MICROALGAE FOR AQUACULTURE:
CONVERTING ART INTO TECHNOLOGY

William van der Riet
CEO Tomalgae, Belgium
TOMALGAE PRODUCES MICROALGAE
(FOR LARVAL AQUACULTURE)
Algae are at the base of the entire aquatic food chain,... Therefore, it is not surprising that the microalgae which compose the phytoplankton play a vital role in the rearing of aquatic animals like molluscs, shrimp, and fish, and have a strategic interest for aquaculture.”

(Arnaud Muller-Feuga 2000)
“Since artificial substitutes are inferior to live microalgae as feed for the critical stages in the life cycles of several aqua-cultural species, a growing demand for microalgae will go hand in hand with the expected growth of aquaculture throughout the world.”

(Amos Richmond 2007)
MICROALGAE IN AQUACULTURE

• “Presently, most aquaculture enterprises produce their own supply of microalgae.” (A. Richmond 2007)

• “Mass production of micro-algae has been recognized as a major bottle-neck to many forms of marine hatchery and nursery production... The problem of high costs of individual hatcheries producing their own algae is compounded by the need of scarce expertise, without which crashes of algae at critical periods occur quite commonly.” (Heasman et al. 2001)

• “This has prompted a search for alternatives to on-site production”

• The most obvious alternative: centralized microalgae facilities that produce, concentrate and preserve the algae.
MICROALGAE FARMING

Production on an industrial scale

Open ponds

Closed Photobioreactors (PBRs)

A pair of 200-foot algae open ponds at Sapphire Energy's Las Cruces, New Mexico R&D Facility. Photo courtesy Sapphire Energy.

MICROALGAE IN AQUACULTURE

Principal applications:
Aquaculture hatcheries (direct or indirect feed source, ‘green water’ technology)
Ingredients for aquatic animal feeds- very limited, expensive (e.g. Spirulina)

Microalgae ‘forms’ used:
— Diatoms (Bacillariophyceae): Chaetoceros, Thalassiosira, Cyclotella, Skeletonema, Phaeodactylum
— Green algae (Chlorophyta): Chlorella, Tetraselmis, Haematococcus, Dunaliella
— Haptophyta: Isochrysis, Pavlova
— Cryptophyta: Rhodonomas
— Eustigmatophyceae: Nannochloropsis
— Cyanophyta: Spirulina
GENERALIZED ANALYSIS OF SELECTED MICROALGAE USED IN AQUACULTURE

Criteria (characteristics) of cultures and their concentrates
— Small cell size
— Fast rate of growth
— Nutritional value
— Digestibility
— Buoyancy
— ‘Resistance’ to grazing
— Concentrate & Drying
— Rehydration and (or) resuspension
MICROALGAE IN (FOR) AQUACULTURE

Haptophyta: *Isochrysis*

- Small cell size
- Fast rate of growth
- Acceptable biochemical quality
- Digestibility
- Buoyancy
- ‘Resistance’ to grazing
- Concentrate and drying
- Rehydration and or resuspension
MICROALGAE IN (FOR) AQUACULTURE

Eustigmatophyceae: *Nannochloropsis*

- Too small cell size
- Fast rate of growth
- Acceptable biochemical quality
- Digestibility
- Buoyancy
- ‘Resistance’ to grazing
- Concentrate and drying
- Rehydration and or resuspension
MICROALGAE IN (FOR) AQUACULTURE

Cryptophyta: *Rhodonomas*

- Small cell size
- Fast rate of growth
- Acceptable biochemical quality
- Digestibility
- Buoyancy
- Resistance’ to grazing
- Concentrate and drying
- Rehydration and or resuspension
Spirulina, Chlorella and Tetraselmis are very poor in PUFAs contents in addition, very low digestibility for Chlorella.

Nannochloropsis contains EPA but Artemia exhibits very low digestibility of Nannochloropsis.
MICROALGAE IN (FOR) AQUACULTURE

BACILLARIOPHYTA (DIATOMS):
THALASSIOSIROID ‘CULTIVAR’ OF TOMALGAE

— Small cell size (specific control)
— Fast rate of growth (specific control)
— Nutritional value (specific control)
— Digestibility
— Buoyancy
— ‘Resistance’ to grazing (rigorous control)
— Concentrate (specific) & Drying (specific)
— Rehydration and (or) resuspension (specific)
OVERALL BIOCHEMICAL COMPOSITION

LONG-CHAIN PUFA’S CONTENT OF MICRO-ALGAE

% of total fatty acids

DHA (22:6n-3)
EPA (20:5n-3)
AA (20:4n-6)

Navicula
Prophyridium
Nannochloropsis
Pavlova
Isochrysis

Chlorella
Dunaliella
Tetraselmis

Brown, CSIRO, Australia, 2002
PRESERVED ALGAE FOR HATCHERIES: OLD IDEA … NUMEROUS ATTEMPTS … LITTLE SUCCESS

“Centralized microalgae facilities which sell (for a high price) frozen pastes or highly concentrated refrigerated stock cultures cover at present only a small part of the aquaculture demand for live microalgae.” (A. Richmond 2007)

And very often (typically)

“… commercial marine hatchery operators throughout the world have tried and discarded substitutes for live micro-algae.” (Heasman 2001)

Obviously, further changes and innovative developments are required in this field…
FOCUS ON DIATOMS  
(BACILLARIOPHYCEAE)

• The most productive and species-rich group of microalgae
• Are the foundation of ocean food web
• The most commonly grown microalgae on-site, specifically at shrimp and shellfish hatcheries and nurseries.

Various marine planktonic diatoms
SELECTING SUITABLE DIATOM CULTIVAR

GENERAL ATTRIBUTES OF MICROALGAE USED IN AQUACULTURE:

- Appropriate size for ingestion
- Readily digested
- Rapid growth rates
- Suitable for mass cultivation
- Stable in culture
- Good nutrient composition
- Absence of toxins

(M. R. Brown 2002)
DIATOMS USED IN AQUACULTURE

Chaetoceros spp.
DIATOMS USED IN AQUACULTURE

*Thalassiosira* spp.
DIATOMS USED IN AQUACULTURE

Cyclotella sp.

Skeletonema sp.
DIATOMS USED IN AQUACULTURE

Phaeodactylum tricornutum
HATCHERY DEMAND ON PRESERVED MICROALGAE

- High cell concentration without damage to cells
- Easy to suspend uniformly in water
- *Neutrally or very slightly negatively buoyant cells*
- Suitable nutritional composition
- Free from pathogens
- *Appropriate packaging and shipping methods*
- Acceptable shelf life
- Regularly available and affordable

R. Shields & I. Lupatsch, 2012
Heasman et al. 2001
CHOICE OF TOMALGAE: A ‘THALASSIOSIROID’ DIATOM (ORDER THALASSIOSIRALES)

Thalassiosiroid diatoms grown commercially, closely related to the cultivar of Tomalgae:

- *Thalassiosira weissflogii*
- *Thalassiosira pseudonana*
- *Cyclotella cryptica*
MICROALGAE FOR AQUACULTURE

SUPPLIERS
- **Reed Mariculture (USA)**; liquid formulations; well recognized. Sound approach
- **A4F (P)**, micro-algae blends to obtain ‘nutritional optimum’
- **Necton/Phytobloom (P)** (*Isochrysis, Nannochloropsis, Tetraselmus, Phaeodactylum*); liquid and freeze-dried formulations
- **Cellana (USA), Algae Spring (NL), Proviron (B), Phytoplankton Marina, Algaenergy (S)**; rotifer culture & GWT

APPLICATIONS
- Green water technology (GWT)
- Rotifer & Artemia culture
- (Zoo plankton) enrichment
- Larval shrimp feed
- Component in manufactured diets
- Natural Astaxanthine (USA/when?)
### OVERVIEW SPECIES AND (INTENDED) USE

<table>
<thead>
<tr>
<th>microalgae species used (‘scalable’)</th>
<th>Used for</th>
<th>Direct feed (shrimp/shellfish)</th>
<th>Indirect feed (fish; enrichment of zoo-plankton)</th>
<th>GWT</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Thalasiosira</em></td>
<td>EPA (+DHA (p))</td>
<td>X</td>
<td>n.a</td>
<td>n.a</td>
</tr>
<tr>
<td><em>Chaetoceros – live algae</em></td>
<td>EPA</td>
<td>X</td>
<td>n.a</td>
<td>n.a</td>
</tr>
<tr>
<td><em>Nannochloropsis</em></td>
<td>EPA/ARA</td>
<td>n.a</td>
<td>rotifer (culture)</td>
<td>X</td>
</tr>
<tr>
<td><em>Tetraselmis</em></td>
<td>EPA</td>
<td>shellfish</td>
<td>rotifer (culture)</td>
<td>X</td>
</tr>
<tr>
<td><em>Phaeodactylum</em></td>
<td>EPA</td>
<td>n.a</td>
<td>r + a enrichment</td>
<td>X</td>
</tr>
<tr>
<td><em>Isochrysis</em></td>
<td>DHA</td>
<td>shellfish</td>
<td>r + a enrichment</td>
<td>X</td>
</tr>
<tr>
<td><em>Pavlova</em></td>
<td>DHA</td>
<td>shellfish</td>
<td>rotifer (enrichment)</td>
<td>n.a</td>
</tr>
<tr>
<td><em>Chlorella</em></td>
<td>unsure</td>
<td>n.a</td>
<td>rotifers (culture)</td>
<td>?</td>
</tr>
<tr>
<td><em>Spirulina</em></td>
<td>unsure</td>
<td>n.a</td>
<td>Rotifers (culture)</td>
<td>?</td>
</tr>
</tbody>
</table>
“Thalassiosira weissflogii” ... is used in the shrimp and shellfish larviculture industry. This algae is considered by several hatcheries to be the single best algae for larval shrimp.”

“Thalassiosira pseudonana” is a small diatom ... that is used in the shrimp and bivalve larviculture industry. This alga has been found to be excellent for larval shrimp.”

REED MARICULTURE (CALIF.) ANOTHER COMMERCIAL MICROALGAE AQUACULTURE FEED PRODUCTS.
SeaAg, Inc., founded 1989 Florida, Joe Weissman and John Benemann, to produce littleneck clams on-shore (grow diatoms in open ponds for seed clams and grow out)

Cyclotella grown in open ponds, fed to clams

From larvae to seed to market size

Clam hatchery - nursery
ACHIEVING ADEQUATE UNDERSTANDING OF CULTIVAR BIOLOGY AND ITS INTERACTION WITH ENVIRONMENT

Some important innovative developments and optimization of growth conditions:

1. Breeding program
2. Temperature factor
3. Rigorous control of culture contamination
4. Nutritious quality control
5. Securing the integrity of cells during concentration, preservation and rehydration
“In most diatom species, after one to several years of size reduction, (*but weeks in our intensive culture*) every surviving individual of every lineage must reproduce sexually or die. Diatoms are some of the most sexual organisms on earth;

**our problem in understanding them is that we do not invade their privacy often enough.**”

Prof. Dr. D.G.Mann, 1999
“We will have to domesticate algae, which has not yet been done. We are still using wild types, that is, strains that come directly out of nature without really any significant improvement or even much selection.”

“... the strains are going to be eventually what drives the industry, not somebody coming up with a better paddlewheel, or a more clever way of injecting CO2, or some kind of fancy new photobioreactor.”
1. BREEDING PROGRAM

Present

Valvar cell size control:
Species-specific range: 3 - 45 µm;

Required for early shrimp larvae (zoea): ≤ 10 µm.

Control over triggering and intensity of sexual reproduction

Future

- Generating new strains and selection;
- Genetic control and manipulations of economically important traits;
- Domestication of the cultivar.

“Cross the best with the best, select the best, and ... hope for the best!”
(John W. Snape, 2004)
1. BREEDING PROGRAM FOR THE CULTIVAR: SEXUAL REPRODUCTION
1. BREEDING PROGRAM FOR THE CULTIVAR:

CELL SIZE REDUCTION - RESTITUTION LIFE CYCLE
CELL SIZE CONTROL AND DYNAMICS

The graph shows the cell diameter in µm over weeks for two different conditions: 19mix F1.2 and PP3 F1 1602.
HOW ABOUT OTHER DIATOM SPECIES WHICH ARE CULTIVATED AT HATCHERIES, WITHOUT RIGOROUS’ ATTENTION TO THE CELL SIZE REDUCTION-RESTITUTION LIFE CYCLE AND ITS CONTROL?
2. TEMPERATURE FACTOR
WHERE TO PRODUCE AT SCALE?
CLIMATIC OPTIMA FOR SHRIMP AND ALGAE CULTURE ARE DIFFERENT

• **Microalgae** – temperature optimum: 18-23°C (Tomalgae cultivar: 15-20°C)
• **Penaeid shrimps** – temperature optimum: 29-33°C

• **Thalapure** – controlled greenhouse conditions, cultivated most of the year at suitable temperature
• **Thalapure** – cultivated in freshwater environment (free of pathogens typical for oceanic water)
3. RIGOROUS CONTROL OF CULTURE CONTAMINATION

Grazers and parasites

‘Weed’ microalgae
4. NUTRITIOUS QUALITY CONTROL – CHLOPOPLASTS
5. SECURING INTEGRITY & PRESERVING THE QUALITY. THE DOWNSTREAM PROCESS

Pre-concentration

Centrifugation
LYOPHILISATION

Freeze-drying works by freezing the material and then reducing the surrounding pressure to allow the frozen water in the material to sublimate directly from the solid phase into the gas phase.

It is gentle and low temperature process resulting in:
- No damage to the substance dried → algae cell integrity maintained
- Biochemical composition (incl. flavour, smell and nutritional content) remain unchanged
- Fast and easy rehydration (reconstitution) due to microscopic pores

Other drying techniques:
- Spray Drying (unsuitable)
- Blast drying (unsuitable)
- Refractance Window Drying (using infrared, test planned)
- With use of Microwave (not tested yet)
Freeze-dried microalgal powder
5. PRESERVING THE QUALITY: FREEZE DRYING & PACKAGING

Distributed by:
THALAPURE: AS FREEZE-DRIED POWDER

After freeze-drying, the nutritional quality of the powder remains as high as the live algae, e.g. (PUFAs):

<table>
<thead>
<tr>
<th>Fatty Acid Description</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>C20:4-omega 6 (AA)</td>
<td>&lt; 0.1</td>
<td>%/fat</td>
</tr>
<tr>
<td>C20:5-omega 3 (EPA)</td>
<td>38.3</td>
<td>%/fat</td>
</tr>
<tr>
<td>C22:0</td>
<td>&lt; 0.1</td>
<td>%/fat</td>
</tr>
<tr>
<td>C22:1</td>
<td>&lt; 0.1</td>
<td>%/fat</td>
</tr>
<tr>
<td>C22:5-omega 3 (DPA)</td>
<td>&lt; 0.1</td>
<td>%/fat</td>
</tr>
<tr>
<td><strong>C22:6-omega 3 (DHA)</strong></td>
<td>8.0</td>
<td>%/fat</td>
</tr>
<tr>
<td>C24:0</td>
<td>&lt; 0.1</td>
<td>%/fat</td>
</tr>
<tr>
<td>C24:1</td>
<td>&lt; 0.1</td>
<td>%/fat</td>
</tr>
<tr>
<td>Sum of saturated fatty acids</td>
<td>15.8</td>
<td>%/fat</td>
</tr>
<tr>
<td>Sum of mono unsaturated fatty acids</td>
<td>26.5</td>
<td>%/fat</td>
</tr>
<tr>
<td><strong>Sum of poly unsaturated fatty acids</strong></td>
<td>57.5</td>
<td>%/fat</td>
</tr>
<tr>
<td>Sum trans fatty acids</td>
<td>0.3</td>
<td>%/fat</td>
</tr>
<tr>
<td>Sum omega 3 - fatty acids</td>
<td>55.0</td>
<td>%/fat</td>
</tr>
<tr>
<td>Sum omega 6 - fatty acids</td>
<td>2.4</td>
<td>%/fat</td>
</tr>
</tbody>
</table>
THALAPURE: KEEPING THE INTEGRITY OF CELLS AT ALL STAGES OF GROWTH AND PROCESSING

Concentrated biomass

Dried biomass

Rehydrated biomass
THALAPURE: AS FREEZE-DRIED POWDER

HOW TO USE

1. Add 1 litre of clean, fresh water to a kitchen blender
2. Switch the blender on
3. Add 20g Thalapure® into moving water
4. Mix for 2 minutes at a moderate speed
5. Rehydrated solution is ready for dilution in tank water
6. The rehydrated cell suspension can be stored at 4°C for a maximum of 12 hours

Correct rehydration

Incorrect rehydration
THALAPURE: DIGESTIBILITY AND CELL INTEGRITY ISSUES – SILICEOUS EXOSKELETON (A FRUSTULE)
ARTEMIA FED WITH THALAPURE
“In healthy larvae showing active feeding and digestion, the hepatopancreas and midgut will be full of small, easily observed bubbles (digestive or “lipid” vacuoles)…”

PACIFIC WHITE SHRIMP FED WITH THALAPURE

hepatopancreas

Zoea II

midgut
MICROALGAE FOR AQUACULTURE: CONVERTING ART INTO TECHNOLOGY

Thalapure for shrimp hatcheries:

Converting ‘microalgae hatchery art’, i.e. diversity of farm- or company-specific methods of on-site microalgae production into technology, i.e. Uniform, well-established and readily reproducible procedures.

The nearest realistic scenario: reducing dependence of hatcheries on live microalgae especially at the most critical stages of larval rearing.
## TOMALGAE: MILESTONES

<table>
<thead>
<tr>
<th>Year</th>
<th>Event Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013 (June - )</td>
<td>Pilot set-up at greenhouse location NL, focus on sustainable production and quality. Test in target markets with selected partner</td>
</tr>
<tr>
<td>2014 (Jan)</td>
<td>Proof of concept first application (direct feed in larval AQ, shrimp)</td>
</tr>
<tr>
<td>2014 (April)</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; commercial RW’s operational at site in Belgium</td>
</tr>
<tr>
<td>2014 (June)</td>
<td>Supply agreement Thailand and China. First shipments</td>
</tr>
<tr>
<td>2014 (Dec)</td>
<td>ISO-9001 and GMP+ certified</td>
</tr>
<tr>
<td>2015 (Feb)</td>
<td>Tomalgae joined the Benchmark group</td>
</tr>
</tbody>
</table>
| 2015 (- present) | Scale-up production facility (Phase 1)  
  - **Upstream**: 5000m<sup>2</sup> (3750m<sup>2</sup> net pond surface)  
  - **Downstream**: 2 x 20m<sup>3</sup> xh<sup>-1</sup> pre-concentration separation technology  
  - **Outsourcing**: Freeze drying and packaging |
A pioneering innovation company operating in the aquaculture, agriculture and animal health sectors

We aim to set the benchmark for sustainable living, starting with food production

Well known in aquaculture:
THANK YOU FOR YOUR ATTENTION

William van der Riet

A Benchmark Company
WHAT DO WE OFFER?

• Controlled batch released specification
• Correct nutritional profile, rich in Omega-3 fatty Acids
• Pathogen free (life algae are principle vector of vibro and protozoal infectins)
• Readily digestible and of suitable cell size
• Full cell integrity (no leaching/ bacterial risk)
• Acceptable shelf life (formulation suitable for storage and cost efficient distribution)
• Natural product, no preservatives used
• Year round available/ sustainable supply source
P. VANNAMEI: ZOEA-2 HEALTH ASSESSMENT IN PHYLAVIVE-TANK:
FAECES (FAecal STRING)
(06.01.2015)
**P. VANNAMEI, ZOEAL_A1_8-6-57-DAY1**

Feces of Zoea1 larvae fed with the algae:

**the composition**

Size (valvar diameter) of cells from feces:
- Range: 5.57 – 9.52 µm
- Average ± SD: 7.56 ± 0.75 µm
- N of measurements: 32

Conclusion:
- Feces basically consist of densely 'packed' remains of algal cells (mainly indigestible siliceous component of cell wall);
- Within cell size range of 5-10 µm, there is no indication of cell size selectivity by Zoea1-larvae (the minor difference between the two means, 7.56 and 7.93 µm, is statistically insignificant).