Optimising lipid nutrition in early developing flatfish larvae

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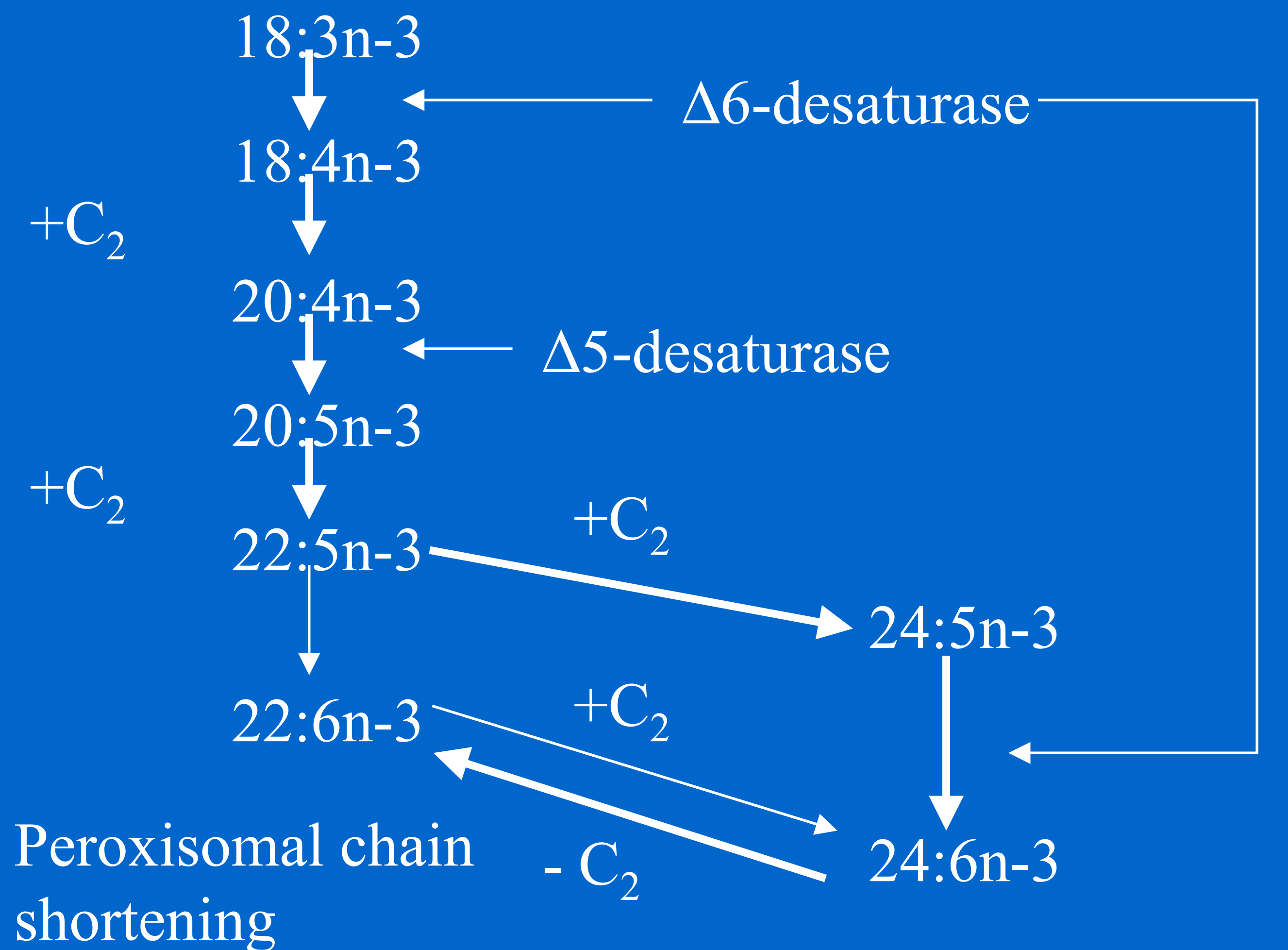
What are essential fatty acids?

- PUFA which cannot be synthesised *de novo* and are required for normal growth and development are EFAs.
- Consequently, they must be supplied, in the correct quantities, in the diet.
- 18:3n-3 (α-linolenic acid) and 18:2n-6 (linoleic acid) are essential for freshwater fish.
- 20:5n-3 (eicosapentaenoic acid) and 22:6n-3 are essential for marine fish.

Pathways of desaturation and elongation of 18:3n-3



Is arachidonic acid (20:4n-6) an EFA for marine fish?

- Marine fish cannot synthesise 20:4n-6 due to lack of Δ5-desaturase/C_{18}-C_{20} elongase.
- 20:4n-6 must be supplied by the diet.
- This requirement is often overlooked when considering diet formulations for marine fish.
- The data of Castell et al. (1994; 1995) confirmed the essentiality of 20:4n-6 for normal growth and survival in juvenile turbot.

Functions of Arachidonic Acid, 20:4n-6

- Present in phospholipids to maintain the structure and function of cell membranes
- Forms the eicosanoids, highly potent local hormones formed by virtually every tissue in the body, generally in response to physiological and environmental stress.

Functions of Eicosapentaenoic Acid, 20:5n-3

- Present in phospholipids to maintain the structure and function of cell membranes
- Competes with arachidonic acid for enzymes forming eicosanoids and thereby depresses the formation of eicosanoids from arachidonic acid

Functions of Docosahexaenoic Acid, 22:6n-3

- Present in phospholipids to maintain the structure and function of cell membranes, particularly in neural tissue, i.e. the brain and the eye, where it is concentrated in nerve synapses

EFA compositions of egg phospholipids in marine fish*

Fatty acid	Cod	Herring	Saithe	Haddock	Whiting	Capelin	Sand eel
20:4n-6	1.9	1.0	1.6	3.7	2.4	1.1	1.9
20:5n-3	15.3	13.7	11.5	12.6	13.3	19.0	16.7
22:6n-3	28.6	31.4	27.7	27.6	30.3	24.6	25.5
DHA/EPA	1.9	2.3	2.4	2.2	2.3	1.3	1.5
EPA/ARA	8.1	13.7	7.2	3.4	5.5	17.3	8.8

Values are weight %. *Data from Tocher and Sargent, 1984.

Target values for DHA/EPA and EPA/ARA ratios in live feeds?

- The DHA/EPA ratio should be 2:1.
- The EPA/ARA ratio should be 8:1.
- Can these values be achieved in available live prey organisms?

HUFA compositions of rotifers

HUFA/enrichment	None[1]	TOO[2]	Super Selco™	TOO + ARA[3]
20:4n-6	trace	1.1	1.4	3.8
20:5n-3	0.2	4.6	13.7	5.2
22:6n-3	0.1	12.7	15.4	10.2
DHA/EPA	0.5	2.8	1.1	2.0
EPA/ARA	-	4.2	9.8	1.4

[1]Rodriguez et al. 1997. [2]TOO = Tuna orbital oil & [3]85% TOO + 15%ARASCO™ all contain 12% soy lecithin, from Estevez et al. 1999.

HUFA compositions of *Artemia*

HUFA/Enrichment	none[1]	TOO[1]	AM 2000™[2]	Super Selco™[2]	DHA Selco™[3]
20:4n-6	1.2	1.8	1.7	1.3	1.5
20:5n-3	5.3	7.6	7.1	16.5	8.3
22:6n-3	0.0	10.0	6.5	9.3	9.4
DHA/EPA	0.0	1.4	0.9	0.6	1.1
EPA/ARA	4.6	4.2	4.1	13.0	5.5

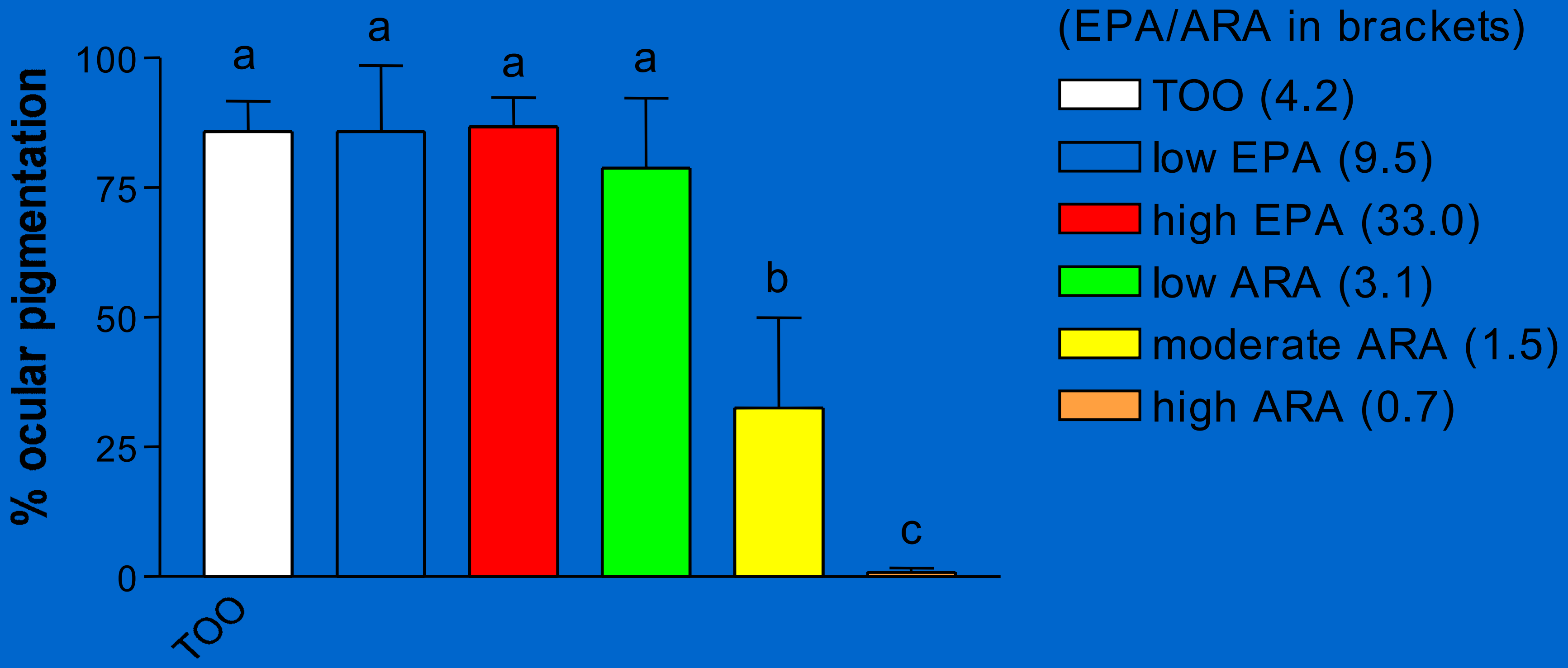
[1]Estevez et al. 1999; [2]Algamac 2000™, Gara et al. 1998; [3]Hamre et al. 2001.

HUFA compositions of copepods

HUFA/species	*E.velox*[1]	*T.furcata*[2]	Mixed nauplii[3]	*A. tonsa*[4]
20:4n-6	1.8	1.7	0.3	0.8
20:5n-3	10.8	11.2	9.2	6.8
22:6n-3	21.8	24.7	39.4	30.3
DHA/EPA	2.0	2.2	4.3	4.5
EPA/ARA	6.0	6.6	30.7	9.2

[1]Shields et al. 1999; [2]Bell et al. 1993; [3]nauplii of *E. affinis, A. teclae* and *C. hamatus;* McEvoy et al. 1998; intensively cultured nauplii, Støttrup et al. 1999.

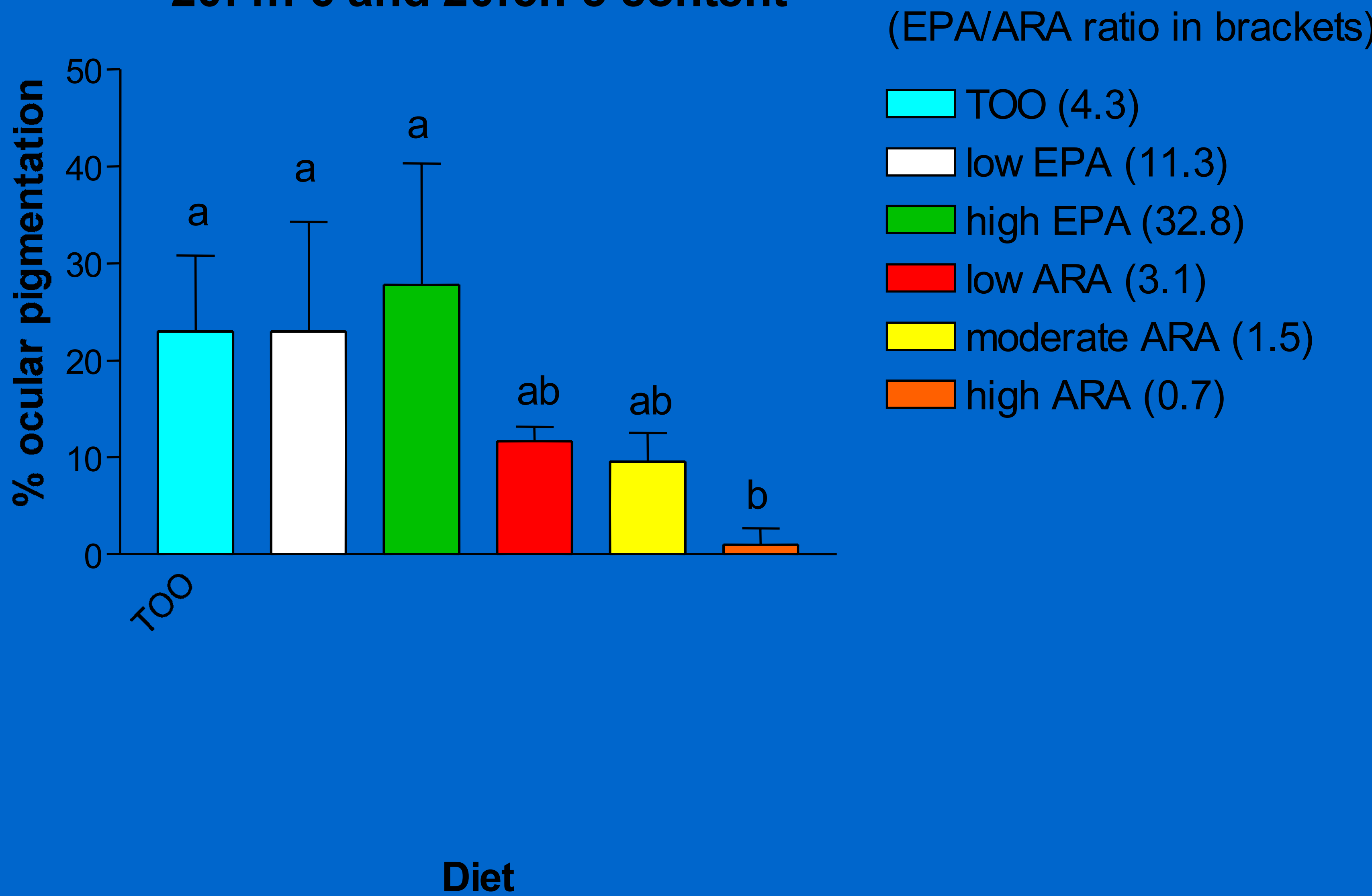
Pigmentation success in turbot fed diets varying in 20:4n-6 and 20:5n-3 content



Diet

Columns assigned a different letter are significantly different ($P < 0.05$).

Pigmentation success in halibut fed diets varying in 20:4n-6 and 20:5n-3 content



Comparison of pigmentation success in turbot and halibut

- Why is pigmentation so much better in turbot compared to halibut given the same diets?
- Turbot were fed initially on rotifers, then *Artemia,* whereas halibut had only *Artemia*.
- Halibut were not fed the experimental diets from first-feeding being weaned onto “control” diet of AM2000™/Super Selco™ (until 488 d^{o}).

Why is the rotifer feeding stage important?

- Dietary phospholipids enhance growth and development in marine fish larvae.
- The HUFA composition of dietary PL will determine their efficacy.
- Having the correct HUFA in the *sn*-2 position will allow direct incorporation of PL into cell membranes of rapidly growing larvae.

HUFA compositions in the total polar lipid fraction of rotifers, *Artemia* nauplii & *C. finmarchicus*

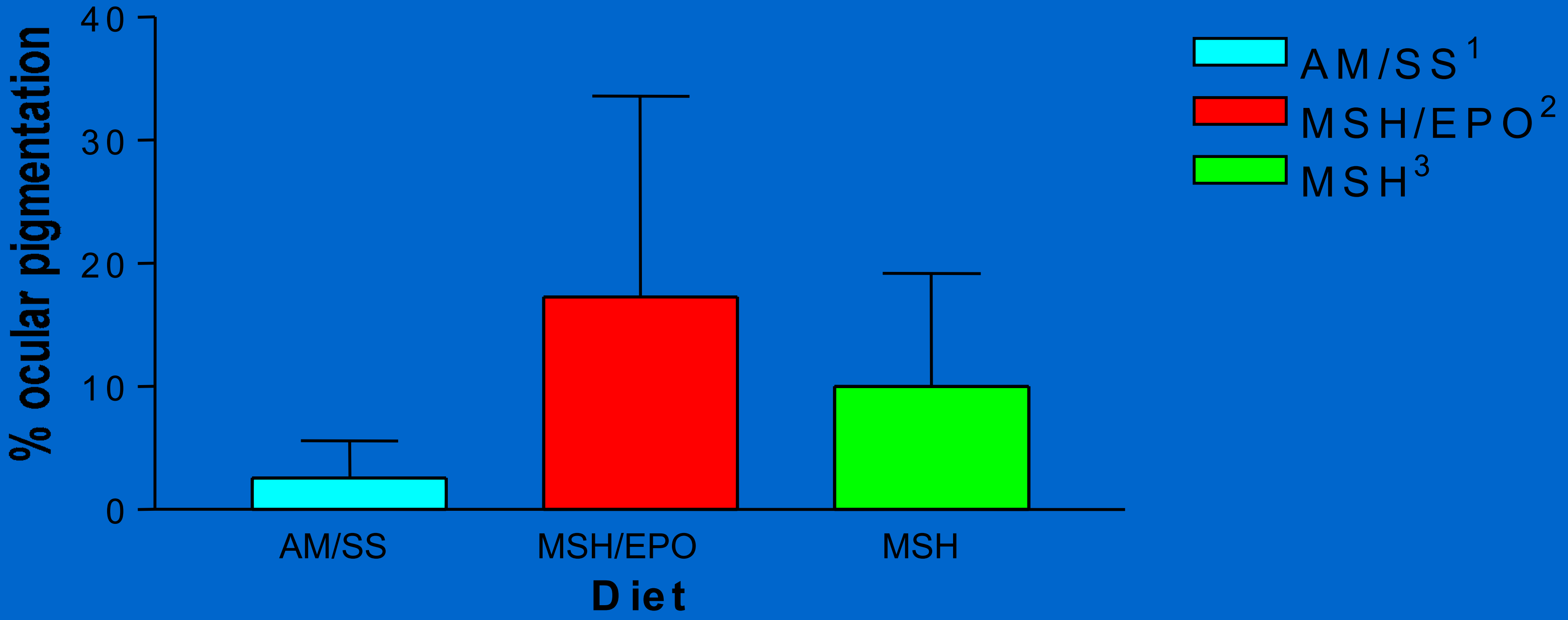
HUFA	Rotifer		*Artemia*		*C. finmarchicus*[1]
enrichment	TOO	SS	TOO	SS	
20:4n-6	2.0	1.4	2.4	1.6	0.6
20:5n-3	8.1	11.1	11.5	14.9	24.0
22:6n-3	12.4	9.8	2.4	0.6	40.6
DHA/EPA	1.5	0.9	0.2	0.0	1.7
EPA/ARA	4.1	7.9	4.8	9.2	40.0

[1]Scott, C. 2001, PhD. thesis.

Is pigmentation linked to eicosanoid production?

- Evidence from previous experiments suggests that diets with EPA/ARA ratios > 5 result in better pigmentation that diets with EPA/ARA < 5.
- If pigmentation is affected by ARA-derived eicosanoids then enrichments containing γ-linolenic acid (18:3n-6) might improve pigmentation.

Pigmentation success in halibut fed *Artemia* enriched with fish oil and evening primrose oil



[1] Algamac™ /Super Selco™ (control)

[2] Fish oil (mackerel/sprat/herring, 50%), EPO concentrate (30%), Krill phospholipid (20%).

[3] Fish oil (mackerel/sprat/herring, 80%), Krill phospholipid (20%).

Summary

- In terms of lipid composition, copepod nauplii are the best live prey for early flatfish larvae.
- Live prey should have a DHA/EPA ratio of >1 and, preferably, close to 2.
- Live prey containing phospholipids, rather than triglycerides, rich in DHA and EPA are beneficial to growth and development.
- For this reason, in the absence of copepods, early rotifer feeding is beneficial in terms of digestibility & availability of essential HUFAs.

Summary continued

- Pigmentation can be improved by using *Artemia* enrichment diets which have a high EPA/ARA ratio (> 5) or rich in 18:3n-6.
- Evidence in halibut suggests that the period before 570 day^{o} is vital in determining pigmentation success, supporting the “pigmentation window” of Naess & Lie, 1998.
- Feeding oils rich in energy-rich monounsaturates after the pigmentation window seems to improve growth & eye migration.

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