Microalgae

1

Microalgae: Systematic and diversity

Microalgae functional definition:

Organisms capable of performing photosynthesis.

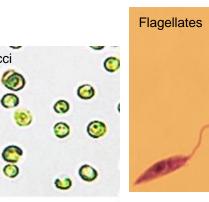
These include:

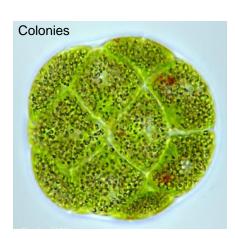
- Prokaryotes (Cyanobacteria & Prochlorobacteria)
- Herbal protists (herbal progenitors)
- Many mixotrophic representatives (auto and heterotrophic)

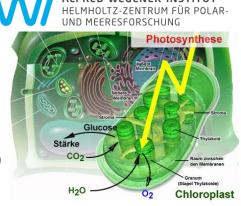
Life forms:

- Trichomes (multicellular filaments)
- Cocci
- Flagellates
- Colonies (three-dimensional)









Microalgae culture

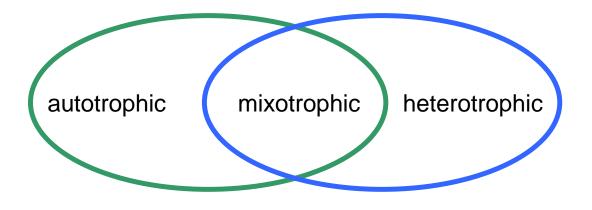






- Autotrophy
 - Energy production by inorganic substrates (phototroph = by light)

- Heterotrophy
 - The ability or requirement to synthesize all metabolites from organic compounds





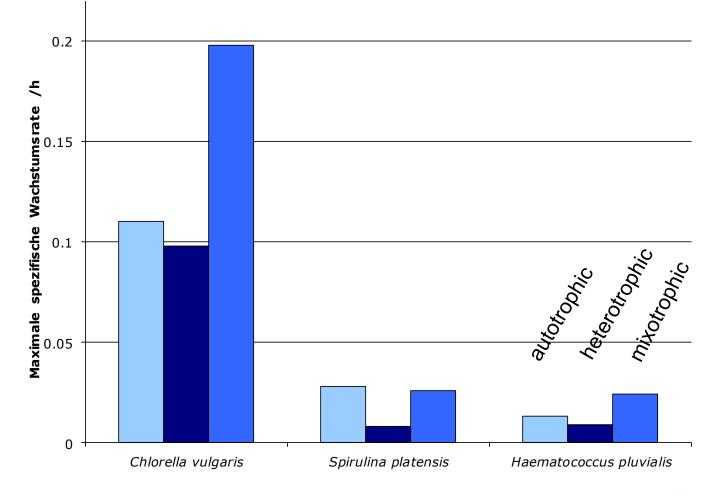






Microalgae culture: Metabolism







Microalgae culture







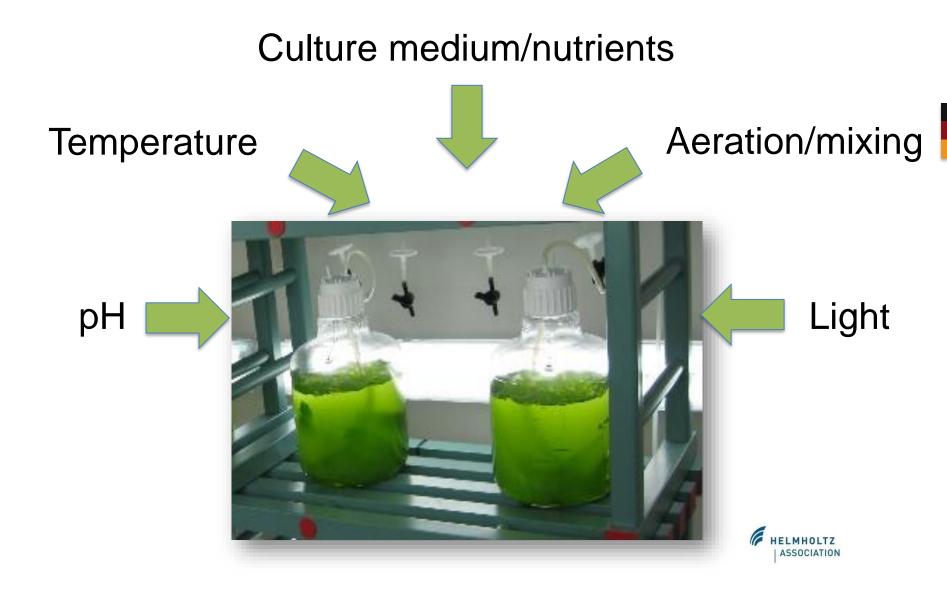






Microalgae culture: Culture parameters

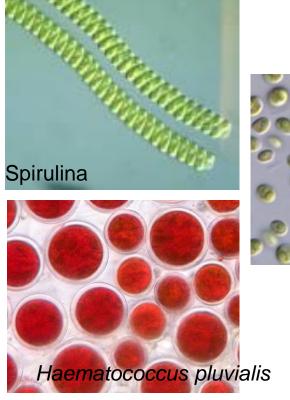




Microalgae culture: Species selection



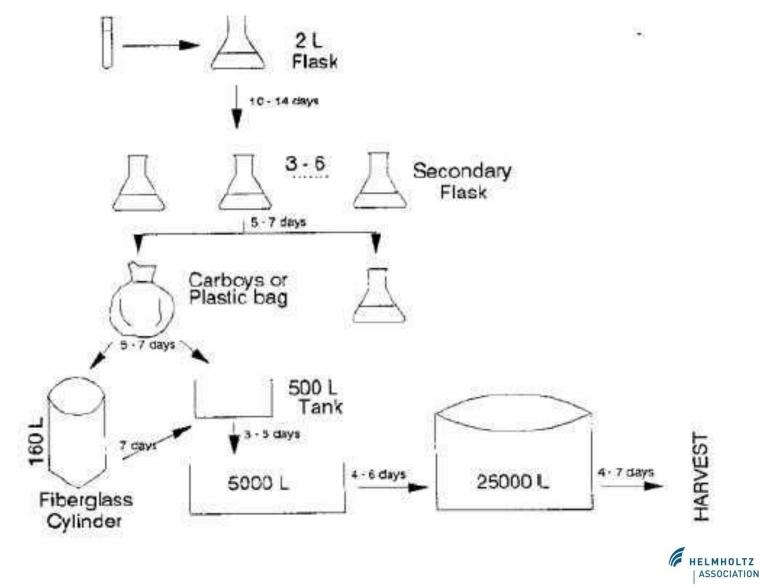
- Species selection
 - Ease of culture in traditional culturing systems
 - High productivity
 - Important bio compounds
 - Fatty acids;
 - Pigments;
 - Proteins/aminoacids
 - High value biomass













OLTZ

- **Open systems**
- exposed to the atmosphere / direct exchange between culture and environment







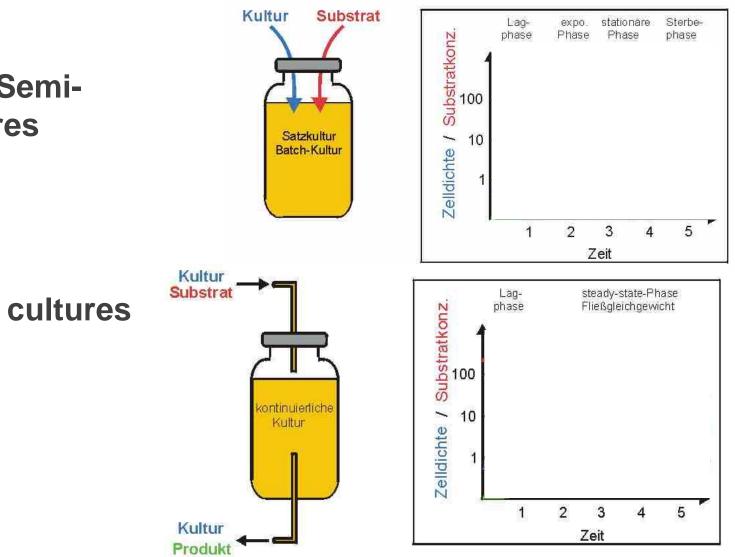


- Closed systems
- largely limited exchange between culture and environment









 Batch- and Semibatch cultures

Continuous cultures

Open systems: natural water bodies



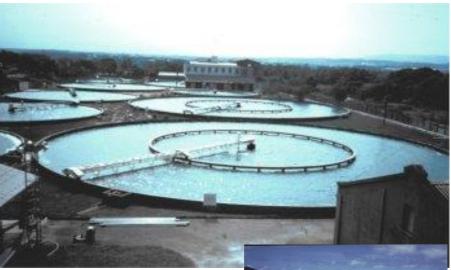


Burma: harvest with buckets from the lake and linen bags



Open systems: open pond or raceway pond



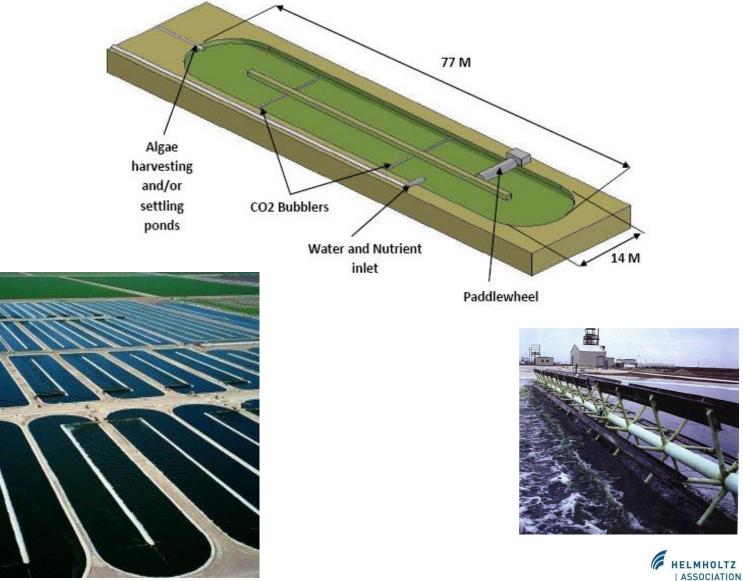


- Low cell densities lead to large amounts of culture
- Frequently extremophilic species
 Dunaliella salina - high salinity
 Spirulina - alkaline medium
 Chlorella - high nutrient concentration
- Simple and cost-effective method



ASSOCIATION







Closed systems: Photobioreactors or fermentors

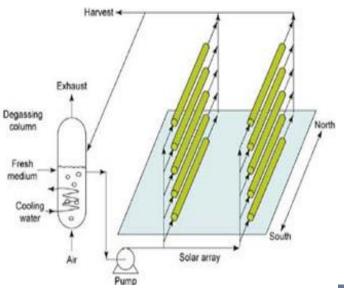
 Circumvented from the atmosphere (Gas exchange prevented - technical supply of gases)
 Tubular systems
 Flat-panel systems
 Plastic bag systems
 etc.
 Fermentors

Optimization on photosynthesis performance





Tubular systems / PBRs







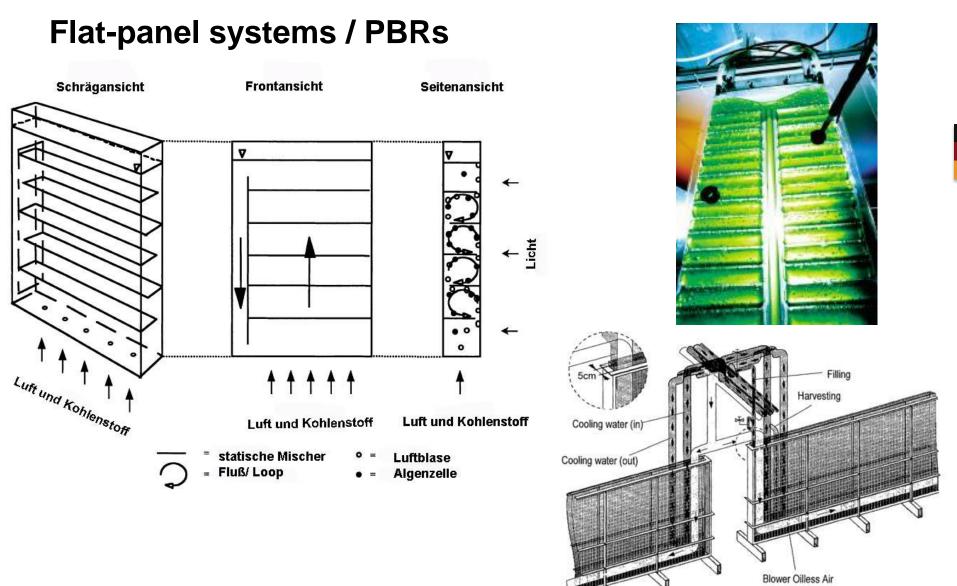














Plastic bag systems / PBRs

















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Merkmal	Offene Systeme	Geschlossene Systeme	
Algenart	Begrenzte Auswahl	Flexibel	
Populationsdichte	Gering	Hoch	
Ernteeffizienz	Gering	Hoch	
Kultivierungszeitraum gemäßigte Breiten	Limitiert (z. B. warme Jahreszeit)	Ganzjährig	
Verunreinigung	sehr groß	Unwahrscheinlich	
Lichtnutzungseffizienz	Gering/mittel	Mittel/sehr gut	
Temperaturkontrolle	Keine	Sehr gut	
Qualität der Biomasse	Anfällig	Nicht anfällig	
Flexibilität der Produktion	Kaum möglich	Problemlos möglich	
Vergleichspräzision	Nicht	Zu gewissem Grad möglich	
Prozesskontrolle	Nicht	Gegeben	
Wetterabhängigkeit	Stark	Insignifikant	
Effektivität des "Behandlungs-" Prozesses	Niedrig, Zeitaufwändig, großes Volumen durch geringe Konzentration	Hoch, kurze Zeit, relativ kleines Volumen	

Microalgae culture: Status quo



Used culturing systems in global ** and European microalgae companies from Vieira & Pulz, not publ., from Pulz, 2007.

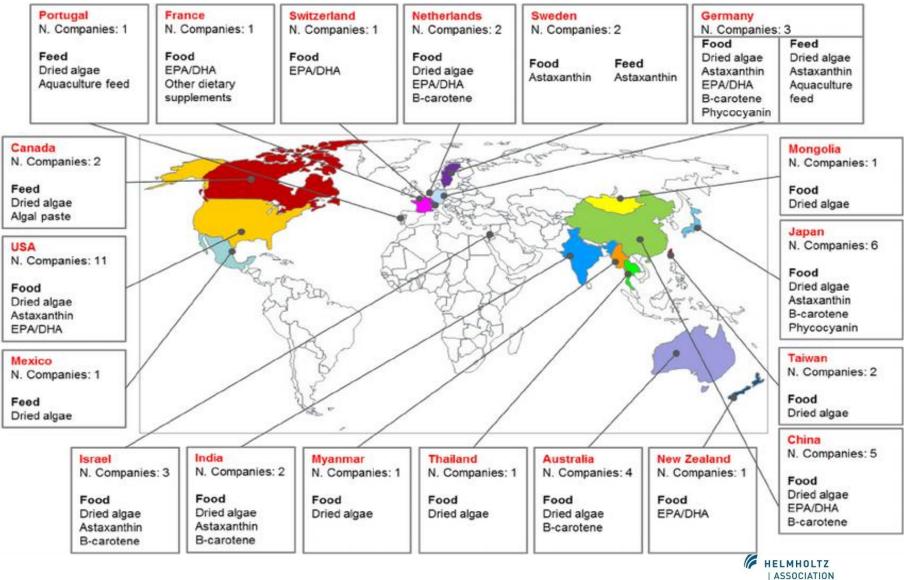
Culture system	Europe	World**
Heterotrophic	4	7
Phototrophic		
- lakes	1 (+2)***	9
- ponds (open ponds and raceway)	13	41
Closed photobioreaktors	10	9
Total	28	66
Failed attempts (for 20 years)	20	64
		HELMHOLTZ

** excl. Europe; *** Production abroad in European possession

Microalgae culture: Status quo



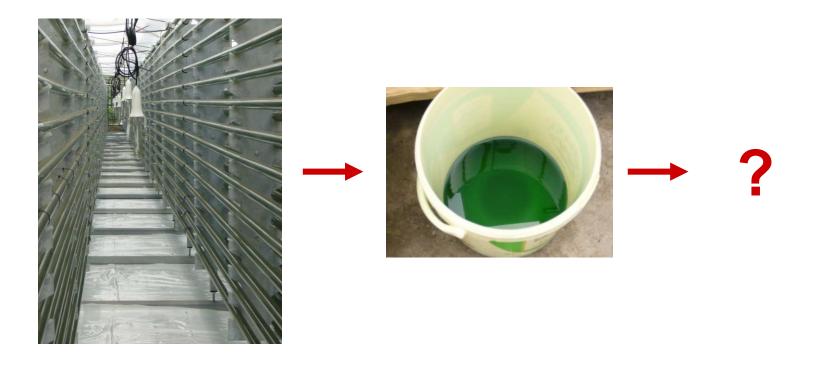
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from Vigani et al. 2015

Microalgae culture: Utilization







Microalgae culture: Utilization



Products and visions

- mostly "high value" products
- isolated products: dyes, fatty acids for the pharmaceutical industry, cosmetics, etc.
- energy extraction
- biomass: food and feed for animals Aquaculture









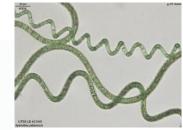














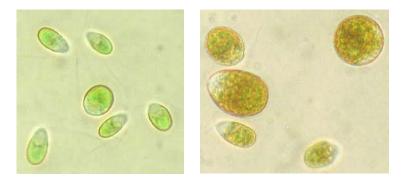


Denvite & Coolundhoit	Lister	nansicht Bilder	galerie Sortie	eren nach: ba	ld endende A	Angebote zuerst	×	Ansicht anpassen
Beauty & Gesundheit (46) Wohlbefinden & Wellness (44)	+	Vergleichen	Artikelbezeichnung	Gebote	Preis*	Versand nach 28203, DEU		Restzeit 🔺
Gewichtsreduzierung & Diät (2) Bücher (3)		۵	Chlorella Vegi-Kaps 120 Stück - Beste HANNES' Qualität	<i>≡Sofort Kaulen</i>	EUR 14,95	EUR 1,95		5Std. 43Min.
Sachbücher & Ratgeber (3) Ratgeber zu ähnlichen			Bärbel Drexel Chlorella Spirulina Guarana WildYams Set	4	EUR 15,60	EUR 5,30	P	5Std. 48Min.
Produkten			Set					
Spirulina platensis Hallo Ebayer Augen a ALGENMASKE FŬR DEN K		Chorels	manako® Chlorella Presslinge 2x250g Dose, 2000 Stück	≅Sofort Kaufen	EUR 36,90 EUR 39,90	EUR 5,90	P	6Std. 02Min.
Alle Ratgeber zu ähnlichen Produkten aufrufen Suchoptionen			Chlorella , ohne Zusätze, rein, 2500 x400mg= 1kg Geprüfte Ware, 1A Qualität!! MHD 4/10	Sofort Kaufen oder Preis vorschlagen	EUR 50,90	EUR 4,90		6Std. 23Min.
Ort		đ	CHLORELLA ALGE 250 KONZENTRAT TABS JE 1000 MG NUR 14,90	⊊Sofort & Neu	EUR 14,90	EUR 7,70 Aus Vereinigte Staaten von Amerika	P	6Std. 31Min.
200 💌 km von 28203		đ	ARNDT, ULRICH: Spirulina, Chlorella, AFA-Algen GEB	⊊Sofort & Neu	EUR 12,90	Kostenlos	P	6Std. 43Min.
Nur anzeigen:			SPIRULINA 2500 TAB A 400 MG, 1 KG+BONUS 60 St CHLORELLA		EUR 29,00	EUR <mark>6,95</mark> Aus Niederlande		6Std. 46Min.
Auktionen			CHLORELLA 4000 Tbl= 1kg SPITZEN Qualität mitNachweis	13	EUR 61,00	EUR 7,00	P	7Std. 31Min.

Suchergebnisse ein	Lister	ansicht Bilder	galerie Sortieren (nach: bald e	ndende Ange	bote zuerst	¥	Ansicht anpasser
Tierwelt (145) Fische (141)	-	Vergleichen	Artikelbezeichnung	Gebote	Preis*	Versand nach 28203, DEU		Restzeit 🔺
Reptilien (5) Hunde (1)		đ	100%ige reine Spirulina-Tabletten - 100ml	-	EUR 4,99	EUR 1,80 Aus Österreich	P	1Std. 12Min.
Beauty & Gesundheit (131) Wohlbefinden & Wellness (124)		đ	Fischfutter Flockenfutter SPIRULINA Premiumqualität 2L.	<i>⊊Sofort</i> & Neu	EUR 15,40	EUR 1,90		1Std. 33Min.
Gewichtsreduzierung & Diät (7)		đ	Fischfutter Flockenfutter SPIRULINA Premiumqualität 4L.	I Sofort & Neu	EUR 24,90	EUR 1,90		1Std. 38Min.
Bücher (13) Sachbücher & Ratgeber (11)		đ	1L Koi Pellets 3mm CypriCo Spirulina Koifutter	<i>⊊Sofort</i> & Neu	EUR 4,30	EUR 2,40		2Std. 50Min.
Studium & Wissen (2) Musik (3) Garten (2)		Ø.	Natura Vitalis Spirulina Herzdose 1700 Spiruletten	8	EUR 29,05	EUR 3,90		3Std. 18Min.
Möbel & Wohnen (1) Ratgeber zu ähnlichen Produkten			Spirulina - green power, 500 Kps. *** SENSATIONSPREIS	14	EUR 8,50	EUR 5,50	P	3Std. 18Min.
100g gesunde Futters Spirulina platensis ALGEN-SCHLANKHEITSUM		đ	Spir <mark>ulina - die Wunderal</mark> ge von Frank Felte (2002)	1	EUR 1,00	EUR 1,00	P	3Std. 20Min.
Alle Ratgeber zu ähnlichen Produkten aufrufen		đ	Spirulina die blaugrüne Wundernahrung-Marianne E. Meyer		EUR 1,99	EUR 1,60	P	4Std. 16Min.
Ort			PRO-S <mark>ANA</mark> Spirulina PUR 500 Tabletten à 400mg	320	EUR 7,69	EUR 3,50	P	4Std. 43Min.

Microalgae culture: Isolated products





Dunaliella salina - Accumulation of carotenoids under stress (orange and red pigments)



Haematococcus pluvialis – red colorant

Astaxanthin

Chlorella - yellow colorant

Lutein





Development of a nutritive and neuroprotective algae combination - the RecAl project



:AWI, University of Bremen, MIAL & CRM

Microalgae culture: Isolated products

RecAl



- Algae properties
 - Health promoting effects of specific ingredients e.g. essential aa, PUFA's, vitamins and minerals
 - Substances suitable for a preventive or therapeutic application against neurodegeneration.
- Project aims
 - Develop a formulated, high-quality food supplement (algae extract) from different algae with special emphasis on the neuroprotective effects.

Microalgae culture: Energy extraction



Possibilities of energy	production
-------------------------	------------



Processes	Final product
Extraction and Transesterfication	Biodiesel
Fermentation	Ethanol
Anaerobic digestion	Methan
Gasification	Hydrogen, Syn gas
Drying	Powder



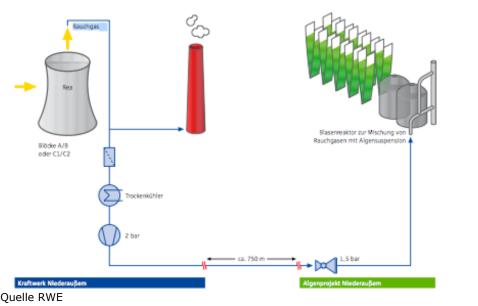
Microalgae culture: Energy extraction

Start: 6.11.08 in Niederaussem

Objective:

- Investigation of options for flue gas utilization
- Optimization of the complete process chain -
- from algae production to the final product using waste heat on flocculated algae to produce dried algae for chemical conversion.

Partners: Phytolutions RWE Jacobs-University, Bremen,







1000 m² photobioreactor installation Phase 1: in house Phase 2 (2011): outdoor

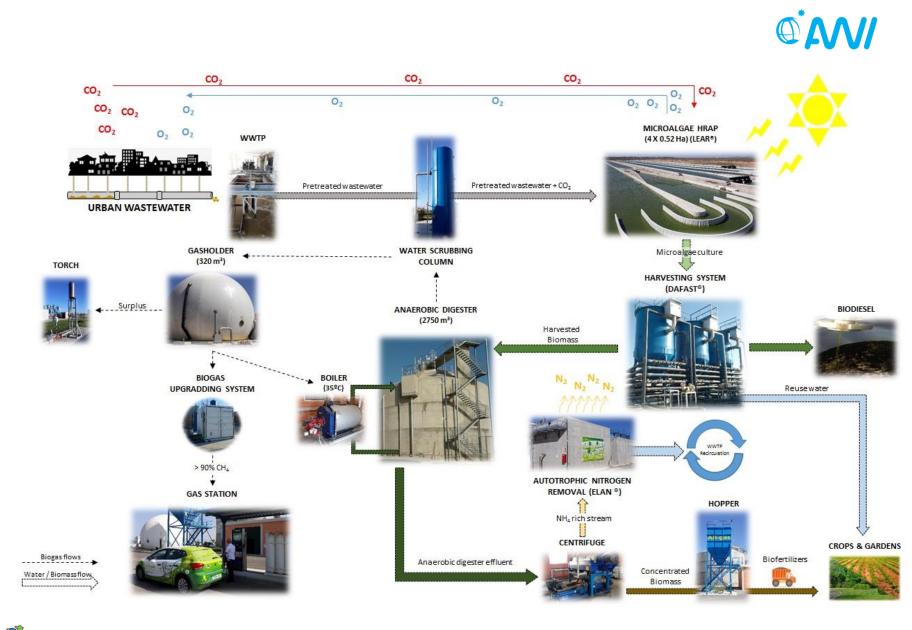
Microalgae culture: Energy extraction project



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Microalgae culture: Energy extraction – Project ALLGAS

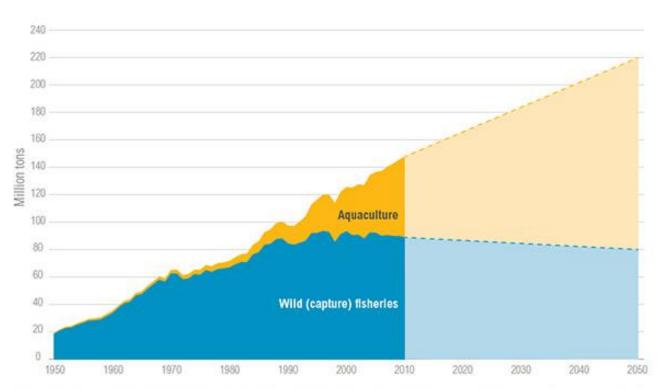






Microalgae culture: Aquaculture





Aquaculture Is Expanding to Meet World Fish Demand

Source: Historical data 1950–2010: FAO. 2014. "FishStatJ." Rome: FAO. Projections 2011–2050: Calculated at WRI, assumes 10 percent reduction in wild fish catch between 2010 and 2050, and linear growth of aquaculture production at an additional 2 million tons per year between 2010 and 2050.

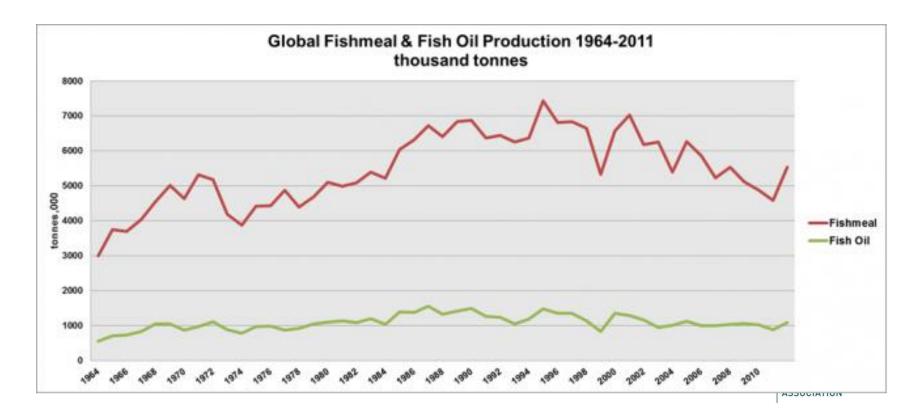
See www.wri.org/publication/improving-aquaculture for full paper.

WORLD RESOURCES INSTITUTE

10% reduction in wild fish catch until 2050! 2 M additional ton per year of aquaculture production from the production for the production of the production

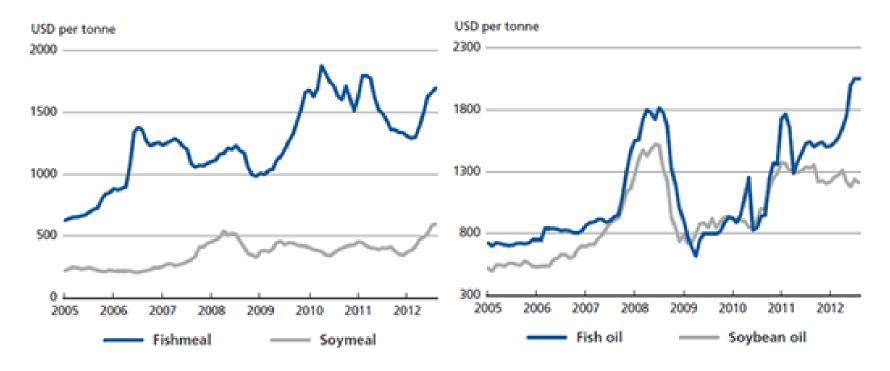


- Fish oil and fishmeal are essential ingredients of aquaculture feed
- Worldwide production of fishmeal and fish oil is stagnating, however...!



- ...prices for fishmeal and fish oil are increasing!
- Prices for alternative protein and lipid sources are also increasing!

OA







The importance of aquaculture

- 2050 an additional **80 M tons** of fish and seafood is required, approx. 50% are fed species!
 - Question: what should we feed?
 - Possible alternatives...
 - Vegetable commodities like soya and lupins
 - Byproducts from fish processing and animal production (e.g. heads, frames, viscera, feather and skin)
 - Insects (e.g. larvae black soldier fly)
 - Microalgae???





Microalgae properties of interest for aquaculture

- Good composition of **amino acids** and **fatty acids**
- Strengthening of the **immune system**
- Improvement of fat metabolism
- Antiviral and antibacterial properties, improves intestinal function
- Increase of the **stress resistance**
- Supply of vitamins, minerals and other bioactive substances





Uses of microalgae in aquaculture

- Replacement of fish oil and/or meal
- Feeding of filter-feeder organisms (e.g. mussels)
- Larval rearing (culture and enrichment of rotatoria, artemia)
- During development stages of shrimp (addition on reaching the Protozoea stage, during the Mysis stages, change to carnivorous lifestyle)
- Integrated Aquaculture (e.g. Aquaponic systems)
- As feed additive / supplement



Microalgae culture: Aquaculture

Tab.: Application of microalgae in aquaculture

Application form	Concentrate/Paste	Powder	Extract
	live	spray dried	water extract
	cooled	drum dried	solution
	frozen	freeze dried	supercritical extraction
Current aquaculture feed	Nursery	Broodstock	Premix feed
	green water	special feed	pigments and omega 3
	crustacean feed	Premix feed	(Astaxanthin, DHA/DPA)
	rotifers feed		
Research		grow-out	grow-out
		fishmeal and fish oil substitute	pigments and omega 3
			(Astaxanthin, DHA/DPA)



- Commercial application of microalgae mainly in niche markets such as larval and broodstock feed so far!
- Reason: Prices for microalgae biomass still so far away from prices of alternative commodities like soya
- BUT!!
 - All major aquaculture feed manufacturers are investing in research to replace fishmeal and fish oil with microalgae!





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Alltech acquires Coppens International, an innovative aquatic feed and nutrition company

🛗 June 10, 2016 📄 Featured, Grain & Wheat News 🥥 No comments



Alltech has acquired Coppens International, a leading international aquatic feed solutions company in the Netherlands. Coppens International is now part of the Alltech family of companies, which includes 14 other companies that Alltech has acquired globally since 2011

For almost 24 years, Coppens International has earned a strong reputation for being an innovative, high-quality aquatic feed producer. The company's specialties include temperate and tropical marine and freshwater diets for a variety of juvenile and adult species. The nany also produces ton-quality ornamental specialty and hait feeds.

The Buzz

range by cutting-e industry May 27, 2016

Always th

in Kentuc

AlgaeIndustryMagazine.com

enmark-based feed company BioMar will start producing fish feed with omega-3 fatty acids obtained from microalgae, in what it says will reduce the stress on marine resources to provide sustainable feed The product was developed in a joint venture with TerraVia Holdings and Bunge and will be produced at their renewable oils manufacturing plant in Brazil, BioMar said in a statement.

BioMar CEO Carlos Diaz calls the

supplies of omega-3 sources



new product a "game changer" as it will reduce stress on limited

BioMar CEO Carlos Diaz has a background in both the aquaculture and pharmaceutical industries

including fishmeal and oil or krill meal, which BioMar uses in its products sold to the aquaculture industry. The added supply will allow the aquaculture industry to grow, he said

"The collaboration with TerraVia and Bunge goes very well in line with our new strategy as a focused aquaculture feed supplier and it will, for sure, be an important step for continuing the development of

Skretting looks to insects and algae as fishmeal alternatives



30-Jun-2014 Last updated on 01-Jul-2014 at 09:37 GMT

Related tags: Skretting, Insect meal, Nutreco, Tilapia, Shrimp, Catfish, Soy, Algae

Nutreco's fish feed division, Skretting, is ploughing a significant amount of its research funds into the development of alternative proteins to fishmeal for the aquaculture sector.

HAMLET 22 PROTEIN

The Dutch group recently organized Aquavision 2014 in Norway around the theme of farmed fish being a key element

in feeding the global population of some nine billion in 2050.

Jose Villalon, corporate sustainability director at Nutreco, told delegates during the three-day event in Stavanger that aquaculture can be used as a model for how protein should be farmed in the future as it has far lower feed conversion ratios compared to pig, chicken, lamb and beef production.

1 comment

BioMar to produce fish feed from microalgae

uct

DSM and Evonik establish Share joint venture for omega-3 fatty acids from natural marine algae for animal nutrition

Kaiseraugst, CH, 08 Mar 2017 08:00 CET

- Breakthrough for animal nutrition to reduce impact on ocean resources and making it more sustainable
- Commercial-scale facility for algal oil for salmon aquaculture and pet food to be built in the United States
- Further develop and produce a high value algal oil with a natural balance of EPA and DHA

Royal DSM and Evonik today announced their intention to establish a joint venture for omega-3 fatty acid products from natural marine algae for animal nutrition. This breakthrough innovation will, for the first time, enable the production of omega-3 fatty acids for animal nutrition without using fish oil from wild caught fish, a finite resource. Evonik and DSM's alternative omega-3 source is the first to offer both EPA and DHA and will be aimed at initial applications in <u>salmon aquaculture</u> and <u>pet food</u>. The companies will together build a commercial-scale production facility in the United States.



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WHAT WE DO

The story of Veramaris starts where all life began - the ocean. Deep down in the sea, we found an original source of the two essential omega-3 fatty acids EPA and DHA. Highly concentrated, our algal oil offers the animal nutrition industry an alternative and reliable source of omega-3.



OMEGA-3 FROM NATURAL MARINE ALGAE

High-quality omega-3 produced from microalgae enables the industry to continue growing within planetary boundaries. Our product is 100% natural and easy to handle.

RESPONSIBLE SOURCING OF OMEGA-3

We produce our algal oil through fermentation using only renewable resources. Our waste-free process has minimal impact on the environment.

https://www.veramaris.com



Assesment criteria

- Nutrient value and amino acid profile
- Digestibility
- Growth / mortality
- Quality of fatty acids (PUFA, DHA)
- Anti-nutritional factors
- Availability and price





Tab.: Raw protein content in fishmeal, microalgae and vegetable raw materials (on a dry weight basis) (Data modified from Shields & Lupatsch 2012).

	% Crude Protein	% Crude Fat	% Crude Carbohydrates	% Ash	% Crude Energy MJ/kg
Fishmeal	63	11	-	15,8	20,1
Poulty meal	58	11,3	-	18,9	19,1
Corn gluten	62	5	18,5	4,8	21,3
Soja bean	44	2,2	39	6,1	18,2
Wheat	12,2	2,9	69	1,6	16,8
Spirulina	58	11,6	10,8	13,4	20,1
Chlorella	52	7,5	24,3	8,2	19,3
Tetraselmis	27,2	14	45,4	11,5	18
Gracilaria sp ¹	34	1,5	37,1	26,9	13,4
Ulva lactuca ¹	37,4	2,8	42,2	17,4	15,7
Schizochytrium ³	12,5	40,2	38,9	8,4	25,6

¹Cultured in effluent of fish tanks

³Commercial product, Martek Biosciences





Tab.: Essential amino acids (in EAA in g 100 g⁻¹ protein) composition of autotrophically and heterotrophically grown *Chlorella regularis* as well as fish meal and soy bean meal. (Data from Ende in press)

AA in g 100g ⁻¹ protein)	Chlorella autotrophic	Chlorella heterotrophic	Fish meal	Soy bean meal	
Isoleucine	4.21	3.35	5.23	2.63	
Leucine	8.08	7.01	6.25	4.18	
Lysine	7.74	9.42	6.79	3.50	
Phenylalanine	5.08	3.19	3.26	2.46	
Methionine	1.25	1.83	2.50	0.99	
Threonine	3.62	3.96	3.97	2.06	
Tryptophan	1.52	1.40	0.84	n.a.	
Valine	5.94	5.05	3.93	1.94	
Arginine	5.75	10.24	5.23	4.18	
Histidine	1.82	2.98	1.97	1.53	

Amino acid composition percent per N x 6.25 in cell weight



© N/

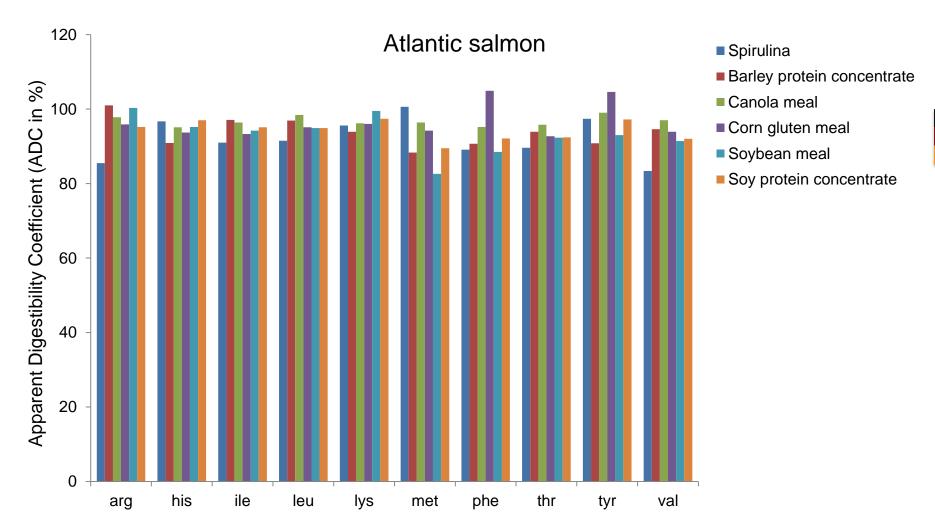


Fig.: **Essential amino acid digestibility** of Spirulina and vegetable raw materials in Atlantic salmon (Data from Burr et al. 2011)





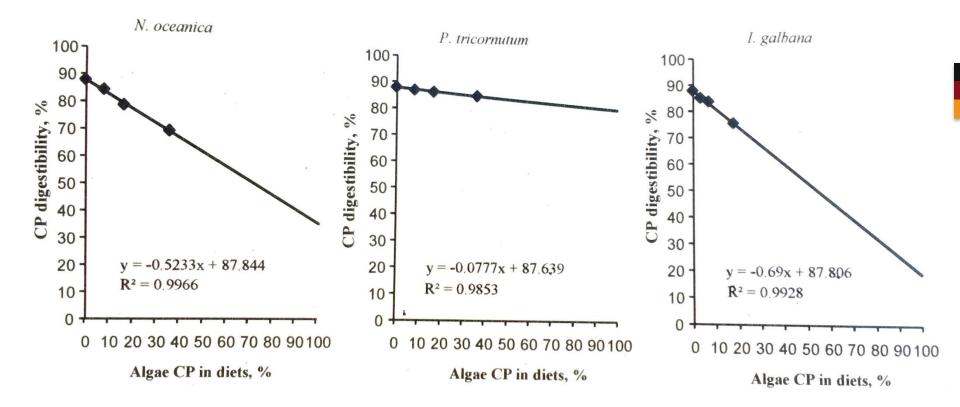


Fig.: **Protein digestibility** as a function of microalgae content in the diet of monogastric animals (mink) including salmonid fish (Data from Skrede et al. 2011)



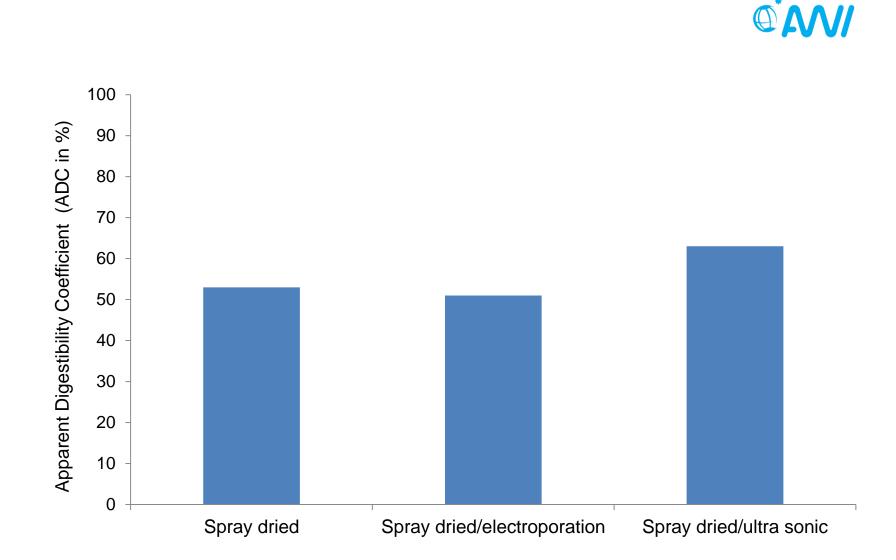


Fig.: **Apparent digestibility coefficient** (ADC in %) of *Chlorella vulgaris* after spray-drying and different pretreatment in rats (Data from Janczyk et al. 2007)





Fish species	Development stage	Microalgae	Content in feed (%)*	Growth**	Reference
Crucian carp	Larvae	I. galbana	100	sign. neg.	Coutinho et al. 2006
Carp	Juvenile	S. platensis	25-100%	sign. pos.	Nandeesha et al. 2001
Sturgeon	Juvenile	S. platensis	50%	sign. pos.	Palmegiano et al. 2005
Tilapia	Juvenile	S. maxima	20-40%	no sign. impact	Olvera-Novoa et al. 1998
Tilapia	Juvenile	S. maxima	>40%	sign. neg.	Olvera-Novoa et al. 1998
Atl. cod	Juvenile	Nannochloropsis+I. sp.	15-30%	sign. neg.	Walker & Berlinsky 2011
Tilapia	Juvenile	Desmochloris sp.	> 50%	sign. pos.	García- Ortega et al. 2015
Atl. salmon	Adult	Nanofrustulum+Tetraselmis	≤ 10%	no sign. impact	Kiron et al. 2012
Carp	Juvenile	Nanofrustulum+Tetraselmis	≤ 10%	no sign. impact	Kiron et al. 2012
Rainbow trout	Juvenile	Spirulina sp	10%	pos. impact	Nikolov 2012
Sturgeon	Juvenile	Spirulina sp	50%	pos. impact	Palmegiano et al. 2005
Shrimp	Juvenile	Nanofrustulum+ Tetraselmis	≤ 10%	no sign. impact	Kiron et al. 2012

* Proportion of fish meal substituted by microalgae

** Compared to fishmeal-based reference feed without microalgae content





Conclusion

- 9 out of 13 studies show no negative or positive impact on fish growth with partial substitution of fishmeal by microalgae
- Some microalgae with a high protein content and comparable amino acid composition
- High digestibility of protein and amino acids (few studies!)
- Pretreatment has a major influence on digestion coefficients (rats)
- The higher the amount of microalgae, the lower the digestibility



Tab.: Recent studies on replacement of microalgae biomass of fish oil for aquaculture feed (Data from Shah et al. 2018).

Author(s)	Microalgae species	Aquaculture species	% replacement of fish oil	Effect of microalgae biomass
Qiao et al. 2014	Nannochloropsis sp Schizochytrium sp.	Olive flounder (Paralichthys olivaceus)	100% replacement of fish oil	No negative effects on growth, feed efficiency or nutritive quality
Kousoulaki et al. 2015	Schizochytrium sp.	Atlantic salmon (<i>S. salar</i>)	Up to 5% replacement of fish oil	No signs of toxicity, stress, inflammation, or any other negative effects of supplementation in diets; fillet quality good
Haas et al. 2016	Pavlova viridis Nannochloropsis sp.	European sea bass (<i>D. labrax</i> L.)	50-100% replacement of fish oil in diets	No negative effects on the growth performance and nutrient utilization of fish
Sarker et al. 2016a	Schizochytrium sp.	Tilapia (<i>O. niloticus</i>)	100% replacement of fish oil	Improved weight gain, feed conversion ratio, and protein efficiency ratio; no significant change in survival rate



Conclusion

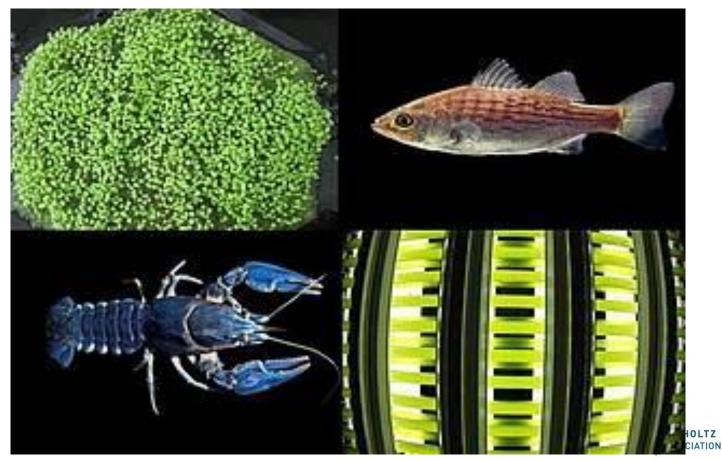
Microalgae biomass as a fishmeal, fish oil substitute or additive mainly dependent on the further development of biotechnology, the resulting availability of microalgae biomass and the price



Microalgae culture: for integrated aquaculture



Fish, crayfish, cropping plants and microalgae in an aquaponic-system - the AquaMoNa project



:AWI, Bell vital & Algatec

Microalgae culture: for feed additive/supplement



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Mareninne as feed additive for aquaculture and nutraceuticals - the CAMAFAN project



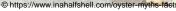


Microalgae culture: for feed additive/supplement

CAMAFAN project

- Marennine properties
 - Size ≈ 10kDa
 - Molecule of polyphenolic nature
 - Antioxidant; (Pouvreau et al., 2008 J. Agr. Food Chem)
 - Antiproliferative; (Gastineau *et al.*, 2012 Aquaculture)
 - Antibacterial;
 - Antiviral;











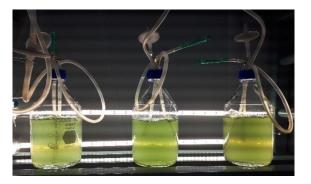


Microalgae culture: for feed additive/supplement

CAMAFAN project



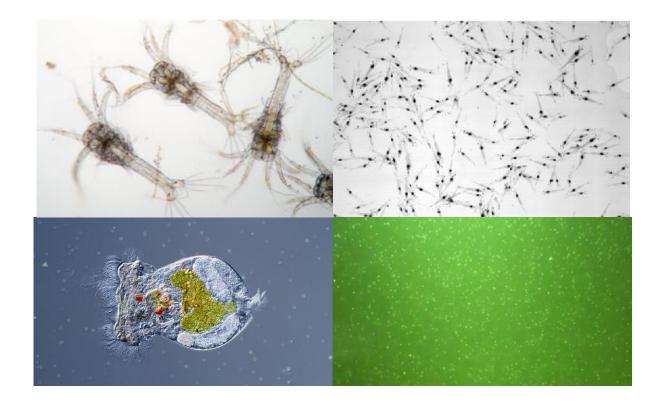
- Project aims
 - Optimization of culturing protocols of Haslea ostrearia and marennine production – light, nutrients, photoperiod, salinity;
 - Use of marennine in nutraceuticals & aquaculture
 - hatcheries Oyster larvae
 - in fish feed Fish juveniles







Application as live feeds in aquaculture





Microalgae culture: Aquaculture







Tab.: Application of microalgae in aquaculture

Application form	Concentrate/Paste	Powder	Extract
	live	spray dried	water extract
	cooled	drum dried	solution
	frozen	freeze dried	supercritical extraction
Current aquaculture feed	Nursery	Broodstock	Premix feed
	green water	special feed	pigments and omega 3
	crustacean feed	Premix feed	(Astaxanthin, DHA/DPA)
	rotifers feed		
Research		grow-out	grow-out
		fishmeal and fish oil substitute	pigments and omega 3
			(Astaxanthin, DHA/DPA)



Three groups of live diets are widely applied in industrial larviculture of fish and shellfish:

- different species of 2 to 20 µm microalgae for:
- bivalves
- penaeid shrimp
- rotifers, copepods,...
- ➤ fish
- the 50 to 200 µm rotifer Brachionus plicatilis for:
- crustaceans
- marine fish
- the 400 to 800 µm brine shrimp Artemia spp. (meta-)nauplii for:
- crustaceans
- ➤ fish







Nutritional value: fatty acids

	5	(III) prome o	\frown			0 00							_
	<i>Chroococcus</i> sp.	<i>Synechococcus</i> sp.	Isochrysis galbana	<i>Pavlova</i> sp.	Phaeodaa tricornuti			<i>Oocystis</i> sp.	Pseudokirchneriella subcapitata	<i>Tetraselmis</i> sp.	<i>Tribonema</i> sp.	Nannochlorop oceanica	51
Saturate	d												Γ
C12:0	2.1	0.7	-	-	-	-	2.0	-	-	_	-	1.2	L
C14:0	0.1	5.6	8.9	7.5	8.8	-	4.1	0.2	0.1	0.5	1.1	16.9	L
C16:0	21.3	3.4	11.5	13.4	16.6	5.9	6.0	3.8	16.2	6.3	2.5	17.2	L
C 18:0	0.3	_	-	0.4	0.6	0.2	0.8	-	1.3	1.2	0.1	1.8	L
C 20:0	0.2	_	-	_	-	_	0.1	-	0.2	_	-	_	L
C 24:0	_	_	-	_	1.6	-	4.0	0.1	0.7	_	-	_	L
Sum	24.0	9.7	20.4	21.3	27.6	6.1	17	4.1	18.5	8.0.	3.7	37.1	L
Monosa	turated												L
C 16:1	1.1	10.8	3.3	12.8	26.0	_	0.4	1.5	1.0	1.3	5.1	18.2	L
C18:1 ^c	0.4	_	13.1	2.9	1.8	0.1	3.4	3.9	31.1	10.7	0.2	4.1	l
C 20:1°	_	_	-		-	-	0.1	-	0.9	0.9	-	0.5	L
C 22:1°	0.1	_	0.6	0.8	0.3	-	0.1	-	0.8	_	-		L
Sum	1.6	10.8	17.0	16.5	28.1	0.1	4.0	5.4	33.8	12.9	5.3	22.8	L
Polyuns	aturated												L
C 18:2 ^b	10.7	_	7.0	2.1	1.5	2.1	11.7	6.4	5.1	2.5	0.2	9.7	L
C 18:3 ^a	1.0	_	3.8	1.8	0.3	-	12.0	8.1	11.4	6.4	-	0.5	L
C 18:4 ^a	_	_	12.5	4.3	3.3	_	5.1	0.7	3.0	4.1	0.1		L
C 20:2 ^b	0.1	-	-		-	0.3	0.1	-	-	_	-	0.5	k
C 20:4 ^b	0.1		-	0.4	2.2	6.0	0.2	0.5	-	0.6	-	3.7	ļ
C 20:5 ^a	_		0.8	18.0	28.4	6.1	4.4	1.1	_	4.8	3.2	23.4	
С22:5 ^ь	_	_	-		1.3	_	0.2	-	2.1	_	-	_	1
C22:6 ^a	_	_	15.8	13.2	0.2	_	_	_	0.1	0.2	-	_	
Sum	11.9	_	39.9	39.8	37.2	14.5	33.7	16.8	21.7	18.6	3.5	37.8	J

Table 3 Fatty acid (FA) profile of the microalgae tested. Data are given as mg g^{-1} of drv weight

Dashes indicate FA not detected

 $^{\mathrm{a}}\omega$ -3 fatty acids

 $^{\rm b}\omega$ -6 fatty acids

 $^{c}\omega$ -9 fatty acids

Patil et al. (2007) Fatty acid composition of 12 microalgae for possible use in aquaculture feed. Aquacult Int. 15:1–9

Microalgae culture: larval rearing



Larvae rearing with rotatoria

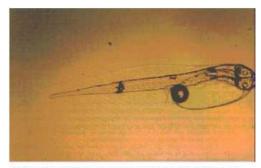
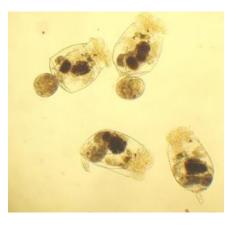


Figure 1.2. Gilthead seabream larva with yolk sac.



Figure 1.3. Atlantic salmon and gilthead seabream larvae at first feeding.



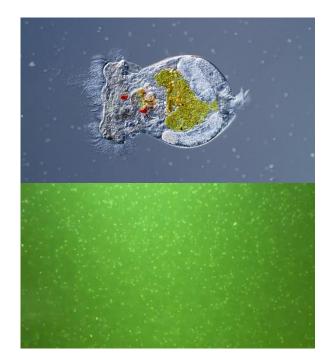




Rotatoria feeding

Brachionus spp.

- 2-20 microns
- Feeds on microalgae
- Own nutritional profile influenced by fed microalgae
- Manipulation of nutritional value by selected combinations of microalgae



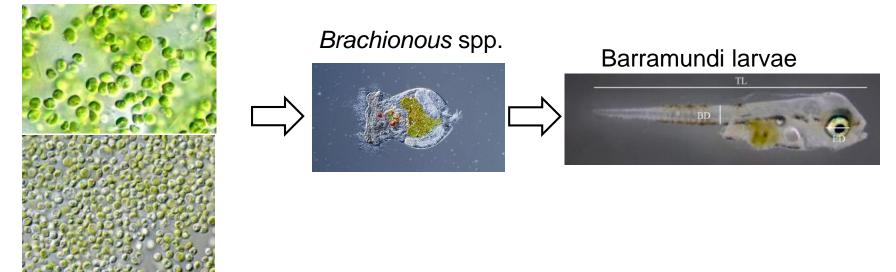
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Microalgae culture: live feeds

Trophic transfer microalgae – Brachionus – fish larvae

Nannochloropsis oculata

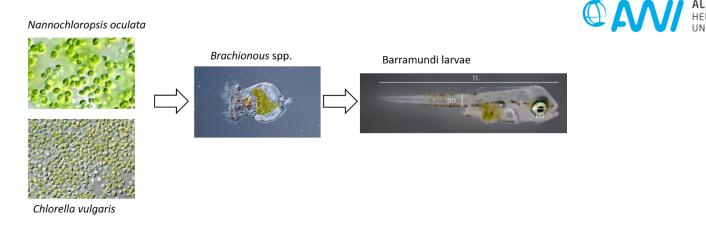


Chlorella vulgaris

Thépot et al. (2016) Rotifers enriched with a mixed algal diet promote survival, growth and development of barramundi larvae, *Lates calcarifer* (Bloch). Aquaculture Reports (3), 147-158



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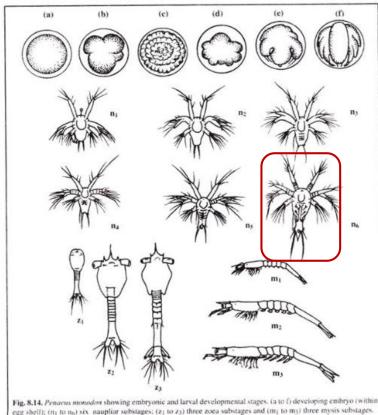


- The **fatty acid content** of the barramundi larvae reflected the fatty acid content of their respective diets
- **Microalgal blends** induced equal or better larval performance compared to the monospecific diets.
- The equiproportional blend of microalgae enhanced growth, development and stress resistance in the barramundi larvae.





Live feeds for shrimp



Penaeid shrimp



Development of digestive track at stage N6 ➤ First feeding





Live feeds for shrimp

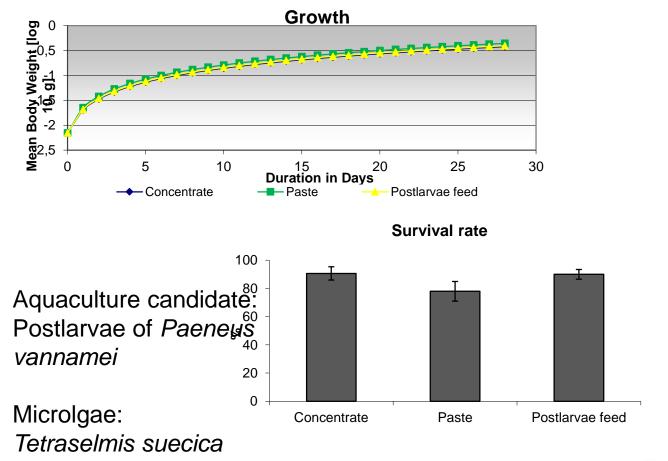
Beneficial effects, direct and indirect:

- enhanced growth and survival also due to the algae's attractant properties that stimulate the appetite of the larvae
- microalgae trigger the digestive system by stimulating the digestive enzymes such as trypsin
- The presence of microalgae in the prawn larval tanks also **positively affects the bacterial population** in the rearing water and thus contributes to a well balanced gut microbial flora in shrimp larvae





Microalgae culture: live feeds

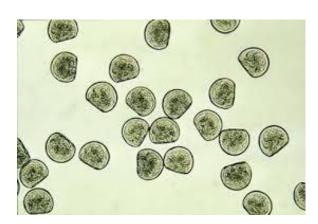






Bivalve culture

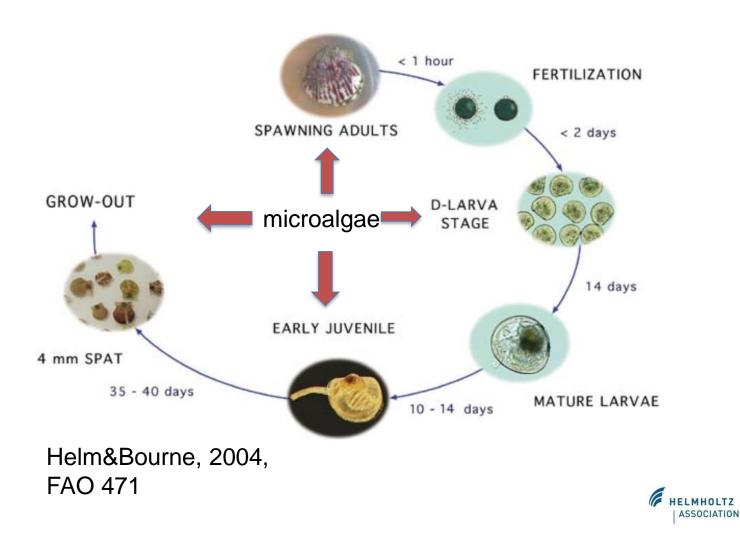
• Microalgae comprises on average 30% of the operating costs in a bivalve hatchery



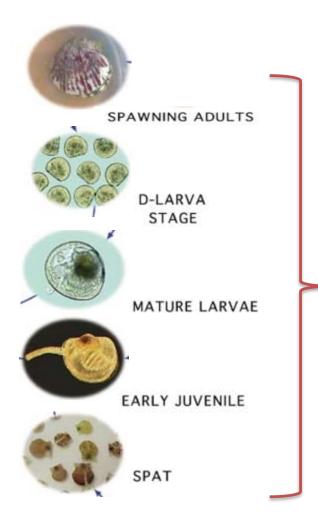










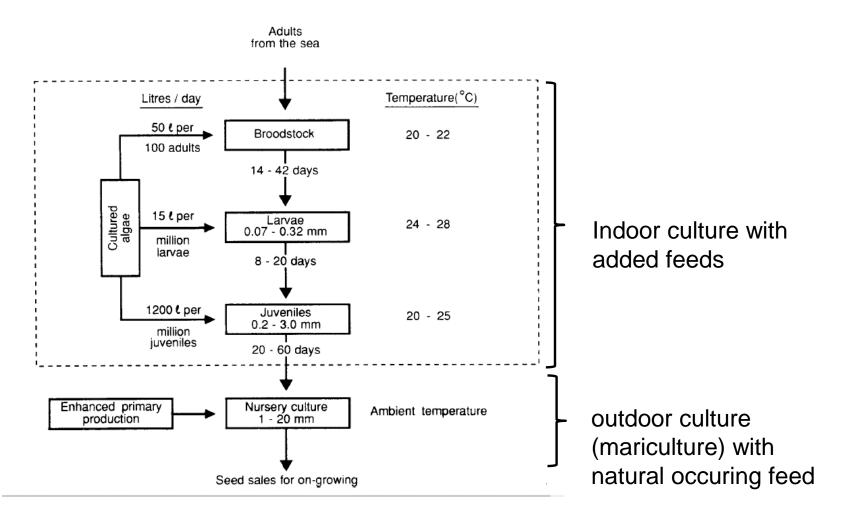


Different life stages require different microalge:

- Size
- Nutritional value
- Nutritional requirements
- High volume production







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Algal species	frequency of use [†]	total daily production n [‡]	volum (m³)	
Isochrysis sp., clone T-Iso	31	18	23.8	
Chaetoceros gracilis	23	11	14.1	
Chaetoceros calcitrans	16	10	6.0	
Tetraselmis suecica	15	10	39.1	
<i>Thalassiosira pseudonana</i> , clone 3H	14	9	112.0	
Pavlova lutheri	11	7	11.7	
Isochrysis galbana	8	5	9.1	
Skeletonema costatum	6	3	58.8	J
Chroomonas salina	5	3	0.76	
Dunaliella tertiolecta	4	2	2.2	
Chaetoceros simplex	3	3	1.76	
Chaetoceros muelleri	3	2	5.0	
Nannochloropsis sp.	3	2	0.20	
Cyclotella sp.	2	1	0.36	
Phaeodactylum tricornutum	2	1	2.0	Alg
Tetraselmis chui	2	0	-	nui
Pavlova salina	1	1	3.18	rep 199
Dicruteria sp.	1	1	4.07	deo
Dunaliella perva	1	1	0.012	and
Chlamydomonas sp.	1	1	0.52	Ad
Chlorella sp.	1	1	0.36	
TOTAL	43	23	295	



Eight species represent 90% of cultured algae in mollusc

Algal species used in hatchery and nursery rearing of bivalve molluscs as reported in an international questionnaire 1991. Species are ranked according to decreasing frequency of use (Coutteau and Sorgeloos, 1992).

Adapted from FAO





- larvae of most bivalve species have similar food preferences;
- Combinations of flagellates and diatoms provide well balanced diet, which will generally accelerate the rate of larval development to metamorphosis in comparison with unialgal diets
- quantity depends on larval density, suitable cell concentrations (expressed as cells.µl⁻¹) are given by each of the following combinations, e.g., :
- Isochrysis galbana; 50
- Chaetoceros calcitrans; 250
- I. galbana/C. calcitrans; 25/125
- ➤ I. galbana/C. calcitrans/ Tetraselmis suecica; 33/83/3.3 (larvae > 120µm)





"Green water" in larval fish rearing

- "green water" polyculture ponds, co-culture of fish larvae, rotifers and microalgae
- microalgae are either suspended or attached to submerged substrata as periphyton (Spataru et al. 1983), "natural food" also includes macrophytes, bacteria, other microbes, and zooplankton
- Enhanced first feeding, survival, growth, stress resistance
- Historically applied in SE Asia in open pond systems for carp culture





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"Green water" in larval fish rearing

beneficial effects green water:

- a direct food source through active uptake
- stimulation of the non-specific immune system in the larvae action as a probiotic
- microbial control by algal exudates in tank water and/or larval gut
- cost of producing "green water" microalgae by the aquaculturists—mostly in SE Asia—is low





Microalgae culture: Biotechnological benefits Summary

- In aquaculture as a farm animal feed: ٠
- Bioremediation (removal of pollutants)
- Food •
- **Cosmetics**
- **Natural Products**
- Energy
- Extraction of functional molecules •



Rotatoria



photobioreactor



Artemia sp.

The future of microalgae industry?





HELMHOLTZ ASSOCIATION

Acknowledgments

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- Poster and oral presentations must include, not only the statement above, but also the project logo and EU logo



