Zebrafish Dry Diet

Wilson Pinto

PhD in Aquaculture Nutrition

Tailoring
your feeds




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New products and tailored nutritional solutions for the aquaculture market

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Business Units

Industrial R&D Services

- Customized R&D feeds
- Nutrition trials with fish & shrimp
- Feed technology tests

Own Products & Services

ZEBRAFEED by sparos

HATCHERY FEEDS by sparos

aquatica by sparos

FEEDNETICS

Markets

Additives

Ingredients

Feeds

Farmers

R&D Institutes

National & European Projects

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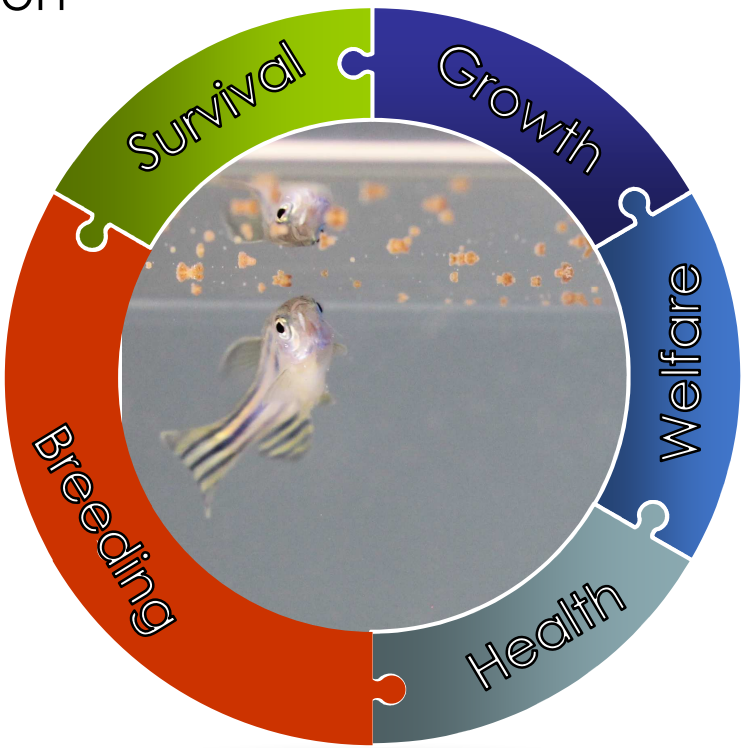
Developing a Zebrafish diet

Tailoring your feeds

Optimal nutrition is key…

Water quality
Physiological/Metabolic status

Metabolic pathways
Gene expression



Stress response
Disease resistance
Skeletal deformities

Fertility
Fecundity
Egg quality

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Challenges in zebrafish nutrition

Limited knowledge on nutritional requirements

- Robusteness of the species

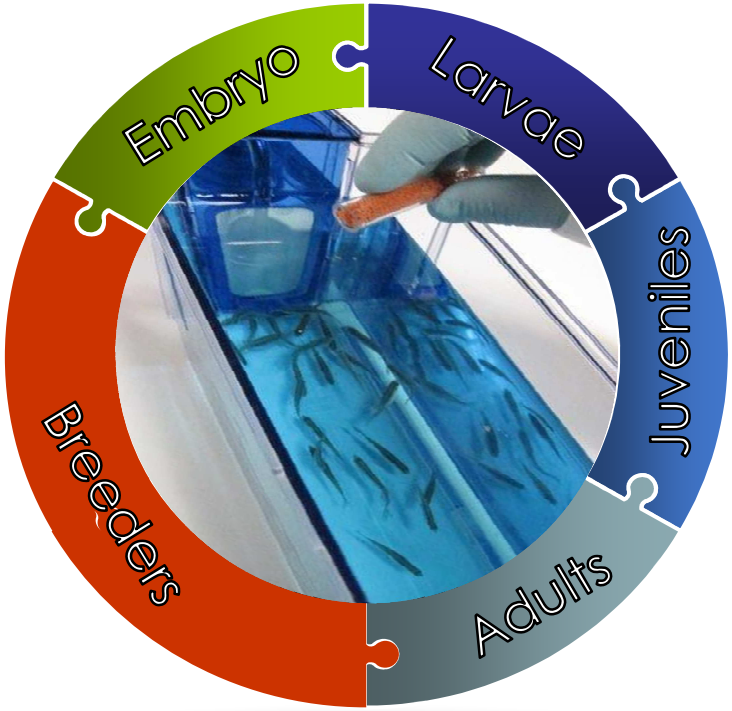
Largest feed particle: ≈ 600 micron

- Technology issues

Variable feeding regimes

Same targets

- Breeding performance
- Survival
- Fast growth
- Water quality



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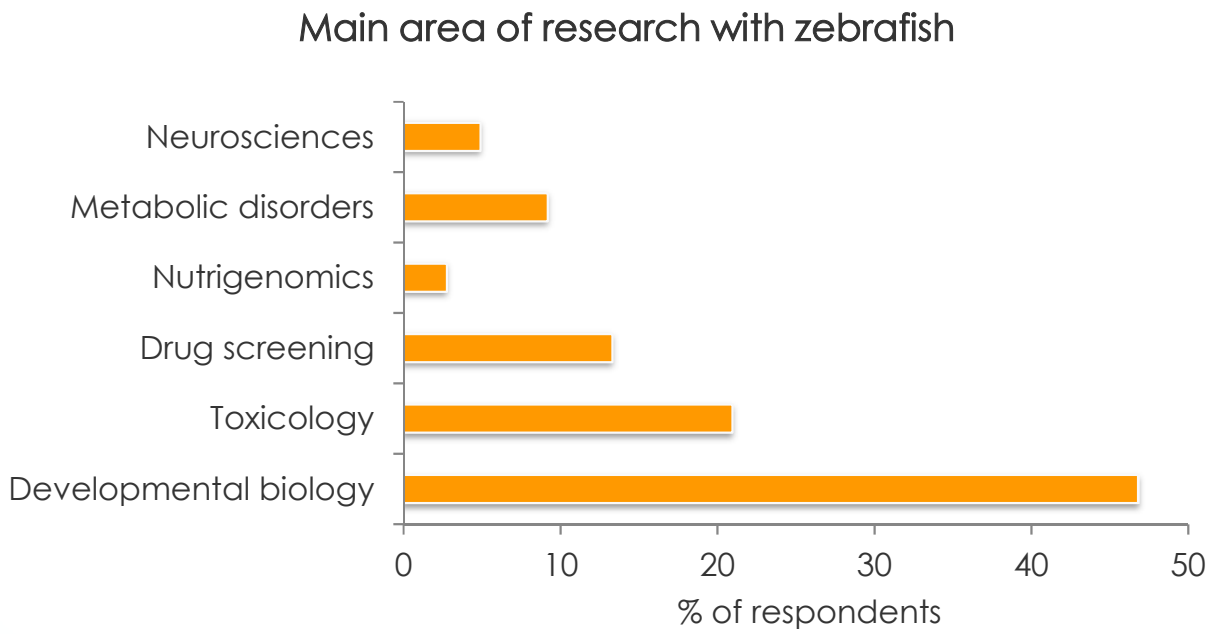
Development of a ZEBRAFISH diet

ZEBRAFEED Project

3-year R&D project between SPAROS & Center of Marine Sciences of Algarve

We started the project by identifiying the needs of the ZF community...

September 2013: online survey with 654 validated responses



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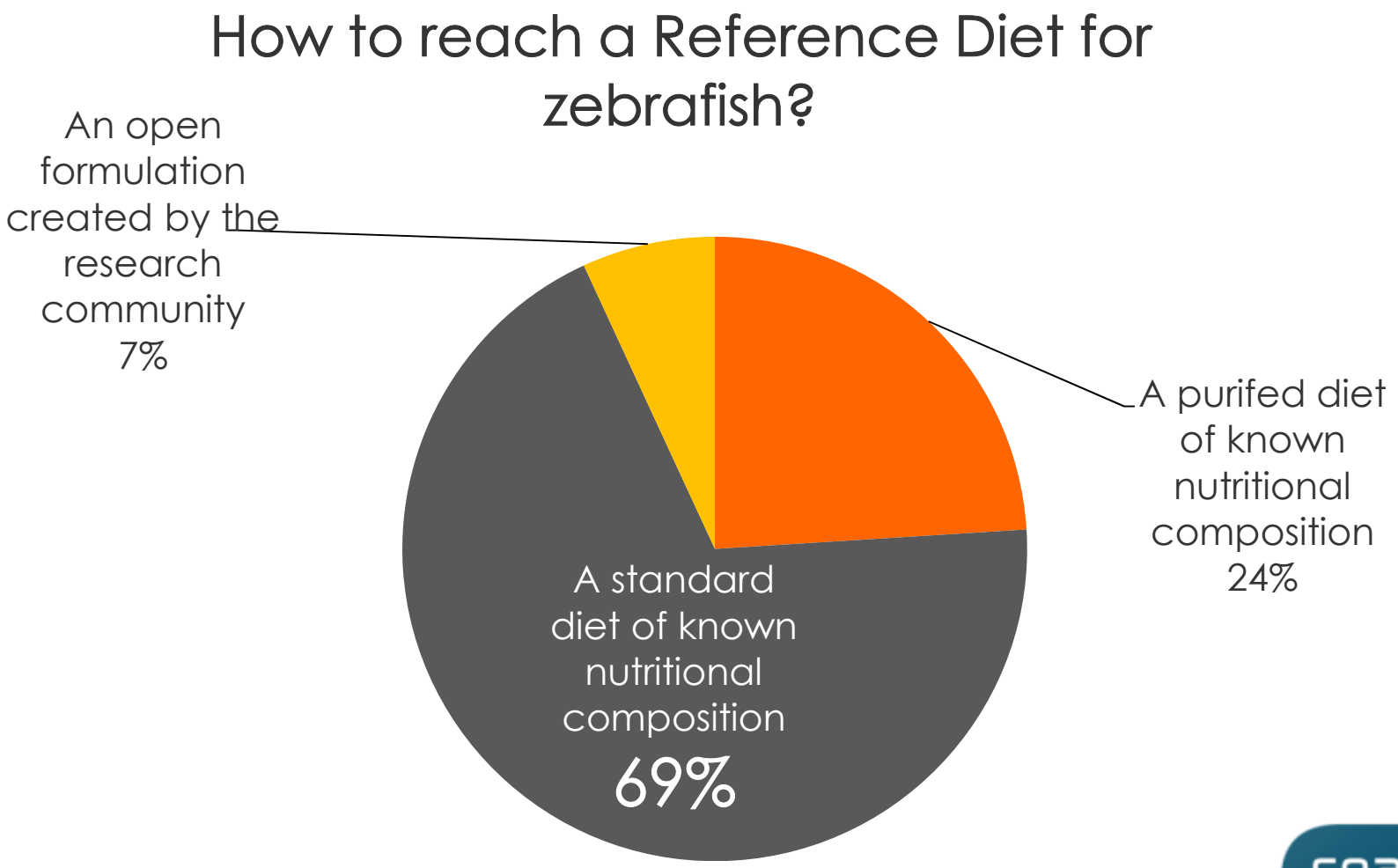
What "you" told us



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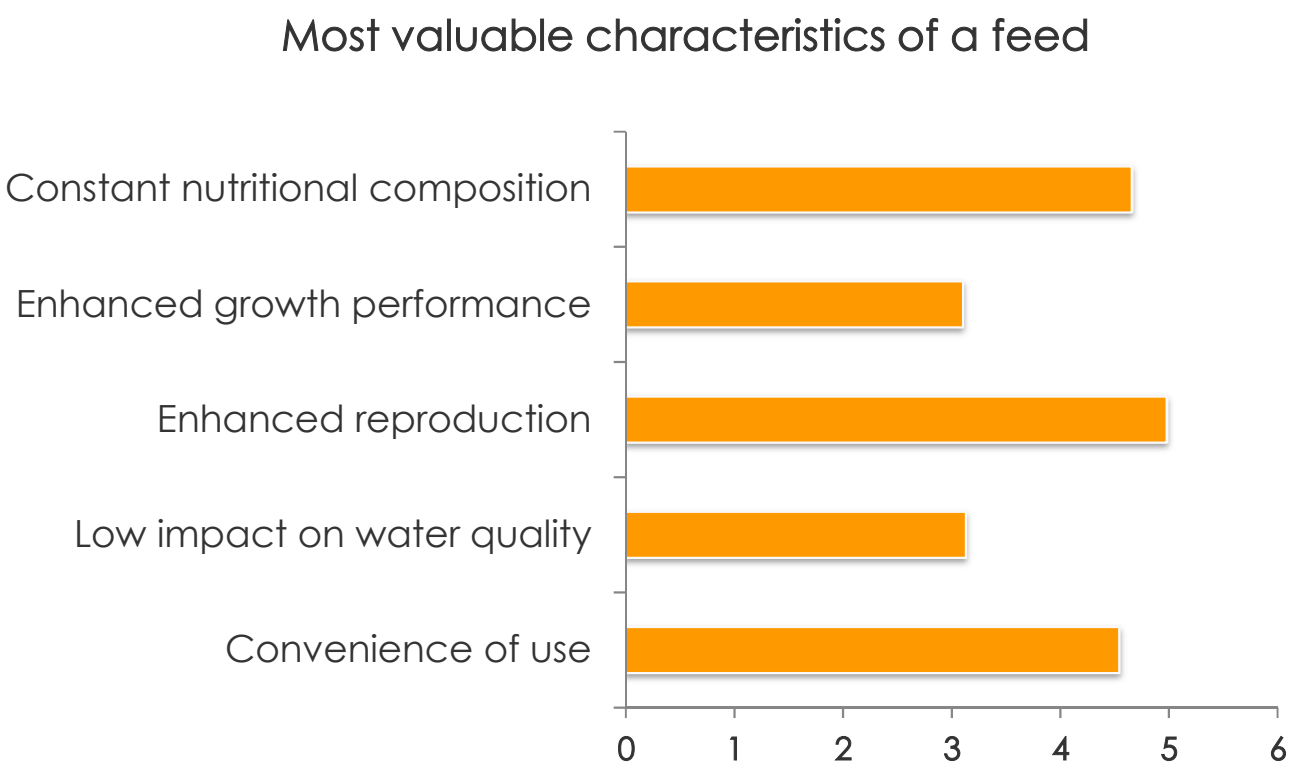
What "you" told us



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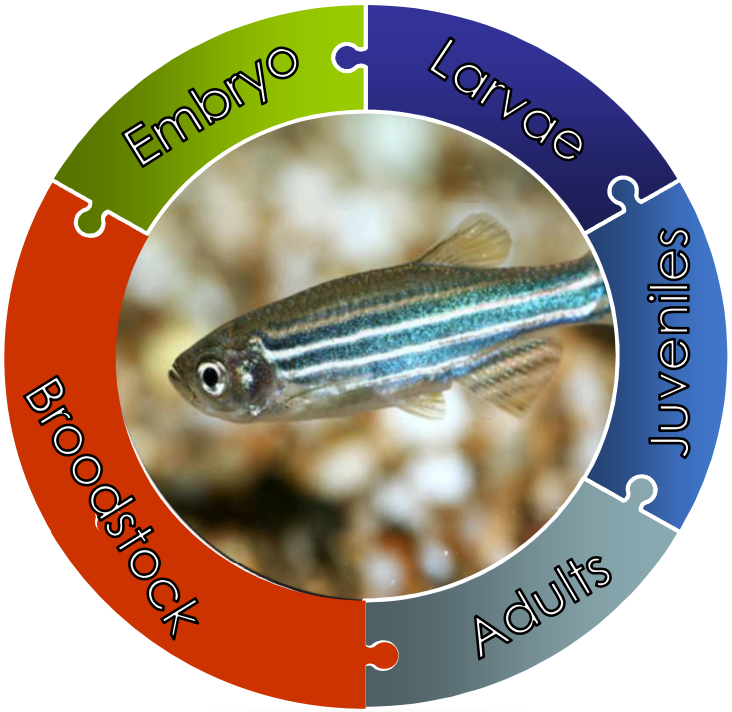
You helped us define our targets

High survival and growth from larvae stages
Enhanced reproductive performance



High welfare standard
Low-impact on water quality
Constant nutritional composition

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Nutritional requirements of zebrafish

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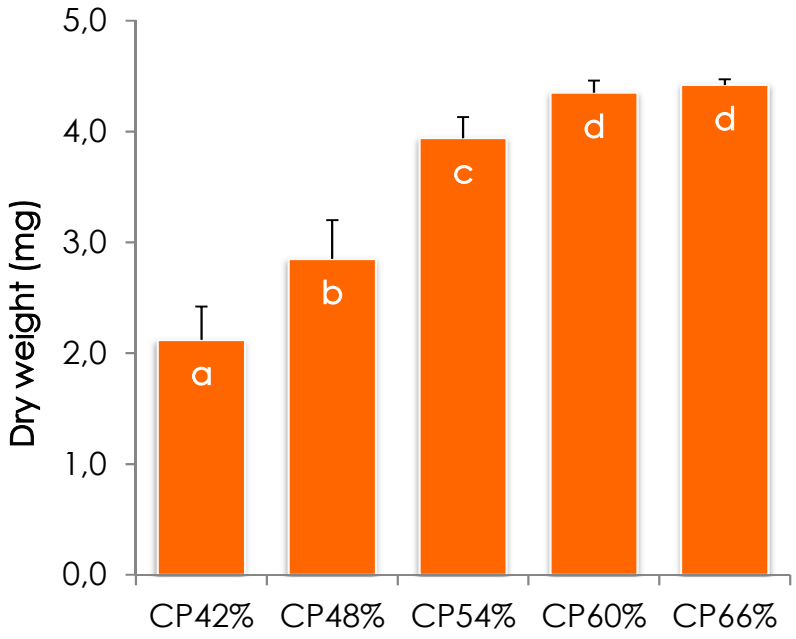
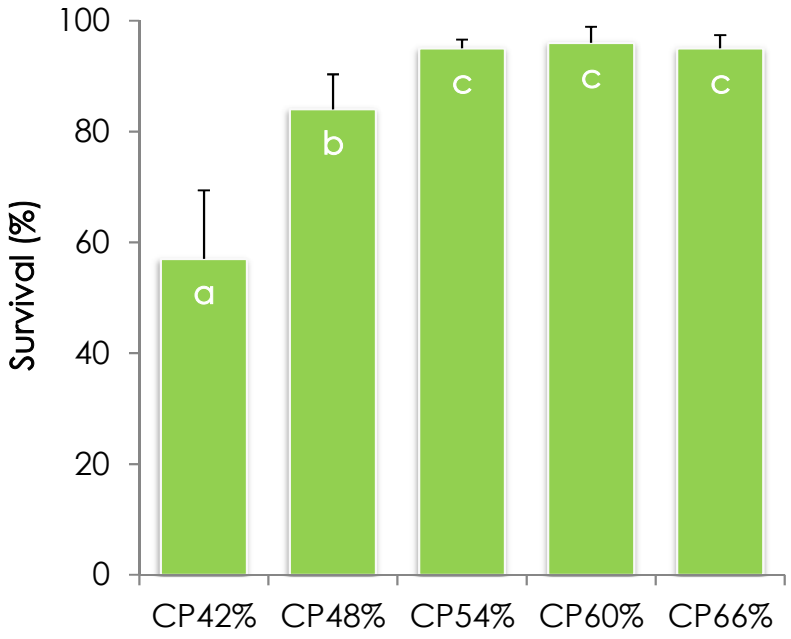
Protein requirements in larvae





Key message

Optimal dietary crude protein level for larval stages (until 30 dpf) is **62%**

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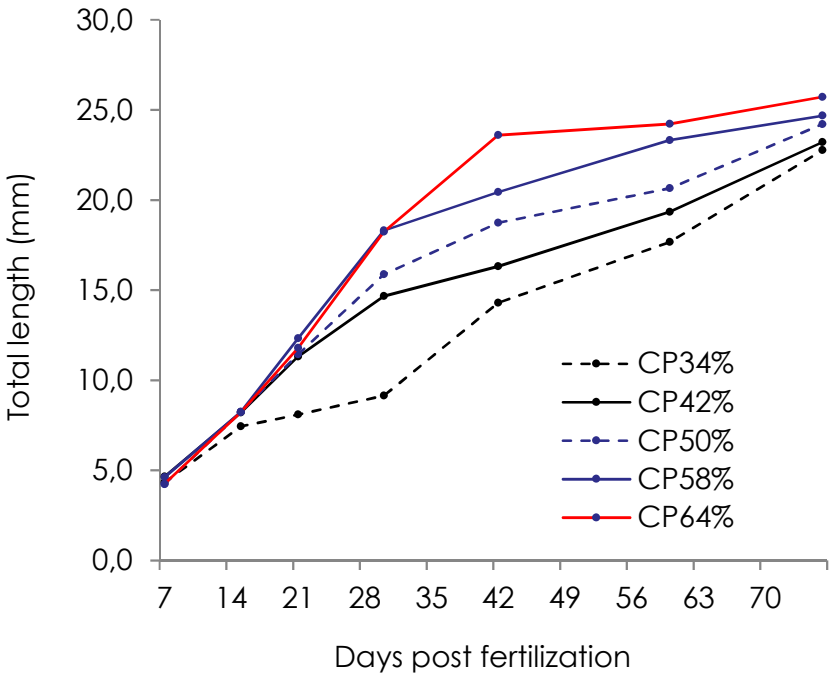
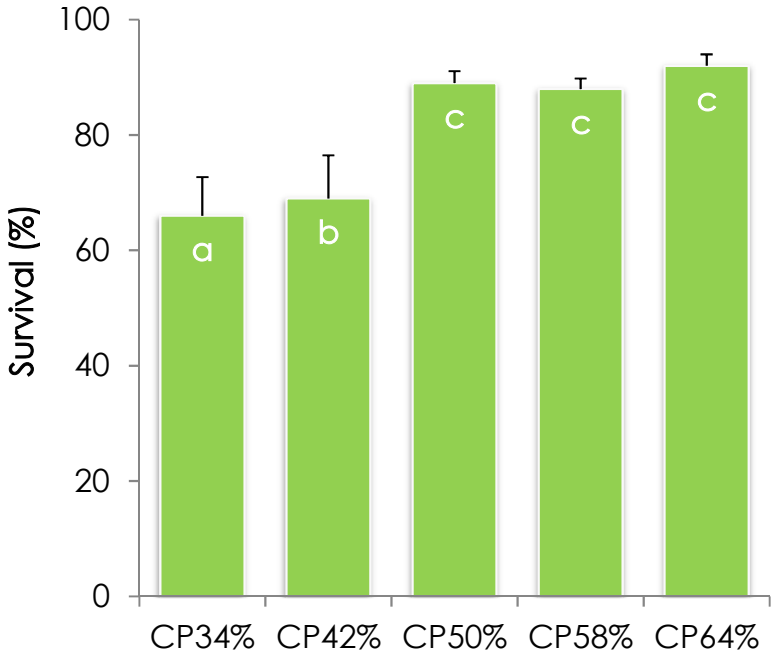
Protein requirements in juveniles/adults





Key message

Adult zebrafish (70 dpf) only need **38-42%** crude protein

...but higher protein levels (58-64%) improve survival, condition factor and reproduction

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ZEBRAFISH
Volume 13, Number 6, 2016

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Fish Haus

Dietary Protein Requirement During Juvenile Growth of Zebrafish (*Danio rerio*)

Helena Fernandes,[1] Helena Peres,[2] and António Paulo Carvalho[1,2]

"Based on dose–response models, the dietary protein requirement of zebrafish juvenile was estimated at **37.6% and 44.8%** for maximum weight gain and maximum protein retention"

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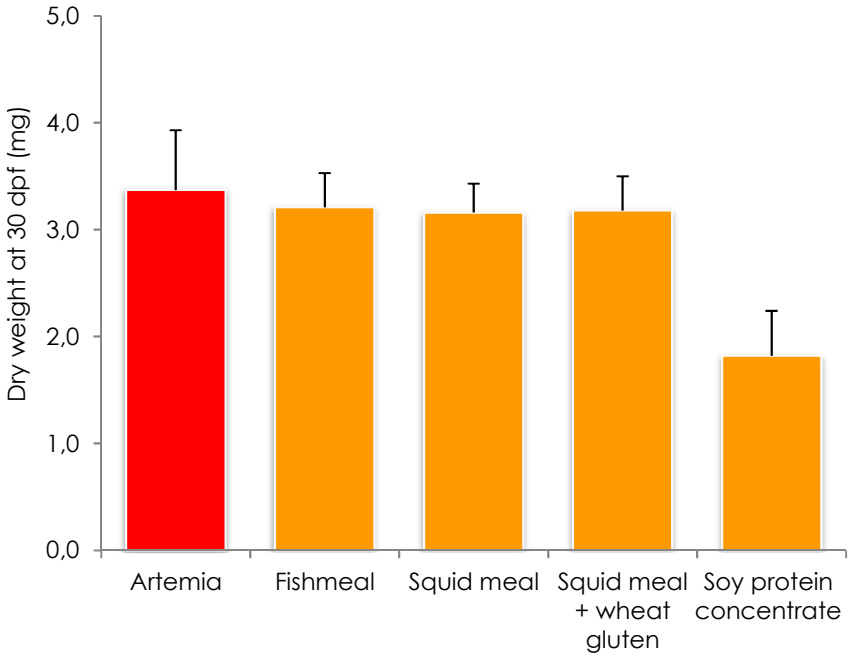
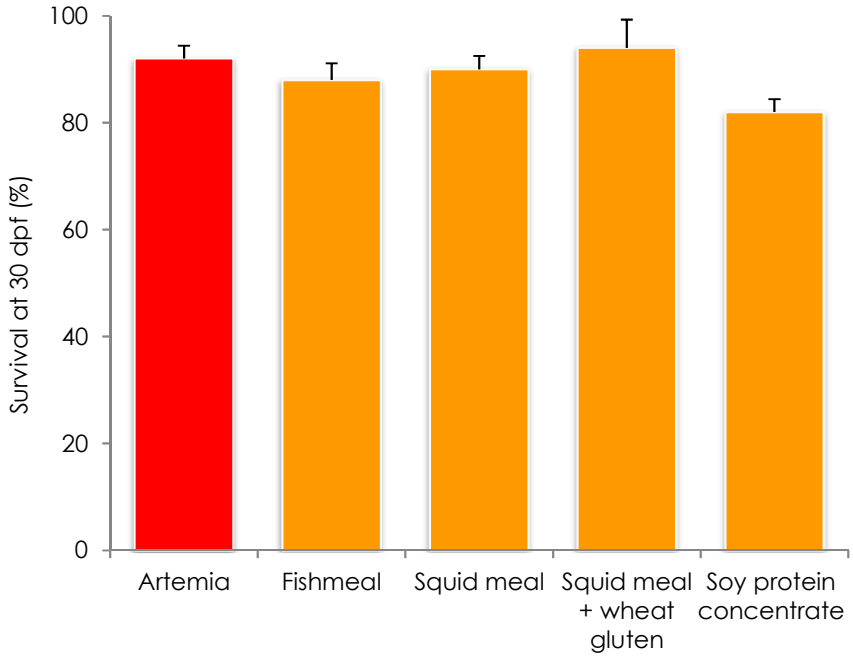
Quality of protein sources





Key message

Good performance with marine ingredients and high quality vegetable proteins (wheat gluten)

But lower performance with soy protein concentrate

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Essential fatty acids and lipid level

As all freshwater teleost, zebrafish show a strict dietary need for the essential PUFAs **linoleic (18:2n-6) and a-linolenic (18:3n-3)** (Tocher et al., 2001).

Arachidonic acid 20:4(ω-6)

Eicosapentanaeoic acid 20:5(ω-3) → Docosahexaenoic acid 22:6 (ω-3)

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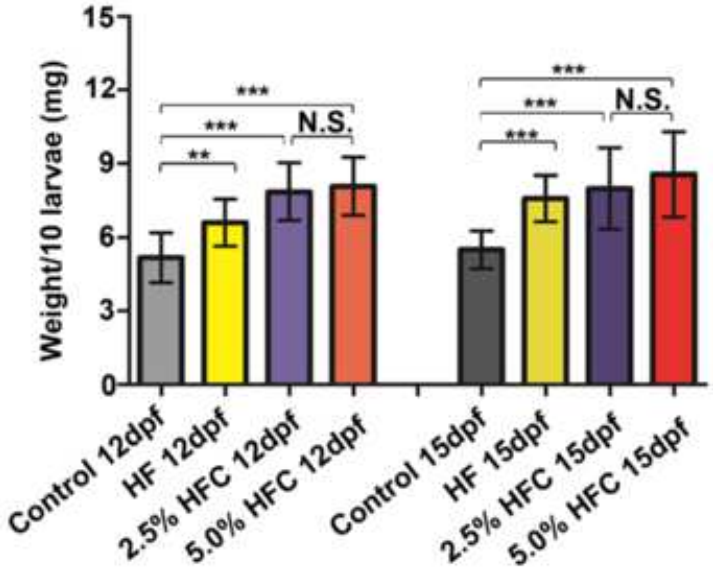
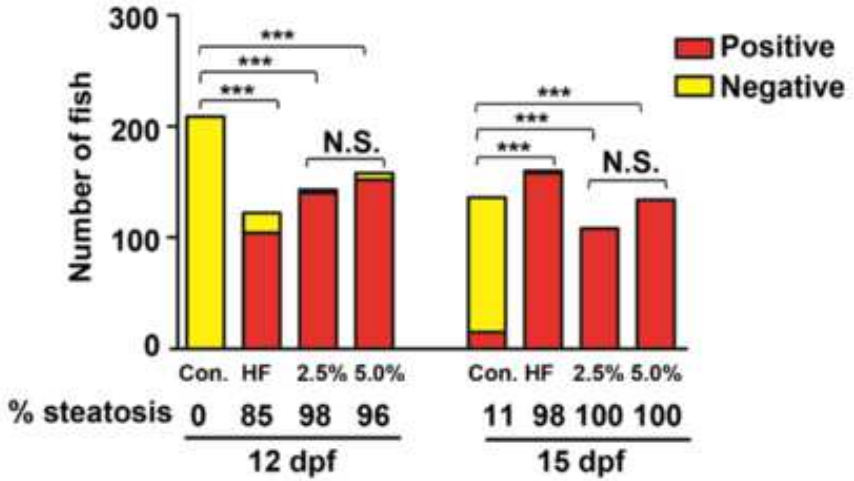
Essential fatty acids and lipid level

As all freshwater teleost, zebrafish show a strict dietary need for the essential PUFAs **linoleic (18:2n-6) and a-linolenic (18:3n-3)** (Tocher et al., 2001).

Dai et al. 2015

Control (Protein: 50 %, Fat: 12 %)
High Fat (Protein: 50 %, Fat: 24 %)
High Fat + Cholesterol at 2.5 % and 5.0 %.





Key message
Zebrafish diets should have moderate lipid levels 14-16%

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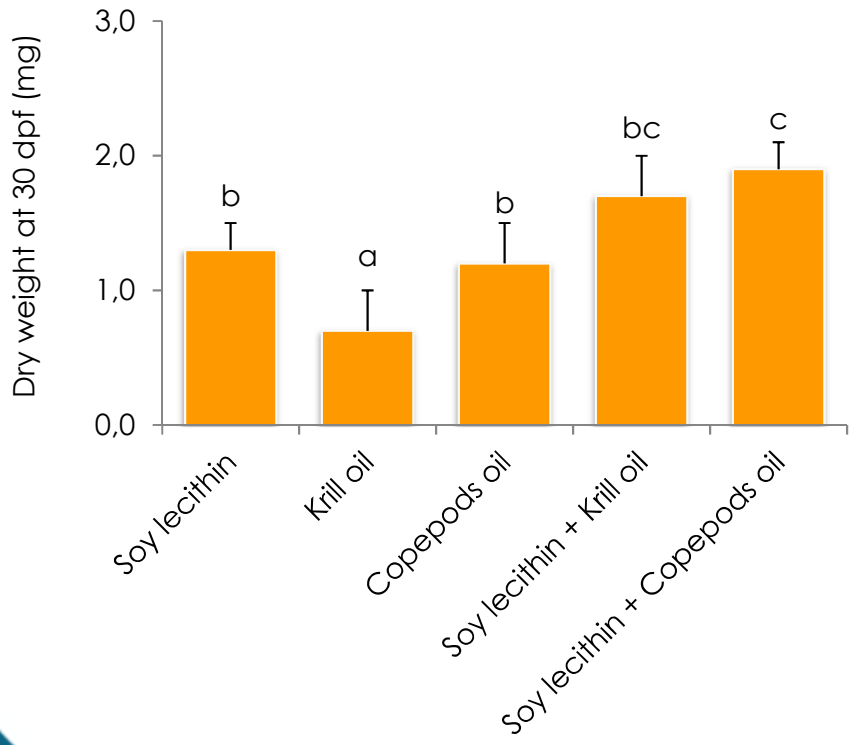
Phospholipids are also essential to fish larvae

The essentiality of phospholipids (PLs) in fish larvae nutrition is clearly established (Cahu et al., 2009).

PLs can be of vegetable (soy, rapeseed and sunflower lecithin) and marine (copepods and krill oil) origin.



Key message

The origin of dietary phospholipids influences zebrafish growth

Best combination seems to be a blend on soy lecithin and marine phospholipids

Other benefits…

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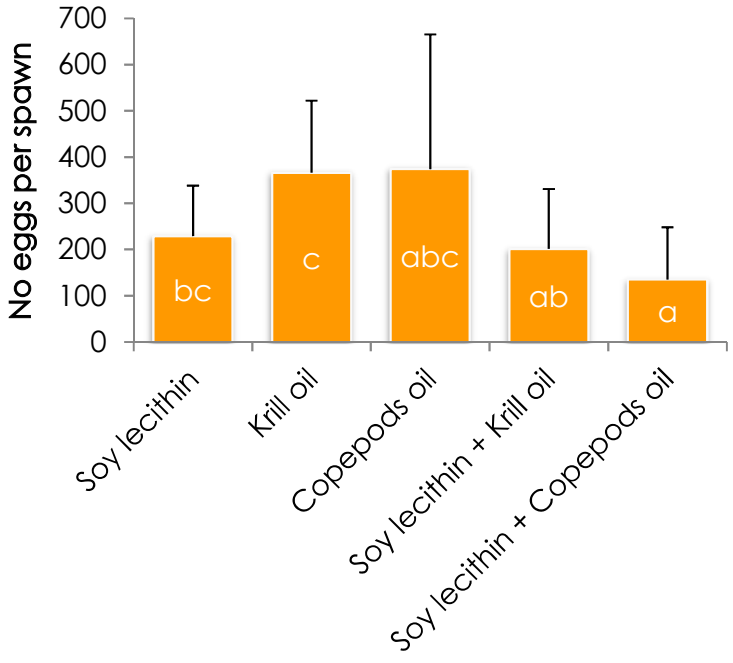
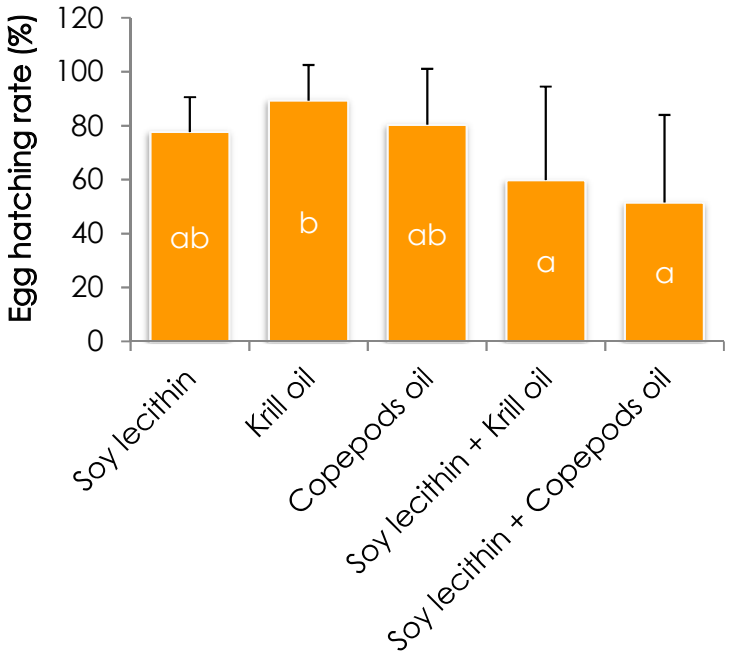
Broodstock performance

Tailoring your feeds

Phospholipids play a key role in reproductive performance

The same diets were used until fish reached the broodstock stage.
A series of mating trials (n=6) were performed to assess reproductive criteria:





Key message
Marine phospholipids have a beneficial effect on spawning performance

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Phospholipids play a key role in reproductive performance

These same diets were used until fish reached the broodstock stage.
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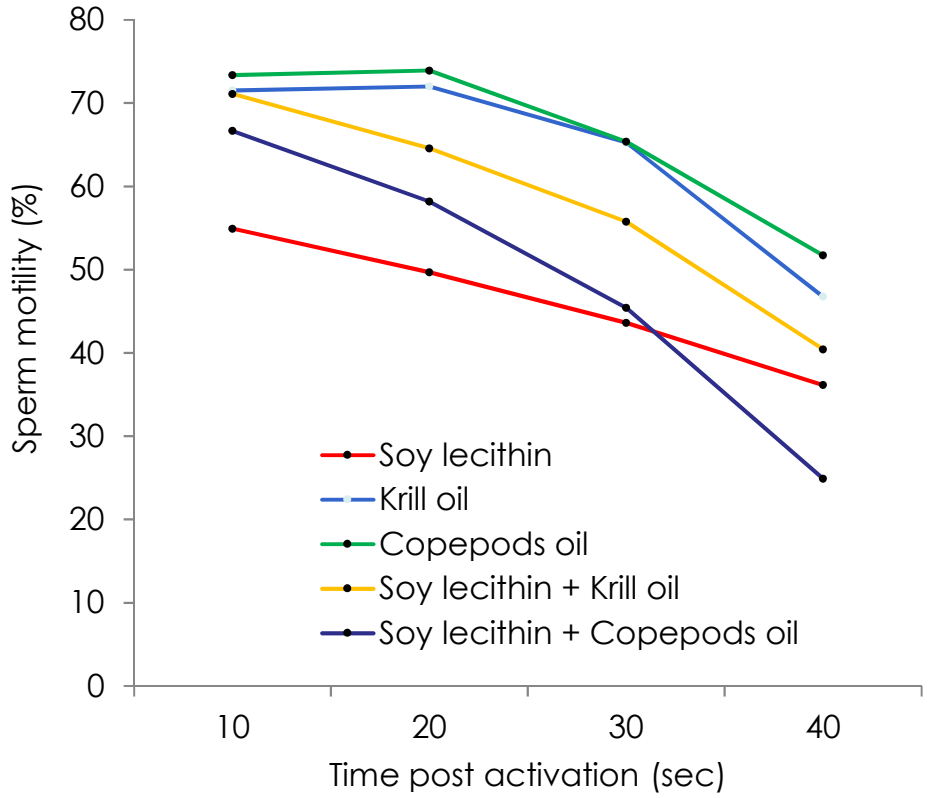

Key message
Phospholipids from krill and copepods oil enhanced sperm motility

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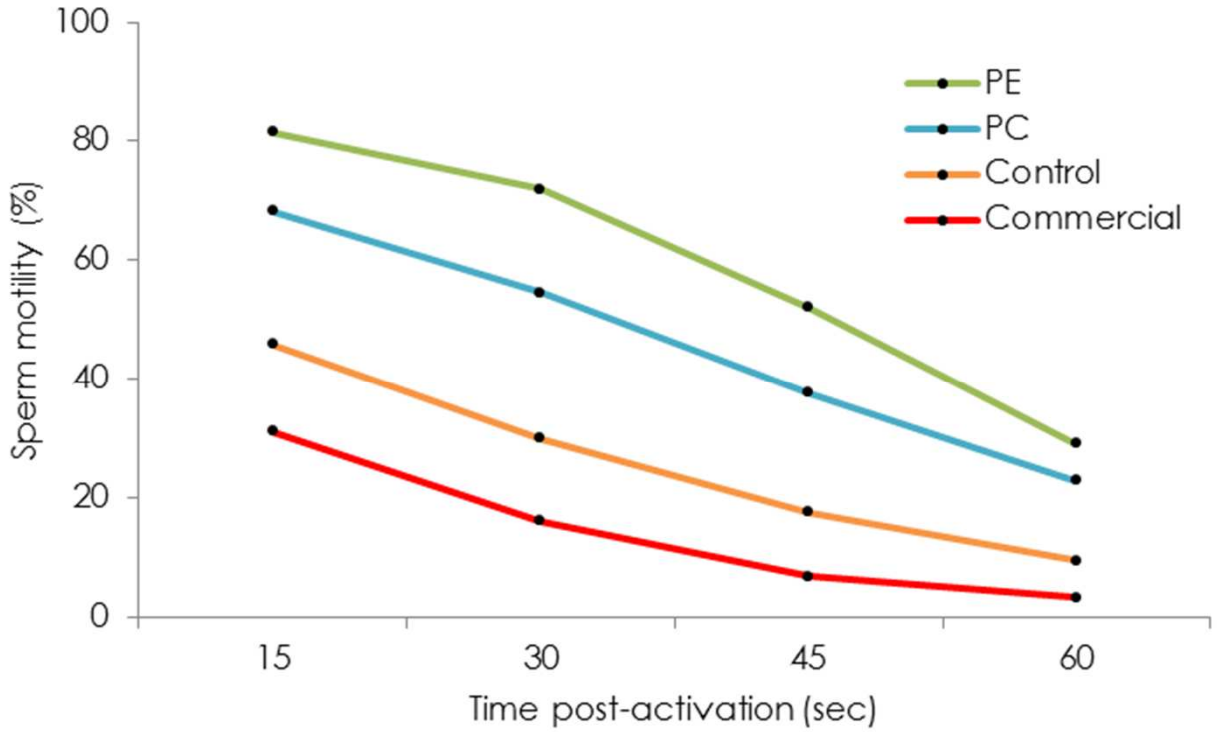
Enhancing reproductive performance



Key message

Supplemental phosphatidylethanolamine (PE at 0.5 g/kg feed) and to a lesser extent phosphatidylcholine (PC) enhanced sperm motility

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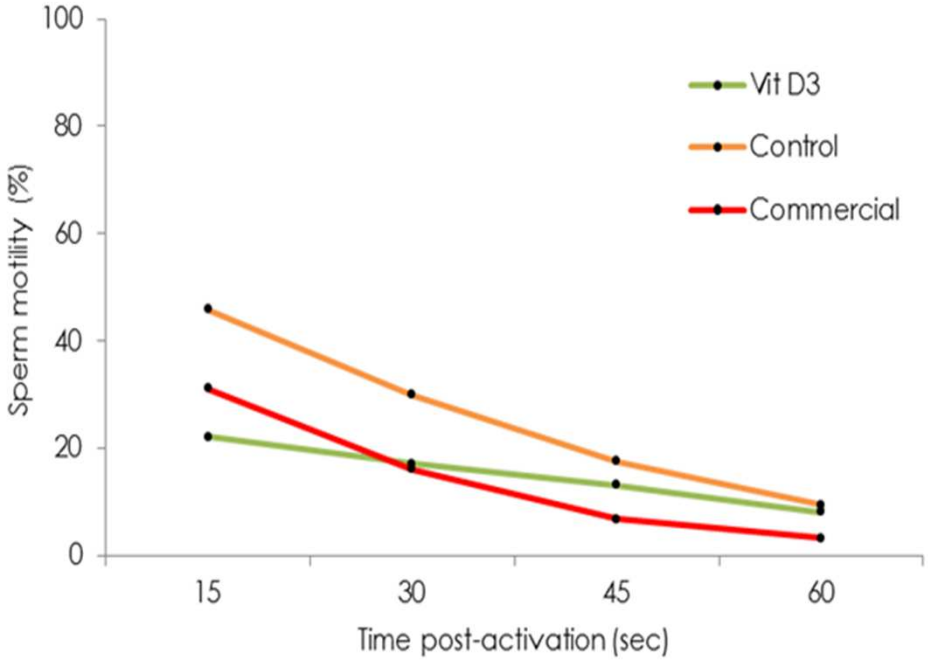
Tailoring your feeds

Enhancing reproductive performance



Key message
Supplemental vitamin D3 (as cholecalciferol) at a dose of 4000 IU/kg feed led to a strong inhibition of sperm motility in zebrafish

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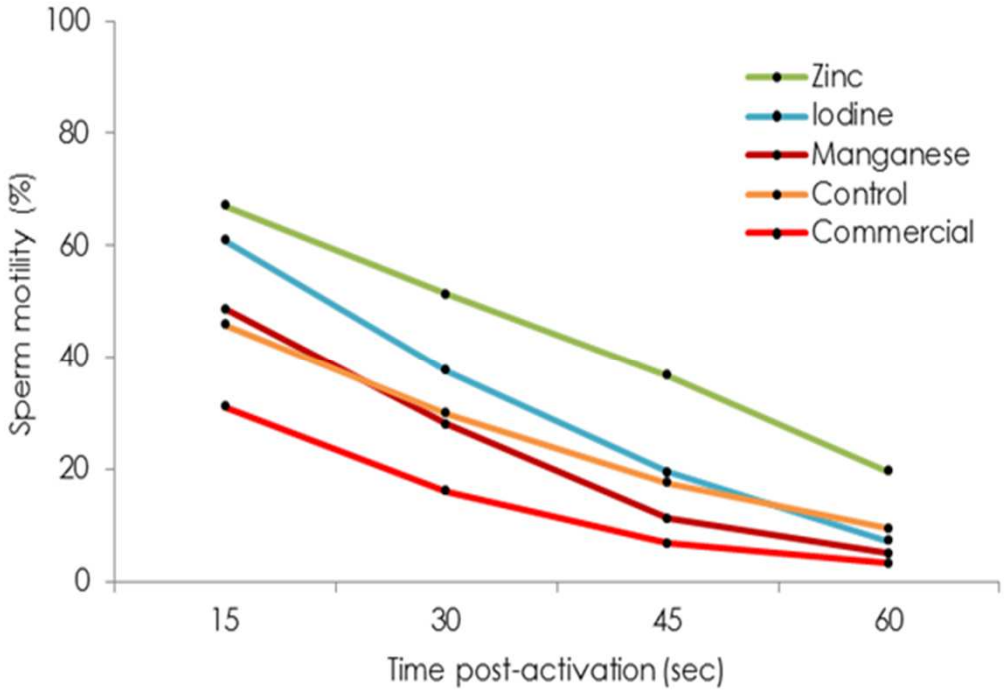
Tailoring your feeds

Enhancing reproductive performance



Key message

Zinc (750 mg/kg) had a positive effect on sperm mobility

Iodine (20 mg/kg) and Manganese (1 mg/kg) did not affect sperm mobility

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Feed Technology

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Feed technology is critical

Technological challenges and solutions:

To guarantee the homogeneity of nutrients in each particle (sizes from 50 to 600 µm)

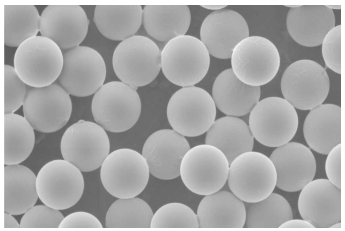
- Ultrafine grinding (pulverization)

To guarantee a high palatability and enhance the water stability of the feed

- Cold-extrusion

To reduce the leaching of highly soluble nutrients (e.g. protein hydrolysates)

- Microencapsulation



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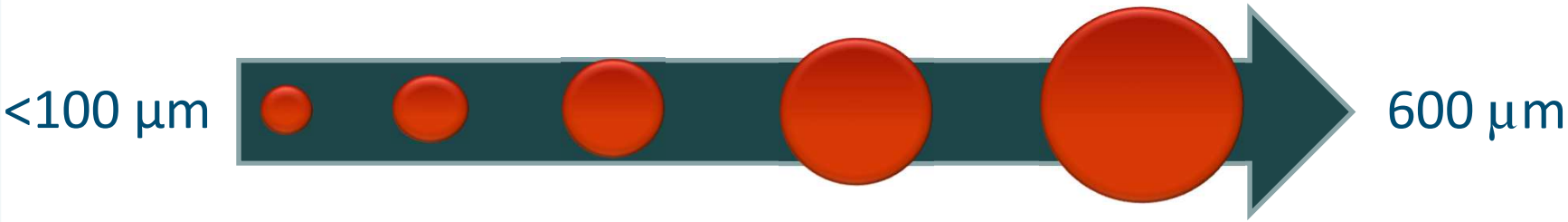
Tailoring your feeds

Microdiet production



Extrusion following:

- **Crumbling**
- **Spheronization**



Including: Protein, lipids, minerals, vitamins...

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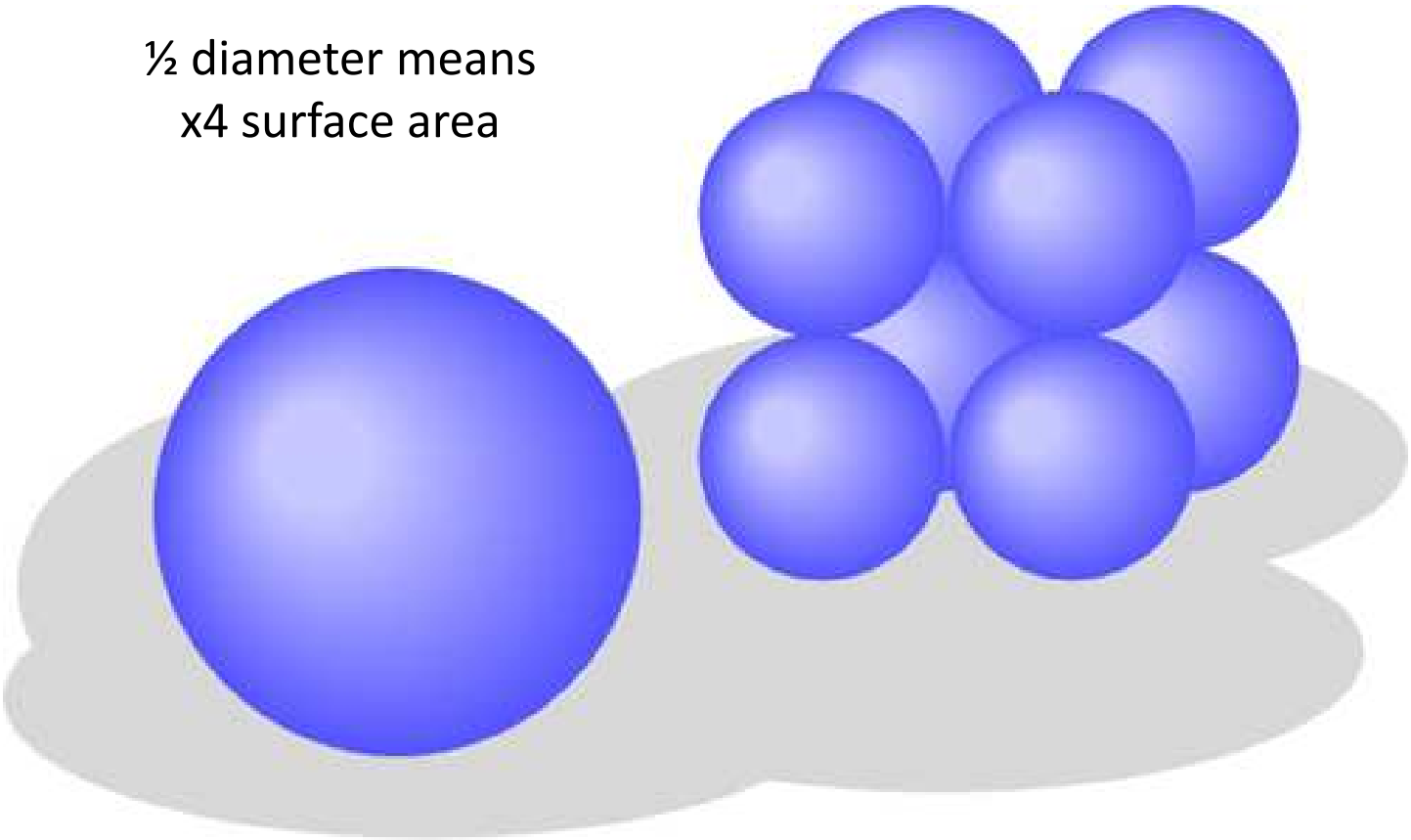
THE PROBLEM…

½ diameter means
x4 surface area



Increase of surface-to-volume ratio

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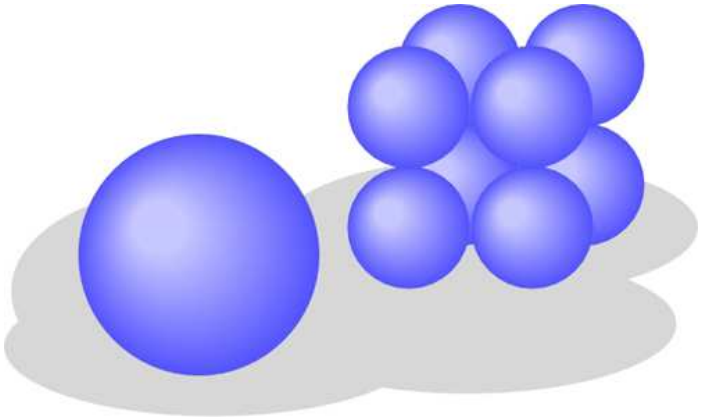
Nutrient leaching

Increase of surface-to-volume ratio



Leaching of water-soluble nutrients

- Loss of nutritional value ⊖
- Deterioration of water quality ⊖
- Attractability ⊕

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Nutrient leaching

Depends on microdiet:

- Production technology (**extrusion**)
- Binder type and concentration
- Nutritional composition
- Size

How BIG/FAST IS OUR PROBLEM?!?

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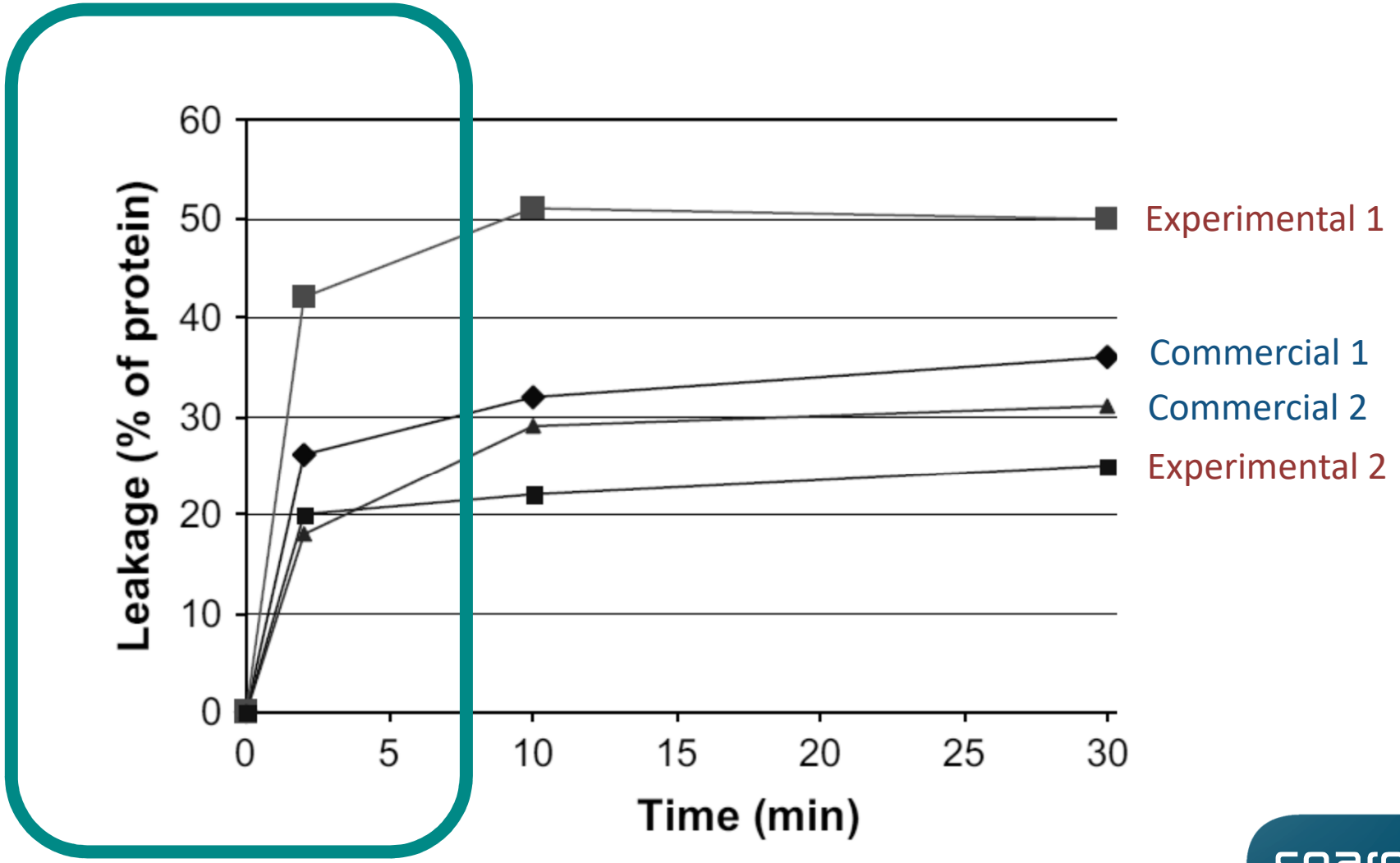
Nutrient leaching - Protein



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Hamre, 2006. ICES Journal of Marine Science, 63: 267-274

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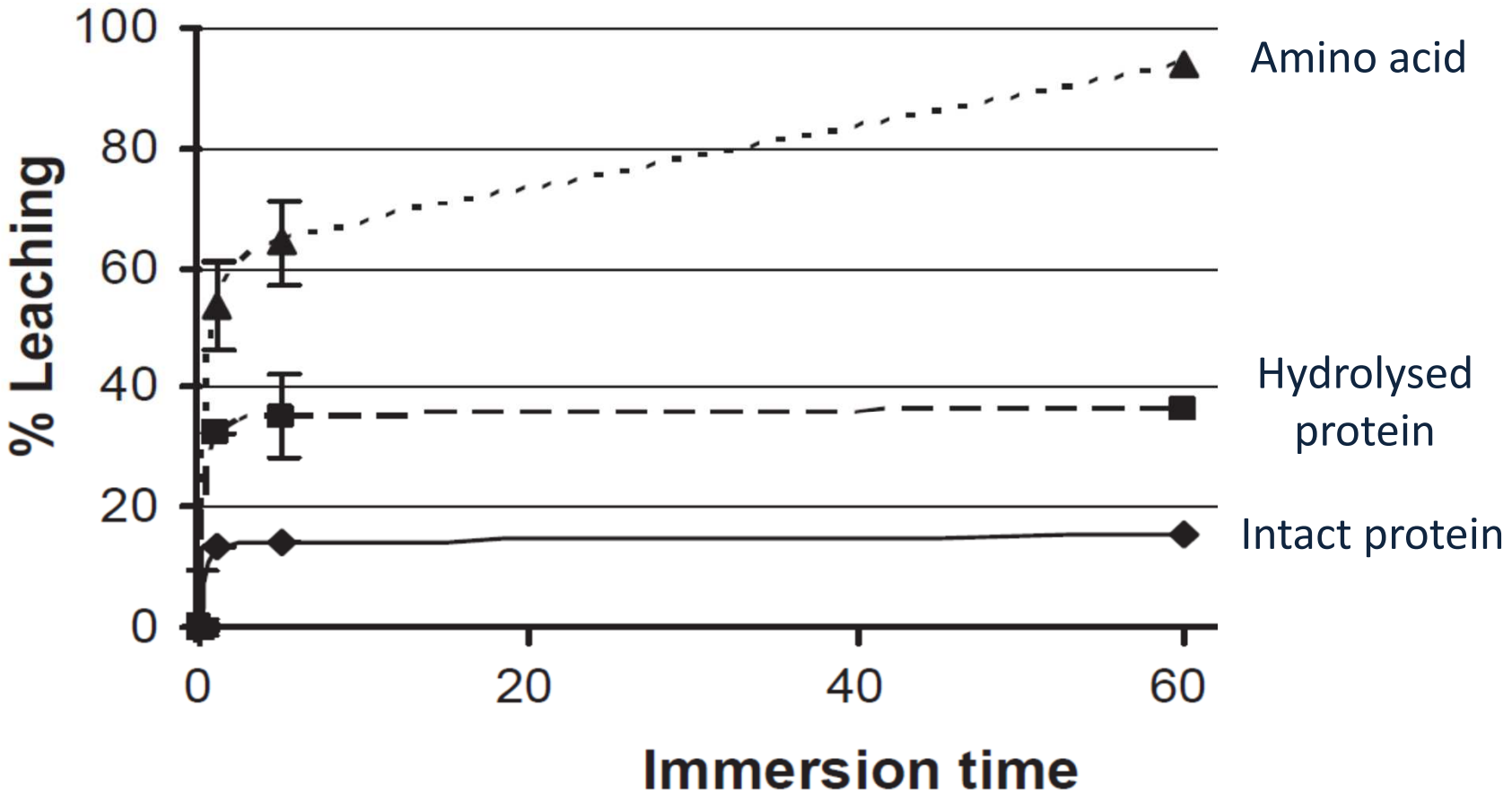
Nutrient leaching - Protein



Kvåle et al., 2006. Aquaculture 251: 402–415

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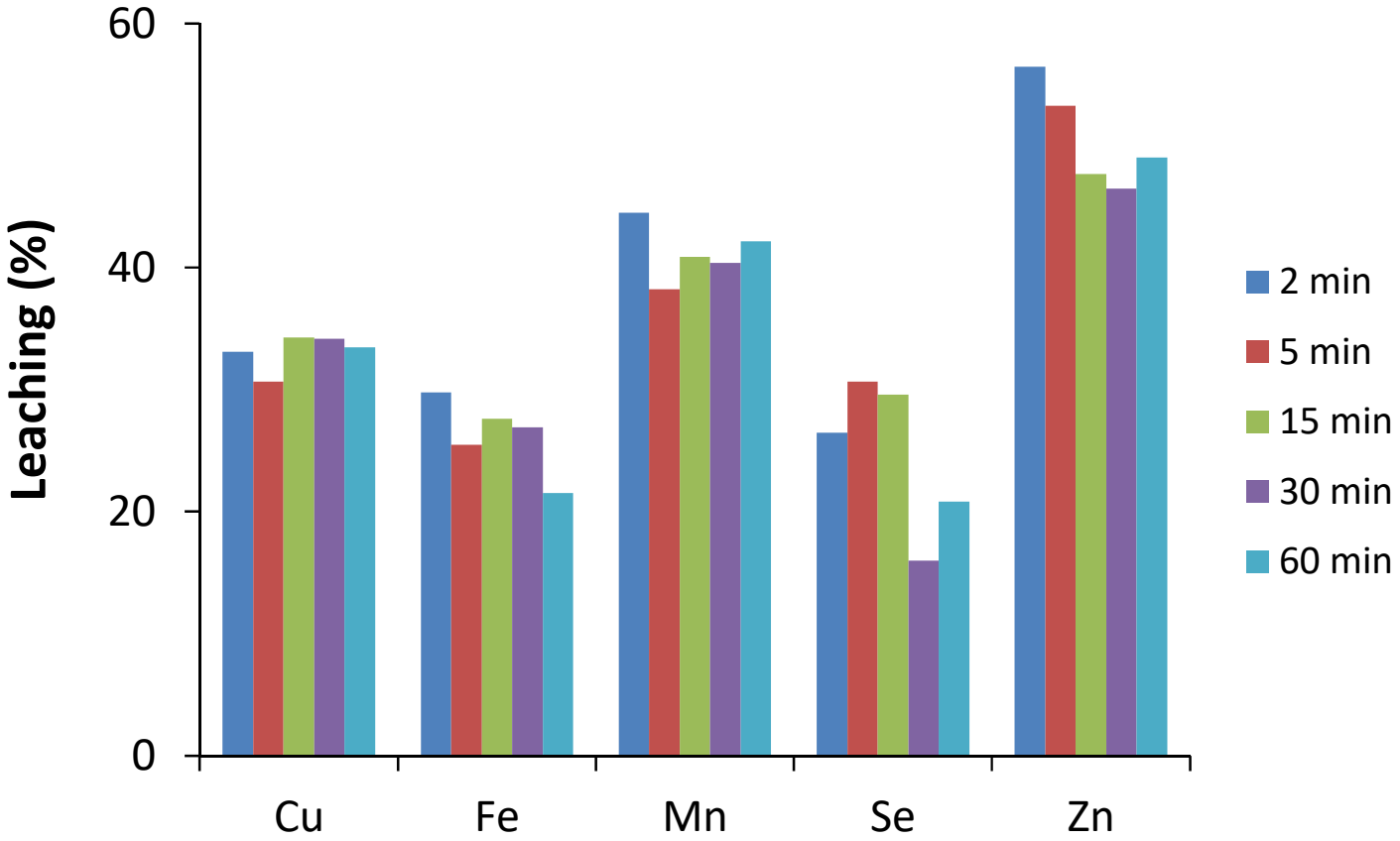
Nutrient leaching – Trace minerals



90 % of water-soluble vitamins leach within 5 min of immersion

Viegas et al, unpublished

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ENCAPSULATION (Micro)

"Process of surrounding or enveloping one ingredient or a mixture of ingredients within a polymer"

Chemistry

Printing and recording, adhesives, pigments and fillers, catalysts

Agriculture

Fungicides/herbicides, insect repellent, biopesticides

Food & feed

Aromas, probiotics, enzyme processing, amino acid protection

Medicine/Pharmacy/Veterinary

Controlled release, taste masking vectorisation, single dose treatment

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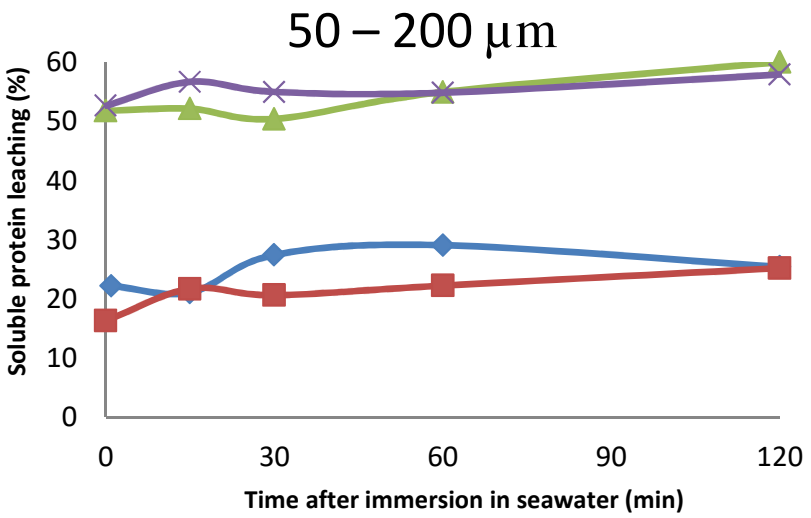
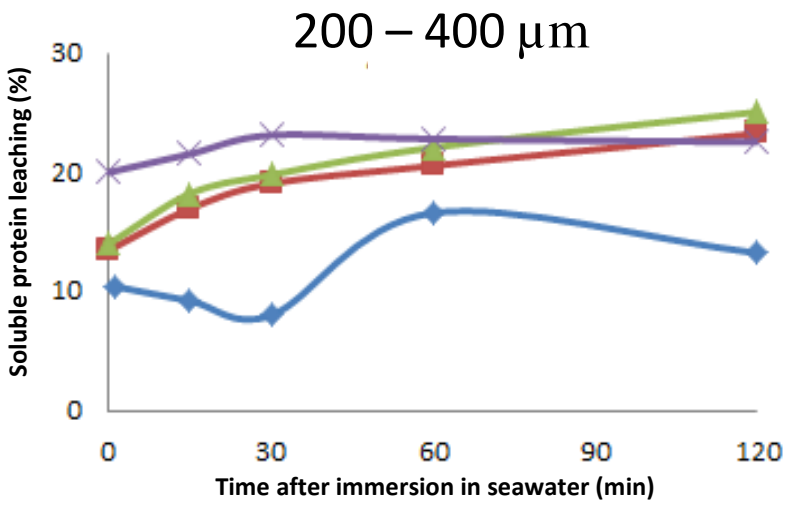
Microencapsulation vs extrusion





Encapsulated prototype 1

Encapsulated prototype 2

Extruded prototype 1

Extruded prototype 2

Technology and particle size → Large influence on product properties

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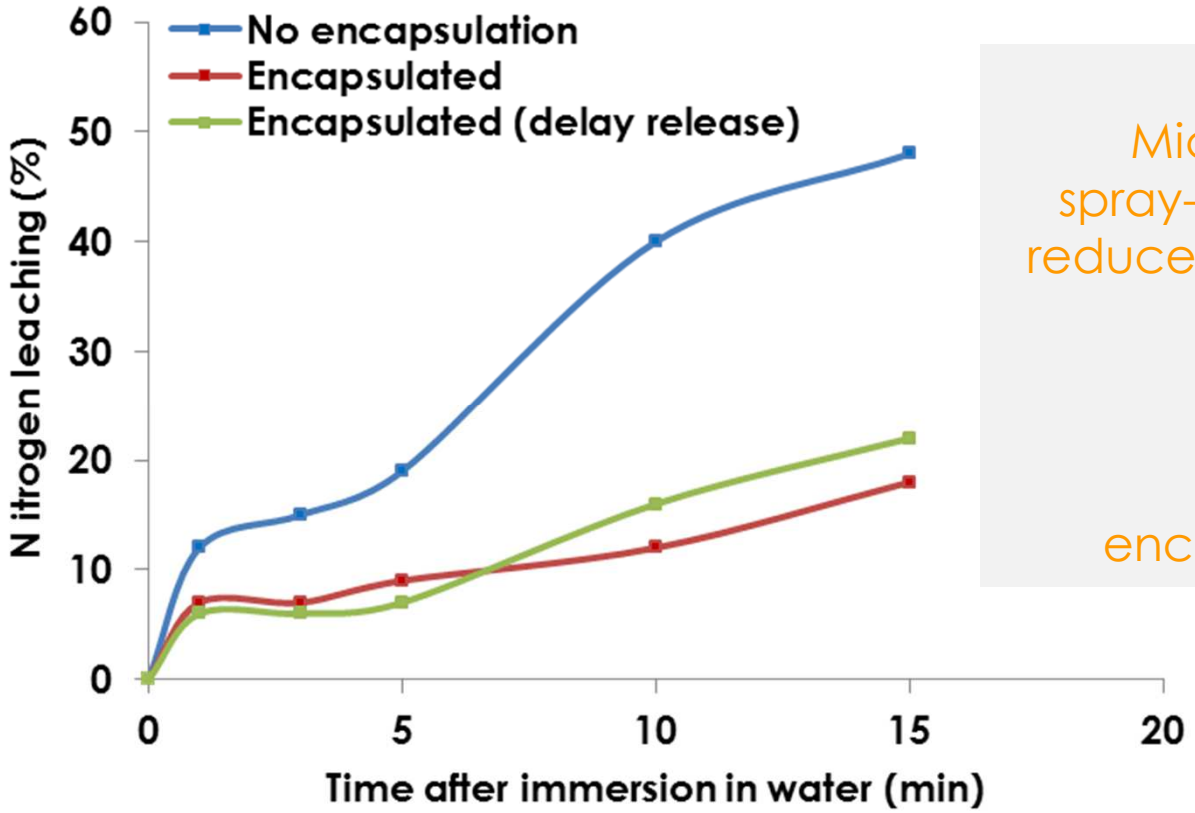
Microencapsulation of protein hydrolysates and amino acids



Key message
Microencapsulation by spray-drying is effective to reduce N leaching losses of the feed

However...
Do they digest the encapsulated nutrients?

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Microencapsulation Processes

Physico-Chemical processes

Coacervation (2-1200 µm)
Solvent evaporation (0.5 – 1000 µm)
Supercritical fluids
Polyelectrolyte multilayer (0.02 – 20 µm)
Hydrogel microspheres
Phase inversion (0.5 – 5 µm)
Hot melt (1 to 1000 µm)

Physico-mechanical processes

Spray drying (5 – 5000 µm)
Fluid-bed technology (20 – 1500 µm)
Pan Coating (600 – 5000 µm)
Spinning disk (5 – 1500 µm)
Co-extrusion (250 -2500 µm)

Chemical processes

Interfacial polymerization (0.5 – 1000 µm)
In situ polymerization (0.5 – 1100 µm)

Many: technologies, terms and classifications

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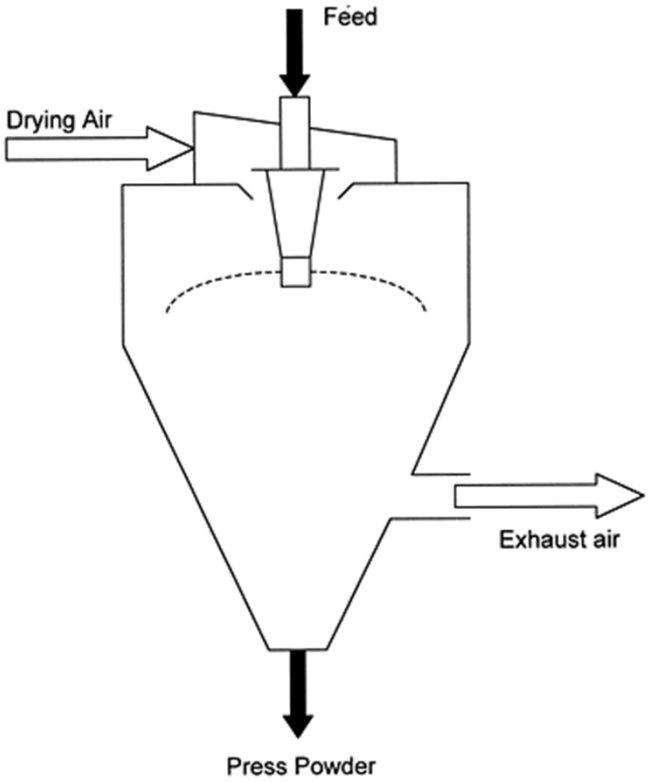
Microencapsulation:
Spray drying



Possible for thermo-sensitive molecules

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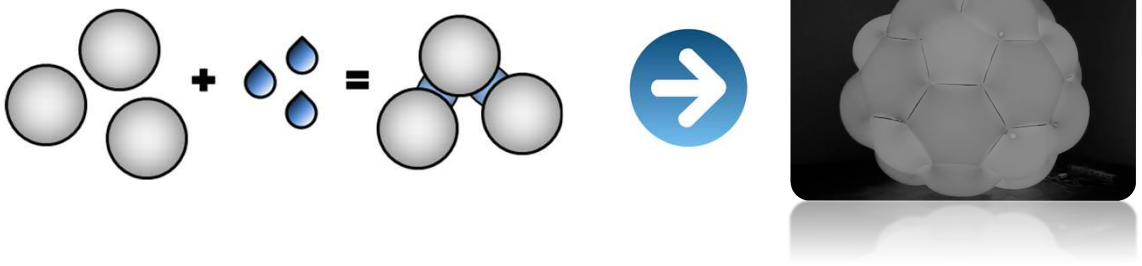
Microencapsulation:
Fluid-bed agglomeration

Top spray



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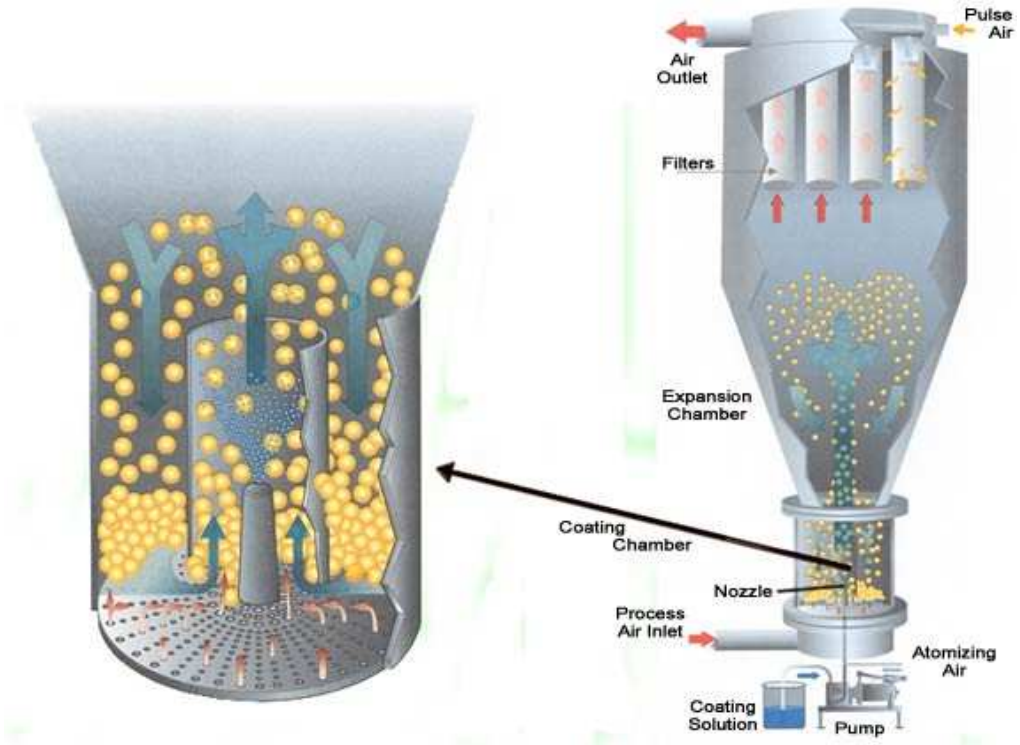
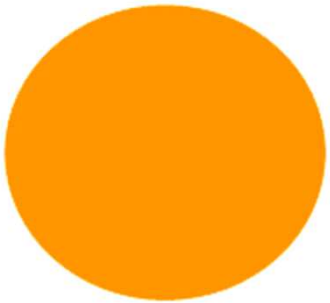
Microencapsulation: Fluid-bed coating



Bottom spray



Extrusion particles
+
Gradual coating deposition

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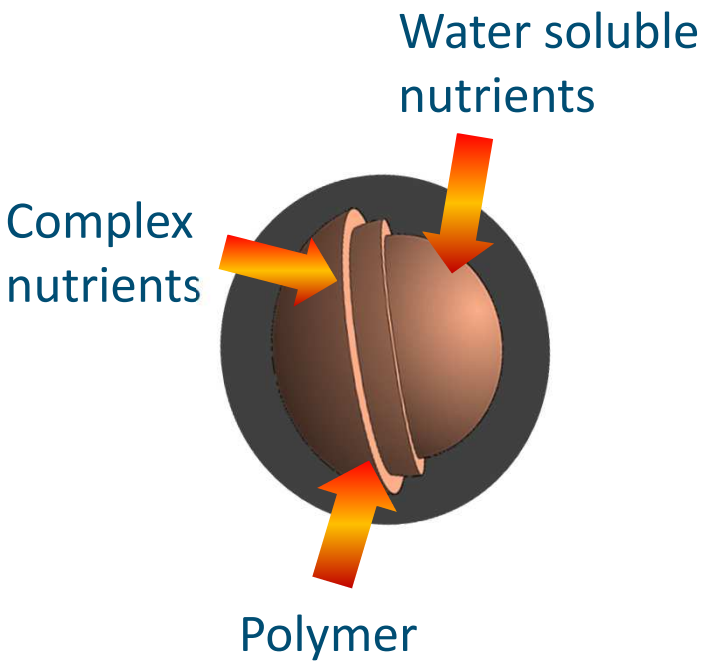
SPAROS approach...

Association of extrusion and microencapsulation:



Advantages

- Complete nutrition
- Low nutrient leaching
- Delivery of attractants/supplements

Disadvantages

- Two step process

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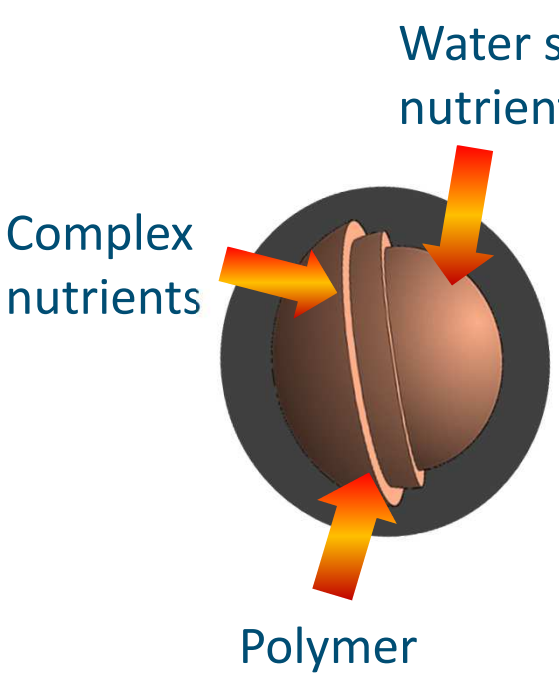
SPAROS approach...

Association of extrusion and microencapsulation:



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What have we learned...

Recommended protein level: 58-62%
Premium marine proteins: squid meal, LT fishmeal, krill meal
High quality plant proteins: wheat gluten, pea protein concentrate
Part of protein must be hydrolysed: fish protein hydrolysate

Recommended fat level: 14-16%
Lipid sources: a blend of marine and vegetable oils
High level of phospholipids: blend of soy lecithin and krill oil

Hydrolysates and selected amino acids should be encapsulated
Cold-extrusion is key to preserve nutrients and enhance water stability of the feed (low pollution)
Adequate dispersion in the water column

Key message
A correct balance all these elements should result in an optimal diet for zebrafish

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Feed tailoring

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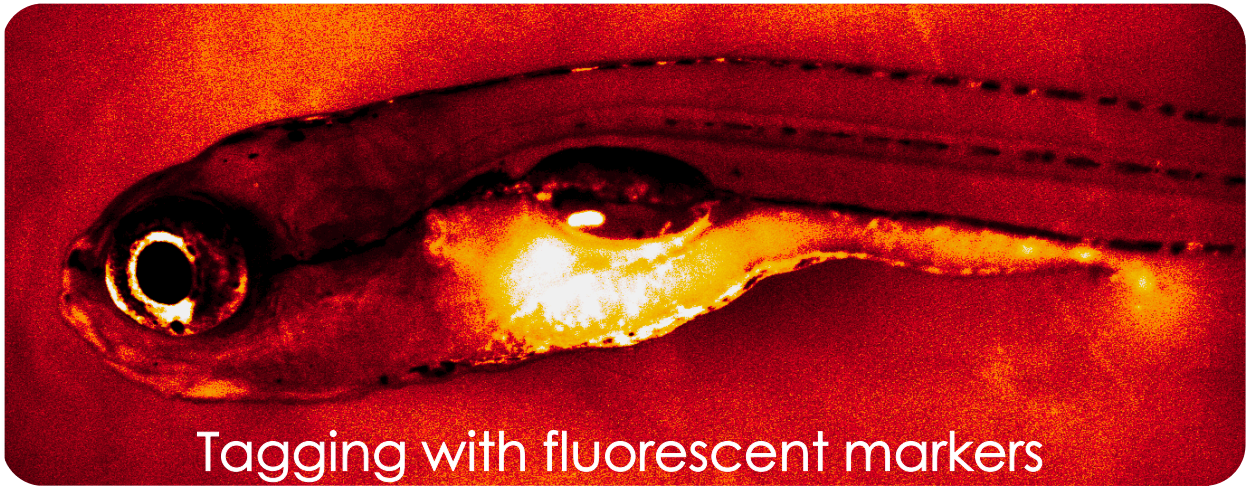
Custom-made zebrafish diets

Controlled changes of specific nutrients

- Additives (probiotics, bioactive extracts
- Compounds for drug screening
- High fat/cholesterol diets

Purifed diets

Others:



Tagging with fluorescent markers

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Thank you

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