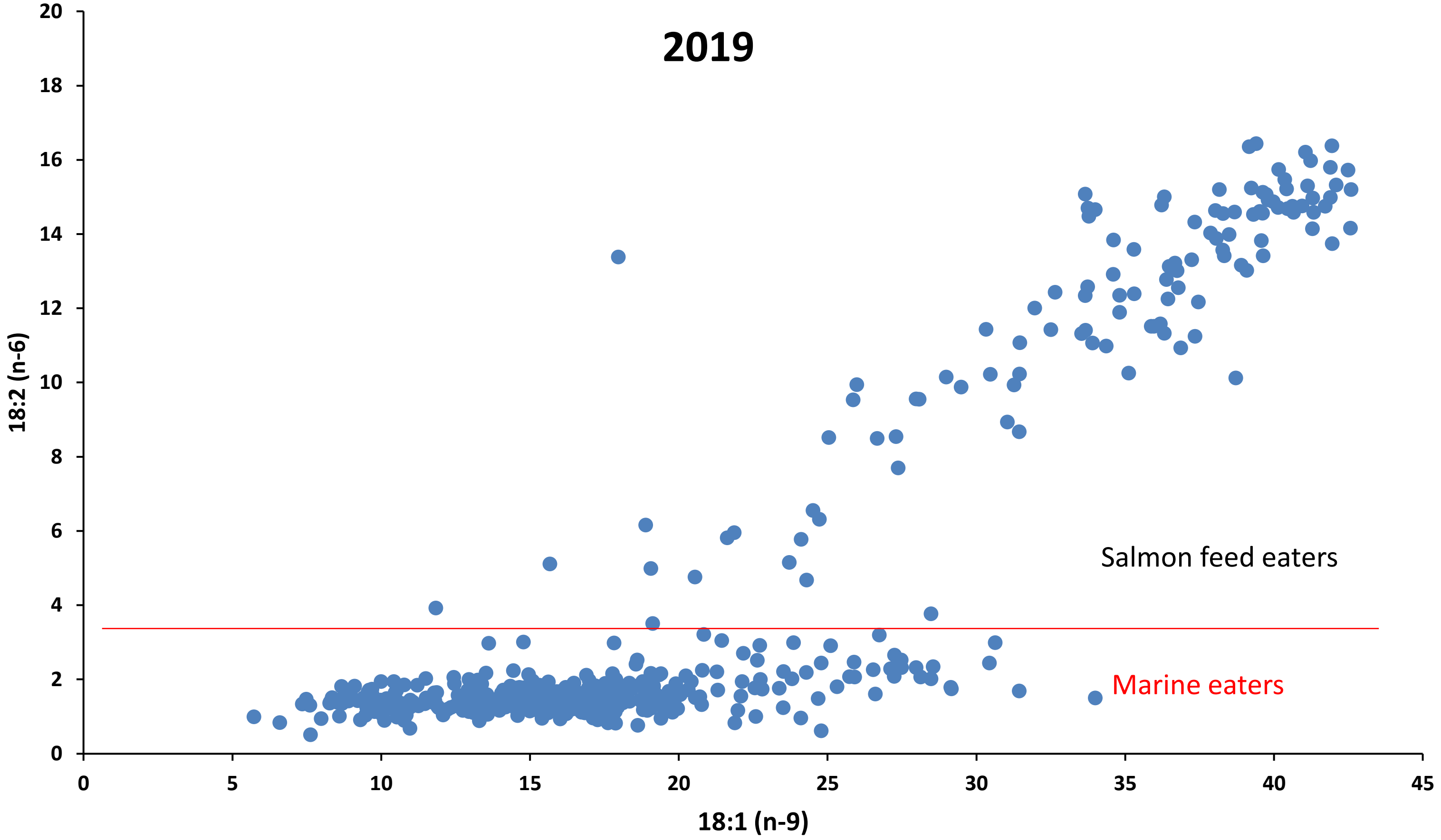
Terrestrial Fatty acids trophic markers (FATMs);

Oleic acids (18:1 (n-9)), typical marine profile (10-25% of total FAs)

Linoleic acid (18:2 (n-6), typical marine profile (1-2 % of total FAs)

α-linolenic acid (18:3 (n-3), typical marine profile (1-3 % of total Fas)

Impacts of salmon farming on Atlantic cod spawning grounds (ICOD, NFR project, 2015-2020)



Wild fish feeding on waste pellets under the fish farms are easy to detect from FA analysis

Ecological Indicators 103 (2019) 43–54

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

ELSEVIER

Fate and longevity of terrestrial fatty acids from caged fin-fish aquaculture in dynamic coastal marine systems

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Institute of Marine Research, Postboks 1870 Nordnes, 5817 Bergen, Norway

OA=18:1 (n-9)
LA=18:2 (n-3)
ALA=18:3 (n-3)

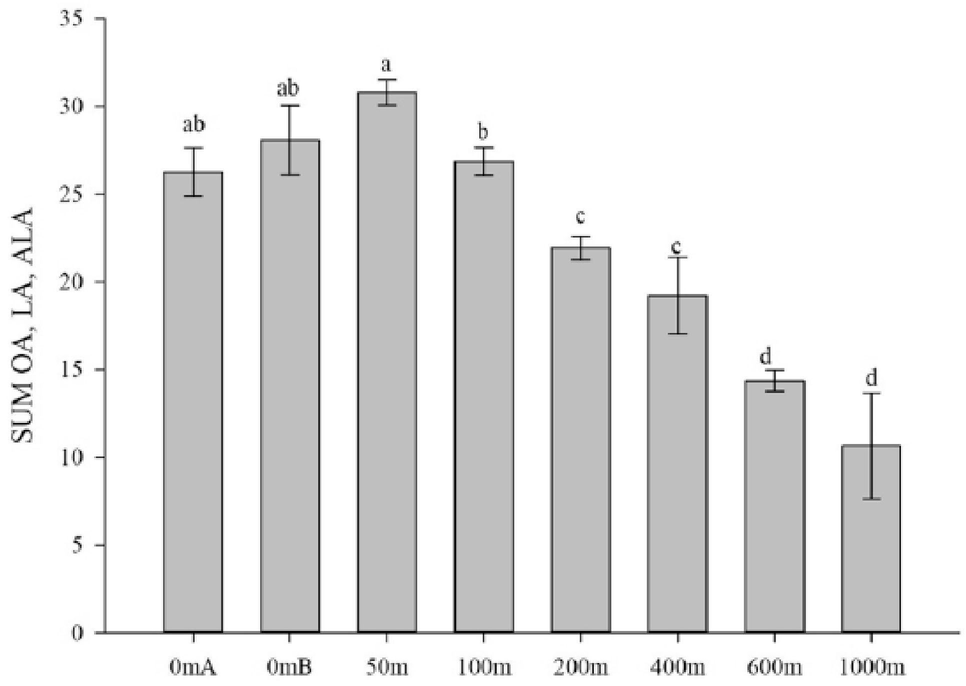

Fig. 7. Comparison of sum of OA, LA and ALA (average ± standard error) of infauna polychaetes sampled during peak production along a transect moving away from a fin-fish farm. Different letters represent significant difference in the sum of OA, LA and ALA.

Lipid methods at IMR

Fatty acids analysis; direct metanolyse (GC-FID)

Journal of Chromatography A, 1104 (2006) 291–298

Validation of a one-step extraction/methylation method for determination of fatty acids and cholesterol in marine tissues

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[a] Institute of Marine Research, P.O. Box 1870, Nordnes, 5817 Bergen, Norway
[b] Norwegian Institute of Fisheries and Aquaculture Research, Bergen, Norway
[c] The Fishery Laboratory of the Faroes, Thorshavn, Faroe Islands
[d] Department of Chemistry, University of Bergen, Bergen, Norway

Lipid classes analysis; TLC/HPLC

J. Exp. Mar. Biol. Ecol., 1989, Vol. 129, pp. 189–197

The rapid analysis of neutral and polar marine lipids using double-development HPTLC and scanning densitometry

R.E. Olsen[1] and R.J. Henderson[2]

[1] *Holt Agricultural Research Station, Tromsø, Norway;* [2] *NERC Unit of Aquatic Biochemistry, Department of Biological Science, University of Stirling, Stirling, U.K.*

Journal of Chromatography B, 877 (2009) 1815–1819

Improved separation and quantification of neutral and polar lipid classes by HPLC–ELSD using a monolithic silica phase: Application to exceptional marine lipids

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Alfred Wegener Institute for Polar and Marine Research, Ecological Chemistry, Am Handelshafen 12, 27570 Bremerhaven, Germany

Their lipid composition of these 4 animals are very different!
It was an analytical challenges and we ended up using 4 different method to characterize the lipid composition

Sponges				Soft corals		Sea anemone	
Polymastia sp.		***Craniella zetlandi***		**Drifa sp**		***hormathia digitata***	
(µg/100 µg ash-free dry weight)							
	4,8		3,6		4,0		15,6
FA profile (% of total Fas)							
16:0	4	i-15:0	4	16:0	18	14:0	3
24:0	2	16:0	4	Iso 17:0	1	16:0	10
16:1 (n-11)	4	Br 17:0 (8Me)	3	18:0	4	16:1 (n-7)	8
16:1 (n-7)	2	Br 17:0 (9Me)	5	16:1 (n-7)	2	18:1 (n-9)	4
22:1 (n-7)	10	Br 17:0 (10Me)	3	18:1 (n-9)	5	18:1 (n-7)	3
24:1 (n-9)	3	Br 19:0 (10Me)	5	20:4 (n-6)	18	20:1 (n-9)	5
24:1 (n-7)	3	Br 19:0 (11Me)	8	24:5 (n-6)	12	22:1 (n-11)	6
20:5 (n-3)	3	16:1 (n-7)	5	20:5 (n-3)	5	20:5 (n-3)	17
26:2 Δ5,9 (NMI)	14	18:1 (n-7)	4	22:6 (n-3)	3	22:5 (n-3)	5
26:3 Δ5,9,19	16	(Br) 27:2 (n-x)	3	24:6 (n-3)	5	22:6 (n-3)	11
∑10 dominating	**63**		**45**		**74**		**73**

- *Polymastia*; (n-7)-MUFA and high levels of NMI-PUFA
- *Craniella zetlandi*; Dominated by bacteria FAs
- Softcoral; Extrem FA profile dominated by (n-6)-PUFA
- Anemone; high wax esters, marine FA profile

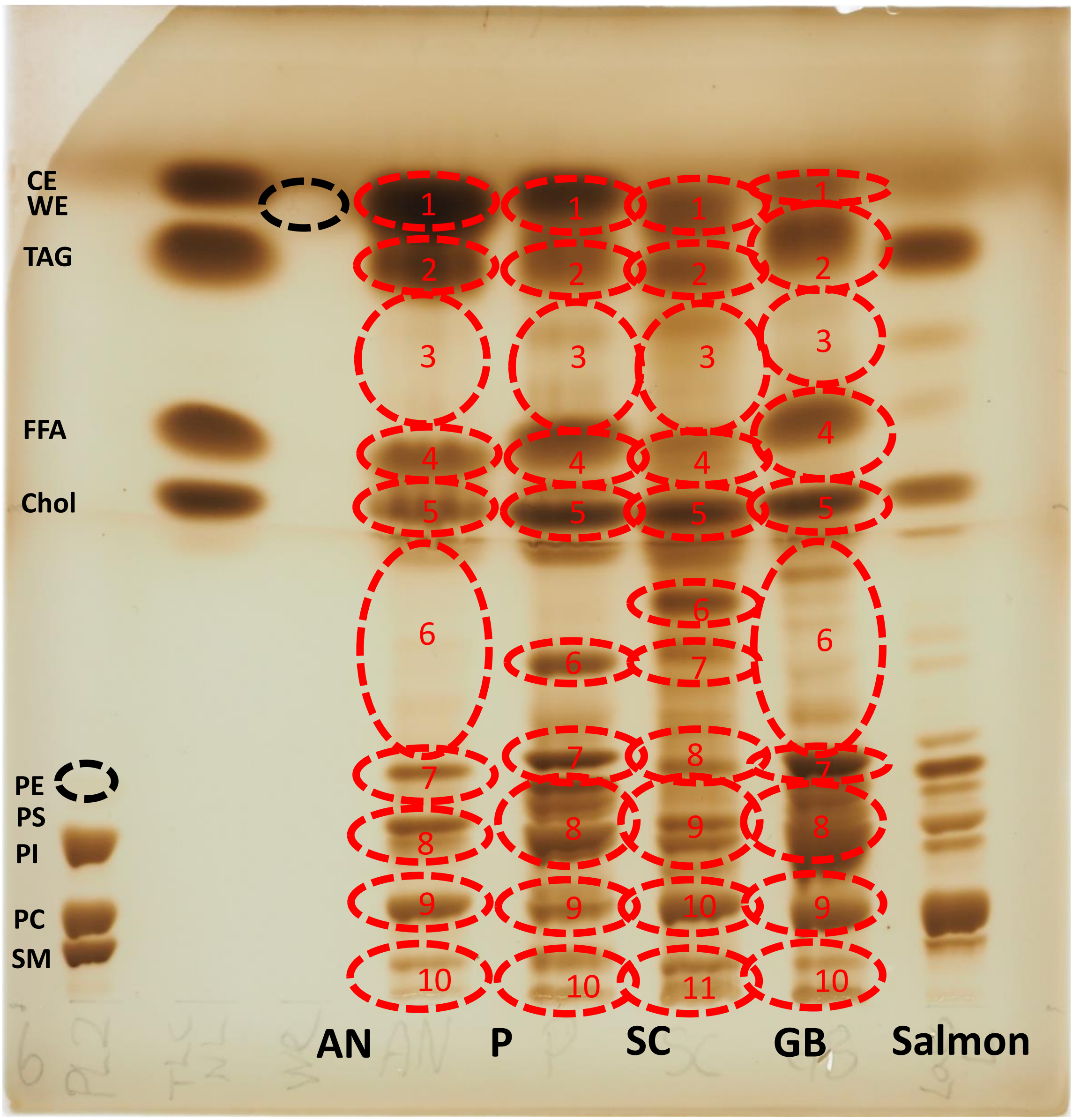


Plate 6

Marine sponges

Polymastia sp.

Characteristics:
These sponges form a yellow or orange cushion, up to 13 cm in diameter. The cushion is covered by tapered, sometimes semi translucent papillae. Sometimes a few papillae grow significantly bigger than the others.
Habitat:
The sponges are usually observed on rocky substrate, deeper than 15 meters.
Distribution:
These sponges are frequently reported from the British Isles and Scandinavia.

- **Kingdom:** *Animalia*
- **Phylum:***Porifera*
- **Class:** *Demospongiae*
- **Order:** *Hadromerida*
- **Family:** *Polymastiidae*
- **Scientific name:** *Polymastia sp.*
- **Norwegian:** -

http://www.seawater.no/fauna/porifera/Polymastia.html

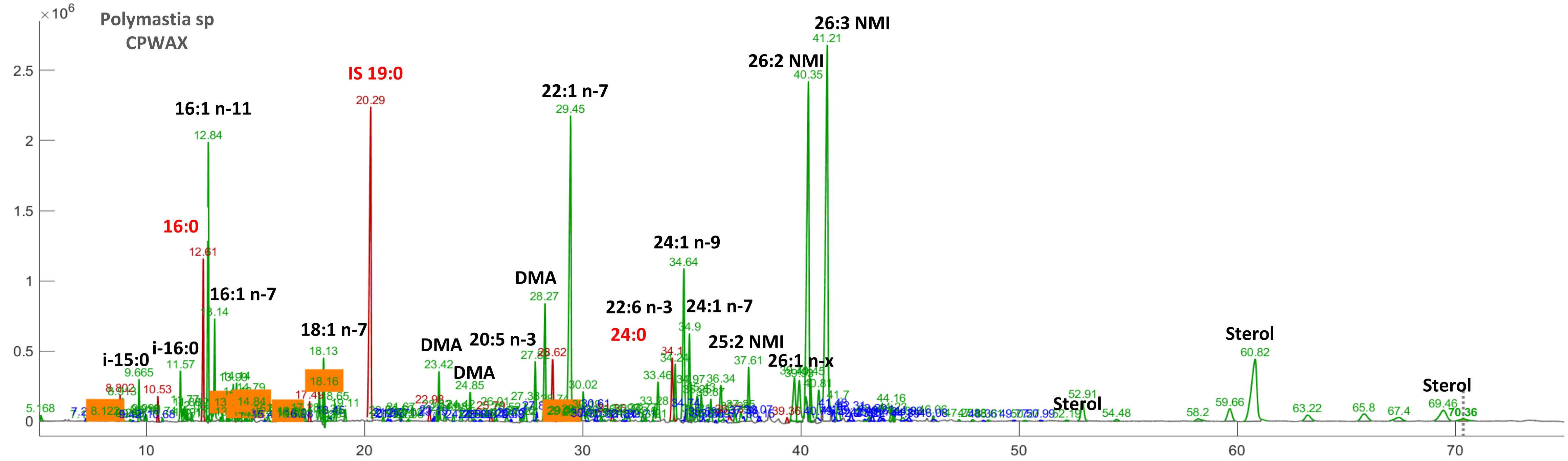


Figure 1. Chromatogram of FA composition of Polymastia

There were identified 91 different FAs in the samples. However, more than 80 % of the total FAs were represented by the 22 FAs (> 1% of the total FAs) shown in Fig3.

The lipids from *Polymasti sp* are dominated by in non-methyl-interrupted (NMI) PUFAs (32-40 % of the total FAs), followed by MUFA (35-37 % of total FAs) and SFA (19-20%), while the PUFAs that normally are very high in marine organism only contributed with 4-6 % of the total FAs.

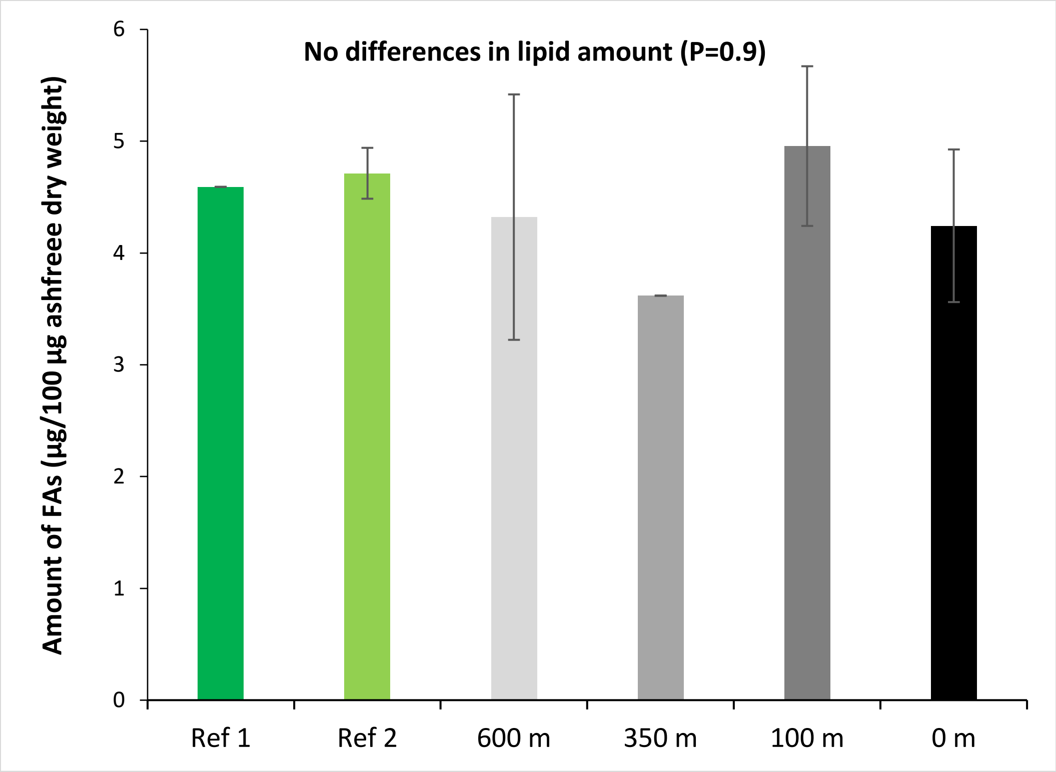


Figure 2. Fatty acids amount in *polymastia* translocated at different distances of the fish farm (right under, 0 m, 100 m, 350 m, 600 m away and at references area 2 km away).

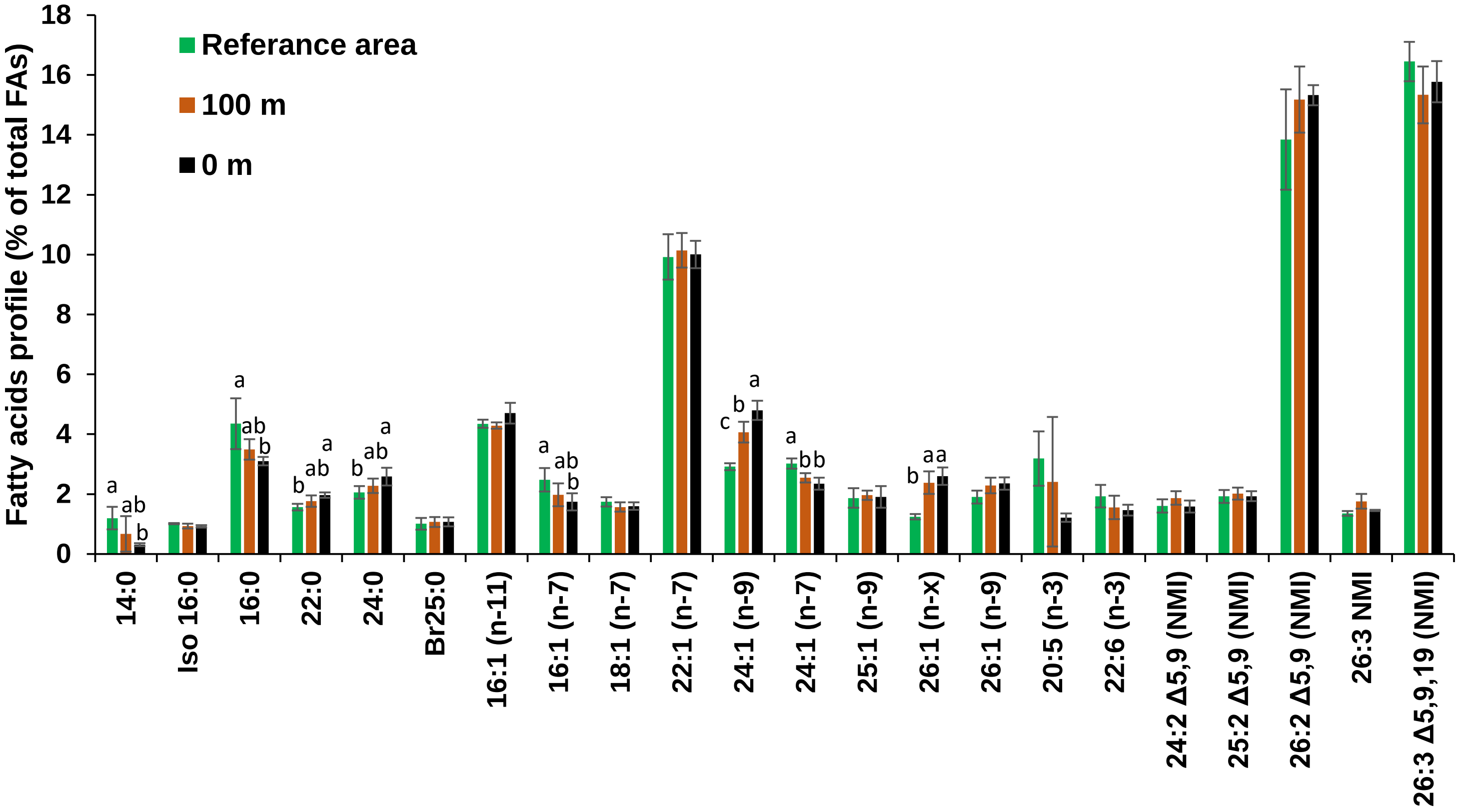


Figure 3. Fatty acids profiles of *polymastia* translocated at different distances of the fish farm (right under, 0 m, 100 m away and at a references area 2 km away).

There were only found some minor differences in the FAs profiles that were related to location from the fish farm.
The sponge at the fish farm (0 m) had lower relative levels of the short chain SFAs (14:0 and 16:0) and higher levels of the long chain SFAs (22:0 and 24:0) compared with the reference's areas. Differences were also found within the MUFAs, where the sponge at the fish farm (0 m) had lower levels of 16:1 (n-7) and 24:1 (n-7) and higher levels of 24:1 (n-9) and 26:1 (n-x) compared with the reference areas.

The FA data suggest a small uptake of FAs from organic waste from the fish farm, and that this are incoporated into the FA synthesis of the sponges; 18:1 (n-9) can be elongated into 24:1 (n-9).

Terrestrial FATM

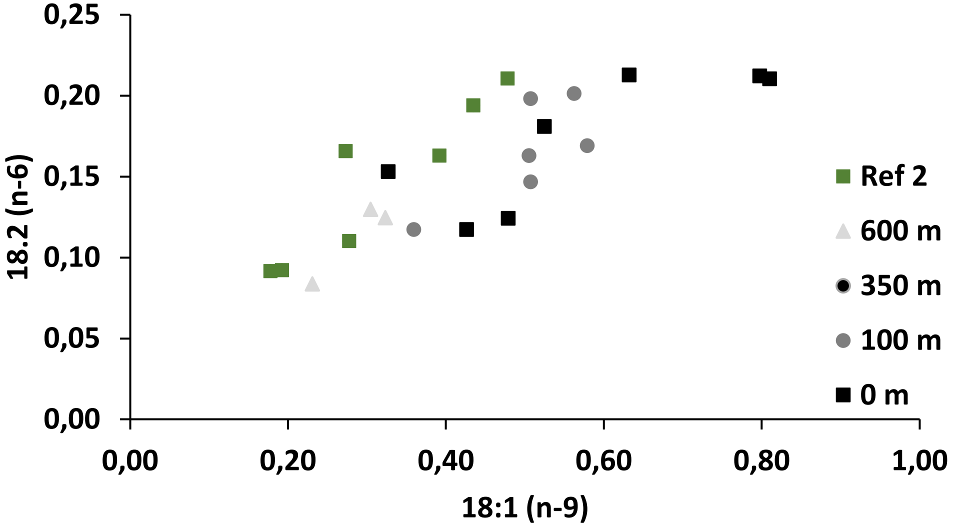


Figure 4. Relative amount of 18:1 (n-9) and 18:2 (n-6)

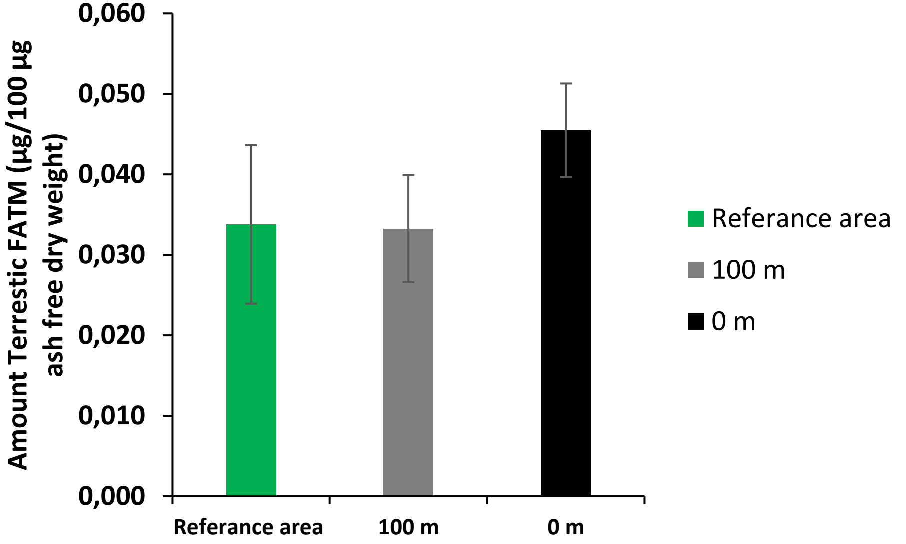
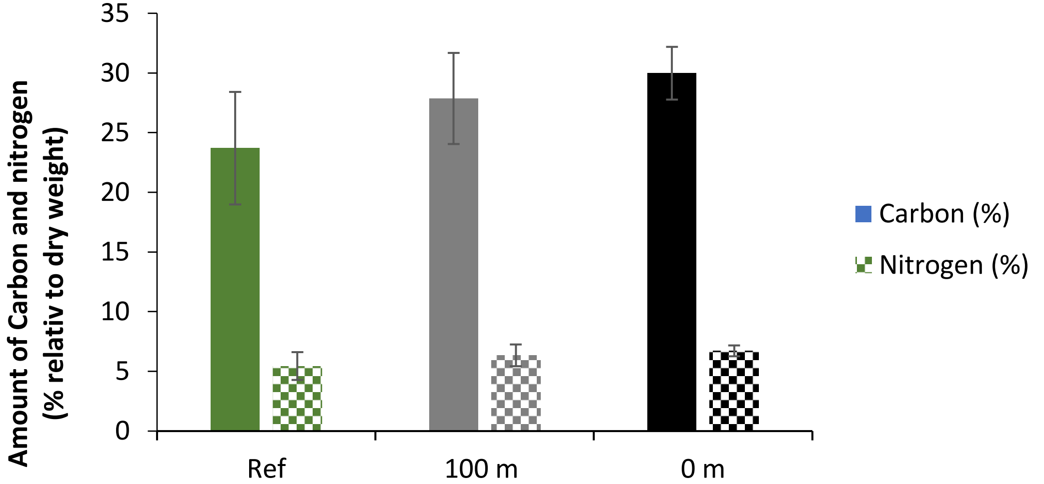


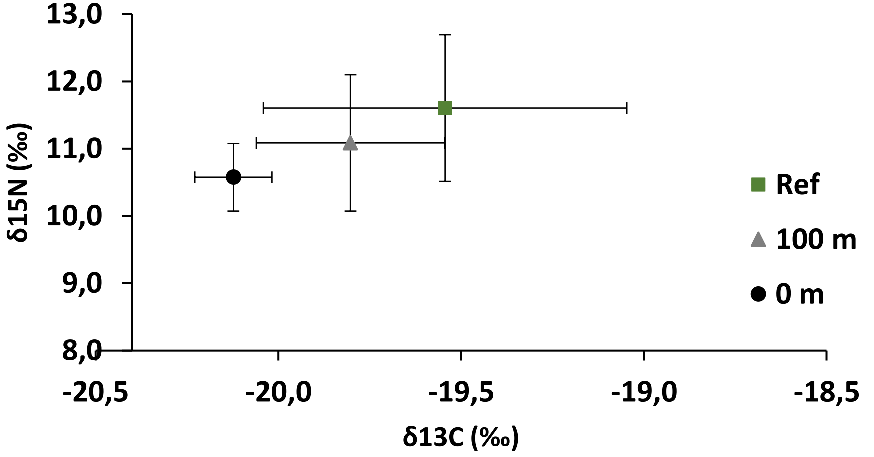
Figure 5. Quantitative amount of the Terrestrial FATM (18:1 (n-9)+18:2 (n-6)+18.3 (n-3)

There are an tendency of higher levels of terrestrial FATM in the animals located at the fish farm.
However, these FAs are very minor contributers (> 1%) to the lipids of *Polymastia*

Amount of carbon and nitrogen relativ to dry weight

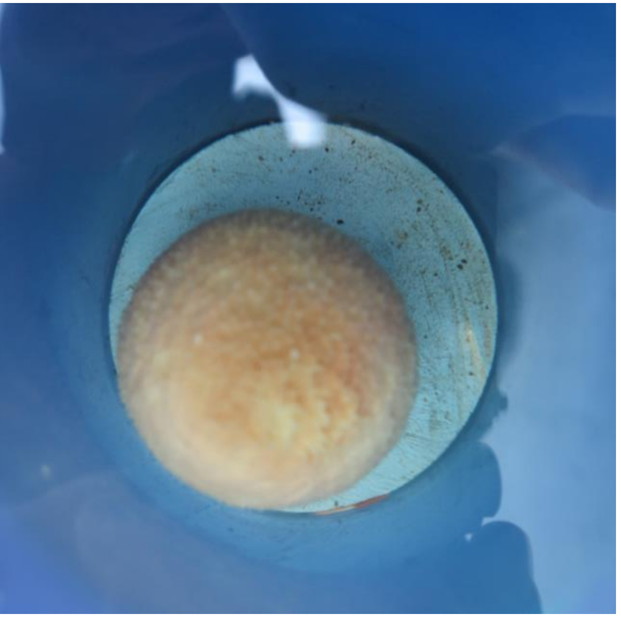


Distribution of stable isotope



Marine sponges

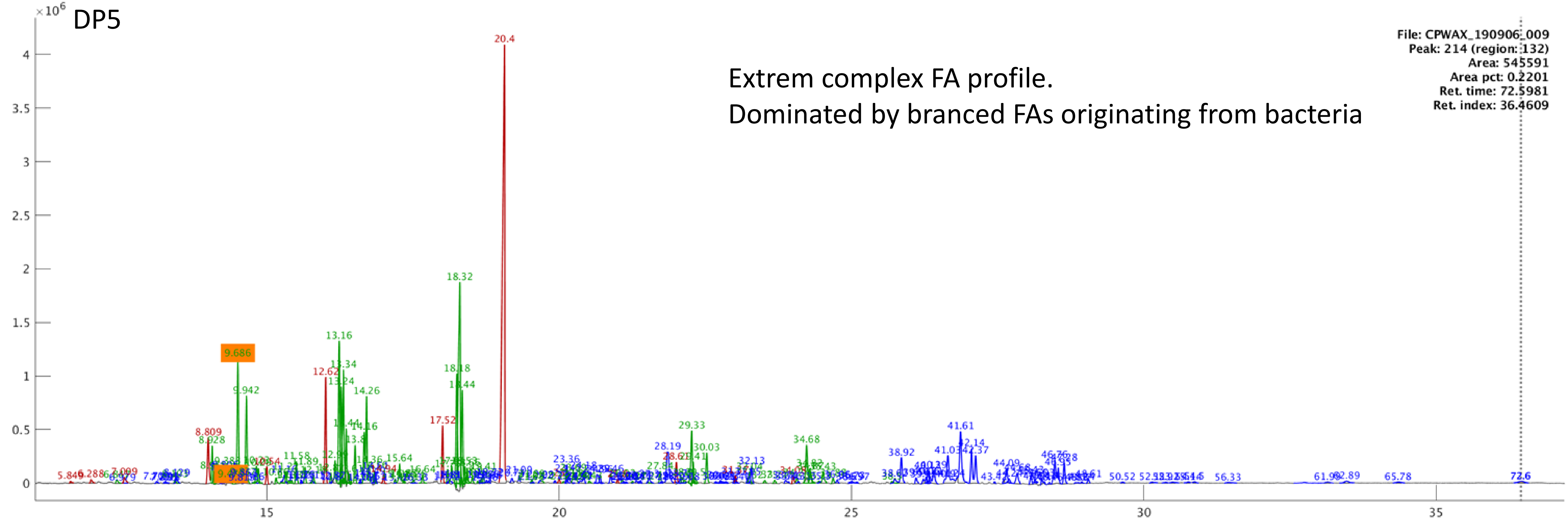
Craniella zetlandica

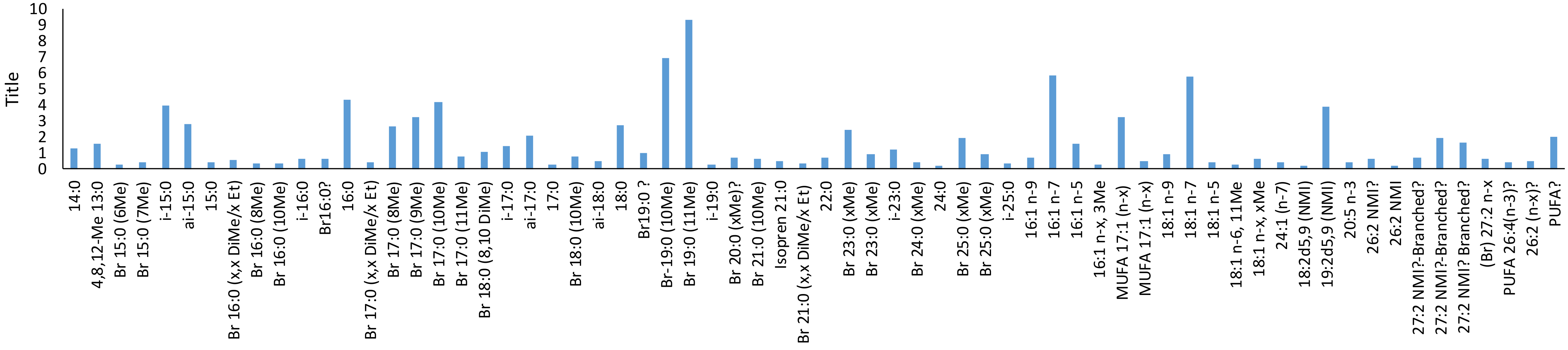


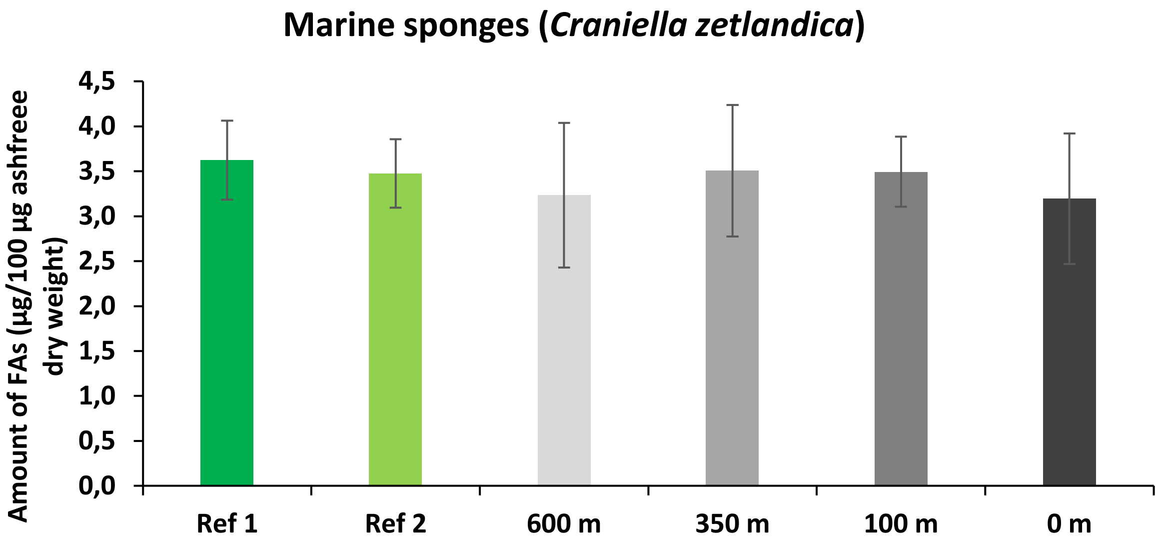
Characteristics:
This spherical or hemispherical sponge can reach a diameter of 6 cm. Live specimens are white. The surface is even, but with a rough, hairy looking texture. It usually have only one or two oscules (openings).
Habitat:
It is found on rocky substrate deeper than 15 meters, but more frequently below 40 meters.
Distribution:
This sponge is reported from most coastlines around the North-East Atlantic, from the Arctic to the British Isles.

- **Kingdom:** *Animalia*
- **Phylum:** *Porifera*
- **Class:** *Demospongiae*
- **Order:** *Teractinellida*
- **Family:** *Tetillidae*
- **Scientific name:** *Craniella zetlandica*
- **Norwegian:** -

http://www.seawater.no/fauna/porifera/zetlandica.html


DP5
Extrem complex FA profile.
Dominated by branced FAs originating from bacteria
File: CPWAX_190906_009
Peak: 214 (region: 132)
Area: 545591
Area pct: 0.2201
Ret. time: 72.5981
Ret. index: 36.4609
×10^6
20.4
18.32
13.16
9.686
12.62
13.34
9.942
14.26
17.52
8.809
8.928
29.33
41.61
34.68
30.03
28.19
38.92
15
20
25
30
35


Title
14:0
4,8,12-Me 13:0
Br 15:0 (6Me)
Br 15:0 (7Me)
i-15:0
ai-15:0
15:0
Br 16:0 (x,x DiMe/x Et)
Br 16:0 (8Me)
Br 16:0 (10Me)
i-16:0
Br16:0?
16:0
Br 17:0 (x,x DiMe/x Et)
Br 17:0 (8Me)
Br 17:0 (9Me)
Br 17:0 (10Me)
Br 17:0 (11Me)
Br 18:0 (8,10 DiMe)
i-17:0
ai-17:0
17:0
Br 18:0 (10Me)
ai-18:0
18:0
Br19:0 ?
Br-19:0 (10Me)
Br 19:0 (11Me)
i-19:0
Br 20:0 (xMe)?
Br 21:0 (10Me)
Isopren 21:0
Br 21:0 (x,x DiMe/x Et)
22:0
Br 23:0 (xMe)
Br 23:0 (xMe)
i-23:0
Br 24:0 (xMe)
24:0
Br 25:0 (xMe)
Br 25:0 (xMe)
i-25:0
16:1 n-9
16:1 n-7
16:1 n-5
16:1 n-x, 3Me
MUFA 17:1 (n-x)
MUFA 17:1 (n-x)
18:1 n-9
18:1 n-7
18:1 n-5
18:1 n-6, 11Me
18:1 n-x, xMe
24:1 (n-7)
18:2d5,9 (NMI)
19:2d5,9 (NMI)
20:5 n-3
26:2 NMI?
26:2 NMI
27:2 NMI?-Branched?
27:2 NMI?-Branched?
27:2 NMI? Branched?
(Br) 27:2 n-x
PUFA 26:4(n-3)?
26:2 (n-x)?
PUFA?



No differences in lipid amount or FA profiles

Figure 9. Fatty acids amount in *Craniella zetlandica* translocated at different distances of the fish farm (right under, 0 m, 100 m, 350 m, 600 m away and at references area 2 km away).

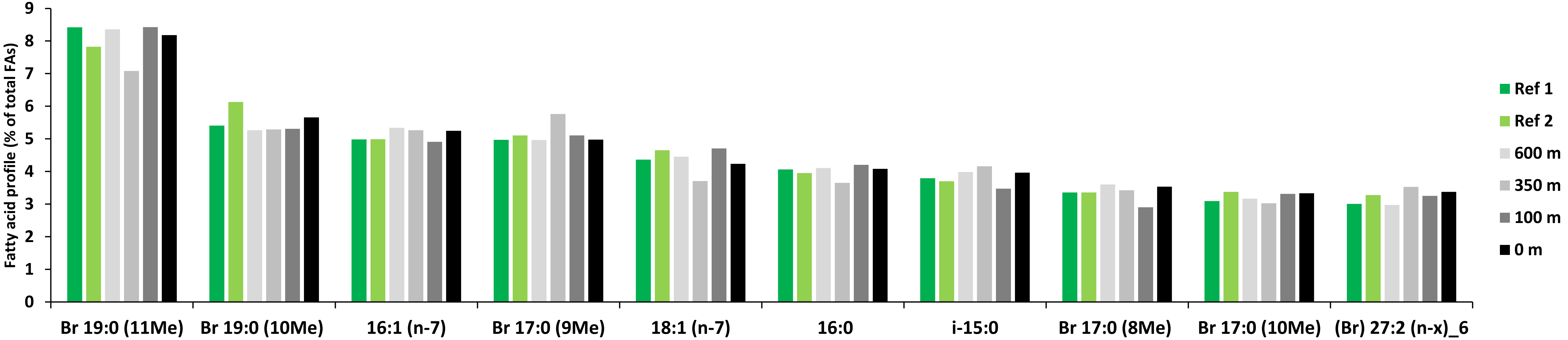
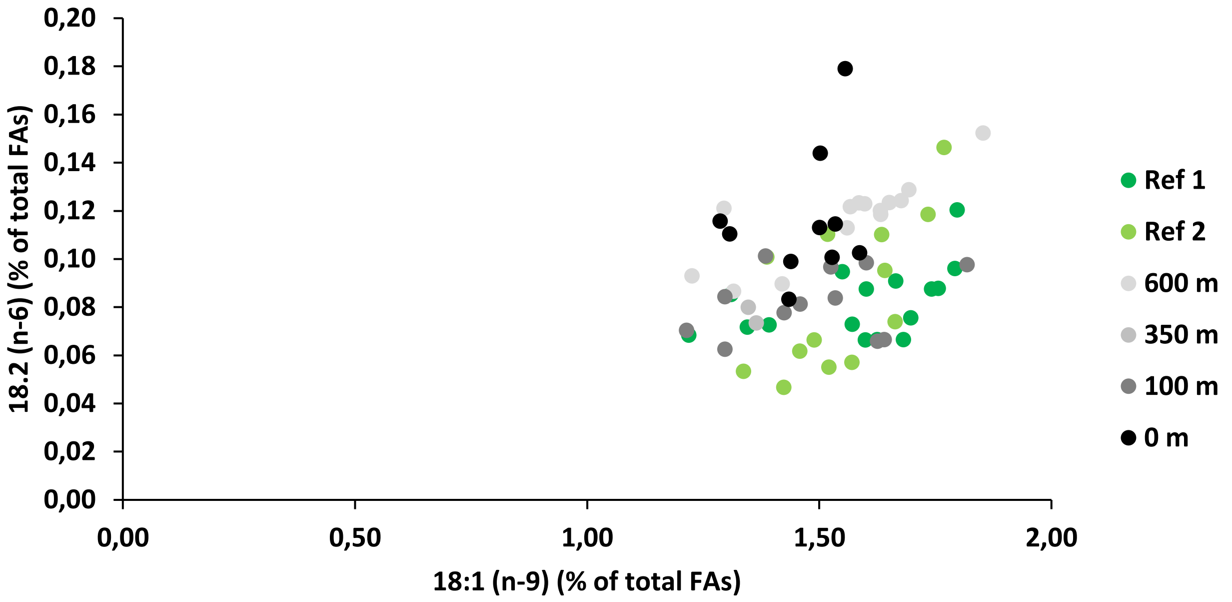
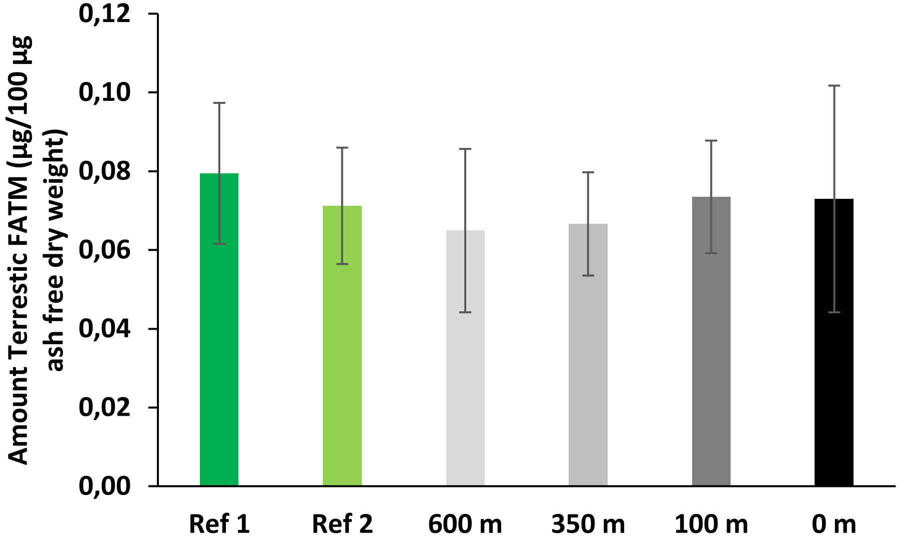


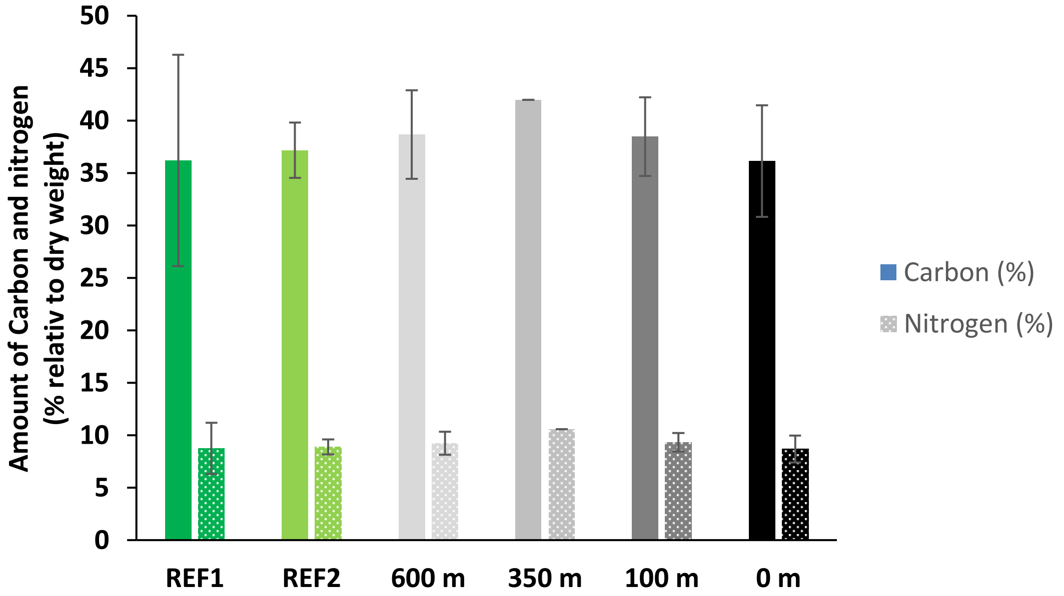
Figure 10. Fatty acids profiles of *Craniella zetlandica* translocated at different distances of the fish farm The 10 most dominating FAs, contribute with 44-45% of the total FAs. The FA profiles are extrem complex in this animals

Terrestrial FATM

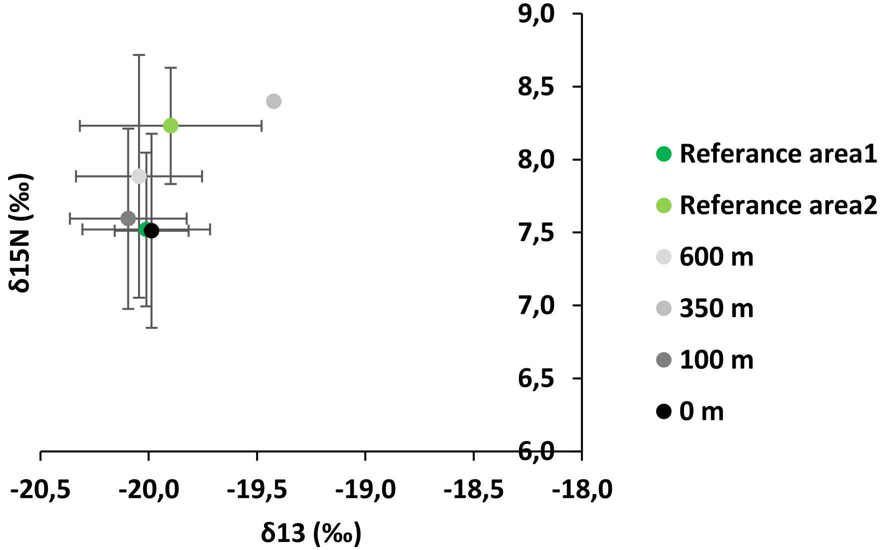




Amount of carbon and nitrogen relativ to dry weight

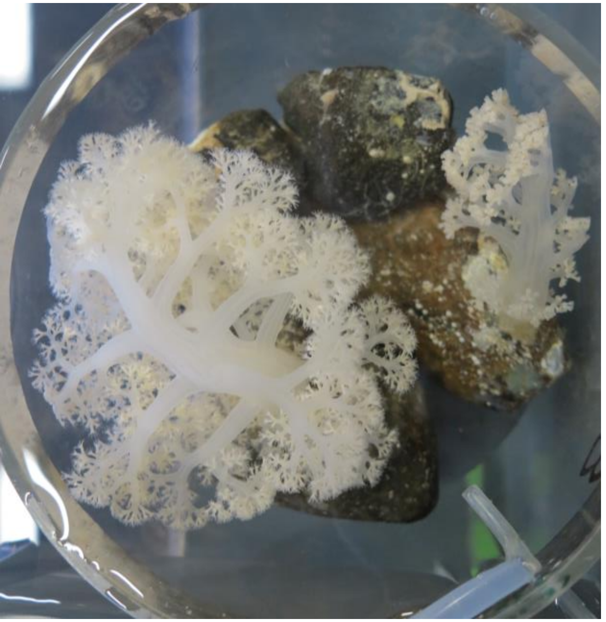


Distribution of stable isotope



Soft coral

Drifa sp.



Blomkålkorallen er myk med polypper som sitter i små hoder (og likner dermed blomkål).

Som de fleste andre koraller liker også blomkålkorallene seg der det er strøm og dermed god fødetilgang. I norske fjorder finnes de på relativt grunt vann, så de er godt kjent for dykkere og er dermed et yndet fotomotiv. De finnes ned til 1500 meters dyp.

Blomkålkorallen finnes i det nordlige Atlanterhavet inkludert rundt Grønland, Svalbard og i Barentshavet – langs norskekysten fra Vestlandet til og med Finnmmark.

- **Kingdom:** *Animalia*
- **Phylum:** *Cnidaria*
- **Class:** *Anthozoa*
- **Order:** *Alcyonacea*
- **Family:** *Nephtheidae*
- **Scientific name:** *Drifa sp.*
- **Norwegian:** Blomkålkorall

https://www.hi.no/hi/temasider/arter/blotkoraller

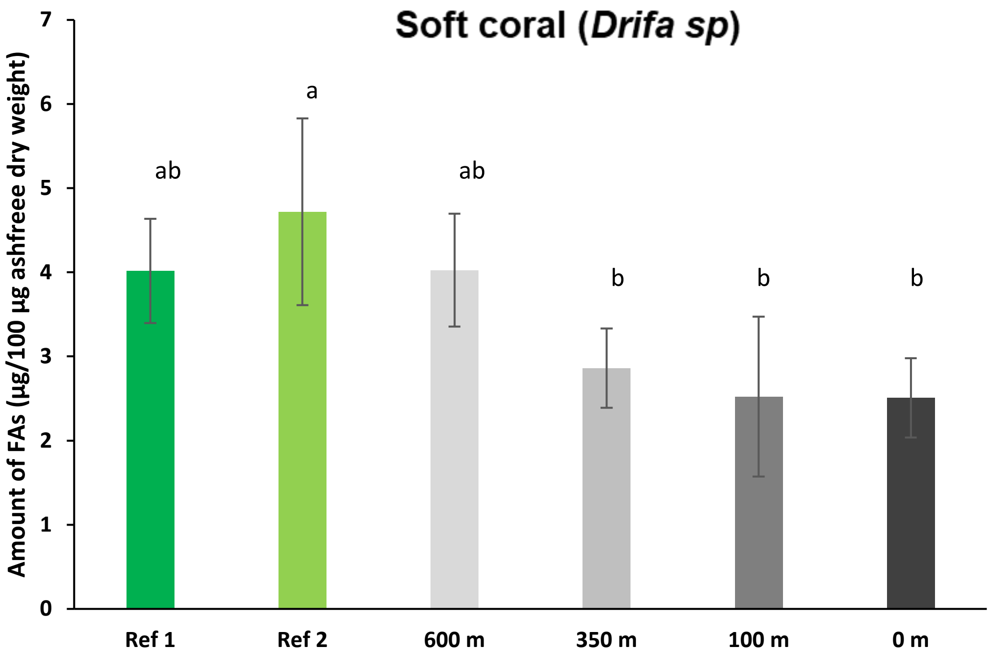
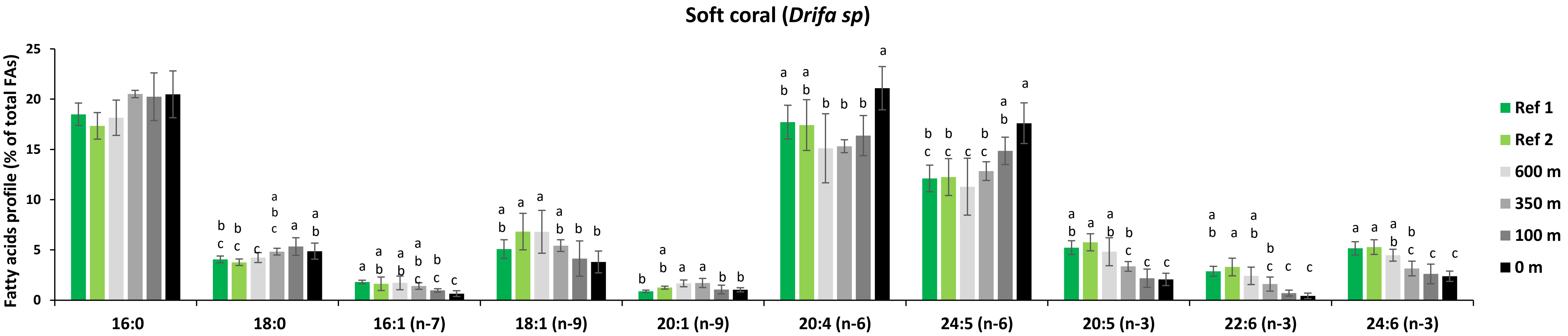


Figure 9. Fatty acids amount in *Soft coral*

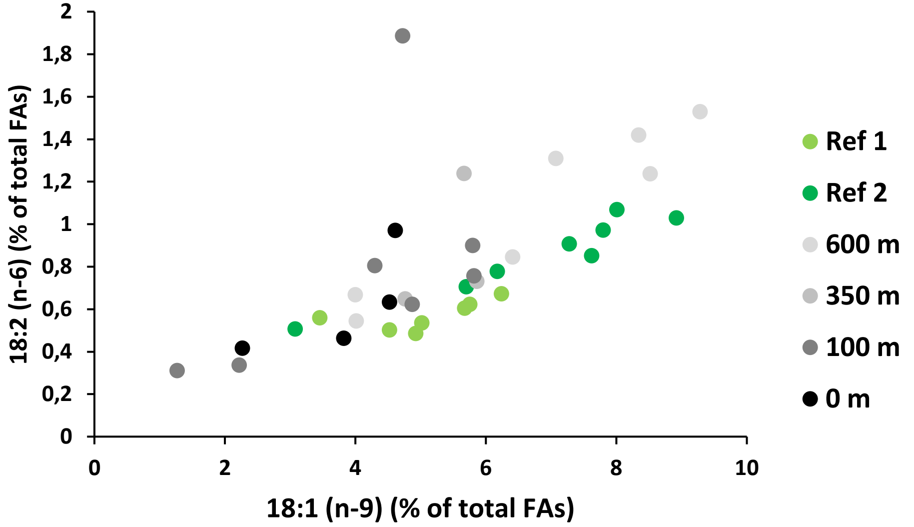
For the soft coral the lipid amount are decreasing as the animal were closer to the salmon farm.
This may be explained by lower feeding rate or higher energy consumtion to overcome strees.

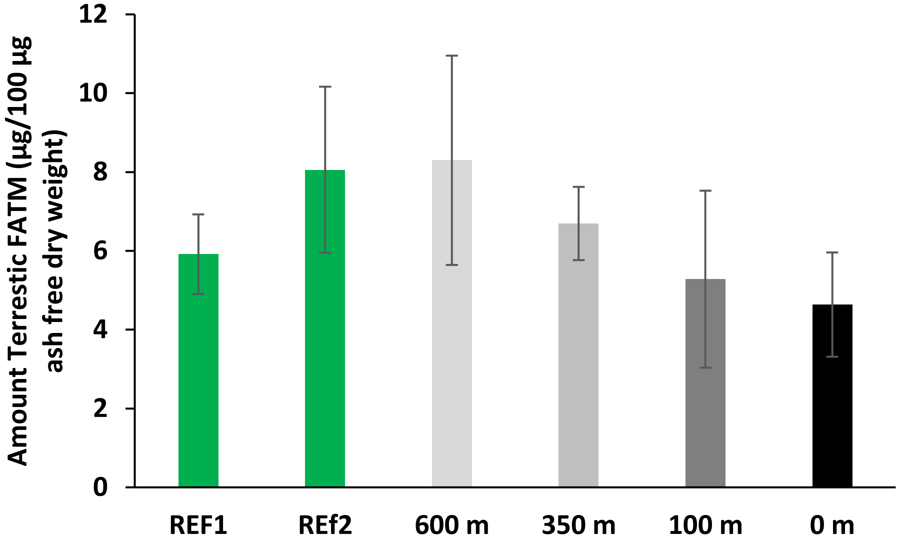
There are also a clear differences in the fatty acids profiles.
The animals close to the farm have low levels of (n-3) PUFAs, but high levels of (n-6) PUFAs



The 10 most dominating FAs, contribute with >70 % of the total FAs

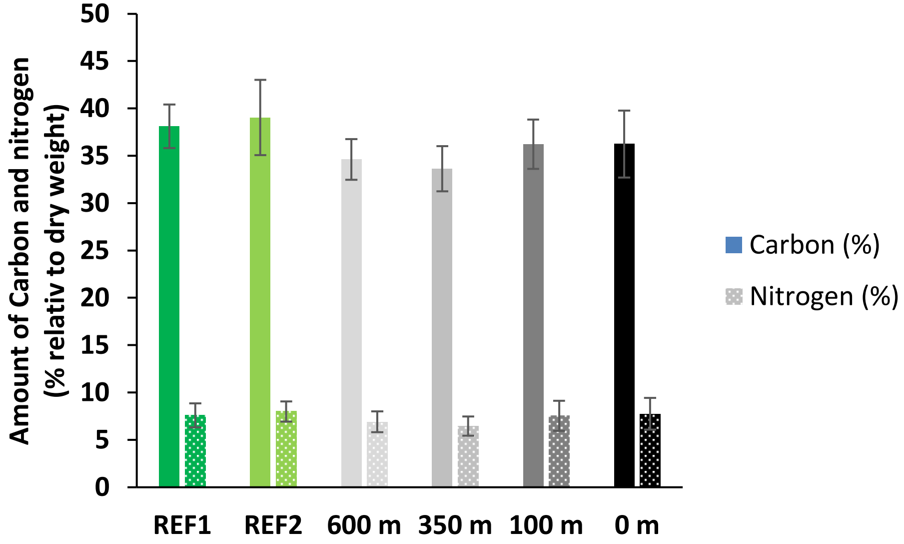
Terrestrial FATM





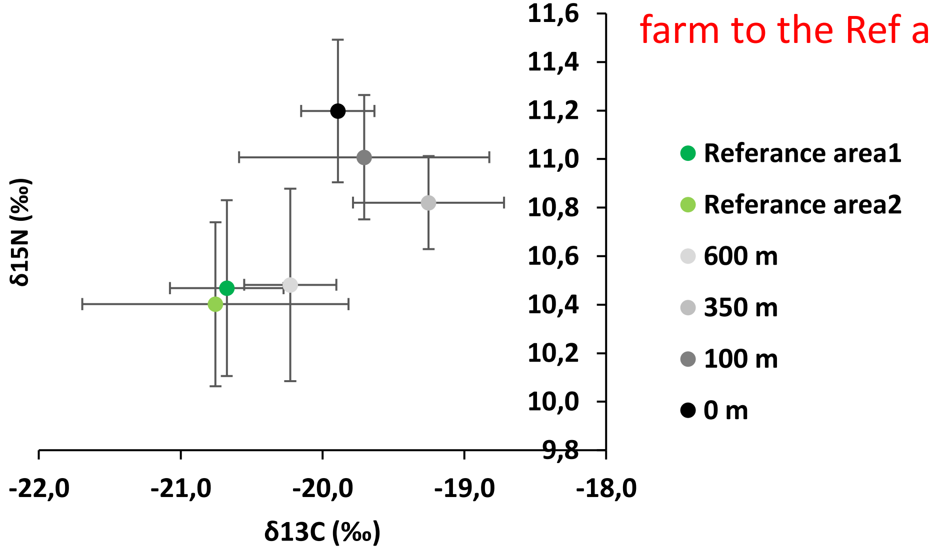
There are no geografic trendt for incorboration of terrestic FAs into the lipids of the soft coral.

Amount of carbon and nitrogen relativ to dry weight



Distribution of stable isotope

There are a gradient in δ15N (‰) from the fish farm to the Ref area



Sea anemone

Hormathia digitata

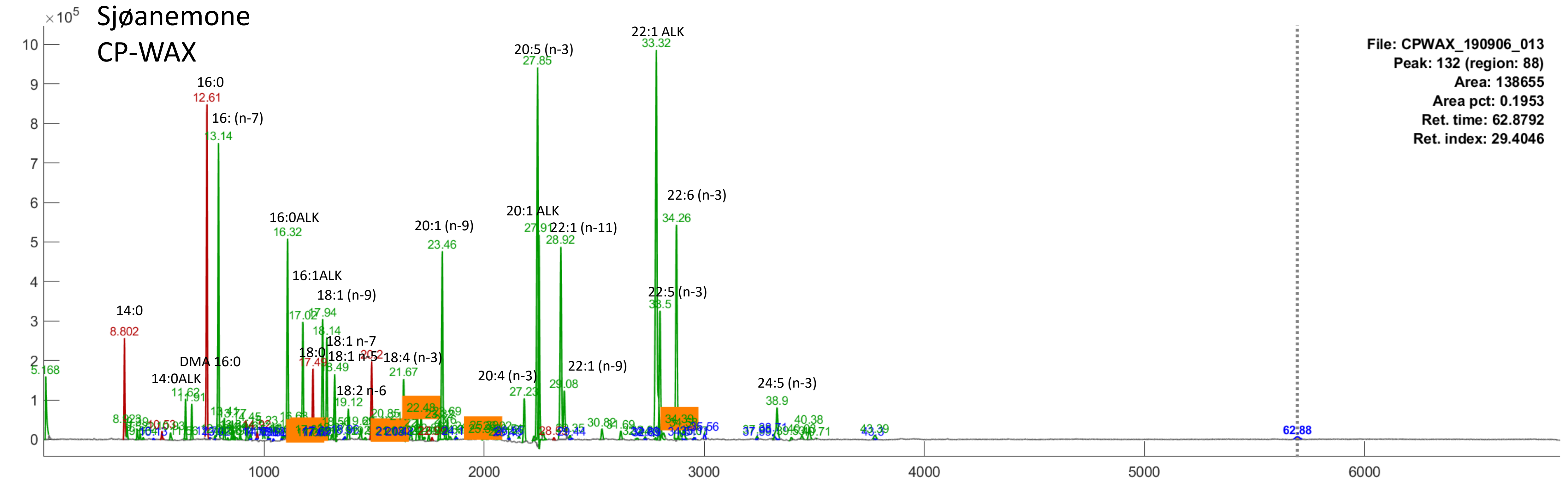
•**Kingdom:** *Animalia*
•**Phylum:** *Cnidaria*
•**Class:** *Anthozoa*
•**Order:** *Actiniari*
•**Family:** *Hormathiidae*
•**Scientific name:** *Hormathia digitata*
•**Norwegian:** -

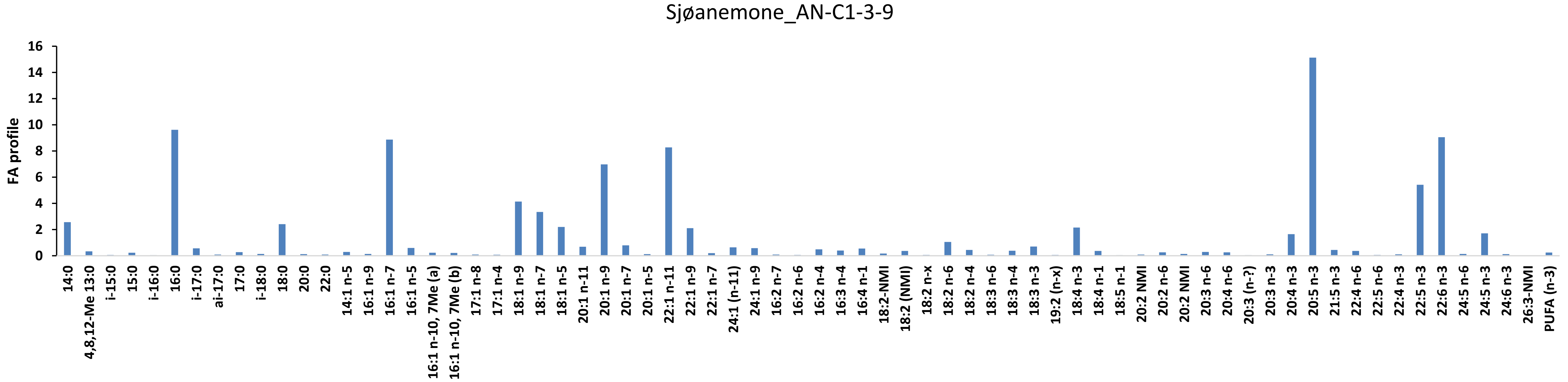
•**Utbredelse:** Den er en lavarktisk boreal art, utbredt fra Labrador til Barentshavet. Finnes i Skagerak og Kattegatt. Fra Hordaland og nordover langs hele kysten.

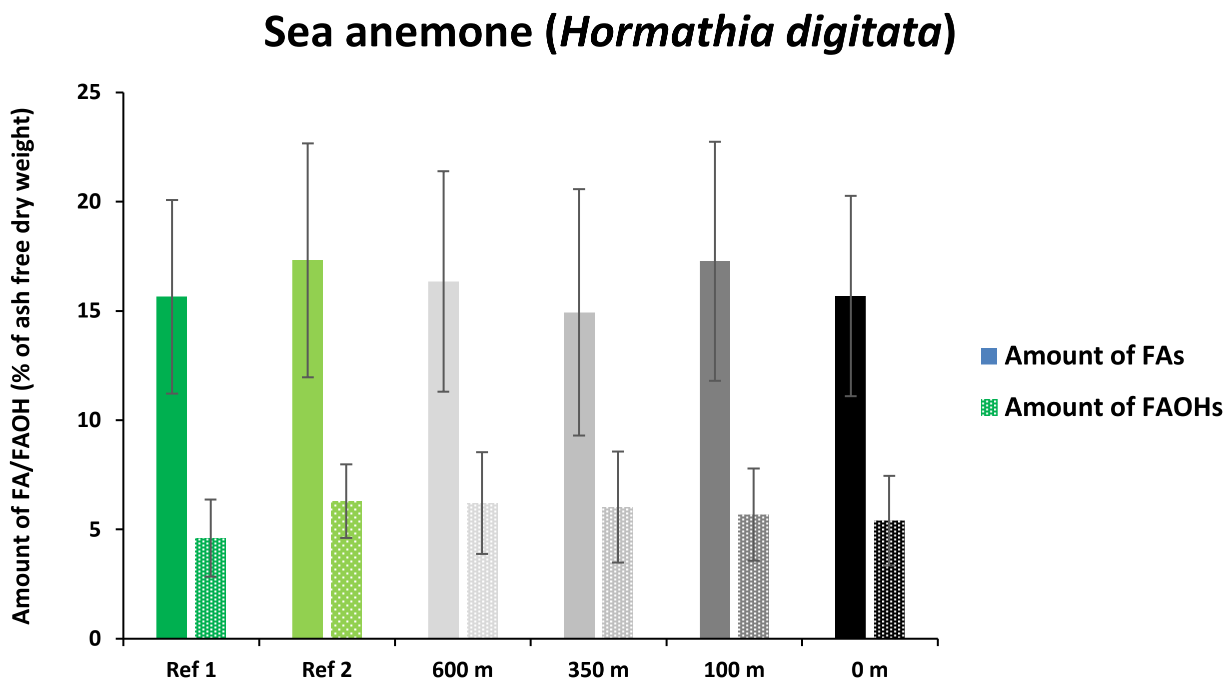
•**Beskrivelse:** De om lag 100 fullstendig retraktile tentaklene har varierende rødfarger. Munnskiven er kjøttfarget eller lyserød. Svelget er orange eller blekt rødgult. Fotskiven bred og flat, ca 80 mm i diameter. Kroppen er tykk og rynket.

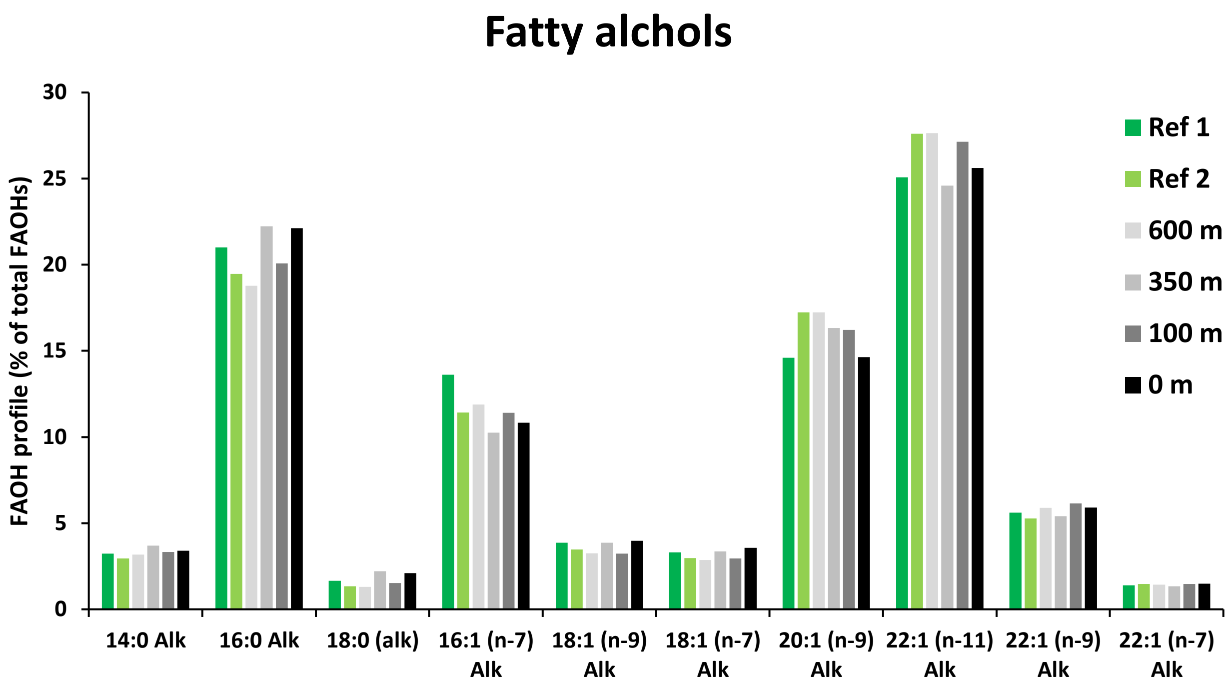
•**Levested:** Ofte ses denne arten festet til skall av levende eller døde bløtdyr, så som stor havsnegl (***Neptunea despecta***) og kongsnegl (***Buccinum undatum***). De døde snegleskallene huser ofte eremittkrepsen av arten ***Eupagurus bernhardus***. Man har også funnet eksemplarer av sjørosen sittende på skall av pyntekrabbe (***Hyas sp***.). Registrert på dyp mellom 25 – 660 m.

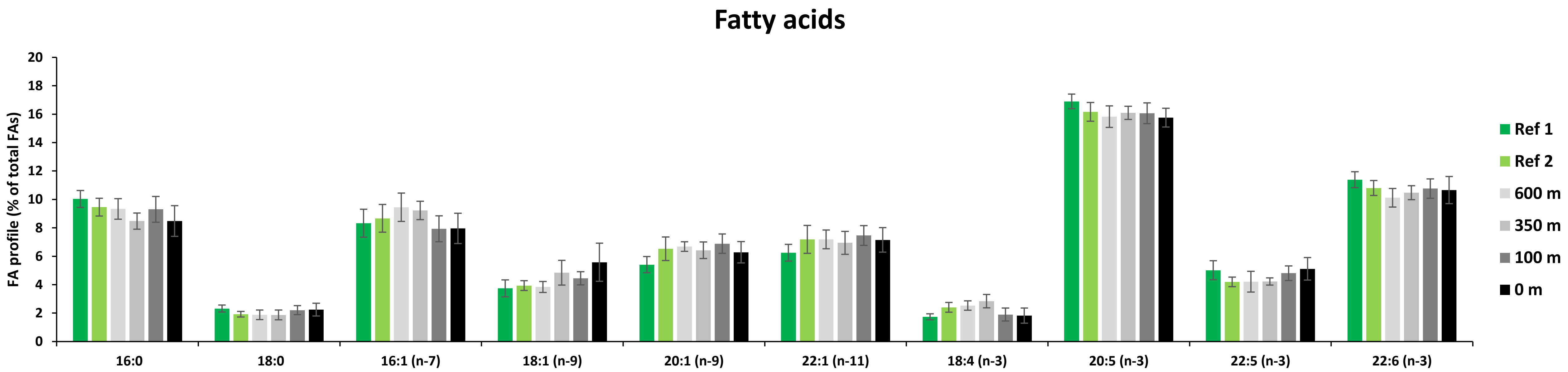
http://www.marinbi.com/cnidaria/hormathia_digitata.html


Sjøanemone
CP-WAX
×10^5
File: CPWAX_190906_013
Peak: 132 (region: 88)
Area: 138655
Area pct: 0.1953
Ret. time: 62.8792
Ret. index: 29.4046
14:0
DMA 16:0
14:0ALK
16:0
16: (n-7)
16:0ALK
16:1ALK
18:1 (n-9)
18:1 n-7
18:0
18:1 n-5
18:4 (n-3)
18:2 n-6
20:1 (n-9)
20:4 (n-3)
20:5 (n-3)
20:1 ALK
22:1 (n-11)
22:1 (n-9)
22:1 ALK
22:6 (n-3)
22:5 (n-3)
24:5 (n-3)


Sjøanemone_AN-C1-3-9
FA profile
14:0
4,8,12-Me 13:0
i-15:0
15:0
i-16:0
16:0
i-17:0
ai-17:0
17:0
i-18:0
18:0
20:0
22:0
14:1 n-5
16:1 n-9
16:1 n-7
16:1 n-5
16:1 n-10, 7Me (a)
16:1 n-10, 7Me (b)
17:1 n-8
17:1 n-4
18:1 n-9
18:1 n-7
18:1 n-5
20:1 n-11
20:1 n-9
20:1 n-7
20:1 n-5
22:1 n-11
22:1 n-9
22:1 n-7
24:1 (n-11)
24:1 n-9
16:2 n-7
16:2 n-6
16:2 n-4
16:3 n-4
16:4 n-1
18:2-NMI
18:2 (NMI)
18:2 n-x
18:2 n-6
18:2 n-4
18:3 n-6
18:3 n-4
18:3 n-3
19:2 (n-x)
18:4 n-3
18:4 n-1
18:5 n-1
20:2 NMI
20:2 n-6
20:2 NMI
20:3 n-6
20:4 n-6
20:3 (n-?)
20:3 n-3
20:4 n-3
20:5 n-3
21:5 n-3
22:4 n-6
22:5 n-6
22:4 n-3
22:5 n-3
22:6 n-3
24:5 n-6
24:5 n-3
24:6 n-3
26:3-NMI
PUFA (n-3)

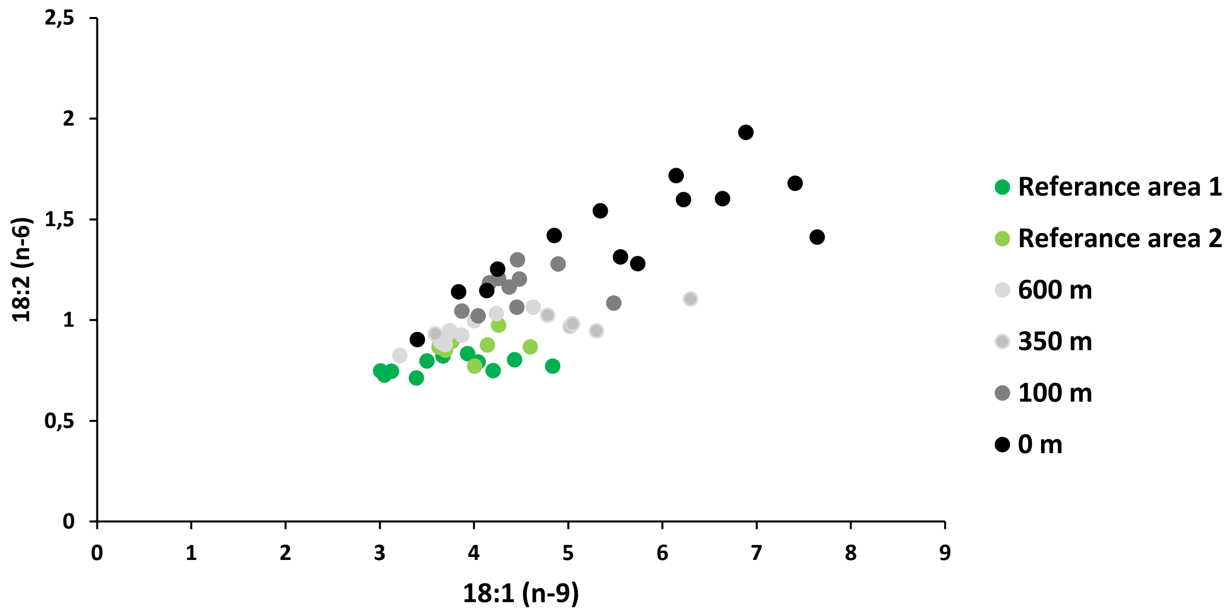


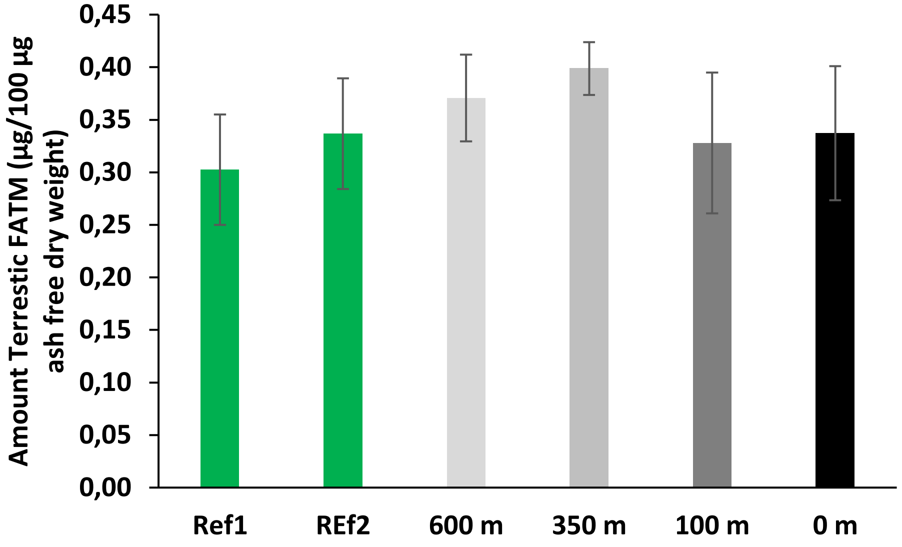




The 10 most dominating FAs, contribute with >70 % of the total FAs

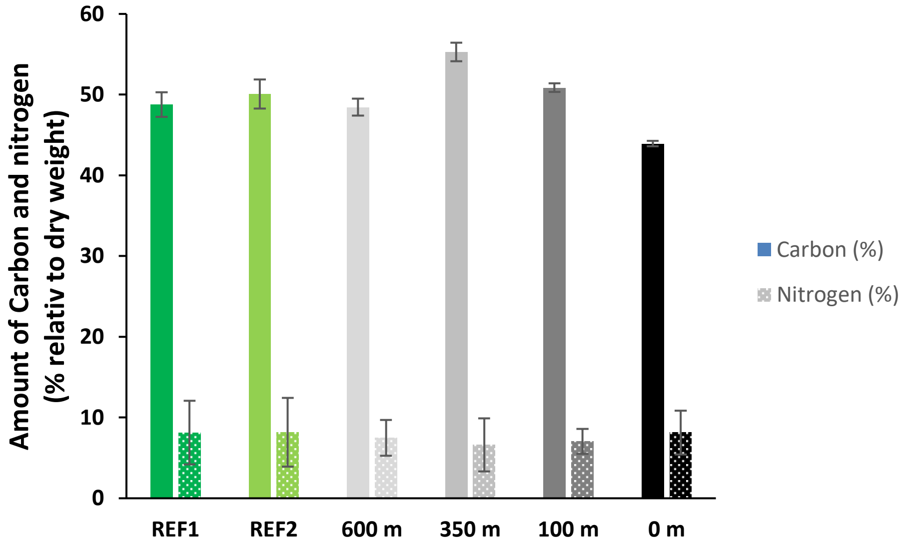
Terrestic FATM



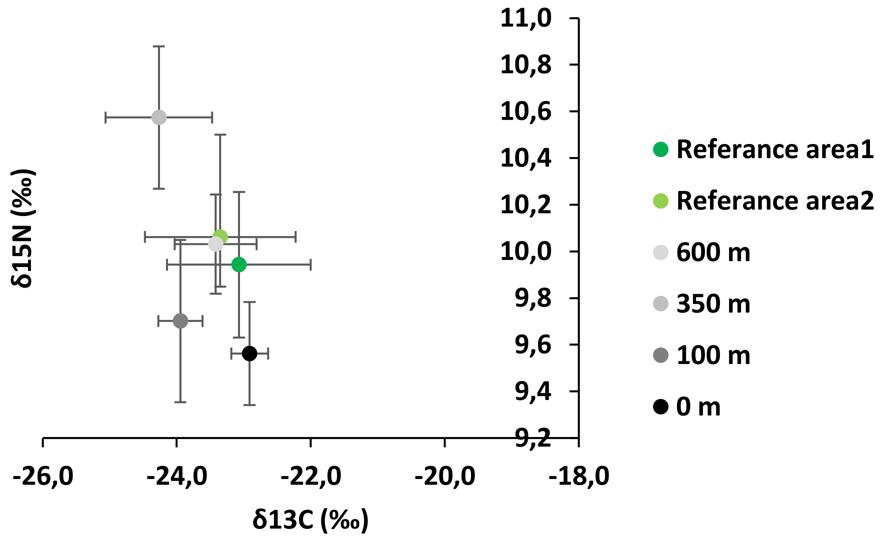


There are a trend of that some of the animals closes to the fish farms have more of the terrestic FATMs, but it is not significant as a group.

Amount of carbon and nitrogen relativ to dry weight



Distribution of stable isotope



Conclusion

1. The benthic organisms in this investigation have a very active lipid synthesis and have very different FA composition
   - *Polymastia*; (n-7)-MUFA and high levels of NMI-PUFA
   - *Craniella zetlandi*; Dominated by bacteria FAs
   - Softcoral; FA profile dominated by (n-6)-PUFA
   - Anemone; High wax esters, marine FA profile (store energy as lipids)

2. There were differences in the FA profile in one of the sponges (polymastia) and the soft coral (drifa) following the transect mowing away from the fish farm. Indicating effects on the lipid metabolism and synthesis.

3. However, there were little evidence that FAs from the fish farm waste were incoporated into the lipids of this animals.

4. The FATM analysis does therefore not seems to be good method for tracing organic effluent from fish farms into these hard-bottom benthic organisms.

5. The stable isotopes (δ13C and δ15N) showed high variation and it was only in the soft coral there were a systematic differences that could be link to the distanse to the fish farm.