Tomalgae

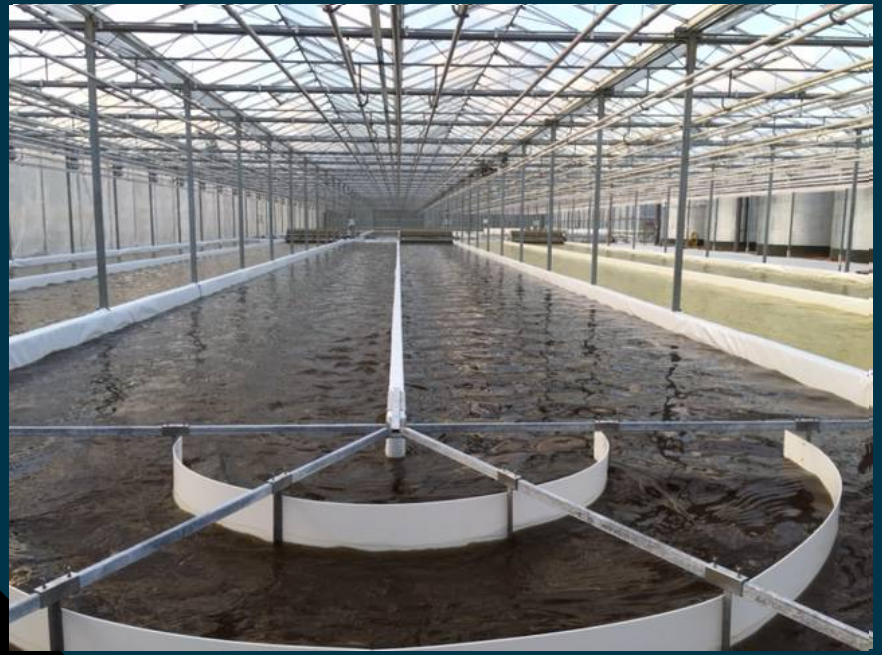
A Benchmark Company

MICROALGAE FOR AQUACULTURE:

CONVERTING ART INTO TECHNOLOGY

William van der Riet
CEO Tomalgae, Belgium

TOMALGAE PRODUCES MICROALGAE
(FOR LARVAL AQUACULTURE)



MICROALGAE IN AQUACULTURE

“Algae are at the base of the entire aquatic food chain,…

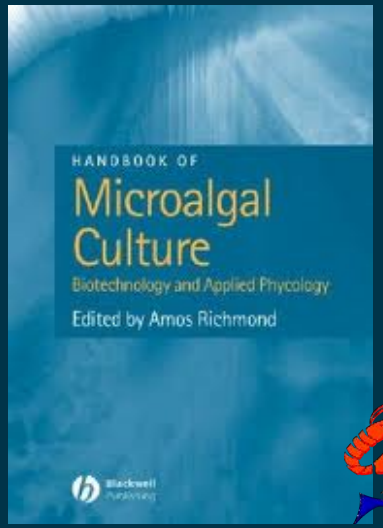
Therefore, it is not surprising that **the microalgae** which compose the phytoplankton **play a vital role in the rearing of aquatic animals** like molluscs, shrimp, and fish, and **have a strategic interest for aquaculture**.”

(Arnaud Muller-Feuga 2000)

MICROALGAE IN AQUACULTURE

"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

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

Closed Photobioreactors (PBRs)

Flat-plate "Acrylic" Photobioreactors. Photo by AzCATI: http://asulightworks.com/blog/azcati-update-plastic-film-vs-acrylic-photobioreactors

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

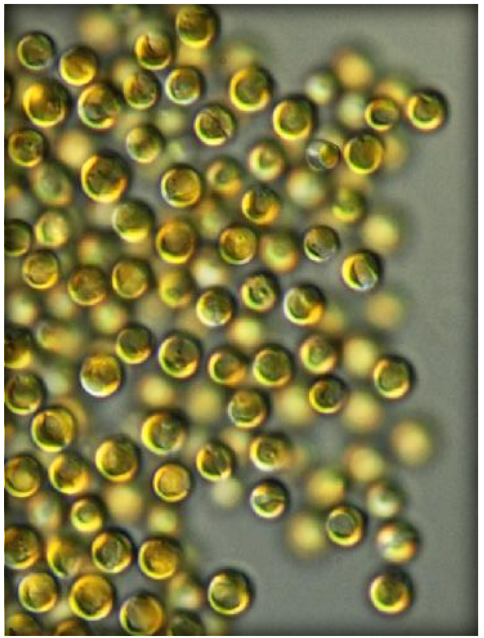
—Concentrate & Drying

—Rehydration and (or) resuspension

MICROALGAE IN (FOR) AQUACULTURE

Haptophyta: *Isochrysis*

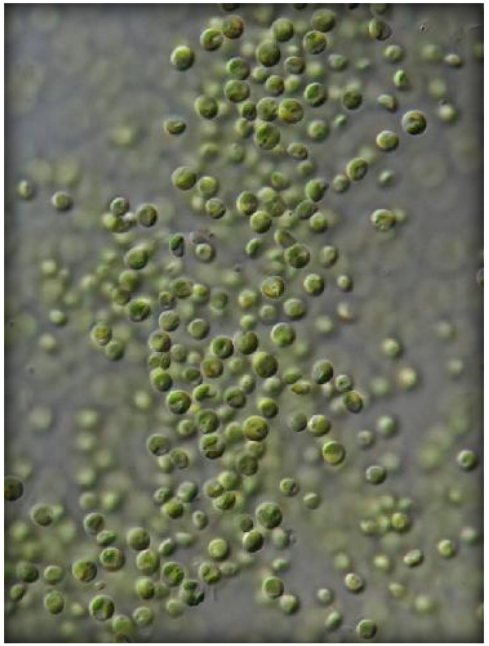
- Small cell size
- Fast rate of growth
- Acceptable biochemical quality
- Digestibility
- Buoyancy
- ‘Resistance’ to grazing
- Concentrate and drying
- Rehydration and or resuspension



MICROALGAE IN (FOR) AQUACULTURE

Eustigmatophyceae: *Nannochloropsis*

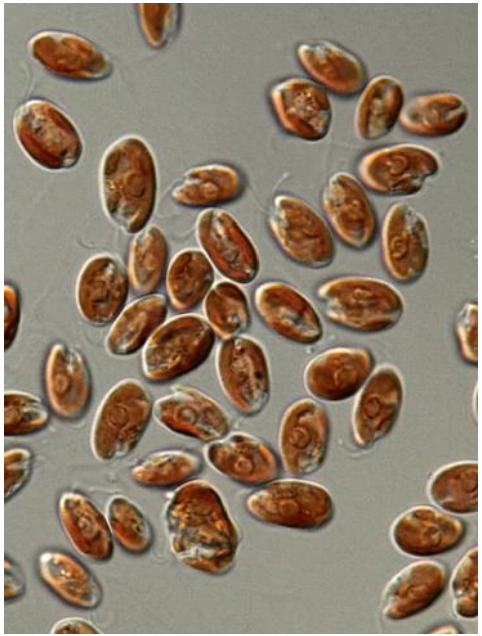
- Too small cell size
- Fast rate of growth
- Acceptable biochemical quality
- Digestibility
- Buoyancy
- ‘Resistance’ to grazing
- Concentrate and drying
- Rehydration and or resuspension



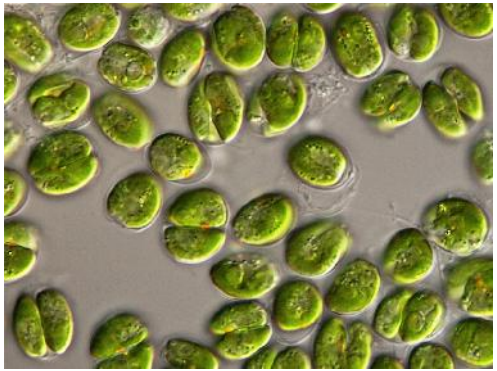
MICROALGAE IN (FOR) AQUACULTURE

Cryptophyta: *Rhodonomas*

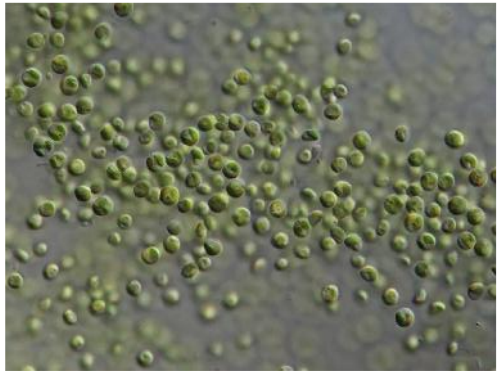
- Small cell size
- Fast rate of growth
- Acceptable biochemical quality
- Digestibility
- Buoyancy
- Resistance' to grazing
- Concentrate and drying
- Rehydration and or resuspension



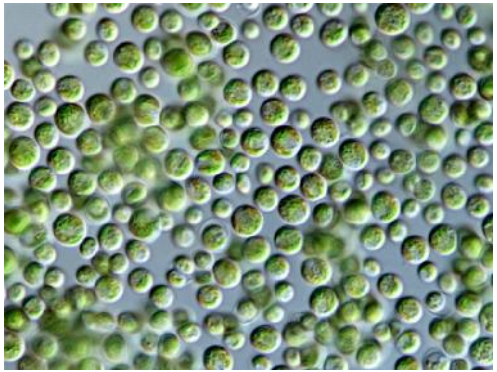
MICROALGAE IN (FOR) AQUACULTURE



Tetraselmis



Nanochloropsis



Chlorella

Spirulina

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)

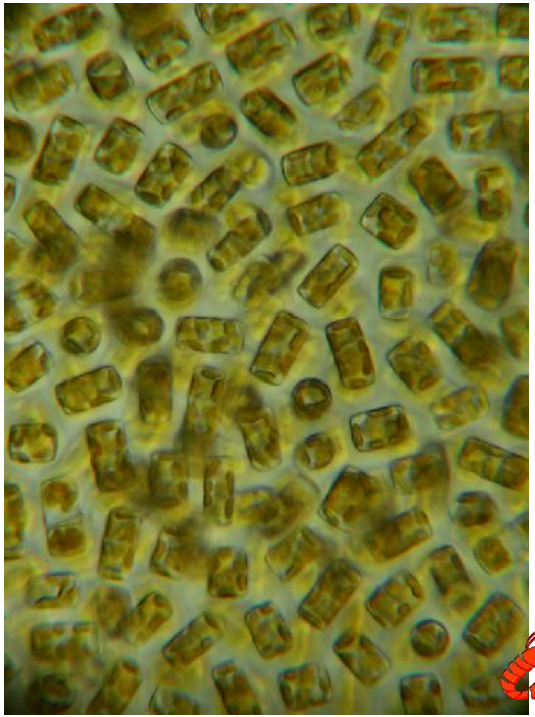
—Digestibility

—Buoyancy

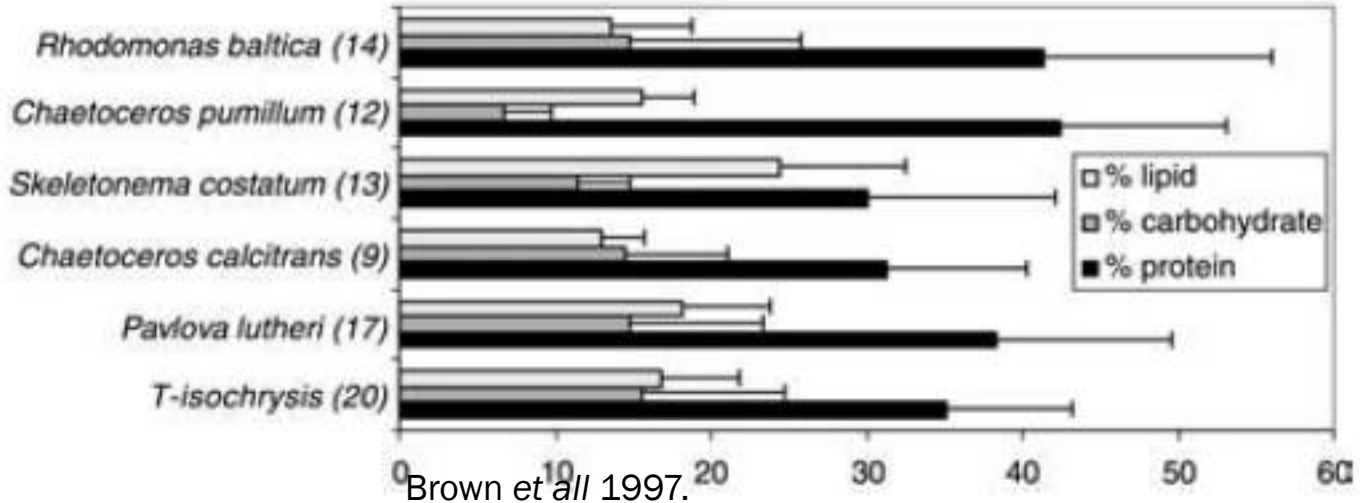
—'Resistance' to grazing (rigorous control)

—Concentrate (specific) & Drying (specific)

—Rehydration and (or) resuspension (specific)

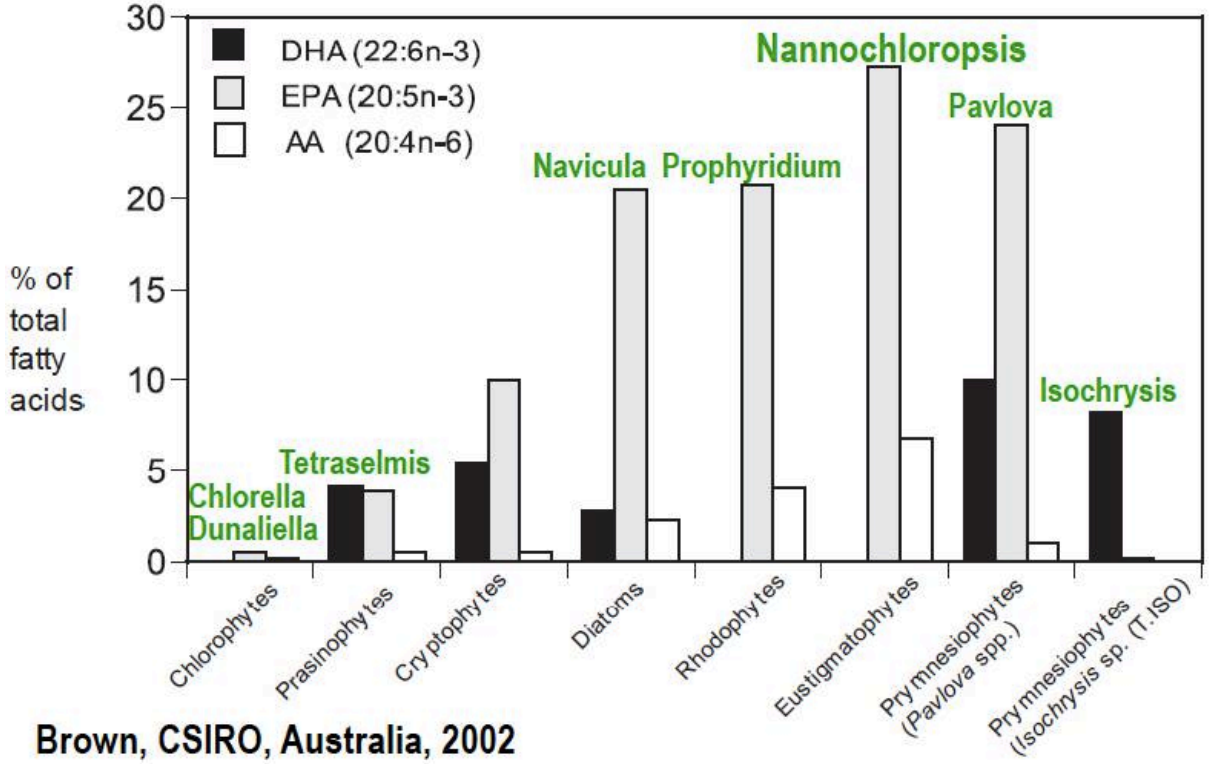


OVERALL BIOCHEMICAL COMPOSITION



Brown *et all* 1997.

LONG-CHAIN PUFA'S CONTENT OF MICRO-ALGAE



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)

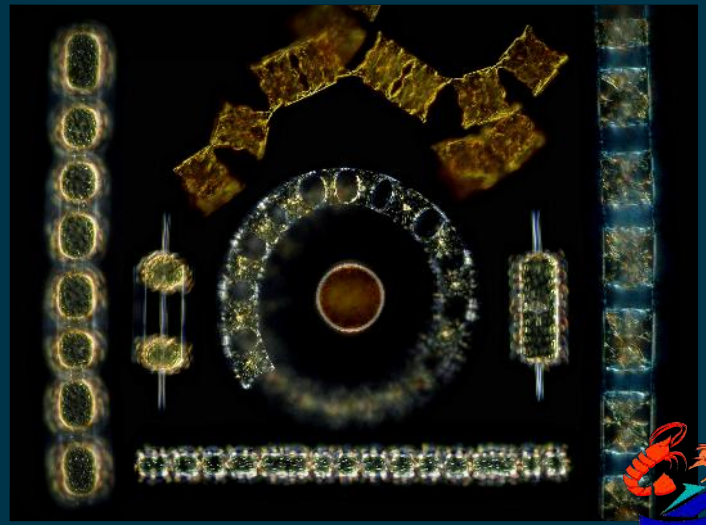
"… commercial marine hatchery operators throughout the world **have tried and discarded substitutes for live micro-algae.**" (Heasman 2001)

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:

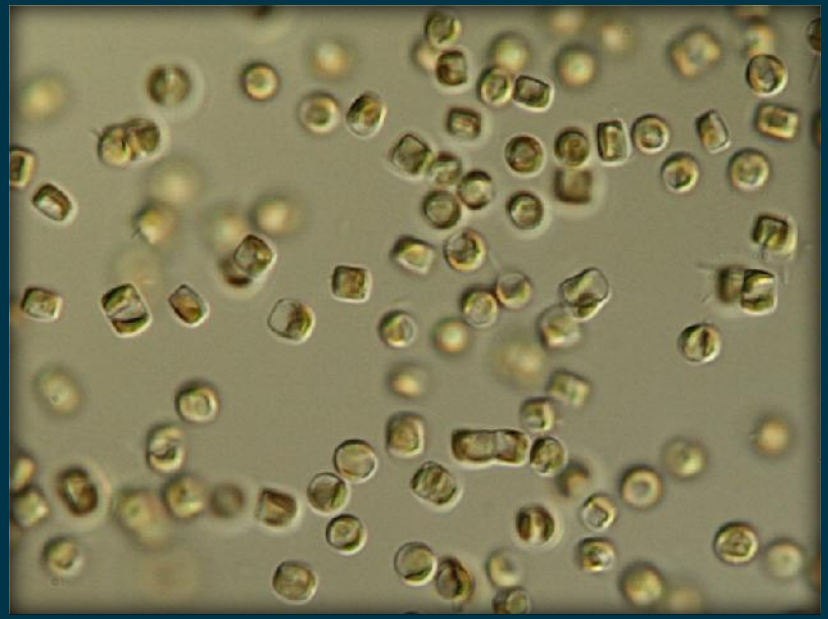
- Appropriate size for ingestion
- Readily digested
- Rapid growth rates
- Suitable for mass cultivation
- Stable in culture
- Good nutrient composition
- Absence of toxins

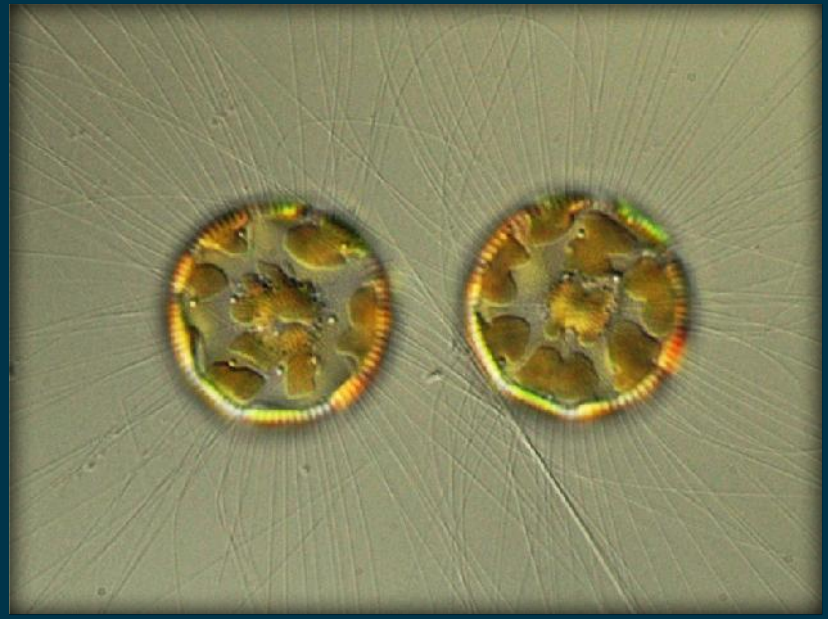
(M. R. Brown 2002)

DIATOMS USED IN AQUCULTURE

Chaetoceros spp.

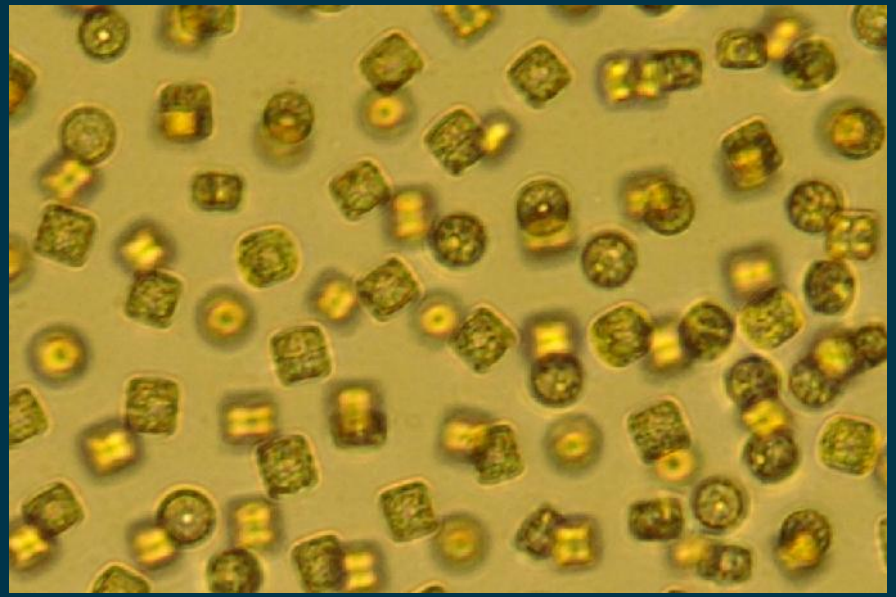
DIATOMS USED IN AQUCULTURE



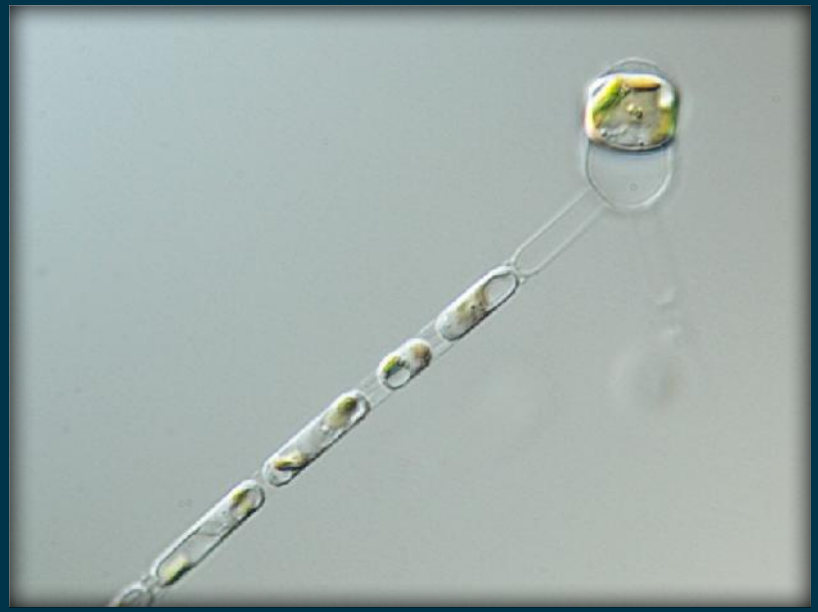


Thalassiosira spp.

DIATOMS USED IN AQUACULTURE

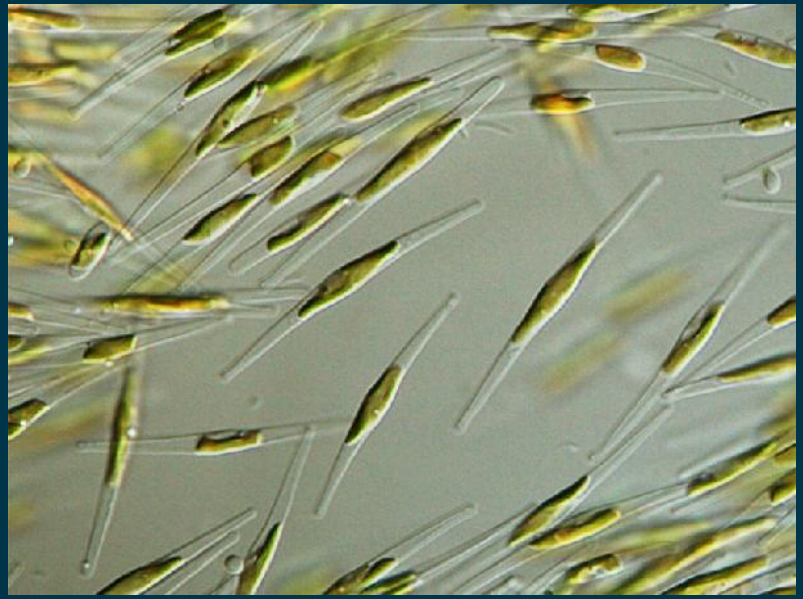


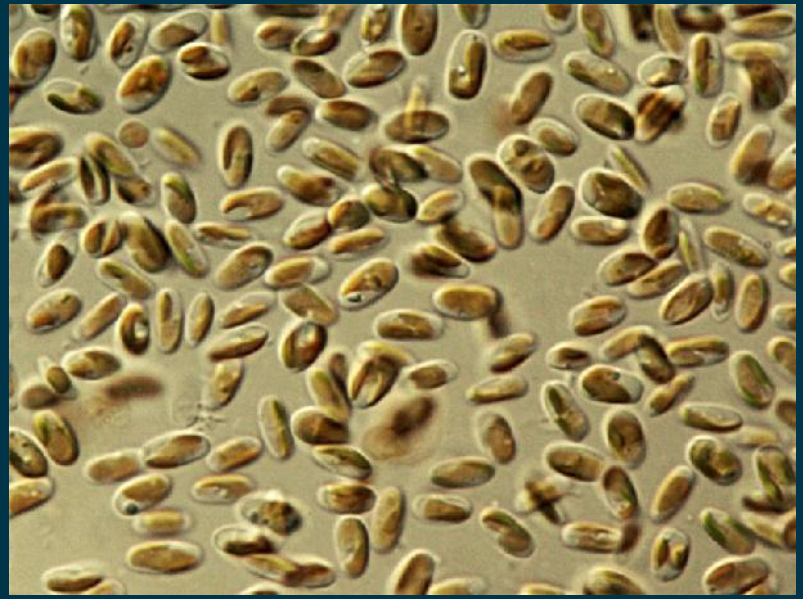
Cyclotella sp.



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

microalgae species used ('scalable')	Used for	Direct feed (shrimp/shellfish)	Indirect feed (fish; enrichment of zoo-plankton)	GWT
Thalasiosira	EPA (+DHA (p))	X	n.a	n.a
Chaetoceros – live algae	EPA	X	n.a	n.a
Nannochloropsis	EPA/ARA	n.a	rotifer (culture)	X
Tetraselmis	EPA	shellfish	rotifer (culture)	X
Phaeodactylum	EPA	n.a	r + a enrichment	X
Isochrysis	DHA	shellfish	r + a enrichment	X
Pavlova	DHA	shellfish	rotifer (enrichment)	n.a
Chlorella	unsure	n.a	rotifers (culture)	?
Spirulina	unsure	n.a	Rotifers (culture)	?

REED MARICULTURE INC

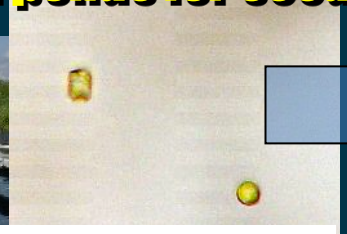
"Thalassiosira weissflogii **… is used in the shrimp and shellfish larviculture industry.** This algae is considered by several hatcheries to be the single best algae for larval shrimp."

"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."

http://reedmariculture.com/product_instant_algae.php

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

Clam hatchery - nursery

From larvae to seed to market size

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

DIATOMS: SEXUAL BREEDING

"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."

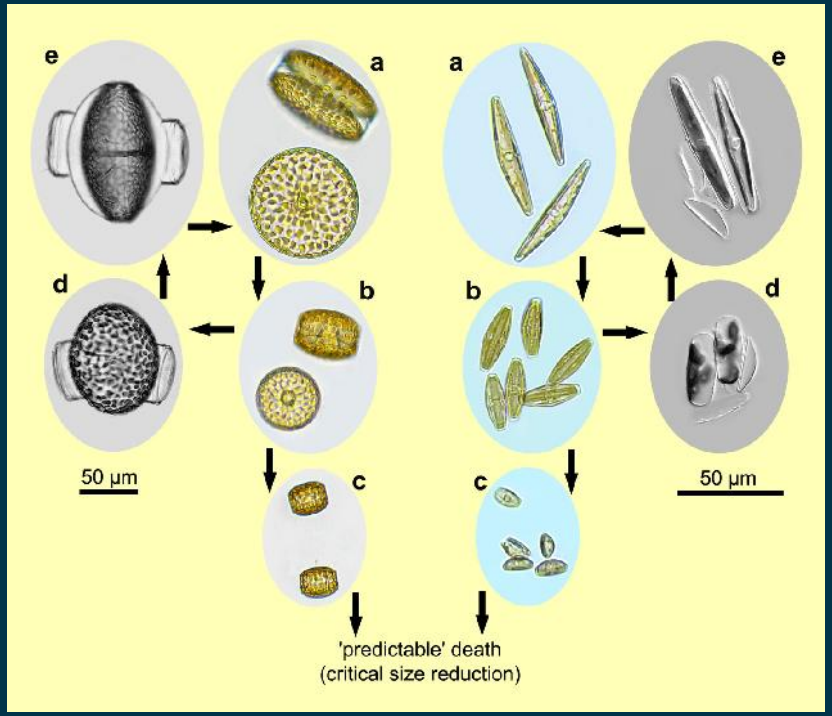
Prof. Dr. D.G.Mann, 1999

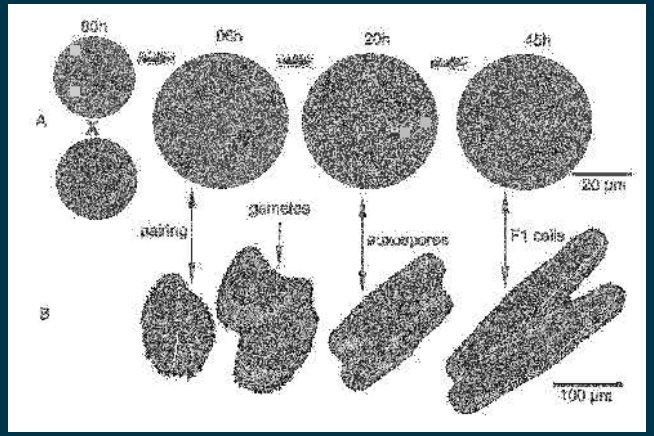
DR. JOHN BENEMANN (2010)

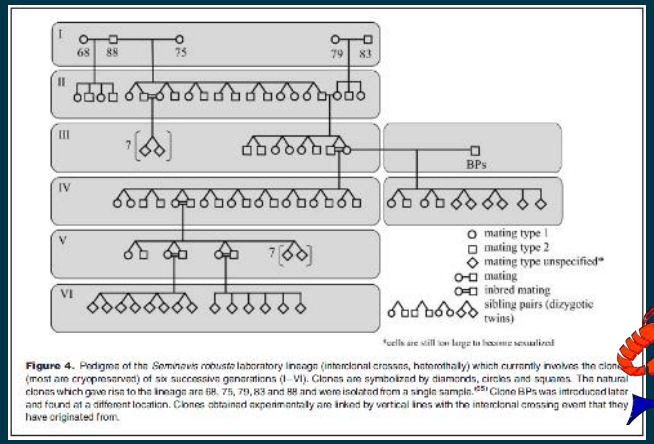
"We will **have to domesticate algae**, which has not yet been done. We are still using wild types, that is, strains that come directly out of nature without really any significant improvement or even much selection."

"… **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."

BASICS ON THE LIFE CYCLE AND SEXUAL BEHAVIOUR IN DIATOMS







Chepurnov V.A., *et al*. (**2008**). In search of new tractable diatoms for experimental biology. ***BioEssays*** 30: 692-702.

1. BREEDING PROGRAM

Present

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

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

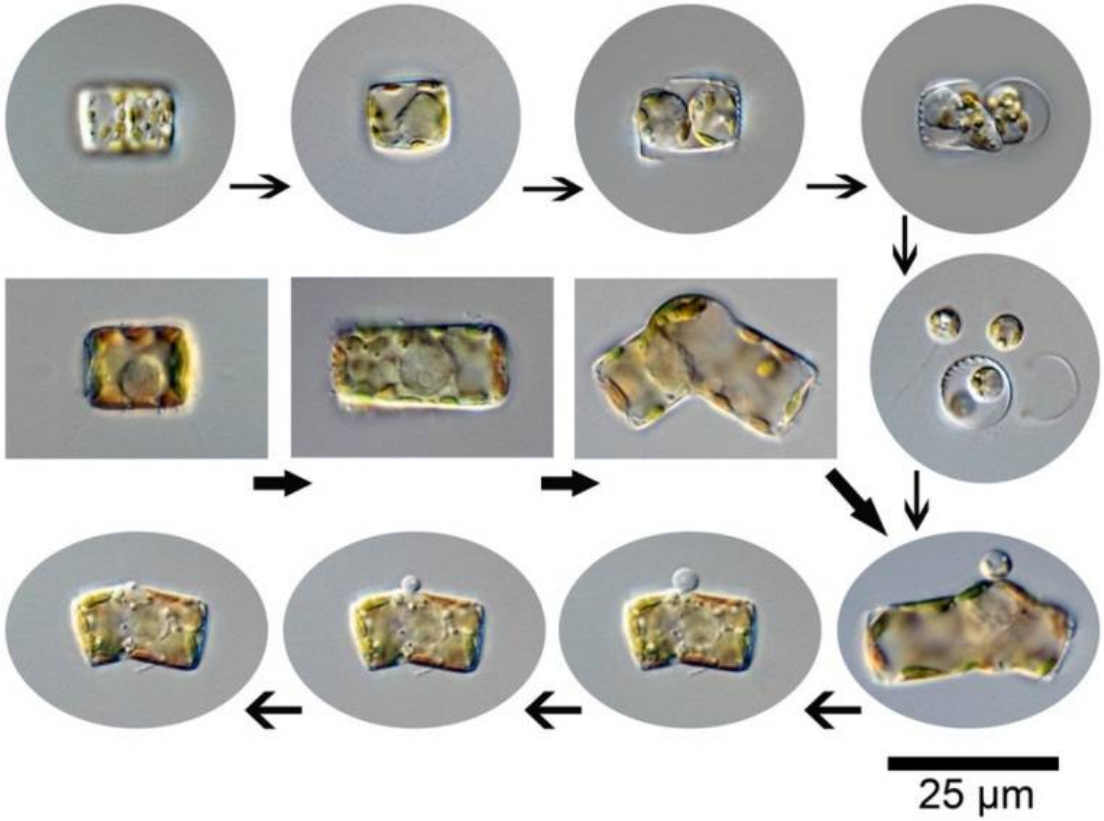
Control over triggering and intensity of sexual reproduction

Future

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

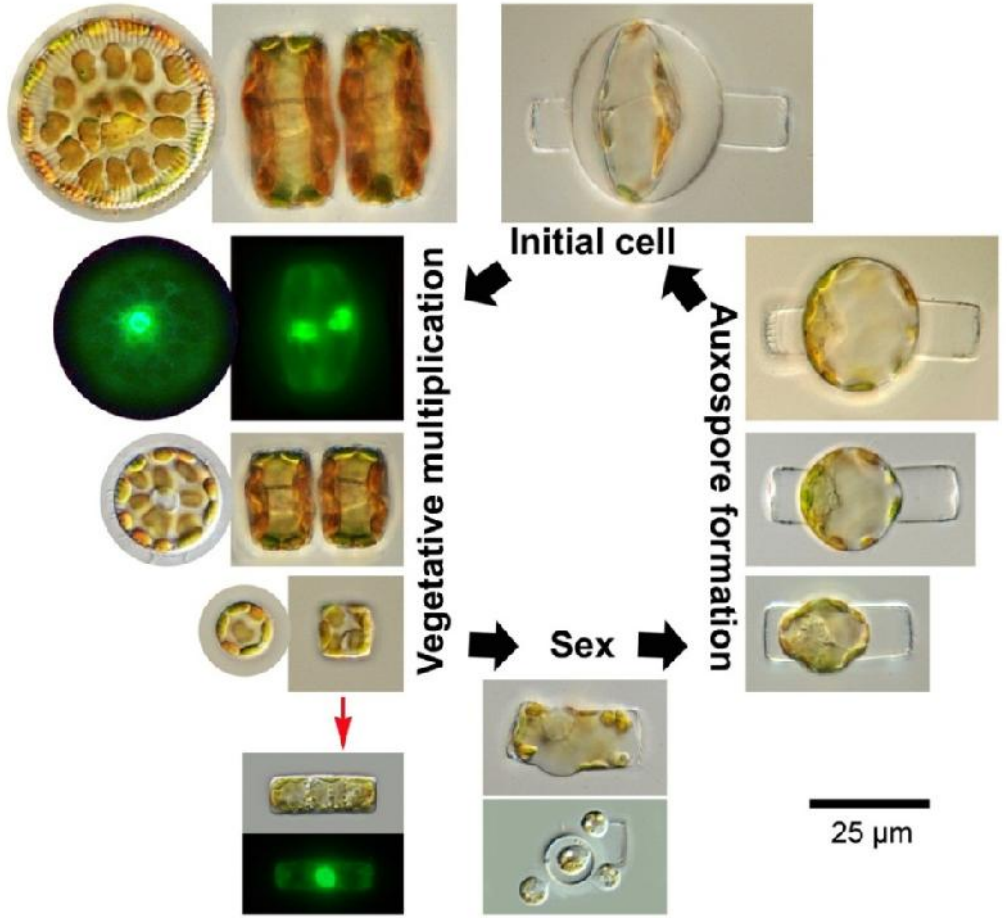
"Cross the best with the best, select the best, and … hope for the best!"
(John W. Snape, 2004)

1. BREEDING PROGRAM FOR THE CULTIVAR: **SEXUAL REPRODUCTION**

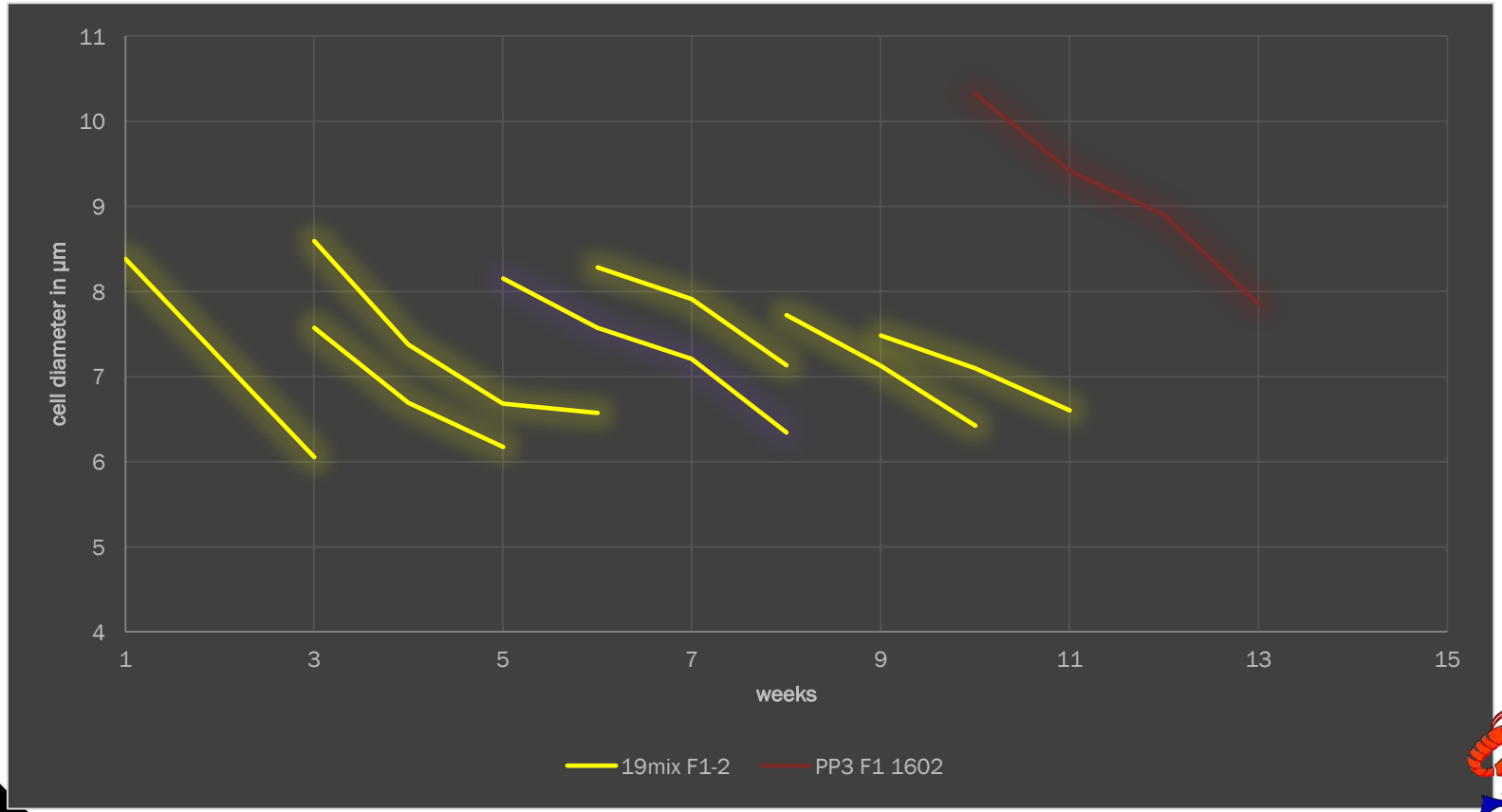


1. BREEDING PROGRAM FOR THE CULTIVAR:

CELL SIZE REDUCTION-RESTITUTION LIFE CYCLE



CELL SIZE CONTROL AND DYNAMICS



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

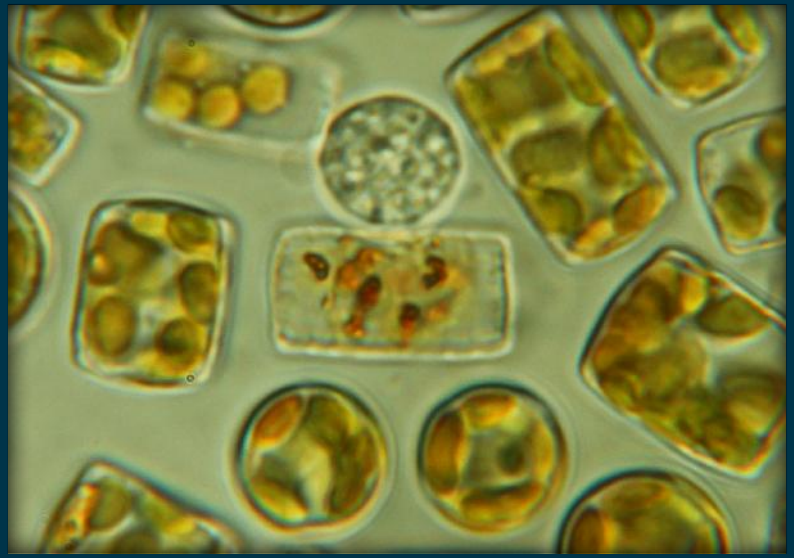
WHERE TO PRODUCE AT SCALE?

CLIMATIC OPTIMA FOR SHRIMP AND ALGAE CULTURE ARE DIFFERENT

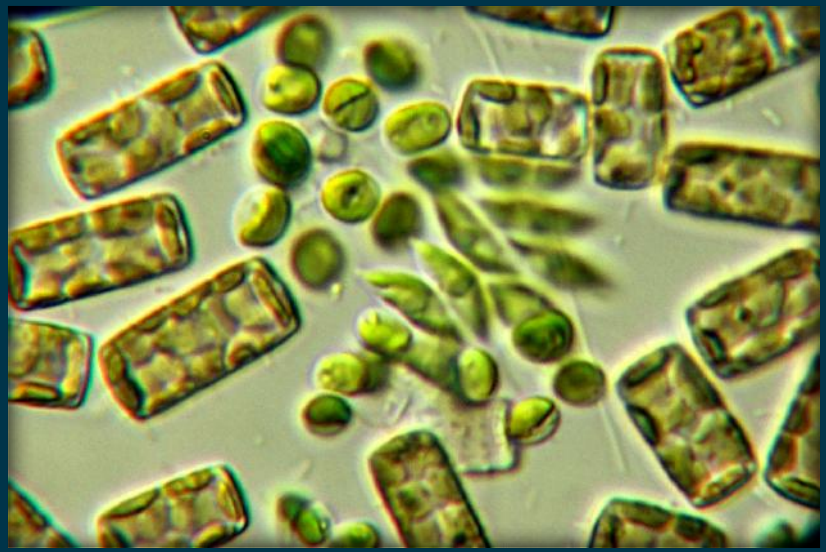
- **Microalgae** – temperature optimum: **18-23 °C** (Tomalgae cultivar: 15-20 °C)
- **Penaeid shrimps** – temperature optimum: **29-33 °C**

- **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:

INVE

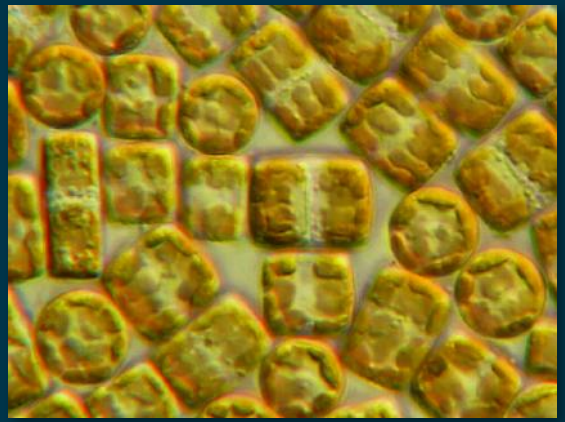
THALAPURE:
AS FREEZE-DRIED POWDER

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

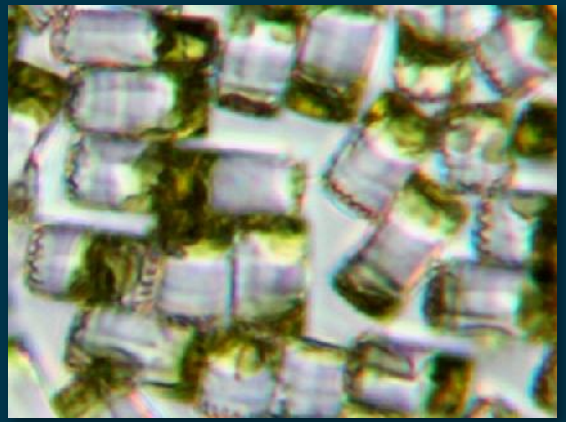
Report: 15-005988
Report date: 09/03/2015
Status: Final Report

C20:4-omega 6 (AA)	< 0.1	%/fat
C20:5-omega 3 (EPA)	38.3	%/fat
C22:0	< 0.1	%/fat
C22:1	< 0.1	%/fat
C22:5-omega 3 (DPA)	< 0.1	%/fat
C22:6-omega 3 (DHA)	8.0	%/fat
C24:0	< 0.1	%/fat
C24:1	< 0.1	%/fat
Sum of saturated fatty acids	15.8	%/fat
Sum of mono unsaturated fatty acids	26.5	%/fat
Sum of poly unsaturated fatty acids	57.5	%/fat
Sum trans fatty acids	0.3	%/fat
Sum omega 3 - fatty acids	55.0	%/fat
Sum omega 6 - fatty acids	2.4	%/fat

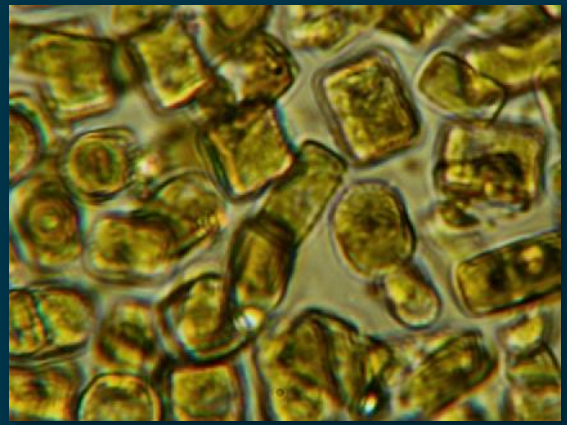
THALAPURE: KEEPING THE INTEGRITY OF CELLS AT ALL STAGES OF GROWTH AND PROCESSING



Concentrated biomass

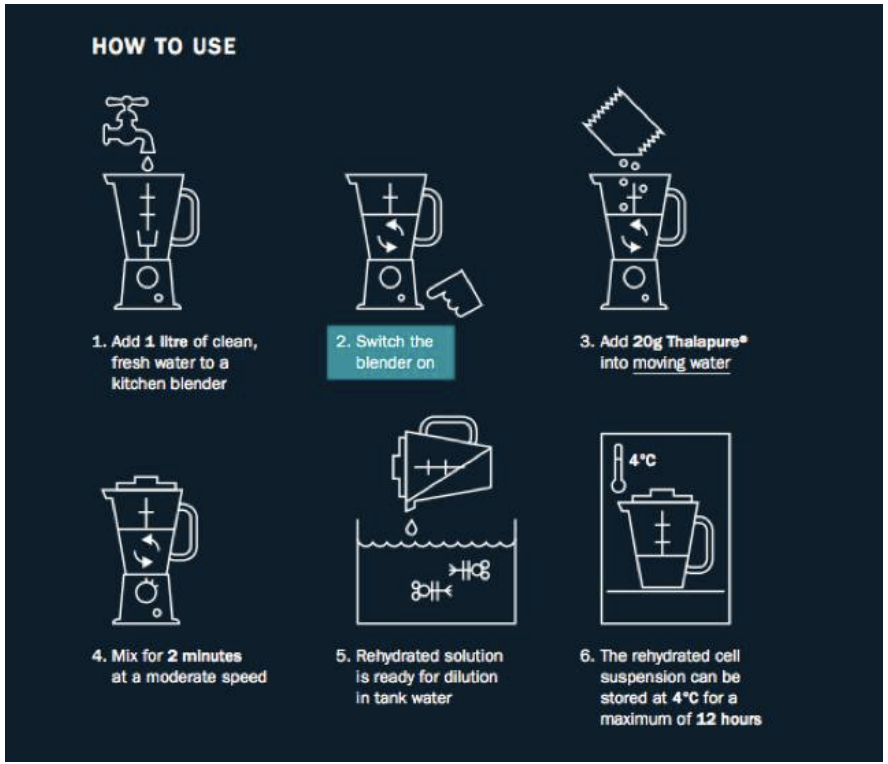


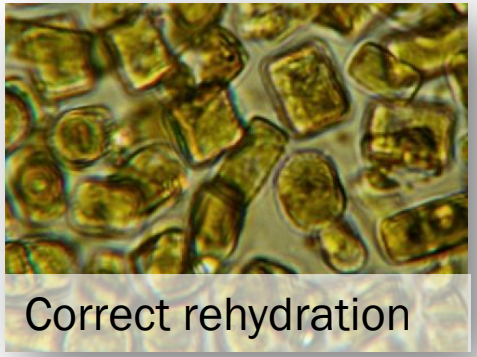
Dried biomass



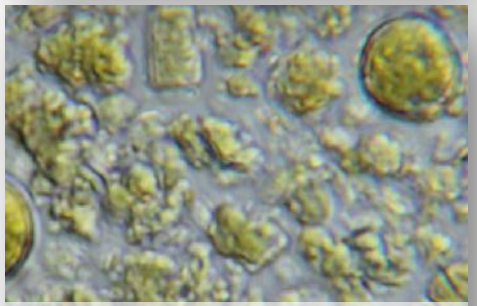
Rehydrated biomass

THALAPURE: AS FREEZE-DRIED POWDER



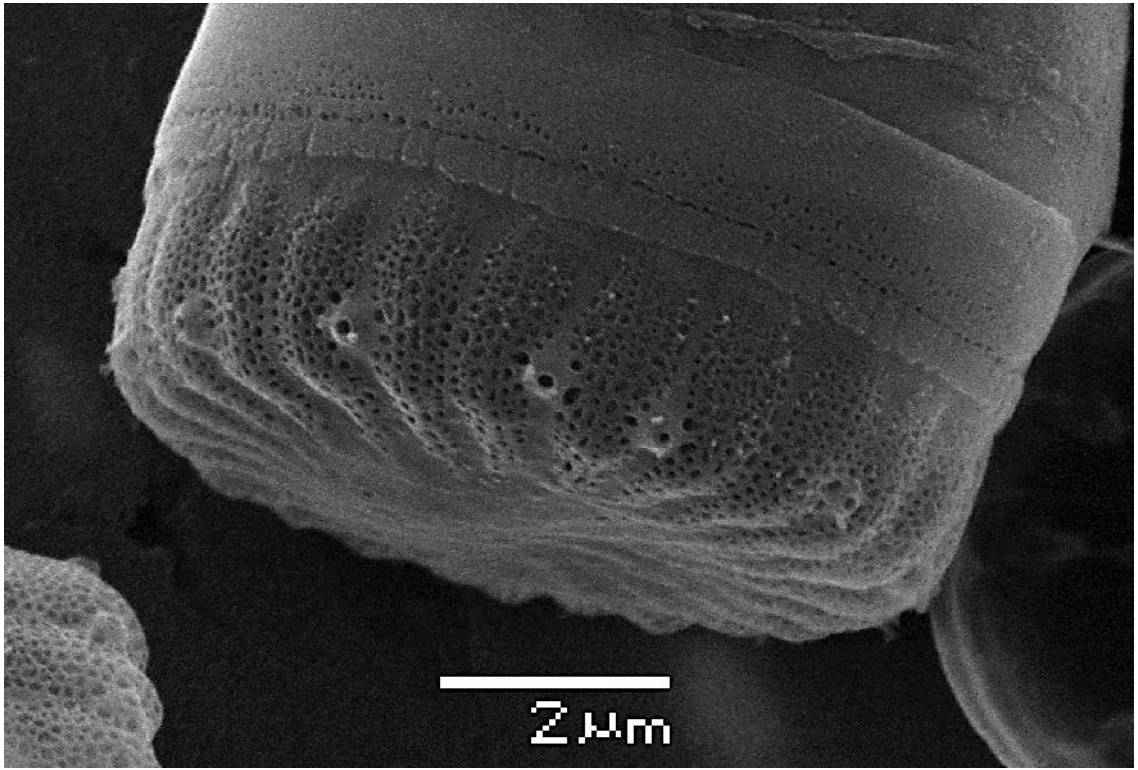


Correct rehydration

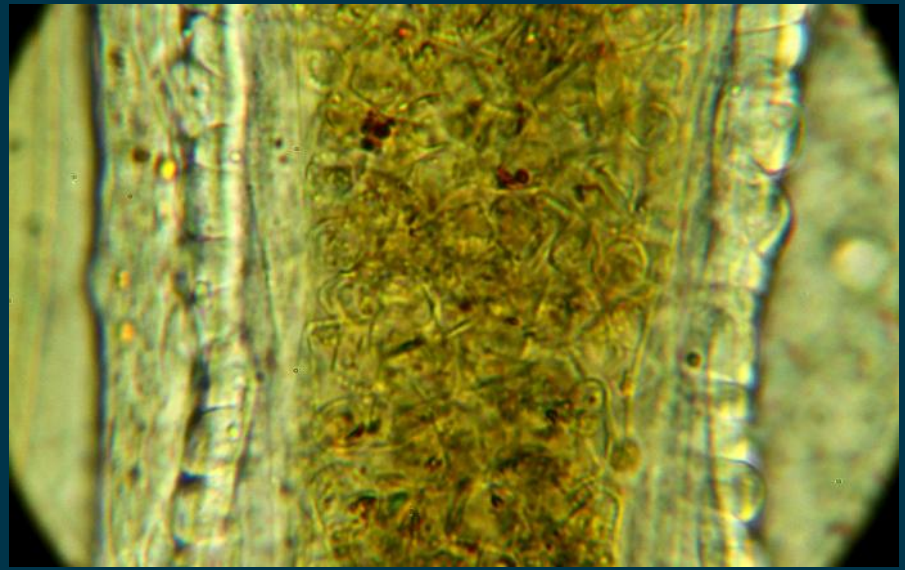


Incorrect rehydration

THALAPURE: DIGESTIBILITY AND CELL INTEGRITY ISSUES – SILICEOUS EXOSKELETON (A FRUSTULE)



ARTEMIA FED WITH THALAPURE

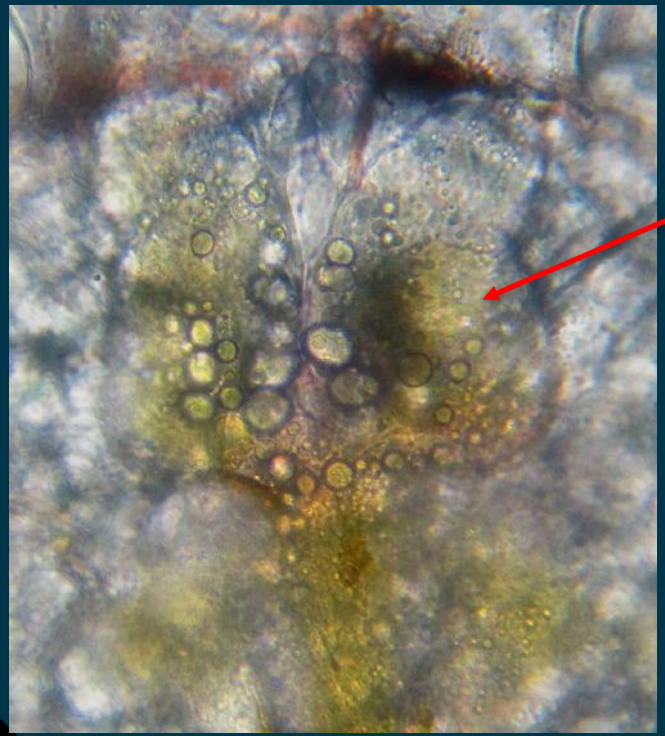


HEALTH ASSESSMENT AND LARVAL CONDITION

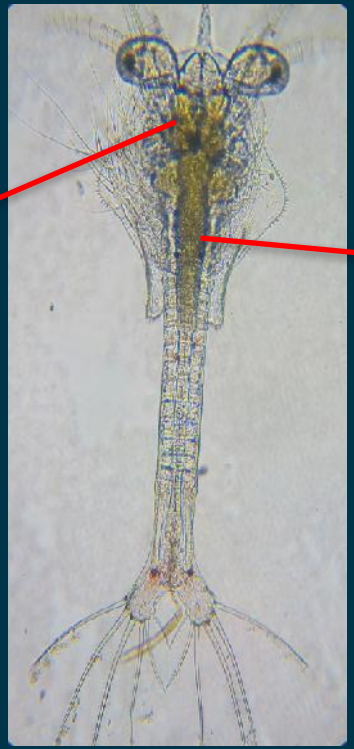
"In healthy larvae showing active feeding and digestion, **the hepatopancreas and midgut** will be full of small, easily observed bubbles (digestive or "lipid" vacuoles)..."

Health management and biosecurity maintenance in white shrimp (*Penaeus vannamei) hatcheries in Latin America,*
FAO Fisheries Technical Paper. No. 450. Rome, FAO. 2003. 62p. http://www.fao.org/docrep/007/y5040e/y5040e00.htm

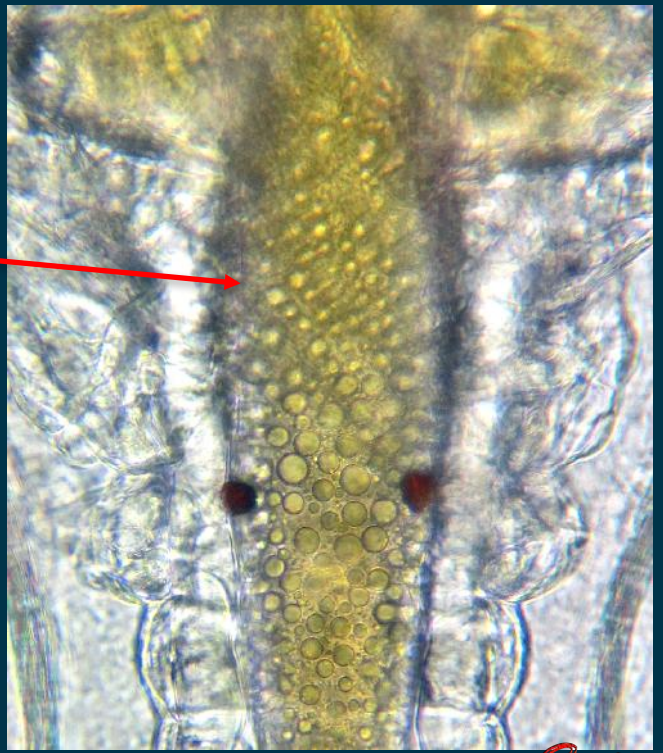
PACIFIC WHITE SHRIMP FED WITH THALAPURE



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

2013 (June -)	Pilot set-up at greenhouse location NL, focus on sustainable production and quality. Test in target markets with selected partner
2014 (Jan)	Proof of concept first application (direct feed in larval AQ, shrimp)
2014 (April)	1st commercial RW's operational at site in Belgium
2014 (June)	Supply agreement Thailand and China. First shipments
2014 (Dec)	ISO-9001 and GMP+ certified
2015 (Feb)	Tomalgae joined the Benchmark group
2015 (- present)	Scale-up production facility (Phase 1) • **Upstream**: 5000m^2 (3750m^2 net pond surface) • **Downstream**: 2 x 20m^3 xh^{-1} pre-concentration separation technology • **Outsourcing**: Freeze drying and packaging

Benchmark Holdings plc

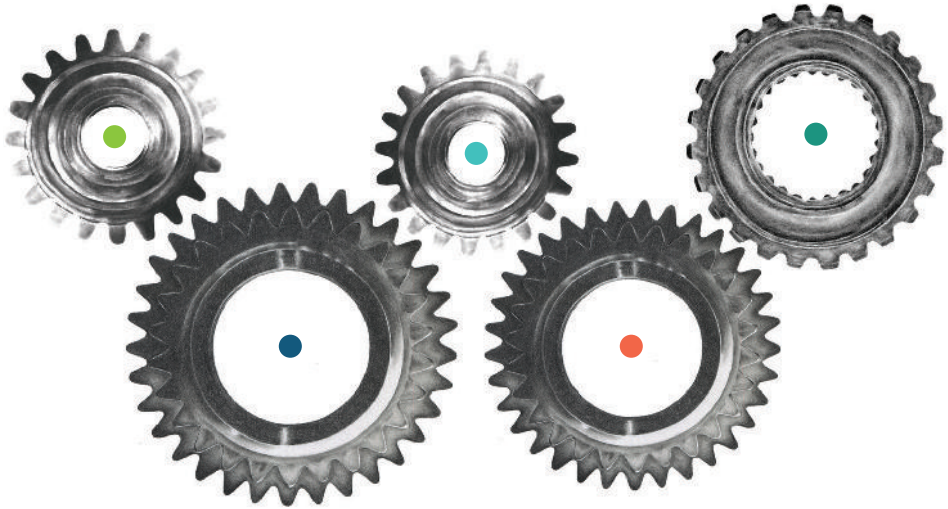
A pioneering innovation company operating in the aquaculture, agriculture and animal health sectors

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

TECHNICAL PUBLISHING

SUSTAINABILITY SCIENCE

ADVANCED ANIMAL NUTRITION*



ANIMAL HEALTH

BREEDING & GENETICS

Well known in aquaculture:

5m Publishing

Benchmark Vaccines Ltd

FishVet Group

fai

Benchmark SalmoBreed

Spring Genetics

INVE

WWW.TOMALGAE.COM
TWITTER: @TOMALGAE
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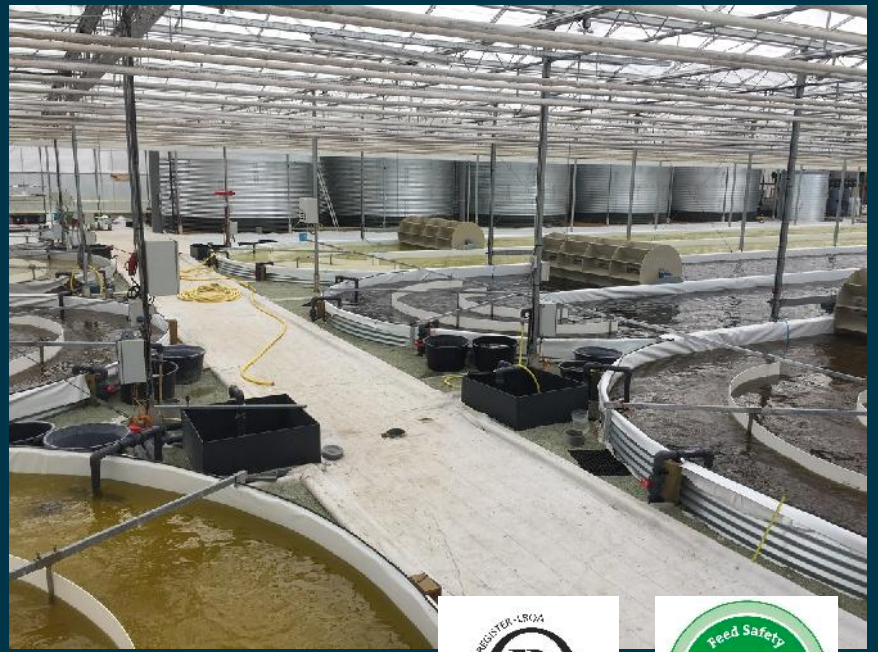


A Benchmark Company

LLOYD'S REGISTER LRQA ISO 9001

Feed Safety GMP+ Assurance

Tomalgae

THANK YOU FOR YOUR ATTENTION

William van der Riet

A Benchmark Company

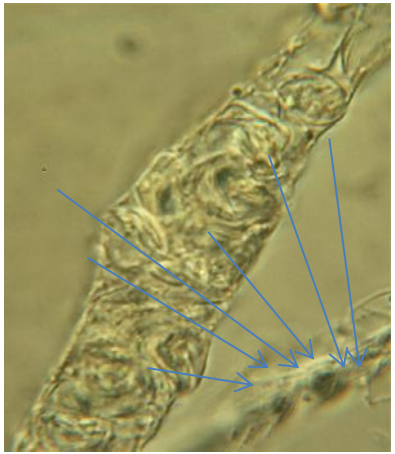
WHAT DO WE OFFER?

- 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, ZOEA1_A1_8-6-57-DAY1

Feces of zoea1 larvae
fed with the algae:
<u>the composition</u>



60

Visible (in focus) remains
of
siliceous cell wall
in feces

<u>Size (valvar diameter) of cells from feces</u>:
Range: 5.57 – 9.52 µm
Average ± SD: 7.56 ± 0.75 µm
N of measurements: 32
<u>Conclusion</u>: - feces besically consist of densely 'packed' remains of algal cells (mainly Indigestible siliceous component of cell wall);
- within cell size rane 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).