Nutritional advances in larvae and juveniles of marine and freshwater fish in Chile

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Commercial fish aquaculture in Chile

75%
Volume of Production
(> 800.000.000 Ton.)
(2015)

- *Salmo salar* (Salmón del atlántico)
- *Oncorhynchus kisutch* (Salmón del pacífico)
- *Oncorhynchus mykiss* (Trucha arcoiris)
- *Psetta máxima* (Turbot)

Species of interest for Chilean Aquaculture

Dissostichus eleginoides
(Bacalao de profundidad)

Cillus gilberti
(Corvina)

Genypterus chilensis
(Congrio colorado)

Genypterus blacodes
(Congrio dorado)

Seriola lalandi
(Palometa)

Seriolella violacea
(Cojinova del norte)

Basiichthys microlepidotus
(Pejerrey chileno)

Eleginops maclovinus
(Robalo)

Galaxias maculatus
(Puye)

Merluccius australis
(Merluza austral)

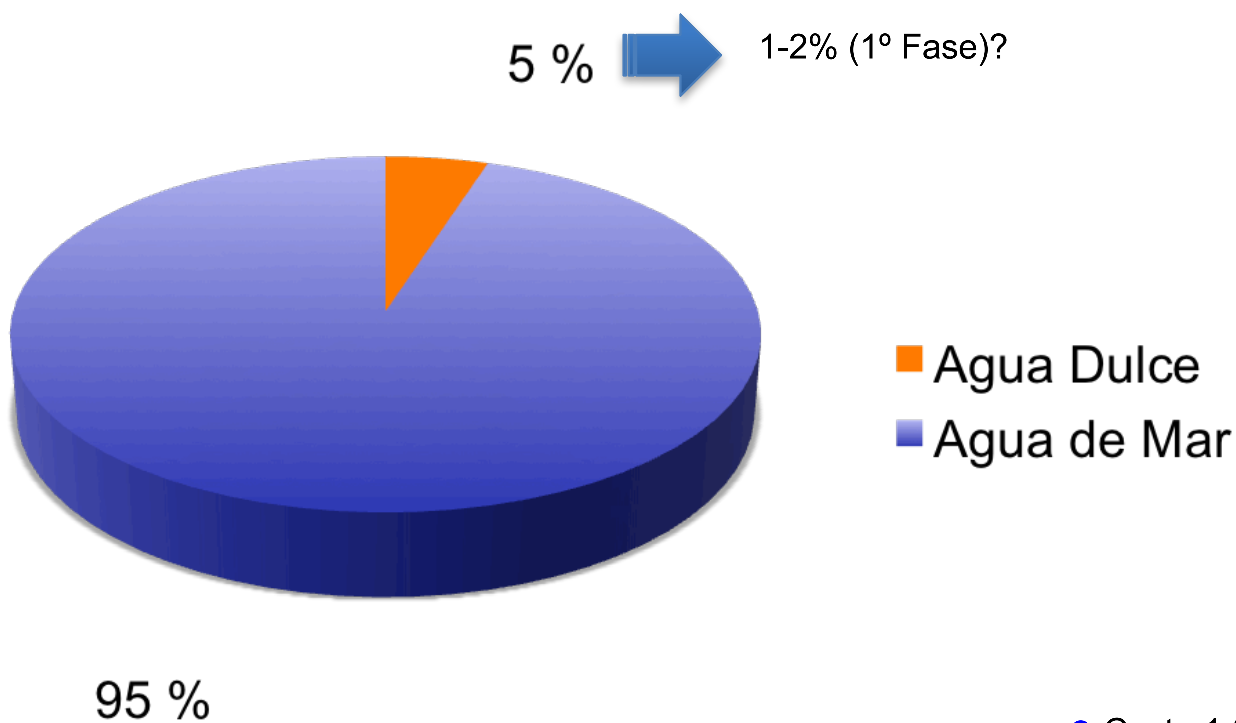
- 1.300.000 toneladas de alimento
- US$ 2.000 millones de dólares

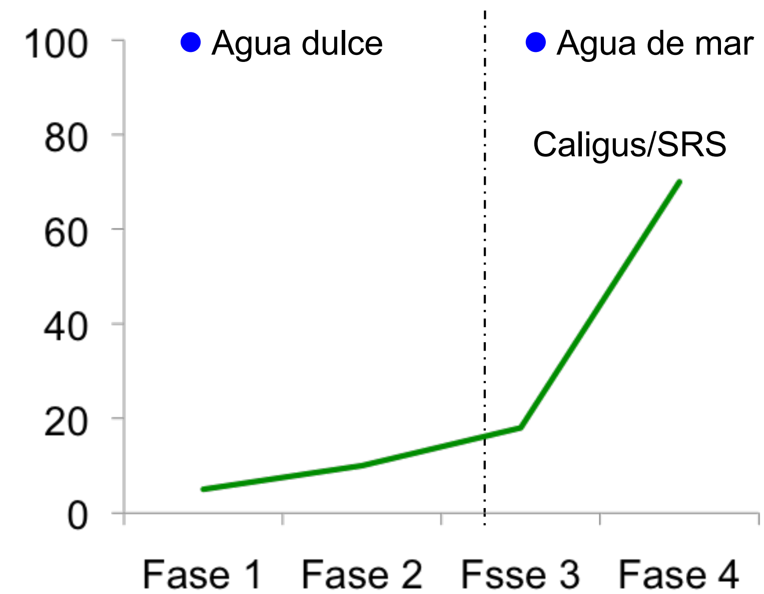
- Alta concentración en pocos actores
(3 compañías se llevan el 75- 80% del mercado)

- Sólidos equipos de investigación y desarrollo

- COSTOS

Volumen de alimento producido





- Costo 1 Smolt : 1,50 – 2,11 US$
Alimento : 0,24 – 0,26 USS
Vcaunas : 0,26 – 0,28 US$ → 12 – 16%

Fuente: Revista AQUA – Marzo 2016

¿What's happening with new marine species in Chile?

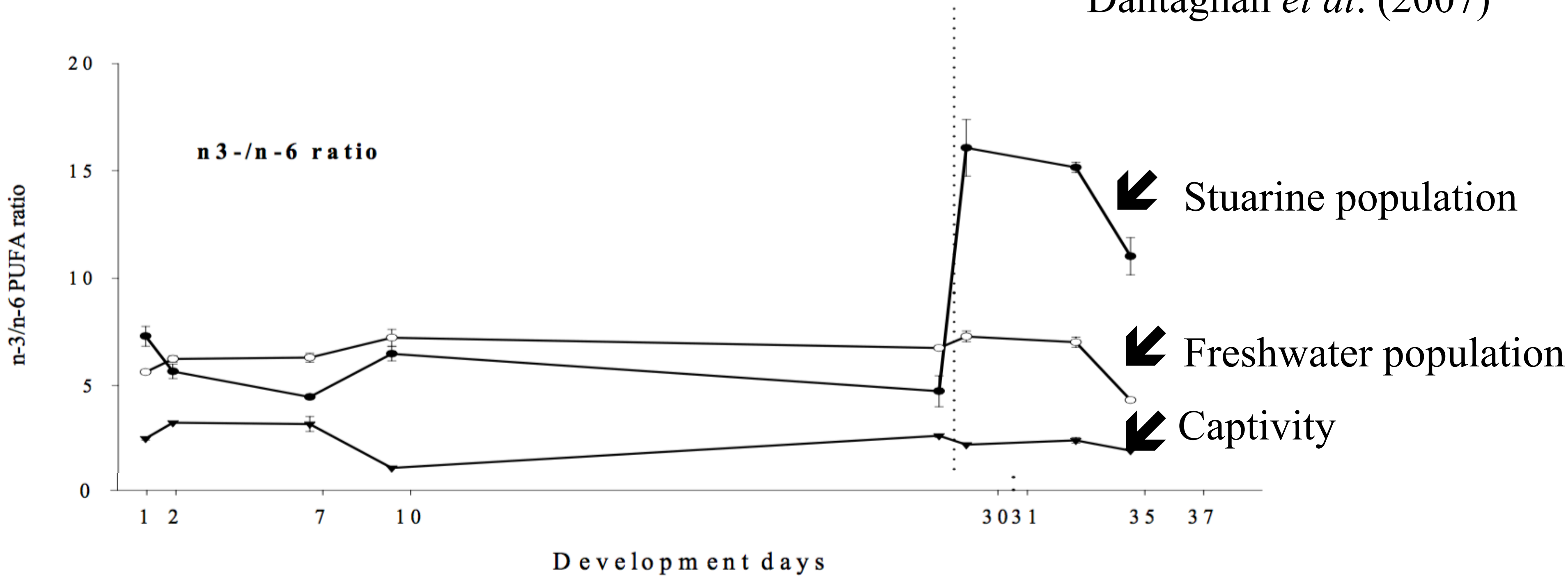
A small number of specied enter pilot-scale production

CRITICAL NUTRITIONAL ASPECTS

- Broodstock (feeding and nutrition)
- Lack of knowledge about reproductive maturity in capivity conditions (Reproductive efficiency - laying quality variability - nutritional requirements, etc.)
- Mass production of larvae (low, no or erratic larval survivals, high level of malformations)
- Production of inert (microdiets) and living diets.
- Lack of knowledge on nutritional requierements throughout larval growth and survival (environmental factors, lack of physiological and morphological information, etc.)
- Limited knowledge on larval deformities and diseases (effects of fatty acids and vitamins)

SOME RESEARCH IN NUTRITION LARVAL AND JUVENILE FISHES IN CHILE

Dantagnan *et al*. (2007)



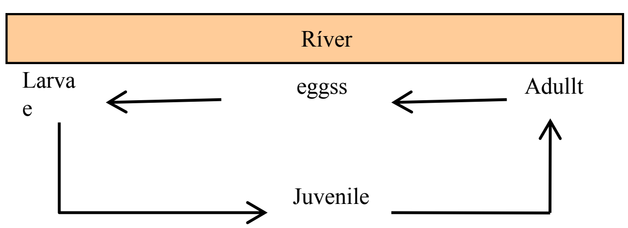
Captivity

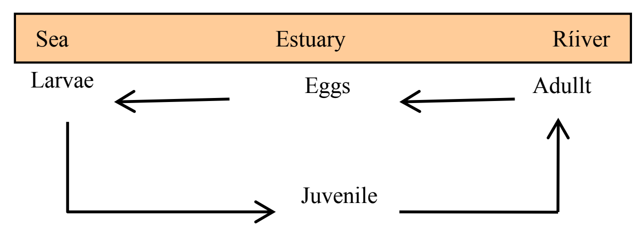
Estuary

Dietary effect

Environmental effect
(Tocher *et al.*, 1995)

- **Different food can cause the same composition if salinity conditions are equal**
- **Similar type of food can cause different composition if salinity conditions change**





Dantagnan *et al*. (2010). ***Effect*** *of EPA/DHA ratios on growth, survival and biochemical composition of Galaxias maculatus (Jenyns, 1842) larvae reared under different salinity Regimes.* Aquaculture Research. 41: e239-e244.

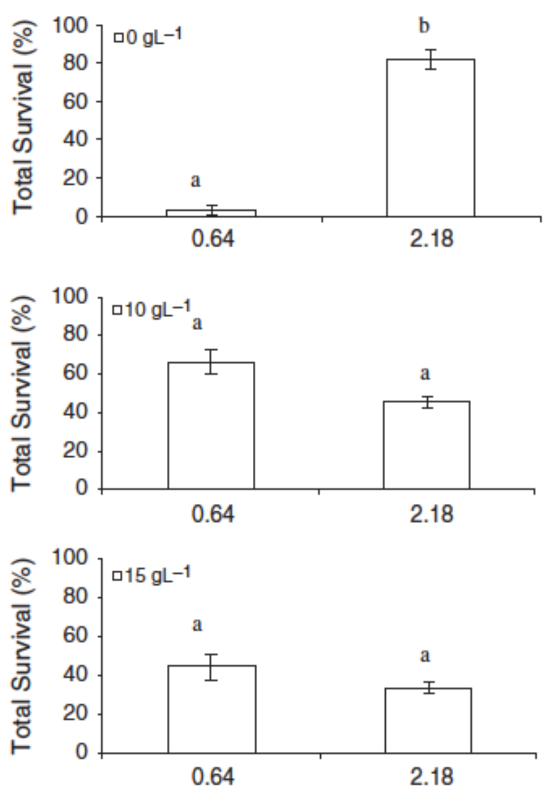


Figure 1 Total survival of *Galaxias maculatus* larvae reared at different salinities and two EPA/DHA ratios (low: 0.64 and high: 2.18). (Data are means ± SD).

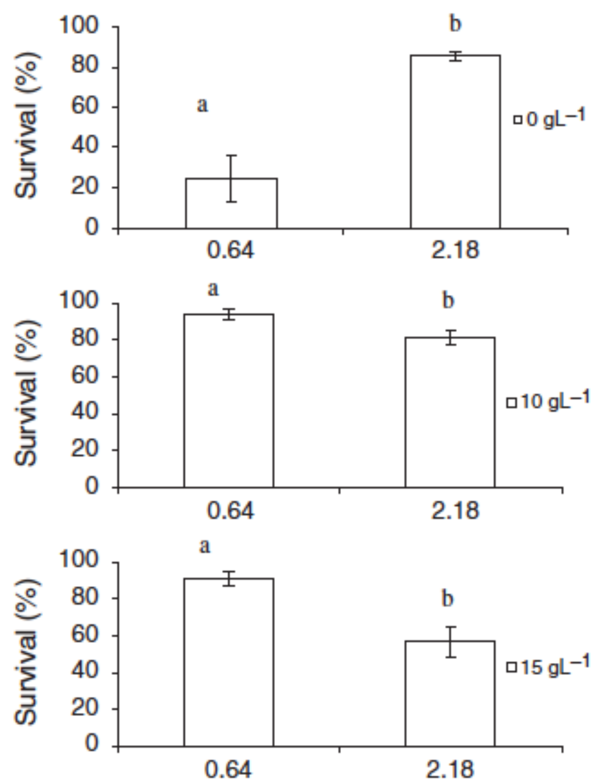


Figure 2 Total survival after a stress stimulus of *Galaxias maculatus* larvae reared at different salinities and two EPA/DHA ratios (low: 0.64 and high: 2.18). (Data are means ± SD).

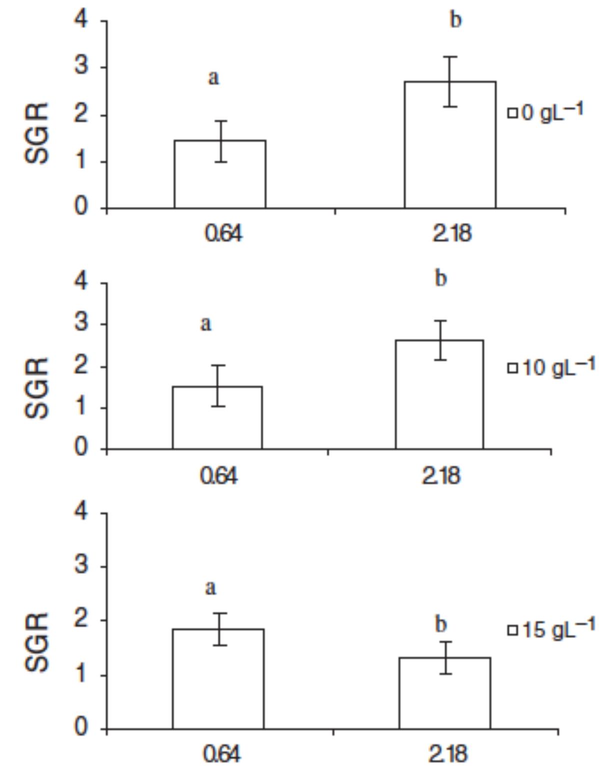


Figure 3 Specific growth ratio (SGR) of *Galaxias maculatus* larvae reared at different salinities and two EPA/DHA ratios (low: 0.64 and high: 2.18). (Data are means ± SD).

A COMMON AND FREQUENT PHENOMENON: MALFORMATIONS

- Skeletal disorders are common in hatcheries and commercial farms that appear in different stages of development (much more abundant in captivity than in nature)

- Malformations are often associated with decreased growth and a high mortality rate.

- Difficulties to detect early: Degree of severity - staining technique and analysis

Tabla 1. Momento de aparición, tipo y frecuencia de malformaciones craneales reportadas en peces cultivados. dpe: días post-eclosión, nm: no menciona.

Especie	Tipo de malformación o parte afectada	Frecuencia (%)	Momento de detección	Referencia
Lates calcarifer	Mandíbula cortas y apretadas	4,24 - 35,71	18 dpe	Fraser & de Nys (2005)
Cyprinus carpio	Boca incapaz de cerrar	0,4 - 36,5	nm	Kocour *et al.* (2006)
Pagrus major	Mandibulas acortadas	68,0	nm	Matsuoka (2003)
Seriola lalandi	Mandibulas superior e inferior	nm	4 - 8 - 16 dpe	Cobcroft *et al.* (2004)
Hippoglossus hippoglossus	Boca abierta	13,2 - 25,0	145 UTA	Morrison & MacDonald (1995)
Pagrus pagrus	Complejo opercular y mandíbula	3,1 - 6,8	4,1 mm	Roo *et al.* (2010)
Paralichthys olivaceus	Mandibulas superior e inferior	20	9 dpe	Haga *et al.* (2003)
Latris lineata	Mandíbulas superior e inferior arco hioideo	nm	post-flexión	Cobcroft *et al.* (2001)
		7,0 - 70	>15 dpe	Cobcroft *et al.* (2012)
		18,0 - 64,0	post-flexión	Cobcroft & Battaglene (2009)
Sparus aurata	Esqueleto hiobranquial complejo opercular	40 - 80	nm	Koumoundouros (2010)
		4,6 - 16,7	21 - 35 dpe	Koumoundouros *et al.* (1997)
		22,7 - 77,3	25 - 40 dpe	Beraldo *et al.* (2003)
		7,5 - 31,9	17 - 20 dpe	Galeotti *et al.* (2000)
Dicentrarchus labrax	Rayos branquioestegales complejo opercular	4,0 - 33,4	nm	Georgakopoulou *et al.* (2007)
		15,7 - 43,9	juveniles	Abdel *et al.* (2004)
Chanos chanos	Membrana branquioestegal complejo opercular	3,0 - 26,0	juveniles	Hilomen-García (1997)
		8,4 - 33,5	40 dpe	Gapasin *et al.* (1998)
		6,8 - 32,4	juveniles	Gapasin & Duray (2001)

Wilfrido Argüello *et al*. (2014)

Tabla 2. Factores causales de malformaciones craneales evaluados en peces de cultivo.

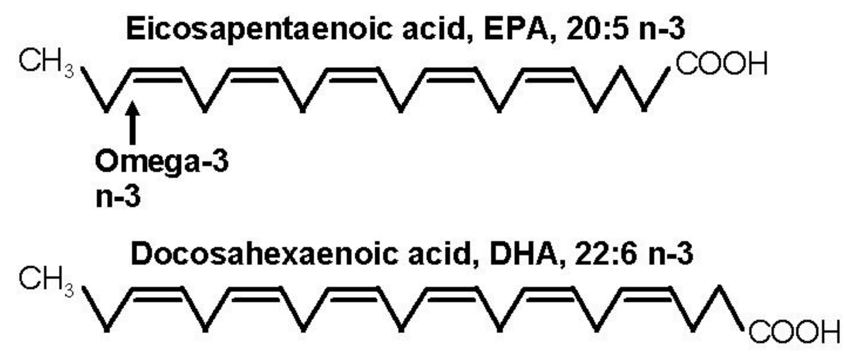
Factor causal asociado	Especie	Estado ontogénico	Tipo de malformación craneal	Referencias
Factores genéticos				
Herencia	*Cyprinus carpio*	Juvenil	Malformaciones bucales	Kocour *et al.* (2006)
	Salmo salar		Pugheadness	Gjerde *et al.* (2005)
Triploidía	*Salmo salar*		Opérculo corto, ausencia de filamentos branquiales, malformaciones mandibulares	Sadler *et al.* (2001)
Expresión de genes twist	*Danio rerio*	Larva	Malformaciones craniofaciales	Germanguz & Gitelman (2012)
Factores nutricionales				
Deficiencia de vitamina C	*Geophagus brasiliensis*	Juvenil (8-10 mm)	Hocico corto	Wimberger (1993)
Desbalance de vitamina A	*Dicentrarchus labrax*	Larva y juvenil	Malformaciones en maxilar, premaxilar, dentario, rayos branquioestegales y opérculo	Mazurais *et al.* (2009) Villeneuve *et al.* (2005)
	Paralichthys olivaceus	Juvenil	Maxilar, premaxilar, dentario extremadamente deprimidos trabécula corta	Haga *et al.* (2003)
Deficiencia de DHA	*Chanos chanos*		Malformaciones operculares	Gapasin & Duray (2001)
Deficiencia de fósforo	*Paralichthys olivaceus*		Opérculo deformado	Uyan *et al.* (2007)
Factores ambientales				
Temperatura	*Dicentrarchus labrax*	Larva	Boca abierta, opérculos anormales. Forma y orientación de los rayos branquiostegales anomarles	Koumoundouros *et al.* (2001) Abdel *et al.* (2004) Georgakopoulou *et al.* (2007)
	Sparus aurata	Larva y juvenil	Opérculo y sub-opérculo plegado	Georgakopoulou *et al.* (2010)
Temperatura y fotoperiodo	*Solea senegalensis*	Larva	Malformación mandibular	Blanco-Vives *et al.* (2010) Dionisio *et al.* (2012)
Disminución de salinidad	*Anguilla japonica*	Larva	Mandíbula inferior curvada hacia abajo	Okamoto *et al.* (2009)
Otros factores				
Intensificación del cultivo	*Pagrus pagrus*	Larva	Torción de la trabécula Cartílago de Meckel curvado hacia abajo	Roo *et al.* (2010)
	Epinephelus marginatus	Larva y juvenil	Malformaciones mandibulares, del complejo opercular y dentario reducido	Boglione *et al.* (2009)
Contaminantes (TBT)	*Sebastiscus marmoratus*	Larva	Mandíbula inferior corta	Zhang *et al.* (2012)
Abrasión de la cabeza e invasión de patógenos	*Hippoglossus hippoglossus*		Boca abierta, cartílago de Meckel plegado	Morrison & MacDonald (1995)
Color de tanque y comportamiento larval	*Latris lineata*		Boca abierta Mandíbula inferior reducida	Cobcroft & Battaglene (2009)
Intensificación del cultivo y wall-nosing	*Pagrus pagrus*	Juvenil	Maxilar superior reducido. Boca tipo mordida en cruz	Izquierdo *et al.* (2010)

Wilfrido Argüello *et al*. (2014)

Dietary LC-PUFA are required to reduce larvae skeletal deformities

- LC-PUFA are key nutrients in Aquaculture due to essential roles in development and metabolism
- Marine fish require a dietary source of LC-PUFA (EPA, DHA and ARA).
- DHA enriched live food reduce skeletal deformities in marine larvae fish

LC-PUFA: Polyunsaturated fatty acids with ≥ C20 and ≥3 double bonds



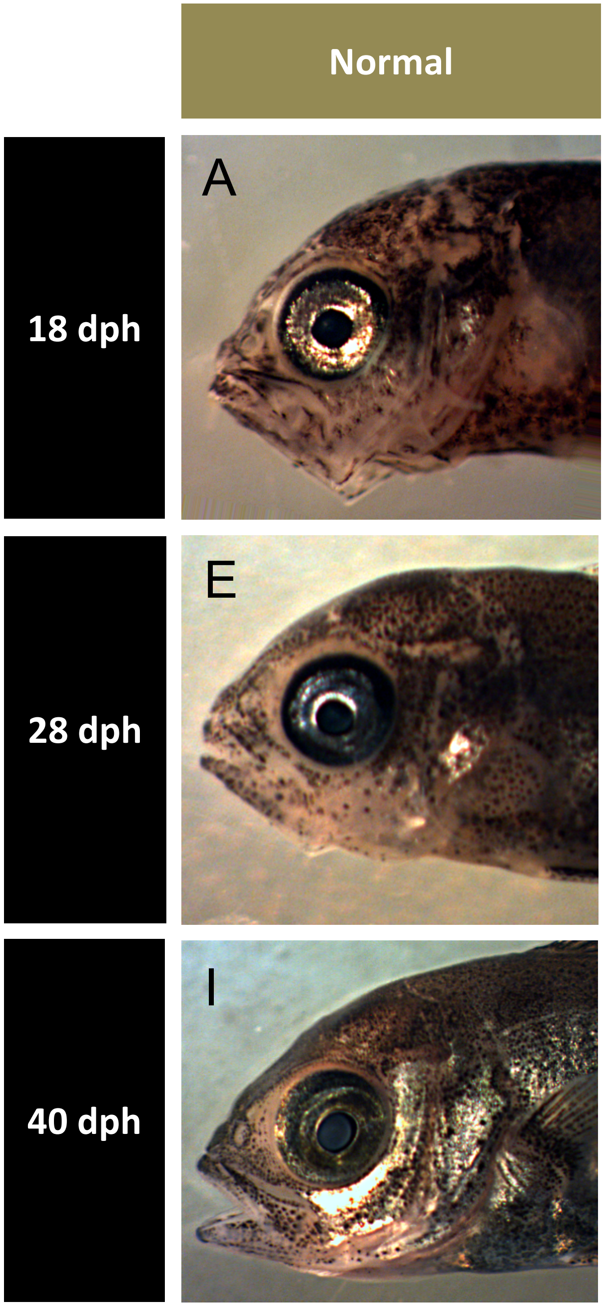
ARA, 20: 4 n-6

(Izquierdo *et al.* 20013, Roo *et al*. 2009, Villeneuve *et al*. 2005, Gapasin and Duray 2001, Lall and Lewis-McCrea 2007)

Objetives

- Cualitative and cuantitative analysis of larvae malformation at comercial hatchery
- Relation between malformation and LC-PUFA content in larva stage, from different spawning.

Cranial deformities in larvae culture



Barra: A – D; 1000 µm, E- H; 2000 µm, (2mm) y I – K; 3500 µm, (3.5 mm).

- **The percentage of deformed larvae is higher in larvae developed from early and middle spawn than late spawn.**

- **The short lower jaw phenotype is not observed at late spawn.**

- **The higher deformities percentage in larvae from early and middle spawn correlate with a lower DHA concentration and DHA/EPA ratio after 10dph compared with larvae from late spawn.**

¿Que sucede con las especies que ya se cultivan en Chile?

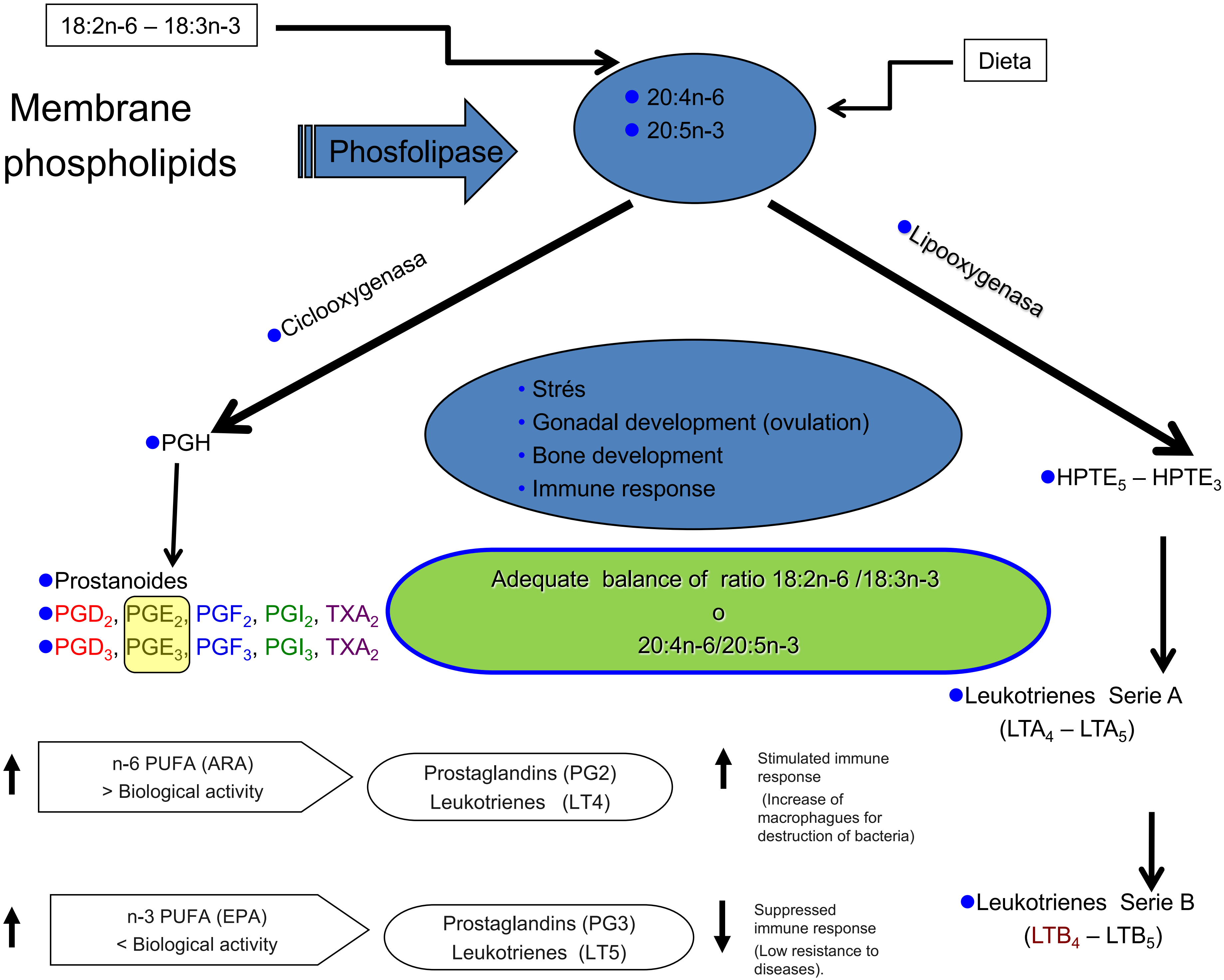
PUNTOS NUTRICIONALES CRITICOS

- Incremento del uso de dietas medicadas (15% del total corresponde a alimento medicado y 1% del total incorpora vacunas orales)
- Altos niveles de malformaciones embrionarias y larvaria e incidencia de enfermedades infecciosas y no infecciosas asociadas a deficiencias nutricionales.
- Bajas evidencias acerca de la incorporación de aditivos o productos bioactivos en las dietas para peces (salud – estrés-reproducción-utilización digestiva – factores antinutricionales)
- Desarrollos reproductivos ineficientes (variabilidad de calidad puesta – efectos nutricionales de los reproductores)
- Desconocimientos del efecto de las sustituciones bajo las condiciones ambientales del país (¿se utilizan o no sustituciones en reproductores?¿tendrá esto que ver con la calidad embrionaria y larval? ¿es posible revertir esta "*mala calidad*")

¡¡¡¡¡balances nutricionales – uso de aditivos !!!!!!!

Nutrition and animal health

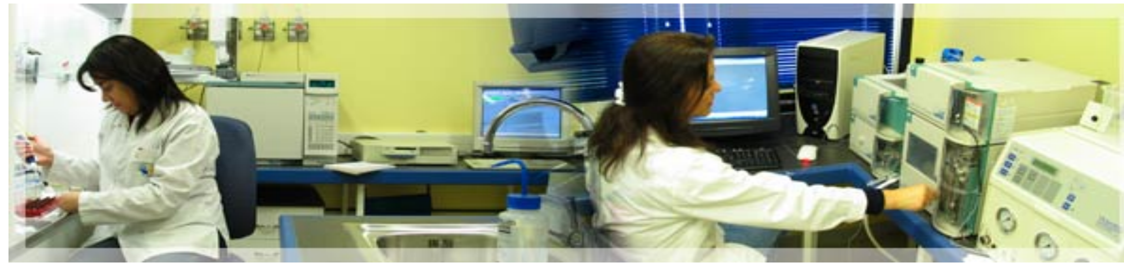
Optimize the inclusion of additives, bioactive compounds and nutritional balances that minimize the impact of substitutions and improve fish defenses and quality



18:2n-6 – 18:3n-3
Dieta
Membrane phospholipids
Phosfolipase
20:4n-6
20:5n-3
Ciclooxygenasa
Lipooxygenasa
PGH
Strés
Gonadal development (ovulation)
Bone development
Immune response
HPTE5 – HPTE3
Prostanoides
PGD2, PGE2, PGF2, PGI2, TXA2
PGD3, PGE3, PGF3, PGI3, TXA2
Adequate balance of ratio 18:2n-6 /18:3n-3
o
20:4n-6/20:5n-3
Leukotrienes Serie A
(LTA4 – LTA5)
n-6 PUFA (ARA)
> Biological activity
Prostaglandins (PG2)
Leukotrienes (LT4)
Stimulated immune response
(Increase of macrophagues for destruction of bacteria)
n-3 PUFA (EPA)
< Biological activity
Prostaglandins (PG3)
Leukotrienes (LT5)
Suppressed immune response
(Low resistance to diseases).
Leukotrienes Serie B
(LTB4 – LTB5)

Commercial diets

Commercial diet	18:2n-6	18:3n-3	20:4n-6	20:5n-3	22:6n-3	DHA /EPA	Vitamin E
1	1,03	0,18	0,20	1,20	2,55	2,12	308 mg/kg
2	1,38	0,20	0,17	2,10	1,66	0,79	405 mg/kg
3	0,70	0,14	0,15	1,34	1,82	1,38	265 mg/kg
4	1,30	0,13	0,14	1,88	2,18	1,15	267 mg/kg

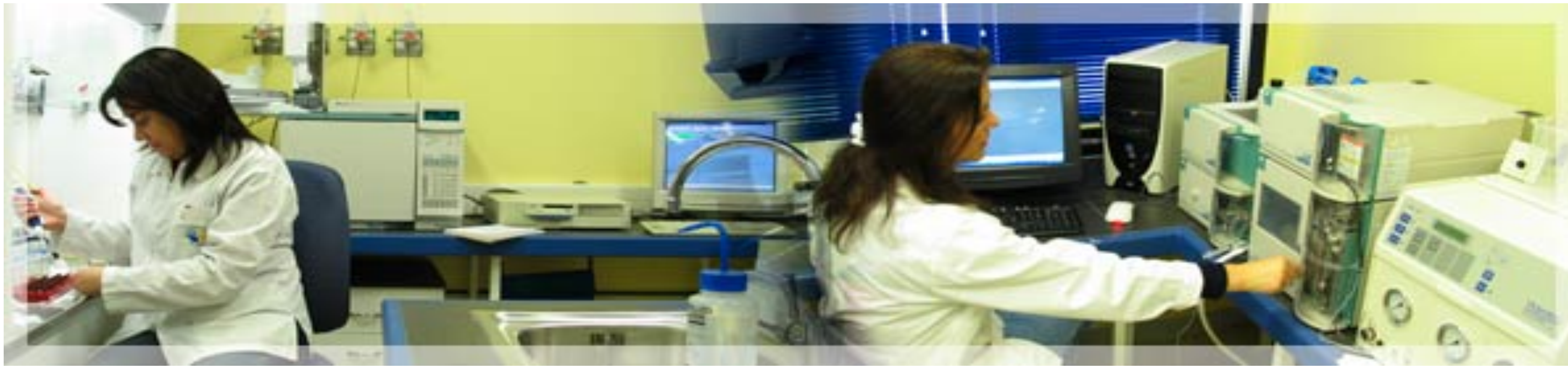


	ARA1/E1	ARA1/E2	ARA2/E1	ARA2/E2	ARA3/E1	ARA3/E2
Proximal composition						
Protein ($N \times 6.25$)	553	553	558	556	556	558
Nitrogen-free extract	120	117	118	122	121	114
Ash	102	102	102	102	102	102
Lipid	219	220	215	213	216	220
α-tocopherol	0.12	0.76	0.19	0.73	0.19	0.72
Fatty acid composition						
C20:4 *n-6*	1.05	1.06	1.77	1.73	3.73	3.75
C20:5 *n-3*	10.16	10.43	12.21	12.55	11.44	12.21
C22:2 *n-6*	0.09	0.10	0.05	0.04	0.04	0.04
C22:6 *n-3*	16.23	16.04	19.11	19.40	17.89	18.87

Dantagnan et al., (2016). Aquaculture Nutrition, doi:10.1111/anu.12438

Table 1 Formulation of experimental diets

Ingredients (g kg^{-1} diet)	ARA1/E1	ARA1/E2	ARA2/E1	ARA2/E2	ARA3/E1	ARA3/E2
Fish meal[1]	619	618	626	625	621	620
Feather meal[2]	50	50	50	50	50	50
Corn gluten[3]	40	40	40	40	40	40
Wheat meal[4]	140	140	140	140	140	140
Astaxanthin[5]	1	1	1	1	1	1
Vitamin premix[6]	2	2	2	2	2	2
Mineral premix[7]	5	5	5	5	5	5
Olive oil[8]	50	50	50	50	40	40
Vevodar® ARA oil[9]	–	–	–	–	15	15
Yardquim oil[10]	–	–	21	21	21	21
ROPUFA® *n-3* INF oil[11]	83	83	65	65	65	65
ROPUFA® *n-3* EPA oil[12]	10	10	–	–	–	–
Vitamin E (α-tocopherol)[13]	0.015	0.65	0.015	0.65	0.015	0.65



- Alevines de 5-6 g
- 110 días
- Evaluación variables productivas
- Evaluación indicadores inmunológicos
- Evaluación exposición al SRS
- Bioacumulación

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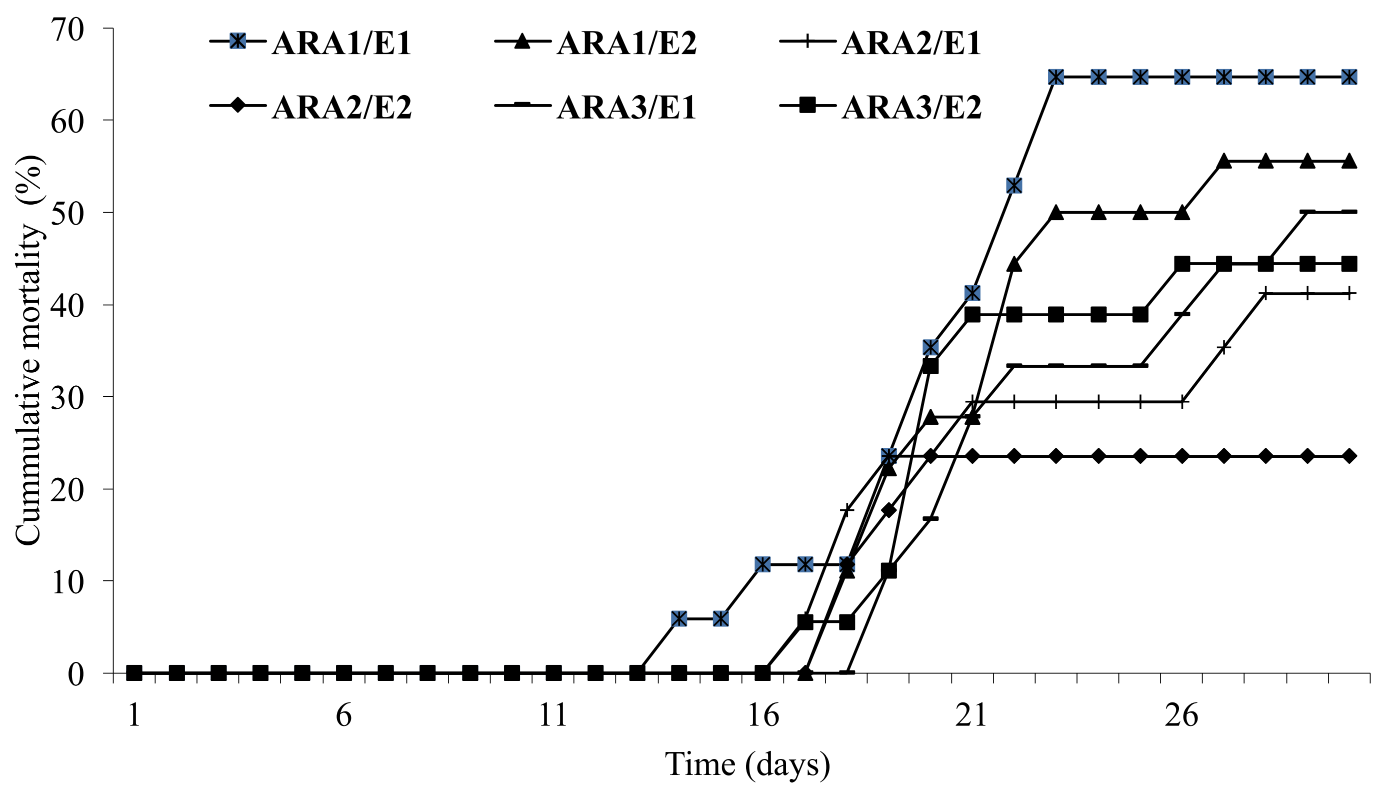
Table 5 Effects of the dietary ARA and vitamin E levels as well as their interaction on the studied immune parameters

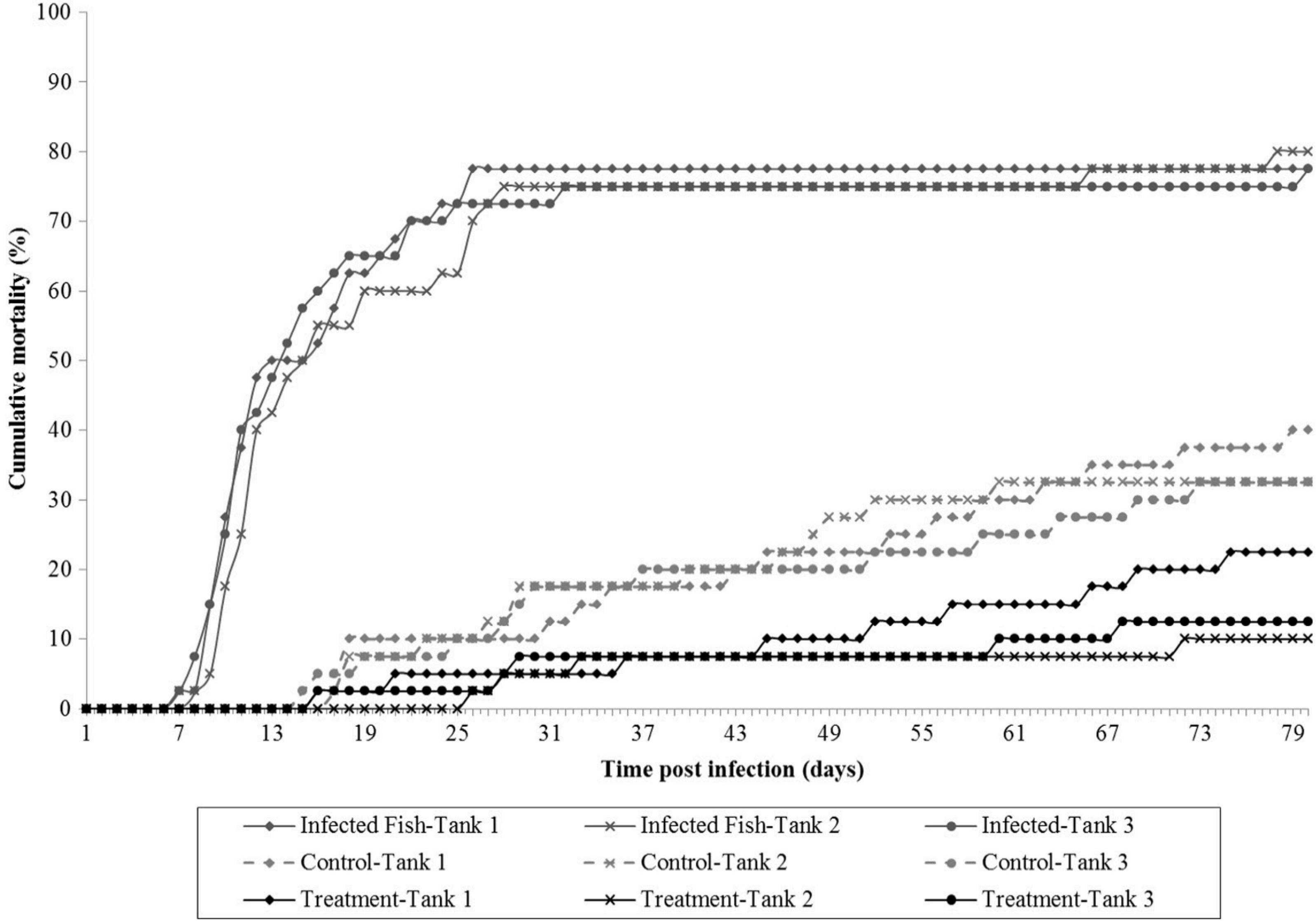
	ARA1/E1	ARA1/E2	ARA2/E1	ARA2/E2	ARA3/E1	ARA3/E2	ARA	Vit E	ARA × VitE
Respiratory burst (%)	0.18 ± 0.01[a]	0.18 ± 0.01[a]	0.23 ± 0.02[b]	0.23 ± 0.02[b]	0.24 ± 0.01[b]	0.24 ± 0.02[b]	**	ns	ns
Phagocytic activity (μ mL^{-1})	19.7 ± 4.5	22.7 ± 5.2	18.6 ± 4.5	16.1 ± 4.9	17.5 ± 4.7	24.0 ± 6.5	ns	ns	ns
Lysozyme activity (OD 630 nm)	0.07 ± 0.03[a]	0.06 ± 0.01[a]	0.12 ± 0.03[b]	0.06 ± 0.03[a]	0.06 ± 0.02[a]	0.09 ± 0.01[a]	ns	ns	**

Asterisks indicate significant differences as $^{**}P < 0.001$; n.s. indicates non-significant differences.

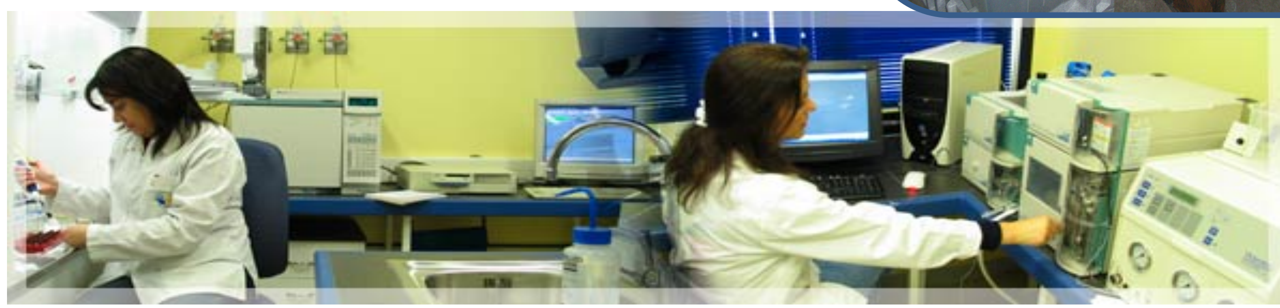


- Futerpenol® is a commercial phytopharmaceutical product. This product has been especially formulated for aquaculture to contain fucoidans and labdane diterpenes as main active compounds deriving from an Acanthaceae family herb and different brown seaweeds. Thus, the aim of this study was to examine the effects of Futerpenol® as a bioactive phytogenic feed additive for rainbow trout.

- *A commercial phytopharmaceutical product branded as Futerpenol® and manufactured by Maqui New Life Inc. (Santiago, Chile)*



Hernández *et al*., 2016. Aqaculture, 454, 109-117



Thank you

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