



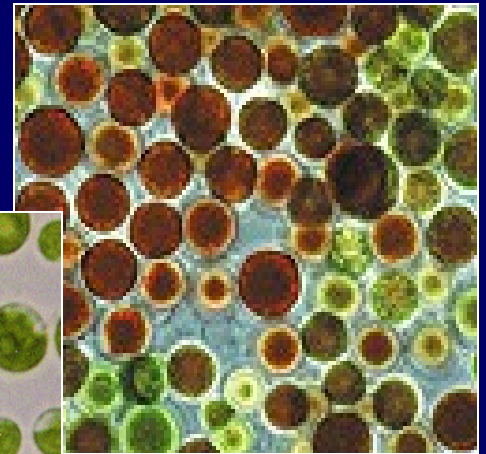
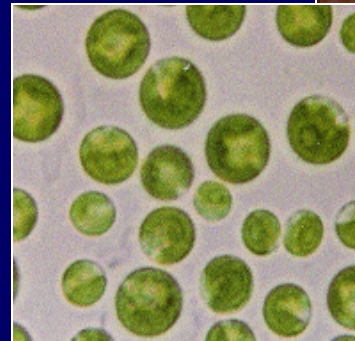
## *El uso de Microalgas en Acuicultura*

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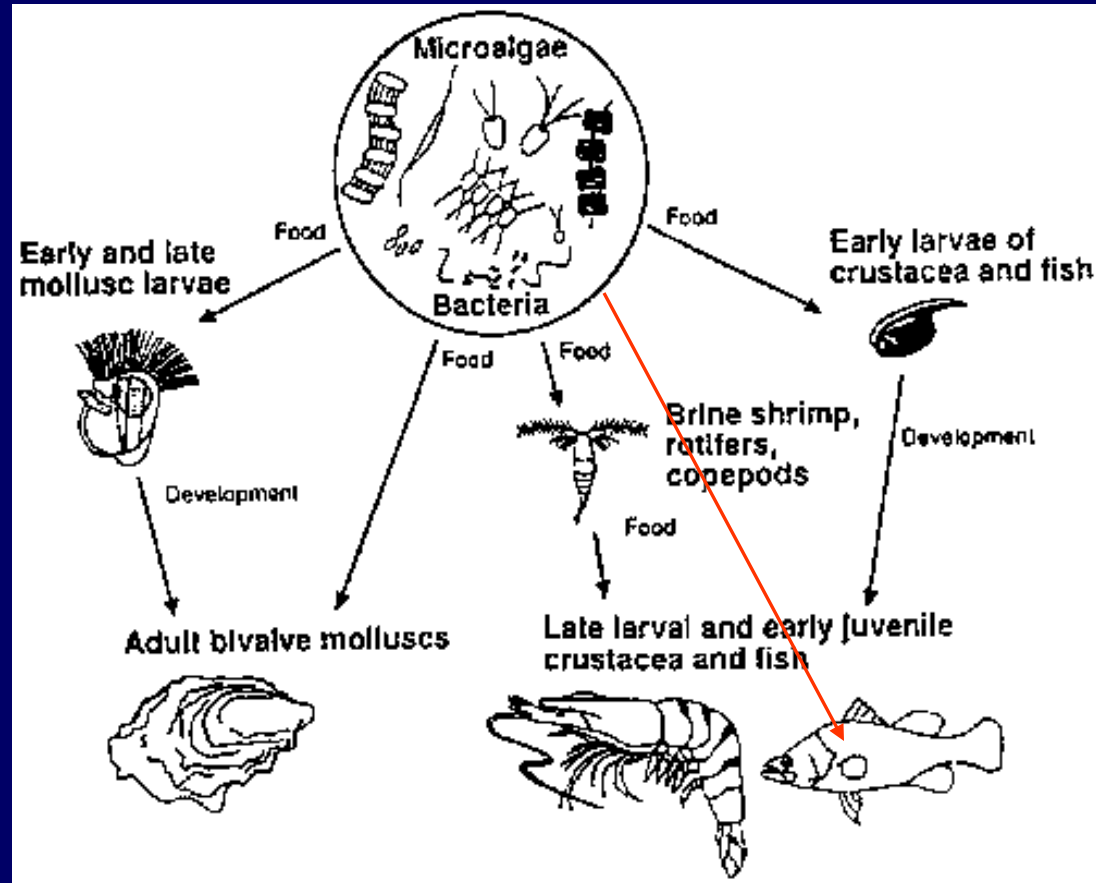
University of Florence

ITALY



# THE CENTRAL ROLE OF MICRO-ALGAE IN AQUACULTURE

(Brown et al., 1989-modified)



The importance of algae in aquaculture is not surprising as algae are the natural food source of these animals. Although several **alternatives** for algae exist such as yeast and microencapsulated feeds, **live algae** are still the best and the preferred food source.

# IMPORTANCE OF MICROALGAE IN AQUACULTURE

- ✓ food source for the reared species
- ✓ together with bacteria they have an important role in the oxygen and carbon balance in the cultures
- ✓ in the green or pseudo-green water technique, suspended microalgae increase feeding by enhancing visual contrast and light dispersion in the tank
- ✓ they are used to colour the flesh of salmonids
- ✓ algae may increase significantly the survival of the larvae by addition of growth factors to the culture medium or by acting as bactericidal agents (probiotics)

# MICROALGAE GENERALLY USED IN AQUACULTURE

Class	Genus	Applications
Bacillariophyceae	<i>Skeletonema</i>	PL, BL, BP
	<i>Thalassiosira</i>	PL, BL, BP
	<i>Phaeodactylum</i>	PL, BL, BP, ML, BS
	<i>Chaetoceros</i>	PL, BL, BP, BS
	<i>Cylinrotheca</i>	PL
	<i>Nitzschia</i>	BS
	<i>Cyclotella</i>	BS
Haptophyceae	<i>Isochrysis</i>	PL, BL, BP, ML, BS
	<i>Coccolithus</i>	BP
Chrysophyceae	<i>Pavlova</i>	BL; BP, BS, MR
Prasinophyceae	<i>Tetraselmis</i>	PL, BL, BP, AL, BS, MR
Cryptophyceae	<i>Chroomonas</i>	BP
	<i>Cryptomonas</i>	BP
	<i>Rhodomonas</i>	BL, BP
Chlorophyceae	<i>Dunaliella</i>	BP, BS, MR
	<i>Chlamydomonas</i>	BL, BP, FZ, MR, BS
	<i>Chlorococcum</i>	BP
	<i>Chlorella</i>	BL, ML, BS, MR, FZ
	<i>Scenedesmus</i>	FZ, MR, BS
	<i>Nannochloris</i>	BP, MR, SC
Eustigmatophyceae	<i>Nannochloropsis</i>	MR
Cyanophyceae	<i>Spirulina</i>	PL, BP, BS, MR

PL: penaeid shrimp larvae  
 BL: bivalve mollusc larvae  
 ML: freshwater prawn larvae  
 BP: bivalve mollusc postlarvae  
 AL: abalone larvae  
 MR: marine rotifers (*Brachionus*)  
 BS: brine shrimp (*Artemia*)  
 FZ: freshwater zooplankton  
 SC: seawater copepods

De Pauw and Persoone  
(1988), modified

**ALGAE BIOMASS REQUIRED BY THE POST-LARVAE OF WORLD AQUACULTURE**  
(1997) – Muller-Feuga (2000), modified

	Molluscs	Shrimp clear water	Shrimp green water	Small larvae fish	Total
World aquaculture production (t/year)	7,442,555	206,416	530,784	169,167	8,348,922
Number of 10 <sup>6</sup> post- larvae per t of final product	0.1	0.3	0.4	0.005	
Number of 10 <sup>6</sup> post- larvae for aquaculture production	744,256	68,804	224,786	845	
Microalgae requirements per 10 <sup>6</sup> post-larvae (Kg d. wt)	14.0	0.06	0.65	60.0	
Microalgae biomass (t d.w./year)	10,420	4	146	51	10,620
Trends	Increase	Decrease	Increase	Increase	

# PRODUCTION SYSTEMS USED IN AQUACULTURE

Algae production level	Usual needs	Type of culture	State of algae	Algae/ consumer relation	Types of enclosures	Culture volumes
<b><i>Extensive</i></b> natural phytoplankton	—	—	Live	Grown together	Sea, lake, lagoon, pond	>1000 m <sup>3</sup> outdoor
<b><i>Semi-intensive</i></b> induced blooms of natural phytoplankton	Enrichment of water	Batch or (semi)-continuous	Live	Grown together or separated	lagoon, pond, tank	>100 m <sup>3</sup> outdoor or greenhouse
<b><i>Intensive</i></b> uni-algal cultures	Preliminary water treatment, inoculation, enrichment of water	Batch or (semi)-continuous	Live or concentrated /preserved	Separated cultures	Tube, Erlenmeyer bottle, carboy, vertical tube, tank	1 m <sup>3</sup> to <100 m <sup>3</sup> indoor or greenhouse

# THE EXTENSIVE APPROACH

Natural phytoplankton is used where large quantities of microalgae are needed as in the case of the culture of bivalve molluscs.

## *Advantages*

- ✓ natural phytoplankton is inexpensive

## *Disadvantages*

- ✓ is uncontrollable and interfering consumers may also grow

# THE SEMI-INTENSIVE APPROACH

The *semi-intensive* production of algae currently used in aquaculture is the stimulation of blooms of natural phytoplankton by **fertilization**.

This technique is used for penaeid shrimp larvae, abalone seed, *Brachionus*, fish, shrimps, bivalve molluscs (larvae and post-larvae) zooplankton.

## *Difficulties*

✓ To find the correct balance and obtain the proper quantities and desirable species



# THE INTENSIVE APPROACH

It consists of culturing **pure strains of selected microalgae**. The majority are marine species.

Cultured microalgae are mostly fed directly (alive) to the consumer, in some cases they are used successfully in a concentrated and preserved form.

## *Advantages*

- ✓ a specific food quality is guaranteed

## *Disadvantages*

- ✓ production cost is very high and scaling-up is difficult

# The large-scale **intensive** production of aquaculture microalgae

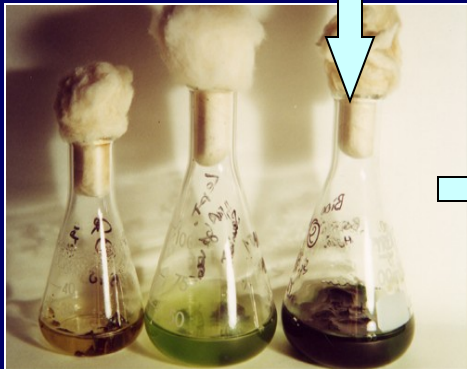
## It is expensive

- Microalgal culture is **labour intensive** and requires a great deal of space. The cost of energy for **lighting, pumping, aeration/mixing and heating/cooling** is very high.
- De Pauw et al (1984) estimated that monospecific algal cultures produced indoors or in a greenhouse range in cost from US\$ **120 to 200/Kg dry wt.**
- According to Benemann (1999) the cost can be as high as **US\$ 1000-2000/Kg dry wt.**

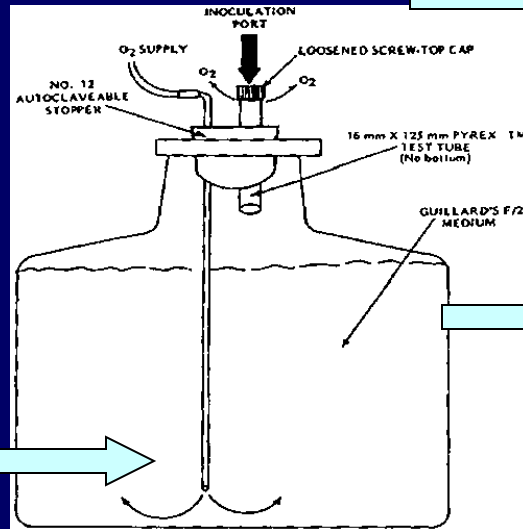
# THE INTENSIVE TRADITIONAL APPROACH



Stock cultures

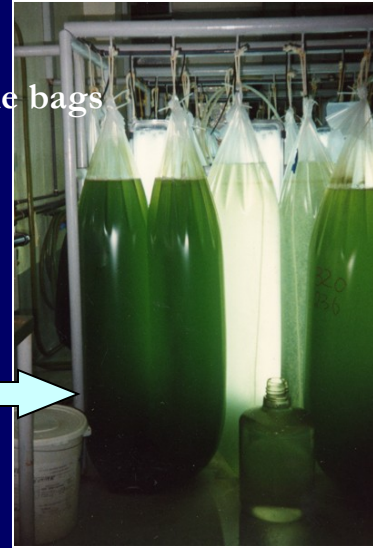


Primary cultures

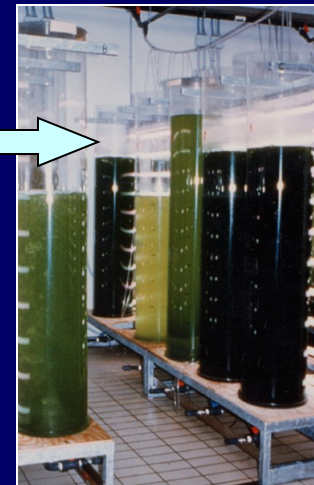


carboy (5 – 10 L)

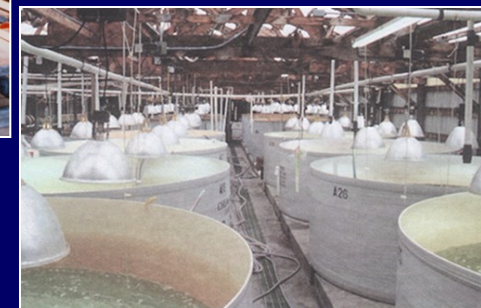
polyethylene bags



polyethylene cylinders



glass fibre cylinders

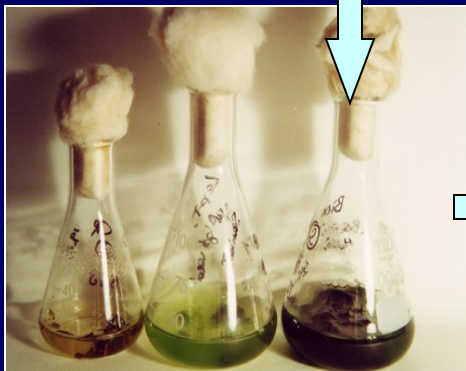


500 – 25000 L tanks

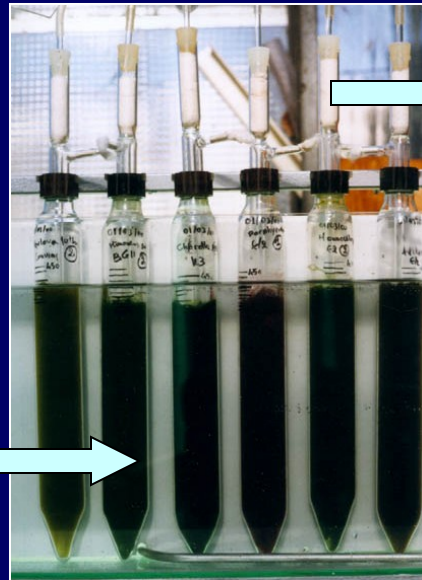
# MICROALGAE SCALING UP AT UNIVERSITY OF FLORENCE



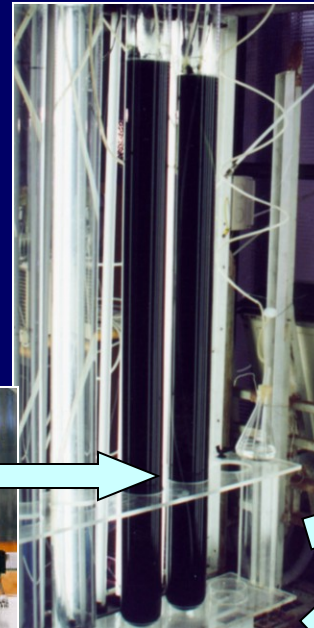
Stock cultures



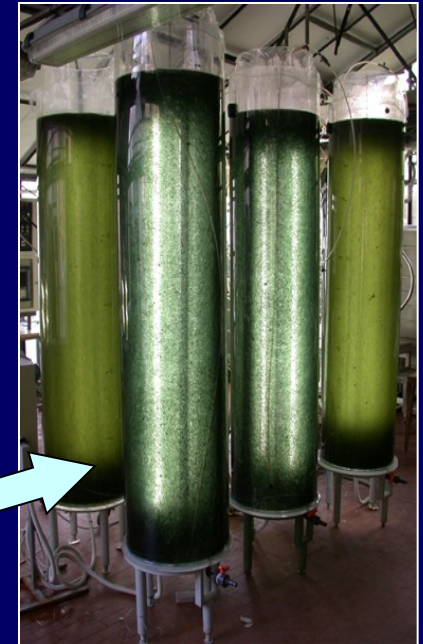
Primary cultures



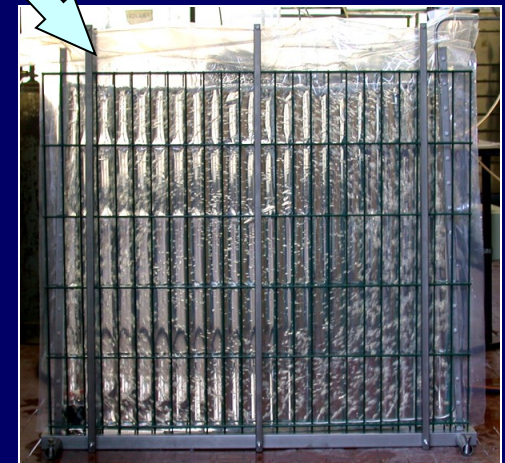
Bubbled tubes  
(500 - 1000 mL)



Bubble tubes  
(10 L)



Annular columns (115 L)



Plastic and wire panel (55 L)



# THE INTERNALLY-LIT ANNULAR REACTOR DEVELOPED AT FLORENCE

Internally-lit cylinders attain higher volumetric productivities and greater efficiencies of light utilisation compared to completely filled columns, since the photon flux provided is completely trapped by the culture. A vertical annular reactor with internal illumination was recently developed at the University of Florence. The reactor consists of two 2-m high Plexiglas® cylinders of different diameter placed one inside the other so as to form a regular annular culture chamber, 3 to 5-cm-thick and 115-150 L in volume. An air/CO<sub>2</sub> mixture is injected at the bottom of the annular chamber for mixing and gas-exchange. To operate the reactor with artificial illumination, lamps or fluorescent tubes are placed inside the inner cylinder. The use of this system for cultivation of *Nannochloropsis* is described.



# Outdoor mass cultivation of *Nannochloropsis* in annular columns (Florence, 2003)

	Dilution rate (d <sup>-1</sup> )				
	10%	30%	40%	50%	60%
Biomass concentration at harvesting (g L <sup>-1</sup> )	2.34	1.25	1.09	0.95	0.73
Cell weight (pg)	8.13	7.15	6.88	6.77	6.21
Cell concentration at harvesting (10 <sup>8</sup> cell mL <sup>-1</sup> )	2.9	1.7	1.6	1.4	1.2
Volumetric productivity (g L <sup>-1</sup> d <sup>-1</sup> )	0.23	0.37	0.44	0.49	0.44
Reactor productivity (g r <sup>-1</sup> d <sup>-1</sup> )	26.91	42.67	50.37	56.58	50.14

*Nannochloropsis oculata* –

INIDEP – Mar del Plata (1000 L round tanks)  
(Lic. Andrea Lopez)

Cell concentration at harvesting	10 <sup>6</sup> cell mL <sup>-1</sup>
Reactor productivity at 50% d.r.	2.5 mg L <sup>-1</sup> d <sup>-1</sup>

# HARVESTING AND **PRESERVATION** OF MICROALGAE

**Dried microalgae**

**Refrigerated microalgal slurries**

**Frozen microalgal pastes**


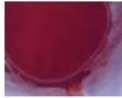

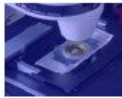




## Welcome to BlueBioTech

BlueBioTech operates in the sphere of marine and aquatic biotechnology ('Blue Biotechnology'). The objective of BlueBioTech is the cultivation of microalgae for commercial and scientific purposes such as biomass or the extraction of active ingredients. Microalgae are already supplied as food and feed supplements, and as feed for aquaculture and aquaristic application, especially ornamental fish. Biomass and extracts are available from quite a number of microalgae.

Biomass and extracts are used:

- as food supplements
- as feed supplements
- as feed for aquaculture and ornamental fish
- as neurophysiological blocker in medical science
- as reference standards for the analysis of seafood
- as active pharmaceutical ingredients (Blue Pharmacy)
- as cosmeceuticals

	<u><a href="#">Food Ingredients</a></u>	<ul style="list-style-type: none"> <li>• Spirulina</li> <li>• Chlorella</li> <li>• BluBio</li> </ul>
	<a href="#">- Shop online</a>	
	<u><a href="#">Feed for Aquaristic</a></u>	<ul style="list-style-type: none"> <li>• Algenia</li> <li>• Astax</li> <li>• SpiruVita</li> </ul>
	<u><a href="#">Feed for aquaculture</a></u>	<ul style="list-style-type: none"> <li>• Nannochloropsis</li> <li>• Isochrysis</li> <li>• Concentrates</li> </ul>
	<u><a href="#">Scientific use</a></u>	<ul style="list-style-type: none"> <li>• Custom Manufacture</li> <li>• Okadaïnsäure</li> <li>• GTX2</li> </ul>
	<u><a href="#">Analysis of seafood</a></u>	<ul style="list-style-type: none"> <li>• Algal Toxins</li> <li>• Standards</li> <li>• Biotoxins</li> </ul>
	<u><a href="#">Pharmaceutical Industry, human / veterinary</a></u>	<ul style="list-style-type: none"> <li>• Exsudates</li> <li>• Polysaccharides</li> <li>• Antioxi dants</li> </ul>
	<u><a href="#">Cosmetic Industry</a></u>	<ul style="list-style-type: none"> <li>• Extracts</li> <li>• Exsudates</li> <li>• Antioxi dants</li> </ul>
	<u><a href="#">Pigments</a></u>	<ul style="list-style-type: none"> <li>• Phycocyanin</li> </ul>
	<u><a href="#">Feed Ingredients</a></u>	<ul style="list-style-type: none"> <li>• Pellets</li> <li>• Granules</li> <li>• Algae powder</li> </ul>



# Feed for aquaculture applications

<http://www.bluebiotech.com/index.html>

BlueBioTech GmbH offers a unique product line of **high quality** microalgae concentrates for your Hatchery applications. These products have been developed through many years of scientific research and are now extensively applied by hatcheries and zoological stations.

<b>Nannochloropsis <i>Starter Concentrate</i></b>	<b>Nannochloropsis <i>Concentrate</i></b>	<b>T-Isochrysis <i>Concentrate</i></b>
6*10 <sup>9</sup> cells/mL	12*10 <sup>9</sup> cells/mL	2*10 <sup>9</sup> cells/mL
2-5µm cell size	2-5µm cell size	4-8µm cell size
77 g/L dry weight	144 g/L dry weight	64 g/L dry weight
EPA: 1% of dry weight	EPA: 1% of dry weight	DHA: 3,5% of dry weight
Live cells in sterile seawater	Fresh cells in sterile seawater	Fresh cells in sterile seawater
2 month storage at 5°C to 10°C	3 month storage at 5°C to 10°C	3 month storage at 5°C to 10°C
<b><i>Innovative product</i></b>	• Rotifer growth	• Rotifer growth
• Large scale starter culture	• EPA enrichment	• DHA enrichment
• Greenwater	• Greenwater	
<b>59 EUR / L</b>	<b>76 EUR / L</b>	<b>48 EUR / L</b>

The **Nannochloropsis *Starter Concentrate*** is a unique product, **exclusively** produced by BlueBioTech with a special cultivation and harvesting technique. This concentrate can be used as a **starter culture** for your **large scale algal** production systems and **greenwater** tanks.

Based on this we offer a **reliable and standardized quality** throughout the year independent from weather conditions or contaminations.

- No biofouling
- No preservatives
- No time consuming prior mixing
- Maximum nutritional quality
- Perfect single-cell suspension

770€/kg

530€/kg

750€/kg

# Mass cultivation of microalgae:



**Open ponds**

**Photobioreactors**



## Drawbacks and limitations of open raceway ponds

- Large open raceway ponds can not be operated at a water level much lower than 15 cm otherwise a severe reduction of flow and turbulence would occur.
- This long light-path results in large areal volumes ( $150 \text{ L m}^{-2}$ ) and cell concentrations of less than  $0.6 \text{ g L}^{-1}$  that facilitate contamination and greatly increase the costs of harvesting.
- Excessive evaporative losses, particularly in hot dry climates, and lack of temperature control are other major drawbacks of open systems.
- Although areal productivities of  $40 \text{ g m}^{-2} \text{ d}^{-1}$  and higher have been reported many times in experimental algal ponds, typically, well managed raceway ponds may achieve  $20\text{-}25 \text{ g m}^{-2} \text{ d}^{-1}$  for short periods, while long-term **productivity** in large commercial raceways rarely exceeds  $12\text{-}13 \text{ g m}^{-2} \text{ d}^{-1}$ .
- Most microalgae cannot be maintained long enough in outdoor open systems because of the risk of **contamination** by fungi, bacteria and protozoa, and competition by other microalgae that tend to dominate regardless of the original species used as inoculum (Richmond, 1999).

## Photobioreactors are necessary for the exploitation of microalgae in aquaculture

- Photobioreactors offer a **closed culture environment**, which is protected from direct fall-out, relatively safe from invasion by competing microorganisms, and where **conditions are better controlled** ensuring dominance of the desired species.

# Commercial scale photobioreactors

- A few commercial-scale photobioreactors have been built and operated, and most were closed after a few months of operation.
- Only three large commercial systems, the plants built by Ökologische Produkte Altmark GmbH in Germany, and by Micro Gaia Inc. and Aquasearch Inc. in Hawaii (USA), are apparently in full operation.
- Unfortunately not much is known about their productivity and general performance. Although not large scale, three other photobioreactors (bio-fence, AAPS and annular columns) are commercialised at present.



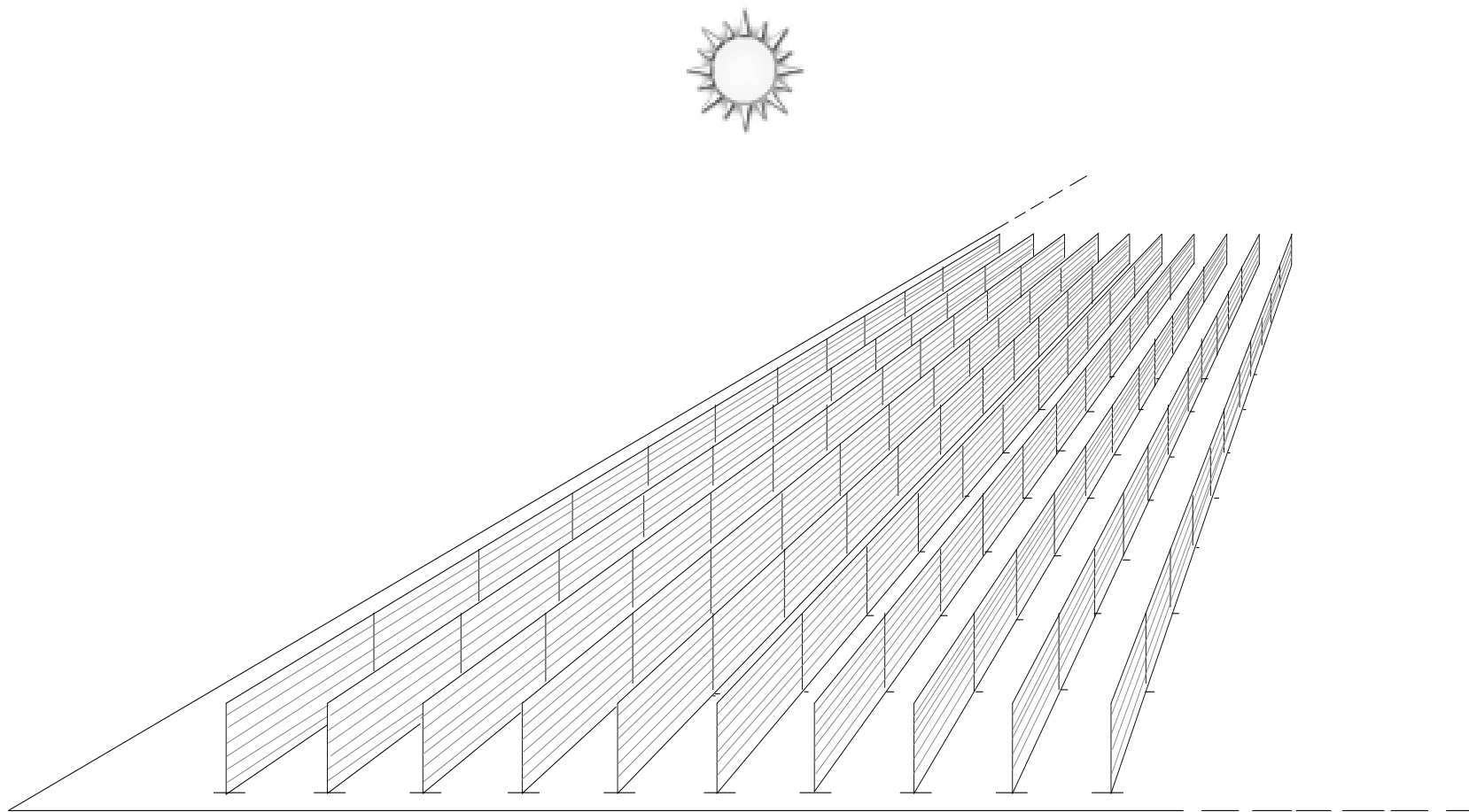
# Photobioreactors at the University of Florence



*(Florence, 1980 - 2006)*

## Ponds vs. vertical photobioreactors: a cost comparison

	Raceway pond	115-L annular column	114 L- 2.5 m <sup>2</sup> P&W panel
Unit cost	-	850 €	180 €
Cost at commercial scale	25 € m <sup>-2</sup>	300 € m <sup>-2</sup>	50 € m <sup>-2</sup>



*P&W panel (Florence, 2006?)*



# CONCLUSIONS

1. A regular supply of live and concentrated microalgae at competitive prices would lead to significant simplifications in hatchery production techniques and savings in hatchery production costs.
2. The costs of preserved microalgae are still too high (see Blue Biotech brochures) and the quality of the product is often poor.
3. The large facilities specialized in commercial microalgae production, which operate highly controlled closed photobioreactors, could produce microalgae at lower costs.

# PHOTOBIOREACTORS VS OPEN PONDS

- ✓ Photobioreactors have much higher S/V ratios than open ponds and can sustain much higher cell concentrations and productivities.
- ✓ The higher population density, together with a more protected environment and a better control over growth parameters (pH,  $pO_2$ ,  $pCO_2$  and temperature), make closed reactors relatively safe from invasion by competing microorganisms.
- ✓ Photobioreactors are much more efficient and productive than open ponds
- ✓ Commercial plants can be constructed at a low cost