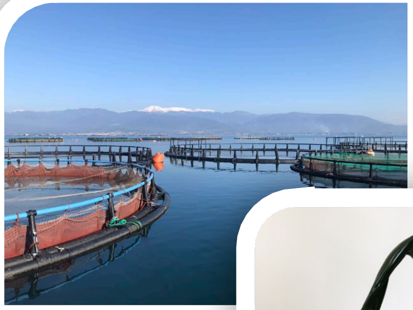
Large-scale cultivation of the microalgae *Tetraselmis striata* and utilization of the biomass produced as alternative raw material in fish feed

Dr. George Triantaphyllidis (gvtrianta@hcmr.gr) – Rome – 25/05/2023



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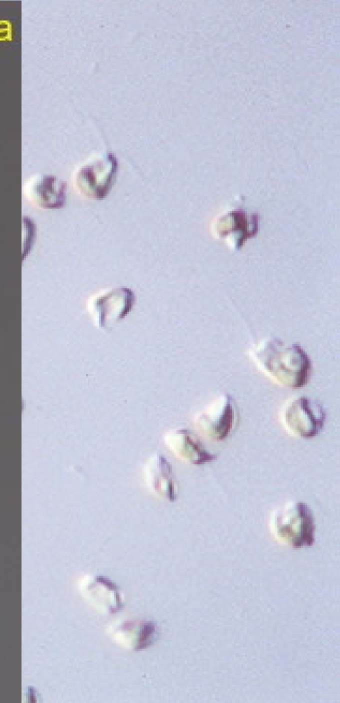
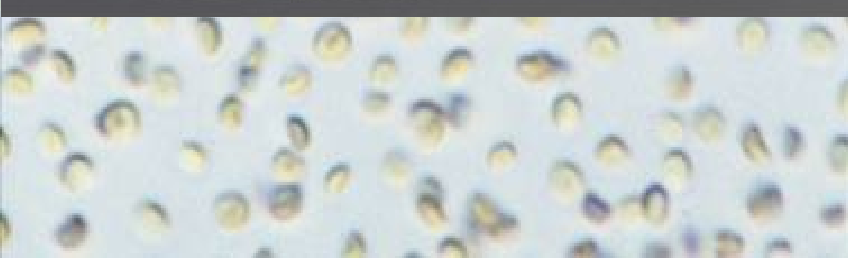
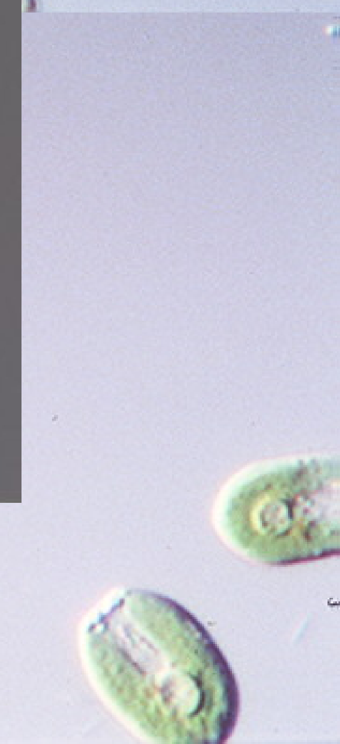
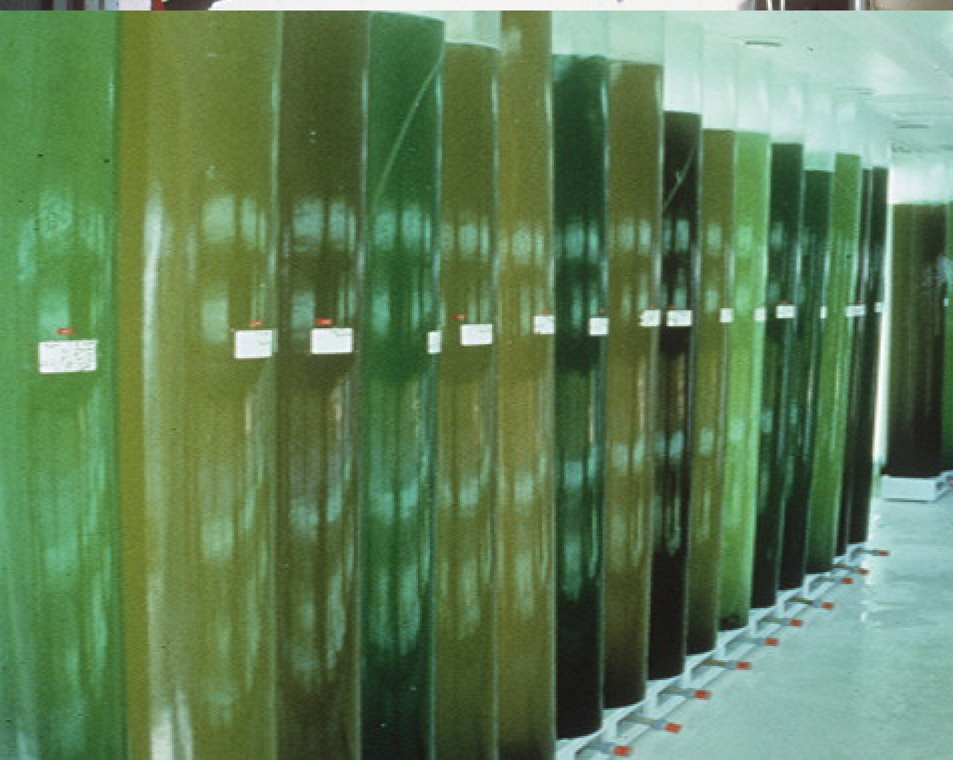
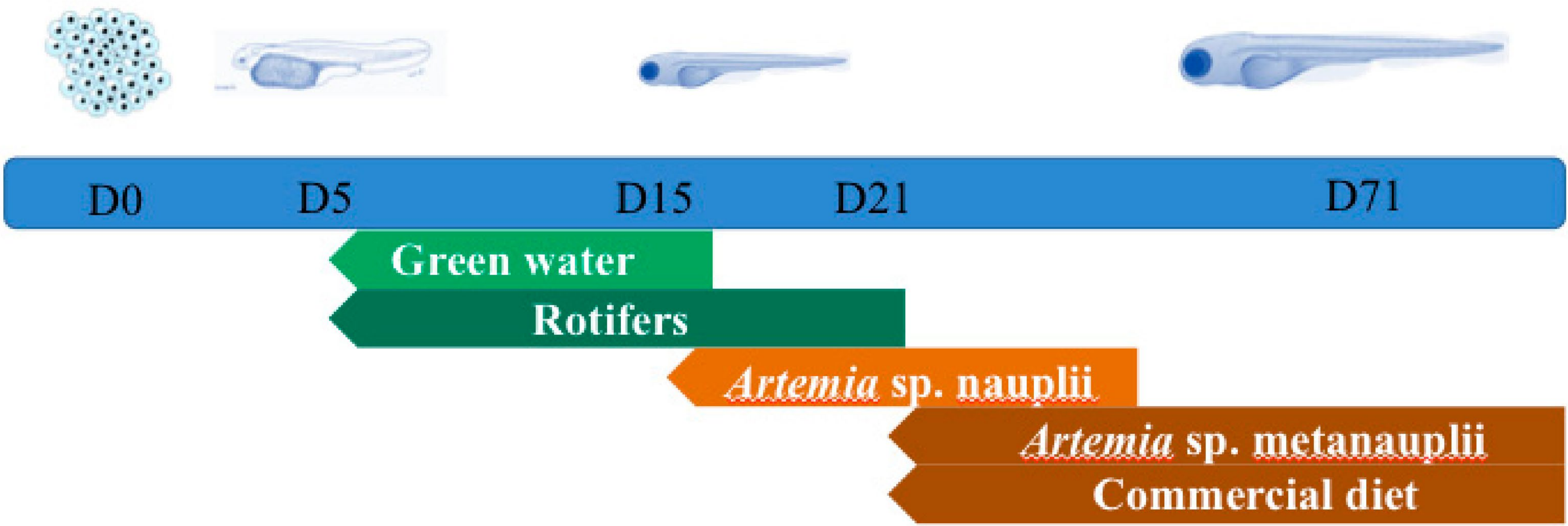






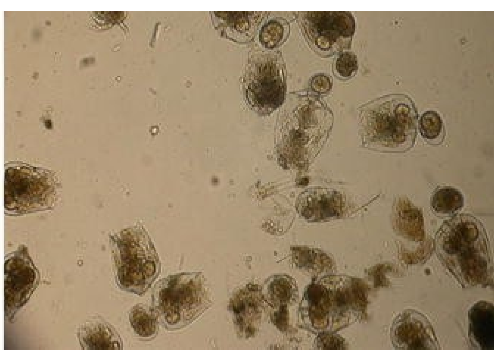
Tetraselmis





Experimental design and trophic stages from day zero (D0) to day 71 (D71) for Gilthead Sea Bream (*Sparus aurata*)

Genes **2019**, *10*(7), 483; **https://doi.org/10.3390/genes10070483**



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Article

Optimization of Cultivation Conditions for *Tetraselmis striata* and Biomass Quality Evaluation for Fish Feed Production

Vasiliki Patrinou [1], Alexandra Daskalaki [2], Dimitris Kampantais [3], Dimitris C. Kanakis [3], Christina N. Economou [4], Dimitris Bokas [5], Yannis Kotzamanis [3], George Aggelis [2], Dimitris V. Vayenas [4] and Athanasia G. Tekerlekopoulou [1,*]

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Water 2022, 14, 3162. https://doi.org/10.3390/w14193162

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Large-scale microalgal cultivation and use of the produced biomass as an alternative raw material in fish

Cultivation of the microalgae *Tetraselmis striata*

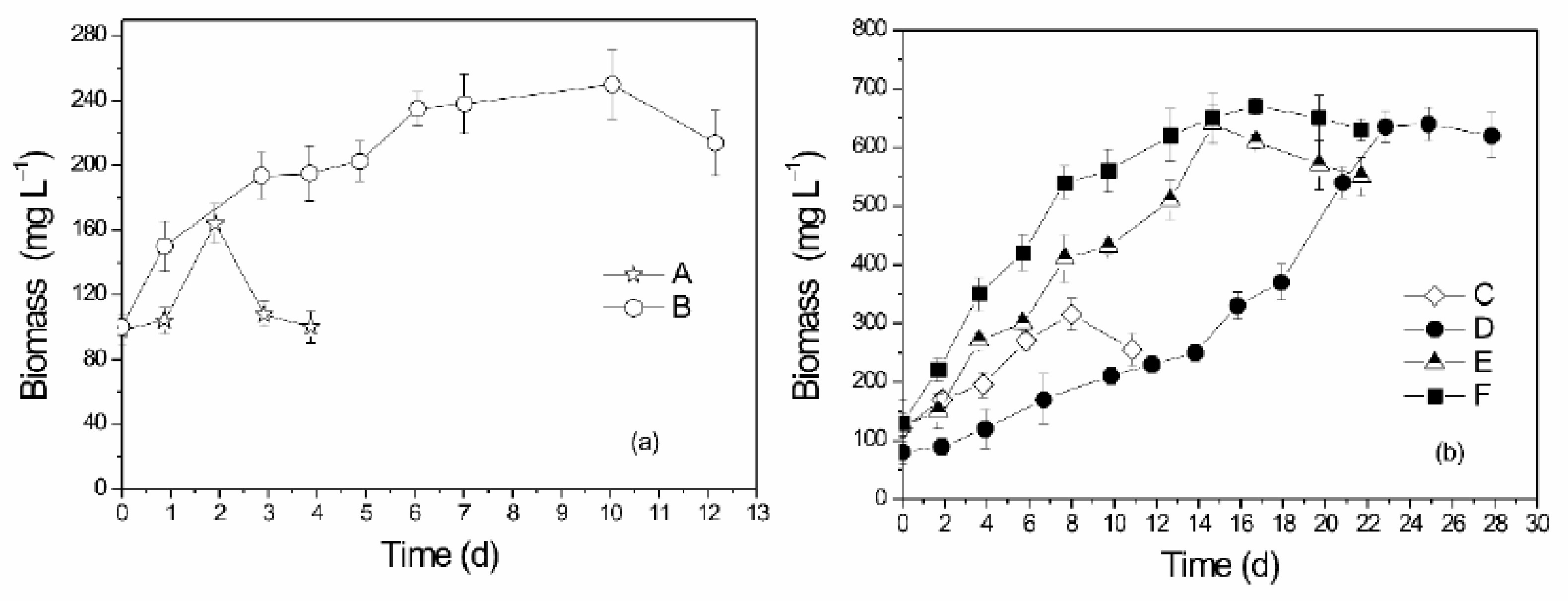

Figure 1. Effect of salinity and substrate composition on the biomass production of *T. striata*. Experimental sets: (a) A (salinity 3.9 ± 0.1%, N:P≈5), B (salinity 3.9 ± 0.1%, N:P≈12), (b) C (salinity 2.8 ± 0.1%, N:P≈12), D: (salinity 2.8 ± 0.1%, modified F/2), E (salinity 2.8 ± 0.1%, Nutri-Leaf 30-10-10 without $NaHCO_3$), and F (salinity 2.8 ± 0.1%, Nutri-Leaf 30-10-10 with $NaHCO_3$).

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Cultivation of the microalgae *Tetraselmis striata*

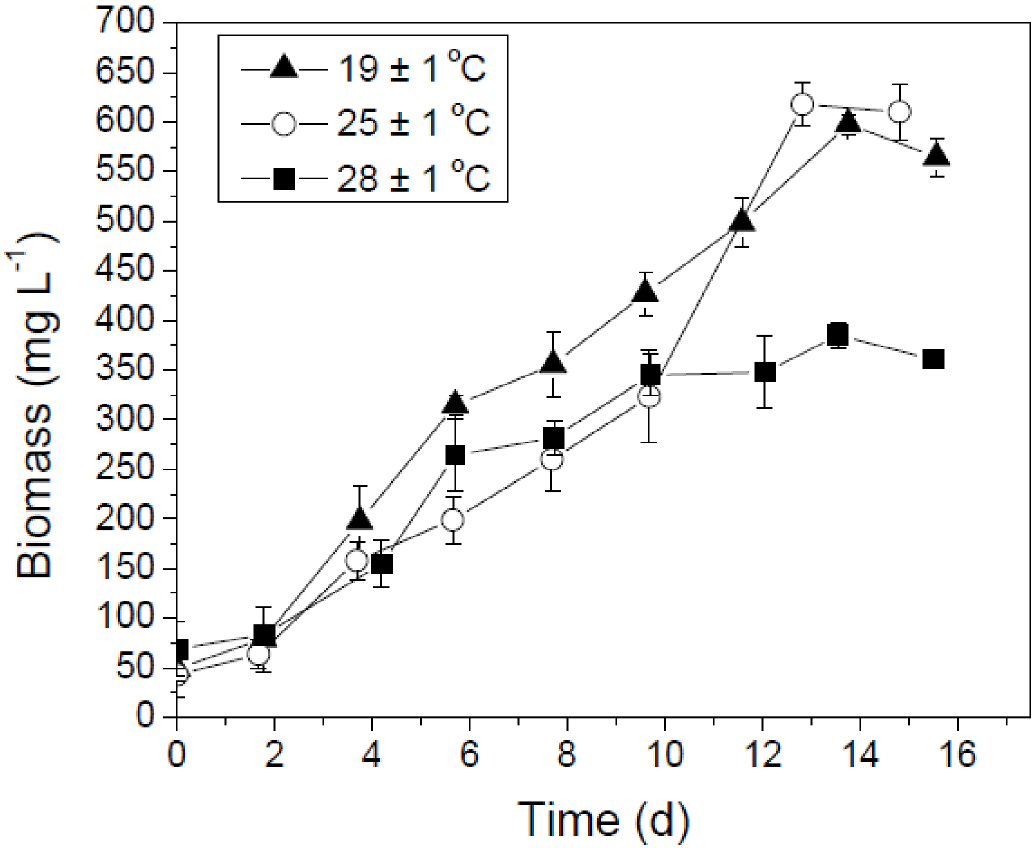
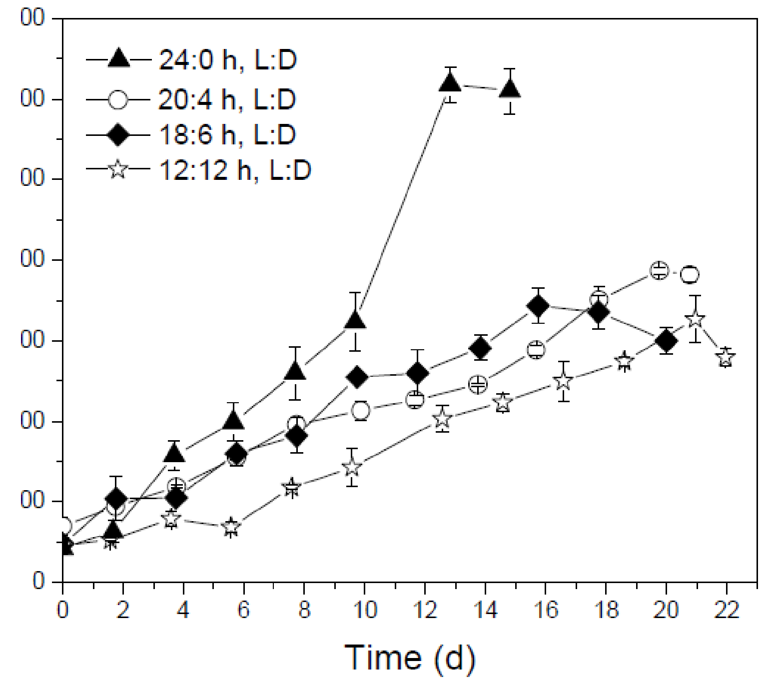


Figure 4. Effect of temperature on the biomass production of *T. striata*.



5. Effect of photoperiod on the biomass production of *T. striata*.

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Cultivation of the microalgae *Tetraselmis striata*

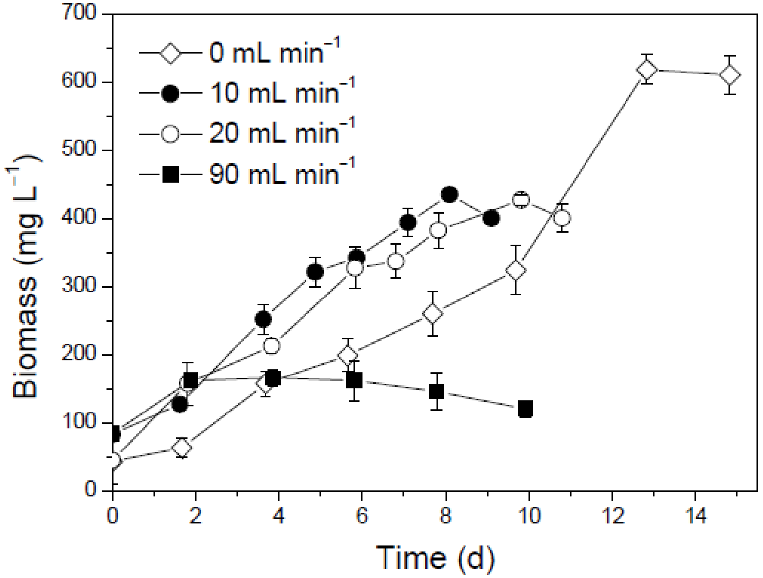
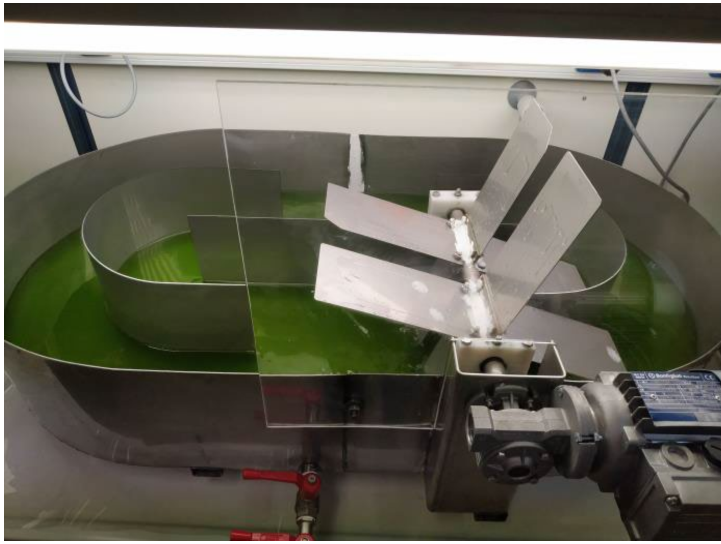


Figure 6. Effect of CO_2 flow rate on the biomass production of *T. striata*.

Table 15. Effect of CO_2 on biomass biochemical composition.

	% d.w. Content				
CO_2 Flow Rate	Proteins	Lipids	Carbohydrates	Total Chlorophylls	Total Carotenoids
10 mL min^{-1}	44.6 ± 0.8	27.3 ± 1.5	10.3 ± 2.6	5.2 ± 0.3	1.13 ± 0.3
20 mL min^{-1}	44.5 ± 0.7	22.5 ± 4.5	11.5 ± 1.0	3.5 ± 0.4	0.78 ± 0.1
90 mL min^{-1}	25.4 ± 0.2	19.0 ± 2.6	11.5 ± 0.7	1.4 ± 0.07	0.19 ± 0.1

Large-scale cultivation of the microalgae *Tetraselmis striata*



Pilot-scale raceway pond of total volume 100 L

15- 45 L

300 L DBPR

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Large-scale cultivation of the microalgae *Tetraselmis striata*

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300 L

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1.1 Biochemical composition and fatty acid composition of the *Tetraselmis striata* produced biomass and the commercial alternative.

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Microalgae Biomass	Produced *T. striata*	Commercial *T. striata*
Crude Protein (%)	39.9	43.03
Crude Fat (%)	9.57	13.25
*Crude Fiber + N-free extract (%)	16.4	12.89
Crude Ash (%)	25.79	26.69
Moisture (%)	8.34	4.14
**Gross energy (MJ kg^{-1})	15.57	17.65

Fatty acid composition as a percentage of total identified fatty acids (%)	Produced *T. striata*	Commercial *T. striata*
C16:0 (Palmitic)	21.16	17.47
C18:1 n9 cis (Oleic)	8.54	6.97
C18:2 n-6 cis (LA) (Linoleic)	3.03	3.11
C18:3 n3 (ALA) (α-Linolenic)	13.58	15.24
C18:4 n3 (Stearidonic)	7.36	8.91
C20:5 n-3 (EPA)	4.40	5.23
C22:6 n-3 (DHA)	0.13	n.d
Σ Saturates	25.95	22.16
Σ Monoenes	14.67	13.24
Σ PUFA n-3	25.83	30.02
Σ PUFA n-6	3.76	4.09
n-3/n-6	6.87	7.34

Essential AA (g/100g feed)	Produced *T. striata*	Commercial *T. striata*
Lysine	2.26	2.60
Methionine	0.47	0.52
Histidine	0.51	0.68
Isoleucine	1.23	1.29
Leucine	2.64	2.76
Phenylanine	1.38	1.74
Threonine	1.50	1.69
Valine	1.74	1.91
Arginine	1.84	2.49

Non Essential AA (g/100g feed)	Produced *T. striata*	Commercial *T. striata*
Taurine	0.52	0.73
Tyrosine	0.80	1.14
Cysteine	0.09	0.13
Hydroxiproline	0.15	0.04
Serine	1.45	1.60
Alanine	2.68	2.83
Proline	1.55	1.66
Glutamic Acid	4.21	4.56
Aspartic Acid	3.27	3.54
Glycine	1.76	1.99

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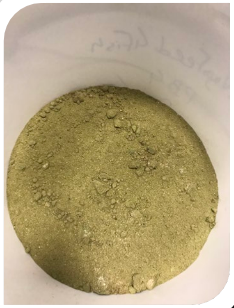
1.2 Feed formulation using *Tetraselmis striata* strains to partially substitute fish meal in the diets of European seabass (*Dicentrarchus labrax*)

Raw Materials	Control	PB 4	CB 2	CB 4	CB 8
Fish meal 67%	32.50	28.50	30.50	28.50	24.50
Produced T. Striata	0.00	4.00	0.00	0.00	0.00
Commercial T. striata	0.00	0.00	2.00	4.00	8.00
Soya Protein Concentrate 60%	10.00	12.10	10.91	11.83	13.65
Wheat flour	14.90	12.48	13.90	12.91	10.92
Soya Cake 44%	10.00	10.00	10.00	10.00	10.00
Corn gluten 60%	8.00	8.00	8.00	8.00	8.00
Wheat gluten	8.00	8.00	8.00	8.00	8.00
Sunflower cake	2.75	2.75	2.75	2.75	2.75
Fish oil	12.92	12.92	12.84	12.77	12.63
L-Lysine	0.00	0.07	0.03	0.06	0.12
Methionine	0.00	0.04	0.02	0.04	0.09
L-Threonine	0.00	0.01	0.00	0.01	0.02
Monocalcium Phosphate	0.69	0.88	0.79	0.89	1.08
Premix Vitamins & Minerals	0.25	0.25	0.25	0.25	0.25



1.3 Biochemical composition of the produced experimental feeds using *Tetraselmis striata* strains to partially substitute fish meal in the diets of European seabass (*Dicentrarchus labrax*)

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Experimental Feeds	Control	PB4	CB2	CB4	CB8
Crude Protein (%)	44.33	44.75	44.86	43.70	43.94
Crude Fat (%)	16.51	16.72	16.38	16.52	16.46
*Crude Fiber + N-free extract (%)	23.84	22.67	23.69	23.66	23.37
Crude Ash (%)	8.11	8.61	8.43	8.84	9.28
Moisture (%)	7.21	7.24	6.64	7.27	6.94
**Gross energy (MJ kg^{-1})	21.09	21.07	21.13	20.91	20.89

Fatty acid composition as a percentage of total identified fatty acids (%)	Control	PB4	CB2	CB4	CB8
C16:0 (Palmitic)	13.60	13.76	14.01	13.73	13.86
C16:1 n7 (Palmitoleic)	4.18	4.16	4.21	4.12	4.09
C18:1 n9 cis (Oleic)	20.95	21.07	21.16	21.03	21.22
C18:2 n-6 cis (LA) (Linoleic)	11.14	11.23	11.18	11.19	11.43
C18:3 n3 (ALA) (α-Linolenic)	2.48	2.69	2.55	2.74	2.99
C18:4 n3 (Stearidonic)	1.69	1.80	1.71	1.81	1.95
C20:5 n-3 (EPA)	6.33	6.17	6.01	6.12	6.09
C22:6 n-3 (DHA)	7.61	7.19	7.04	7.43	7.24
Σ PUFA n-3	19.37	19.07	18.51	19.32	19.47
Σ PUFA n-6	12.26	12.34	12.21	12.31	12.51
n-3/n-6	1.58	1.55	1.52	1.57	1.56
Σ Saturates	22.44	22.57	22.94	22.22	22.32
Σ Monoenes	39.40	39.33	39.57	39.33	39.14

Five isonitrogenous and isoenergetic diets produced

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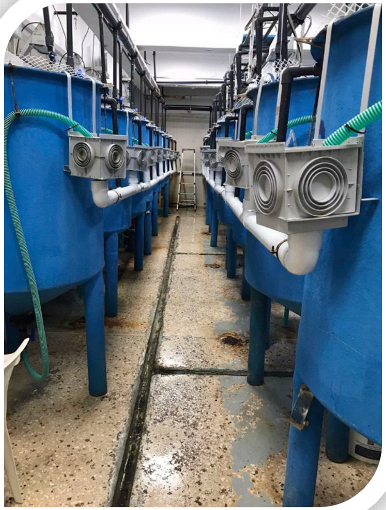
1.4 Experimental Design of the feeding trial

- Fish were transferred from commercial aquaculture open cages to our experimental facilities
- Were left to acclimatize for 10 days,
- Afterwards, fish were individually weighted to calculate the fish initial population frequencies
- 15 Fish with an average weight of 25.46g were separated in 15 cylindroconical tanks with a volume of 1m^3 (triplicates) in a programmable logic controller (PLC) controlled Recirculating aquaculture system (RAS)
- The fish were fed to apparent satiation (*ad libitum)* for approximately 2 months.



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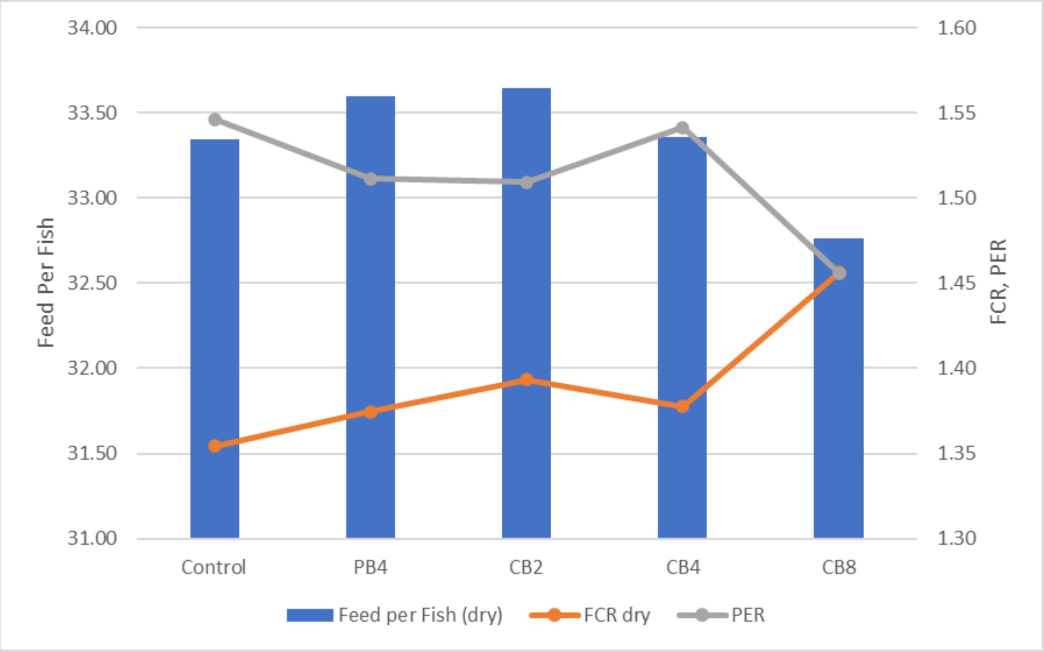
1.5 Growth Performance indices

Experimental Feeds	Control	PB4	CB2	CB4	CB8
Initial Fish Weight	25.42	25.55	25.62	25.44	25.28
Final Fish Weight	50.07	50.09	50.10	49.68	47.82
Fish Weight Gain	24.64	24.53	24.47	24.24	22.54
Biomass gain (%)	96.88	95.91	95.71	95.26	89.12
Survival (%)	100	100	100	100	100
TGC	0.57	0.56	0.56	0.56	0.53
DGI	1.31	1.30	1.29	1.29	1.22

Experimental Feeds	Control	PB4	CB2	CB4	CB8
Feed per fish wet	35.93	36.22	36.04	35.97	35.21
Feed per Fish (dry)	33.34	33.59	33.65	33.36	32.76
FCR wet	1.46	1.48	1.49	1.49	1.56
FCR dry	1.35	1.37	1.39	1.38	1.46
SGR	1.19	1.18	1.17	1.17	1.12
PER	1.55	1.51	1.51	1.54	1.46
PER dry	1.67	1.63	1.62	1.66	1.56
DFI wet	8.37	8.51	8.42	8.39	8.25
DFI dry	7.76	7.90	7.87	7.78	7.67

1.6 Conclusions

- Fish growth and health were satisfactory in all experimental diets and zootechnical indicators representative of fish size and species
- The addition of microalgal biomass in the aquafeeds didn't seem to affect the palatability of the experimental feeds
- Commercial and produced biomass of the *Tetraselmis striata* strain didn't present any statistically significant differences in terms of growth
- In the targeted fishmeal substitution levels (2, 4 and 8%), the *T. striata* could successfully partially replace fish meal as the results showed a comparable growth within the 2 month feeding trial



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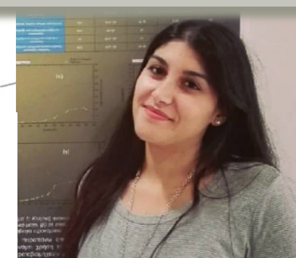

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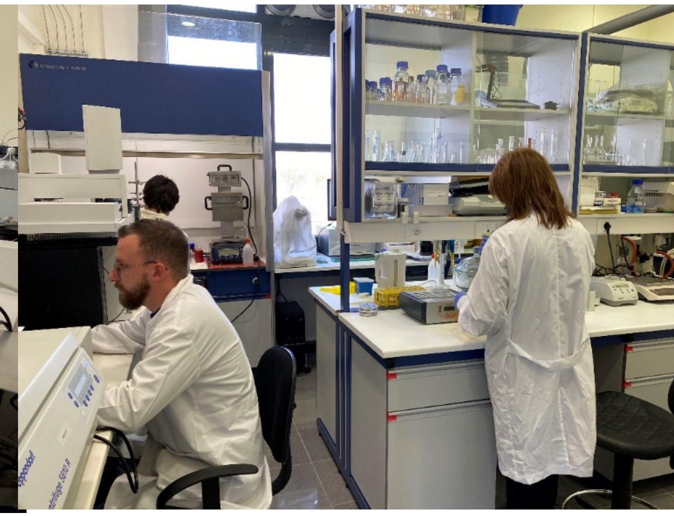

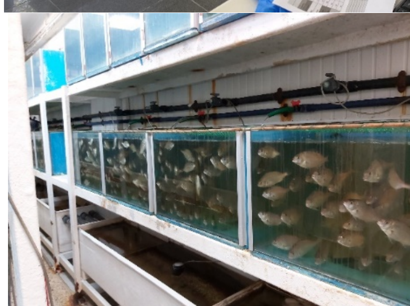

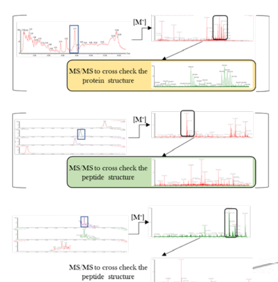

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Large-scale cultivation of the microalgae *Tetraselmis striata* and utilization of the biomass produced as alternative raw material in fish feed

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