

# **COMPARATIVE STUDY OF THE CHEMICAL COMPOSITION OF BLACK SEA *CHAETOMORPHA LINUM* AND *CYSTOSEIRA CRINITA***

Veselina Panayotova  
Mona Stancheva

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# Why Algae?

- ✓ Algae have been used since ancient times as food, fodder, fertilizer and source of medicine
- ✓ Seaweeds are increasingly viewed as potential sources of bioactive compounds with immense pharmaceutical, biomedical and nutraceutical applications
- ✓ Benthic food webs may receive considerable inputs from macroalgae (seaweeds), either grazed directly or, as is mostly the case, enter the detrital food webs via microheterotrophs



# Aim of Study

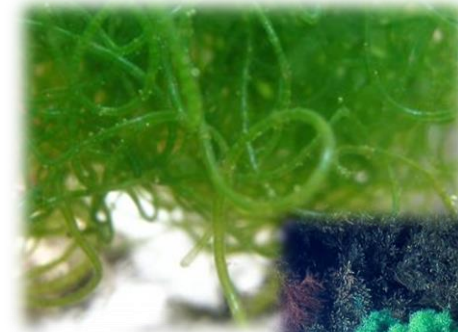
- Total lipids and fatty acid composition
- Astaxanthin
- $\beta$ -carotene
- $\alpha$ -tocopherol
- Ergocalciferol



# Objects

## Chlorophyta

*Chaetomorpha linum* (O.F.Müller) Kützing



## Rhaeophyta

*Cystoseira crinita* Duby



# Total lipids and fatty acid composition

- ✓ Moisture content - 105 °C, AOAC, 2000
- ✓ Lipids extraction - Bligh and Dyer, 1959
  - i. Homogenized algal tissue – chloroform:methanol (1:2, v/v)
  - ii. Residue – thrice with small portions of chloroform:methanol (1:1, v/v)
  - iii. Chloroform and water (1:1, v/v) for phase separation
  - iv. Total lipids were determined gravimetrically
- ✓ Base-catalyzed transmethylation 2M KOH/CH<sub>3</sub>OH (BDS EN 5509:2000)



# Total lipids and fatty acid composition

## ***Apparatus***

- ✓ FOCUS Gas chromatograph  
equipped with Polaris Q Mass detector, Thermo Scientific
- ✓ Capillary column TR-5 MS, 30m x 0.25mm
- ✓ Temperature program

## ***Peak identification***

- ✓ FAME mix standard (SUPELCO F.A.M.E. Mix C4-C24)
- ✓ Mass spectral library
- ✓ ***Quantification*** – external calibration



# Total lipids and fatty acid composition

- 💧 Saturated ( $C_{8:0} - C_{24:0}$ );
- 💧 Monounsaturated ( $C_{14:1} - C_{24:1}$ );
- 💧 Polyunsaturated

## 😊 $\omega$ -3

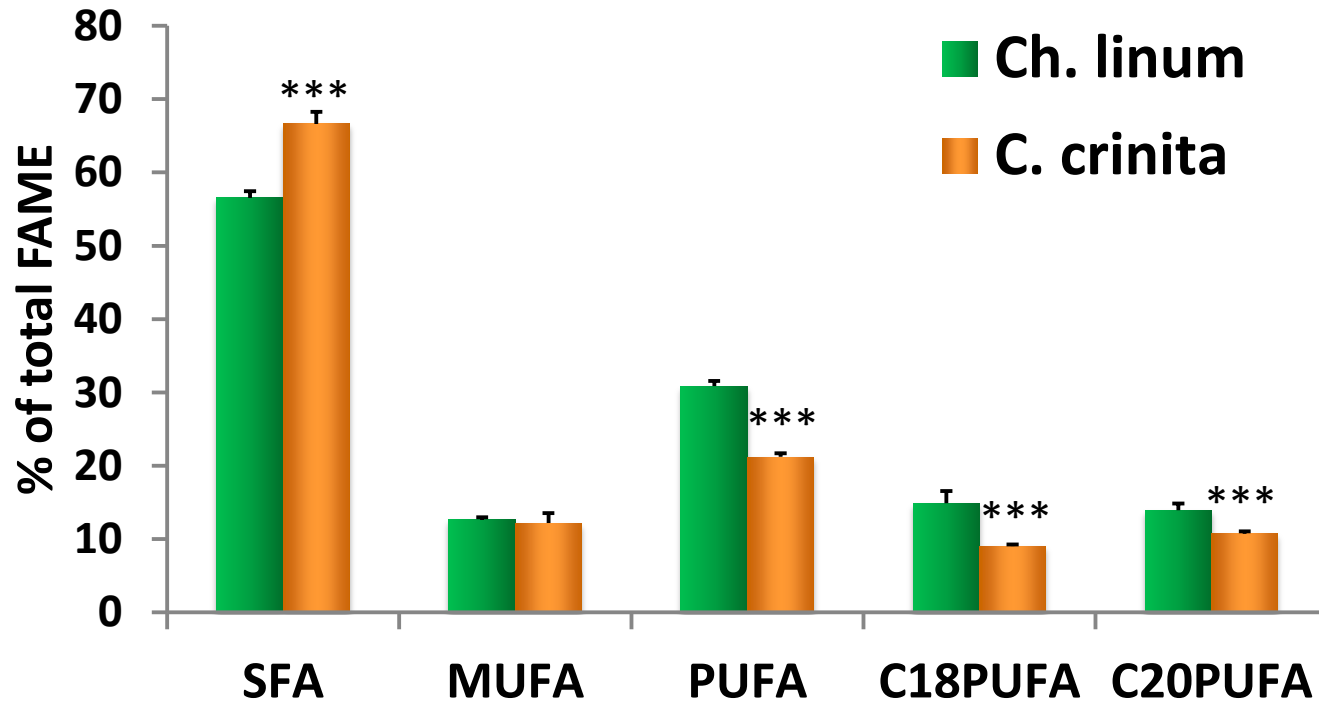
C 18:3 n3 (ALA )  
C 20:3 n3  
C 20:5 n3 (EPA)  
C 22:6 n3 (DHA)

## $\omega$ -6

C 18:2 n6 (LA )  
C 18:3 n6  
C 20:3 n6  
C 20:4 n6 (ARA)



# Total lipids and fatty acid composition



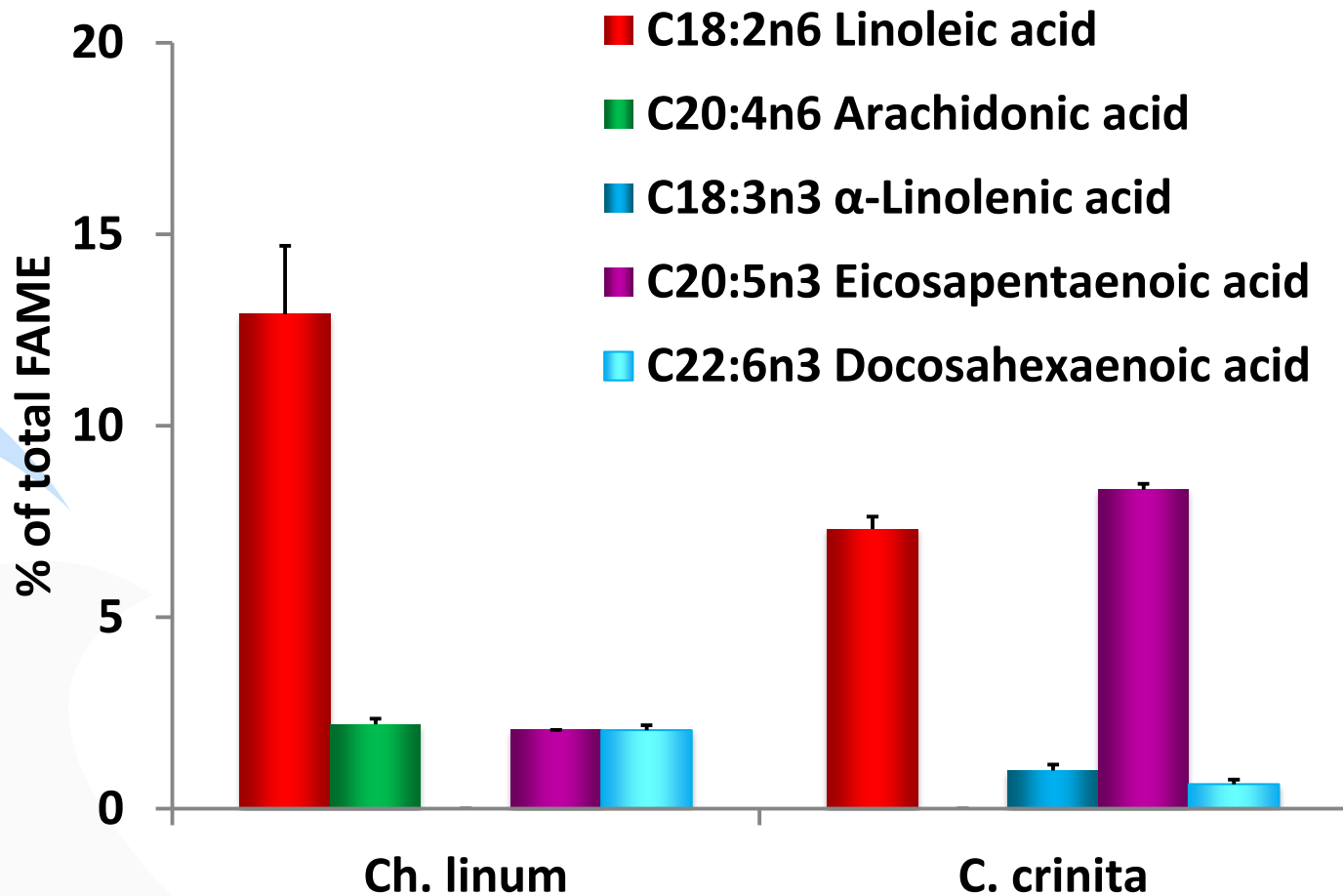
Fatty acid profile of *Chaetomorpha linum* and *Cystoseira crinita*

Significance level  $p < 0.05$





# Total lipids and fatty acid composition

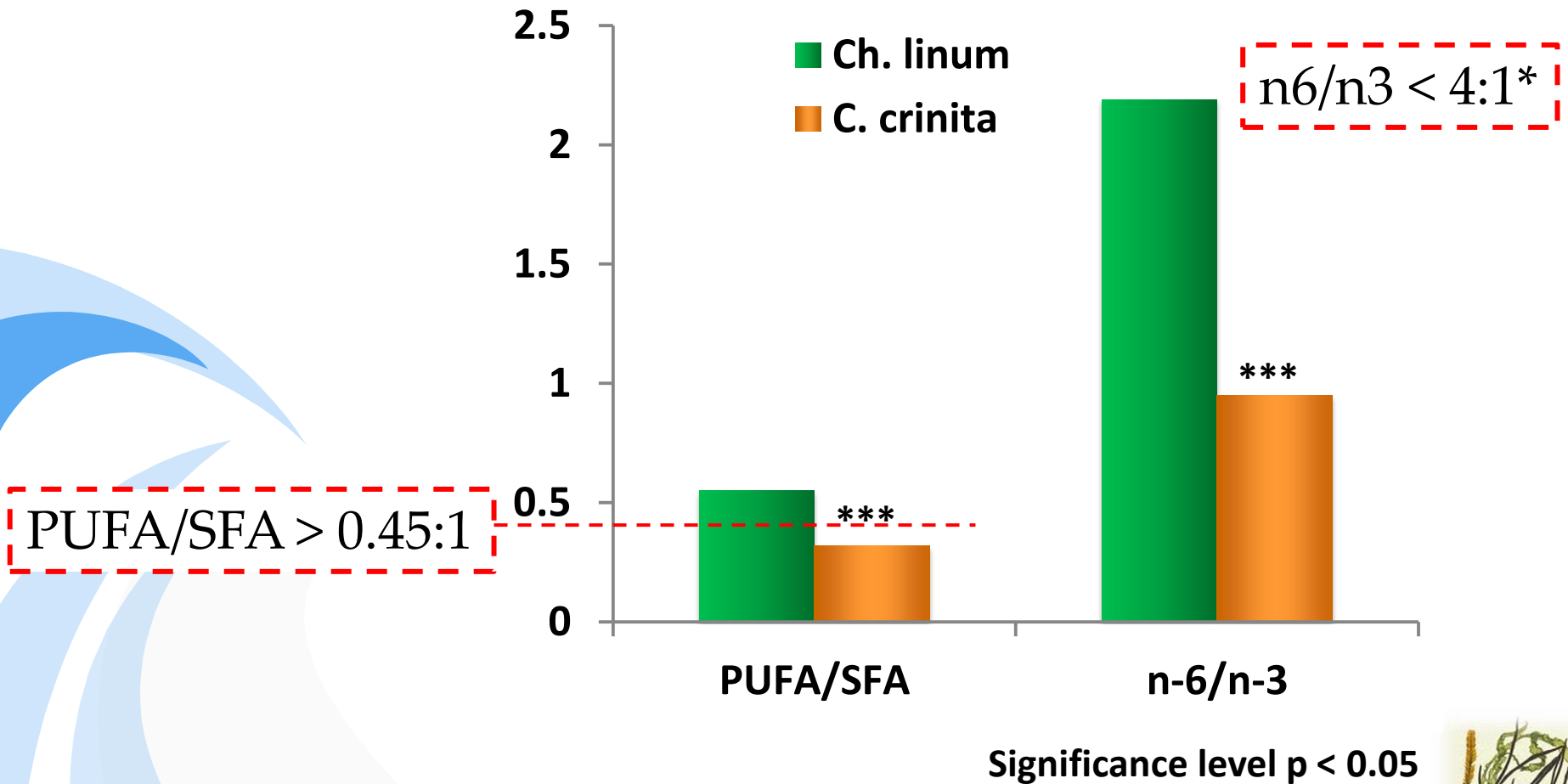


Fatty acid profile of *Chaetomorpha linum* and *Cystoseira crinita*

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# Total lipids and fatty acid composition



# Fat soluble vitamins and carotenoids

- ✓ Homogenized tissue - 1% methanolic L-ascorbic acid and 0.3M methanolic potassium hydroxide
- ✓ Saponification at 40°C for 30 min
- ✓ Extraction with n-hexane:dichloromethane
- ✓ Evaporation under nitrogen



# Fat soluble vitamins and carotenoids

## *Apparatus*

- ✓ Thermo Scientific Spectra SYSTEM HPLC/UV/FL
- ✓ RP Synergi 4 $\mu$  Hydro-RP 80A pore 250x4,6 mm column
- ✓ Gradient

## *Detection*

- ✓ UV detector: astaxanthin ( $\lambda=474\text{nm}$ ),  $\beta$ -carotene ( $\lambda=450\text{nm}$ ), ergocalciferol ( $\lambda=265\text{ nm}$ ), cholesterol ( $\lambda=208\text{ nm}$ )
- ✓ FL detector:  $\alpha$ -tocopherol ( $\lambda_{\text{ex}}=288\text{ nm}$ ;  $\lambda_{\text{em}}=332\text{ nm}$ )
- ✓ **Quantification** – External calibration



# Fat soluble vitamins and carotenoids

	Chaetomorpha linum	Cystoseira crinita
Astaxanthin	0.17 ± 0.01	6.68 ± 0.57***
β-carotene	0.18 ± 0.03	8.99 ± 1.49***
Retinol equivalent	0.03	1.50
α-tocopherol	12.40 ± 0.73	59.24 ± 1.03***
Ergocalciferol	0.28 ± 0.08	0.42 ± 0.11*

+Results are expressed as *mg per 100g dw*

Significance level  $p < 0.05$



# Conclusions

- ✓ Three classes of fatty acids were identified in Black Sea *Chaetomorpha linum* and *Cystoseira crinita* in the following order:

**SFA > PUFA > MUFA**

- ✓ Although lipid content was generally low, *Cystoseira crinita* contained high proportions of the “fish fatty acid” eicosapentaenoic acids (EPA, C20:5n3), which plays crucial role in proper development of the nervous system and prevention of cardiovascular diseases



# Conclusions

- ✓ Marine macroalgae form a good, durable and virtually inexhaustible source of PUFA with n6/n3 ratio between 1.0 and 2.5. This ratio is recommended by the WHO to be less than 4 in order to prevent inflammatory, cardiovascular and nervous system disorders.
- ✓ A possibility for the utilization of marine plants, especially fatty acids and  $\alpha$ -tocopherol-rich species could be in the development of novel foods such as “functional foods”
- ✓ High concentrations of  $\alpha$ -tocopherol,  $\beta$ -carotene, polyunsaturated fatty acids and the presence of the powerful antioxidant astaxanthin demonstrate possible application of *Cystoseira crinita* as supplements for use in food, pharmaceutical industry and cosmetics







- ✓ Total lipids – *Chaetomorpha linum* ( $1.07 \pm 0.12\text{g}$  per 100g fw),  
*Cystoseira crinita* ( $0.70 \pm 0.05\text{g}$  per 100g fw)
- ✓ Moisture content - *Chaetomorpha linum* ( $90.82 \pm 0.79\%$ ),  
*Cystoseira crinita* ( $79.14 \pm 0.91\%$ )
- ✓ Gradient - Solvent A: methanol-water (93:7), solvent B:  
acetonitrile and solvent C: 2-propanol
- ✓ The gradient changed as follows: 0-16.0 min, 100 % solvent A,  
20.0-30.0 min, 60% solvent B and 40 % solvent C, 30.0-40.0 min,  
50 % of solvent B and 50% solvent C. The gradient was then  
returned to 100 % of solvent A

