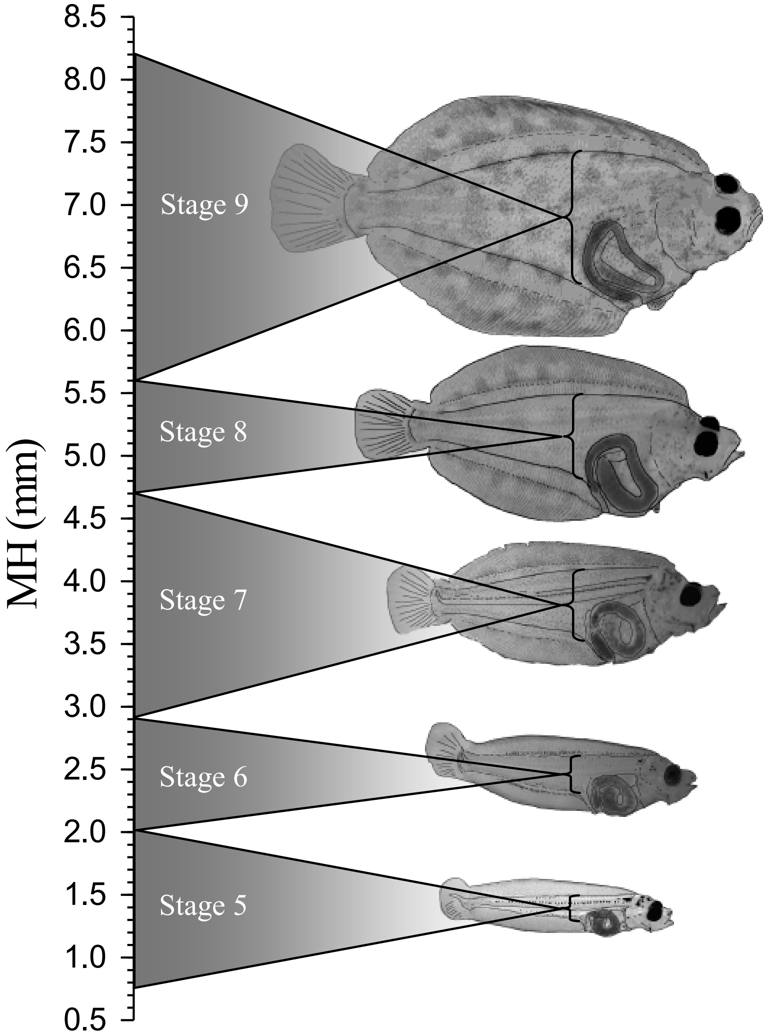


Drawing: Øystein Sæle (2003)

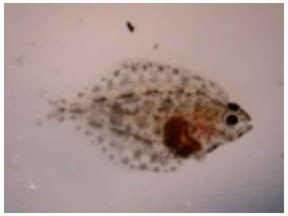
Atlantic halibut larval nutrition and the drivers of pigmentation and eye migration in flounders

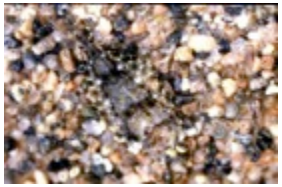
Kristin Hamre

Developmental malformations in farmed halibut juveniles

Larva 14dpff

Normal juvenile





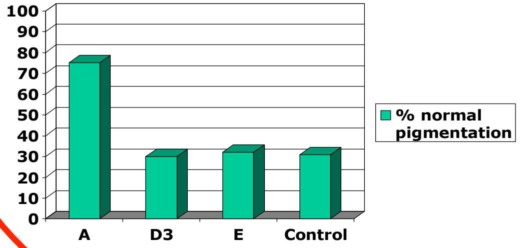
No eye migration

Malpigmented

Thyroid hormone is the driver of metamorphosis

Too high ARA and too low DHA give malpigmentation

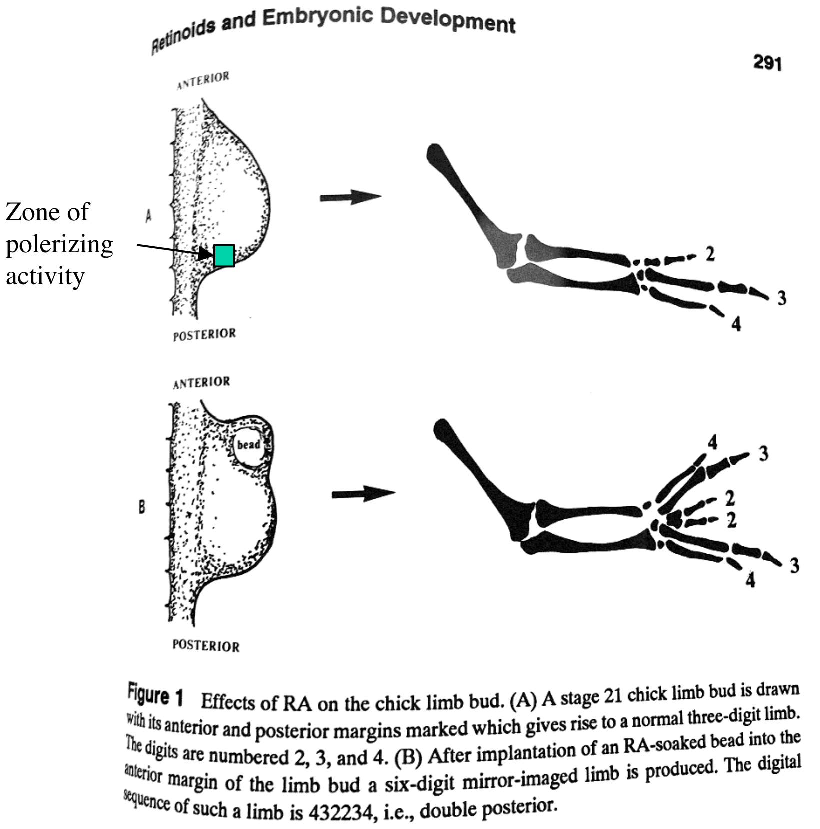
Vitamin A affects pigmentation in Japanese flounder larvae



Miki et al., 1990

Photos: Kristin Hamre, Øystein Sæle, Karin Pittman, IMR

Retinoic acid in development of the chick wing


Retinoids and Embryonic Development

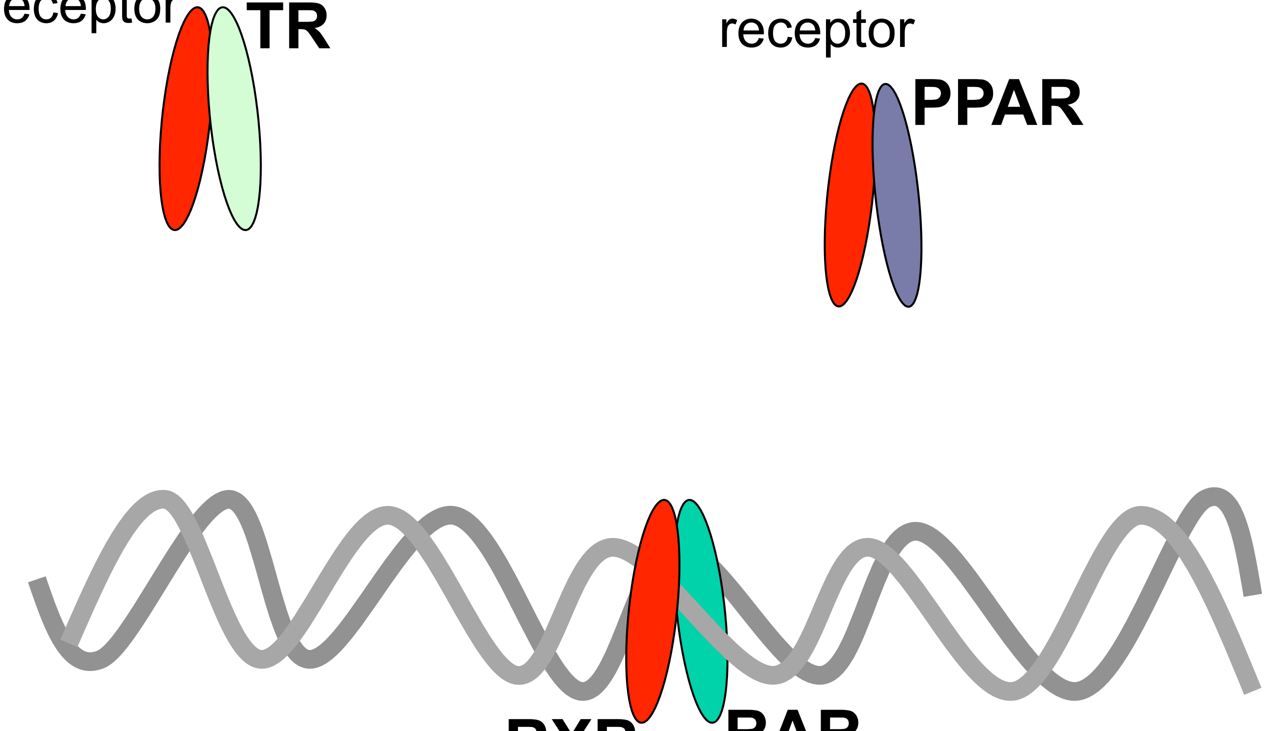
291

Figure 1 Effects of RA on the chick limb bud. (A) A stage 21 chick limb bud is drawn with its anterior and posterior margins marked which gives rise to a normal three-digit limb. The digits are numbered 2, 3, and 4. (B) After implantation of an RA-soaked bead into the anterior margin of the limb bud a six-digit mirror-imaged limb is produced. The digital sequence of such a limb is 432234, i.e., double posterior.

Maden et al.,1994

→ **Hypothesis**

Interactions between vitamin A, thyroid hormone and fatty acids on the gene expression level



Thyroid hormone activated receptor

TR

Peroxisomal proliferator-activated receptor

PPAR

RXR

RAR

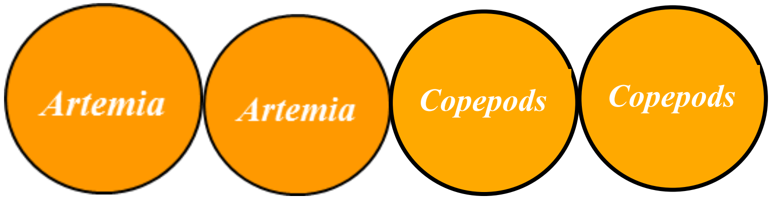
Retinoid X activated receptor

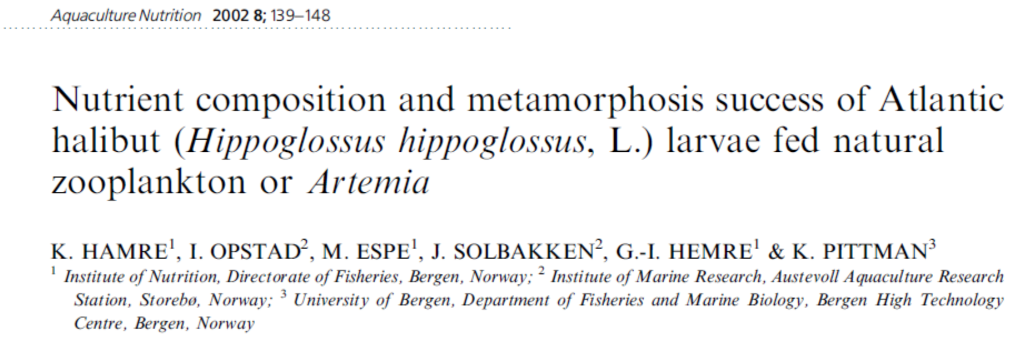
Retinoic acid activated receptor

Hypothesis (1999):

Fatty acids, vitamin A and nutrients necessary for synthesis of thyroid hormone (iodine, phenylalanine/tyrosine, selenium) act alone or in interaction to promote normal development in Atlantic halibut larvae

Experimental




Aquaculture Nutrition 2002 8; 139–148

Nutrient composition and metamorphosis success of Atlantic halibut (*Hippoglossus hippoglossus*, L.) larvae fed natural zooplankton or *Artemia*

K. HAMRE[1], I. OPSTAD[2], M. ESPE[1], J. SOLBAKKEN[2], G.-I. HEMRE[1] & K. PITTMAN[3]
[1] *Institute of Nutrition, Directorate of Fisheries, Bergen, Norway;* [2] *Institute of Marine Research, Austevoll Aquaculture Research Station, Storebø, Norway;* [3] *University of Bergen, Department of Fisheries and Marine Biology, Bergen High Technology Centre, Bergen, Norway*

- ***Artemia* was enriched with DHA-selco and fed according to standard methods**
- **Copepods were harvested from a fertilized pond (Svartatjønn, Austevoll)**
- **Experimental period: day 0-60 after firstfeeding**

Pigmentation and eye migration

	Artemia	Zooplankton
Normal pigmentation (%)	7 (depigmentation)	68 (ambicoloration)
Complete eye migration (%)	10	88
Final weight (mg dry wt.)	35	55

Photo: IMR

Depigmentation

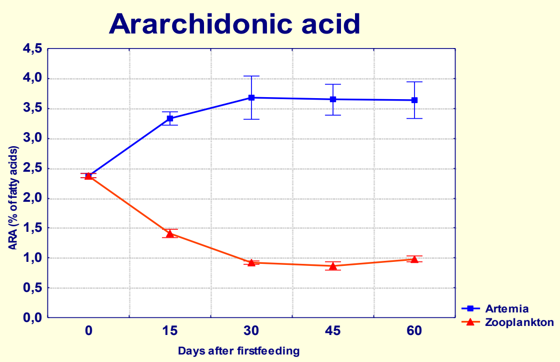
Photo: Jostein Solbakken

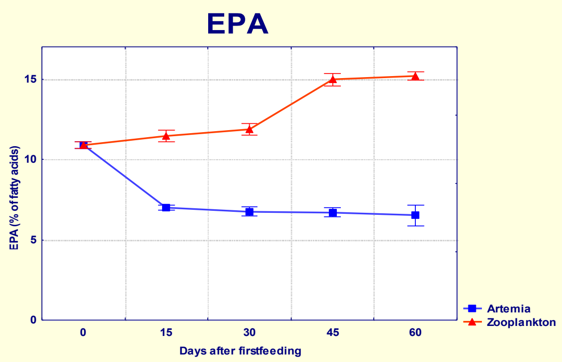
Eye migration

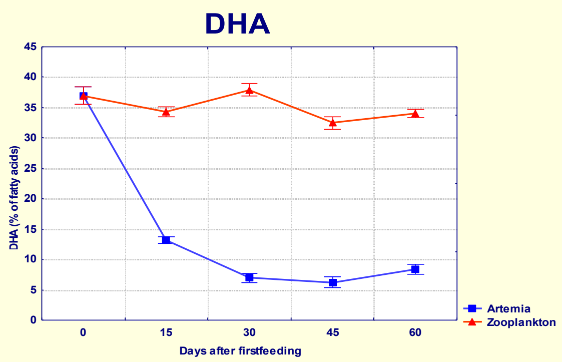
Fatty acids in *Artemia* and copepods

%	*Artemia*	Copepods
ARA	2,1±0,1^{a}	0,7±0,2^{b}
EPA	4,9±0,3^{a}	18,6±3,2^{b}
DHA	6,3±0,9^{a}	28,5±4,8^{b}

Fatty acids in larvae







Effect of fatty acids on growth and survival

Aquaculture 277 (2008) 239–243

Contents lists available at ScienceDirect

Aquaculture

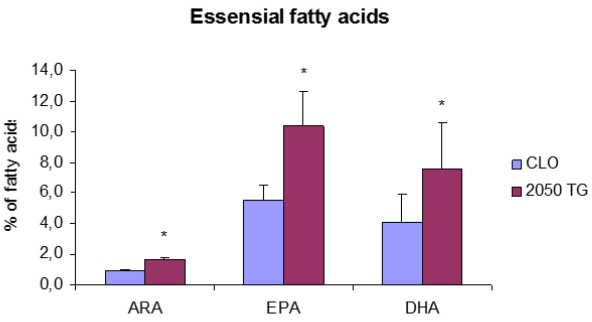
journal homepage: www.elsevier.com/locate/aqua-online

Artemia enriched with high n-3 HUFA may give a large improvement in performance of Atlantic halibut (*Hippoglossus hippoglossus* L.) larvae

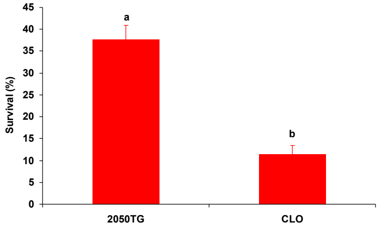
Kristin Hamre [a],*, Torstein Harboe [b]

[a] National Institute of Nutrition and Seafood Research (NIFES), PO Box 2029, 5817 Bergen, Norway
[b] Institute of Marine Research, Austevoll Aquaculture Station, 5392 Storebø, Norway

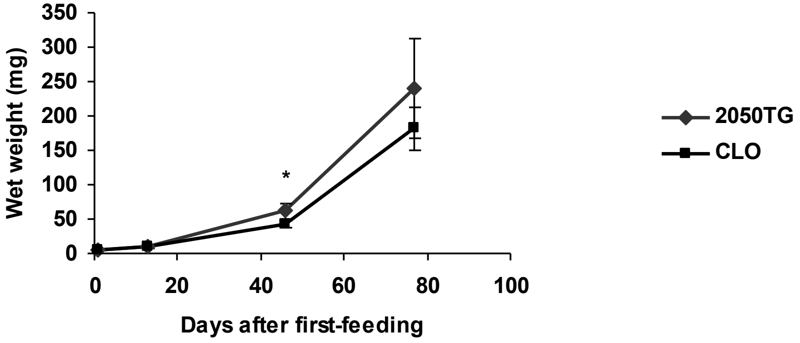
Artemia



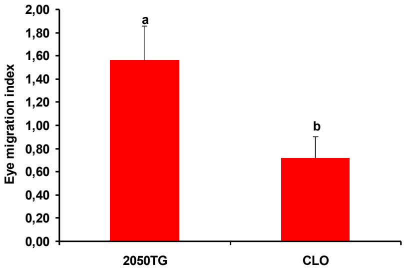
Larval survival



Larval growth

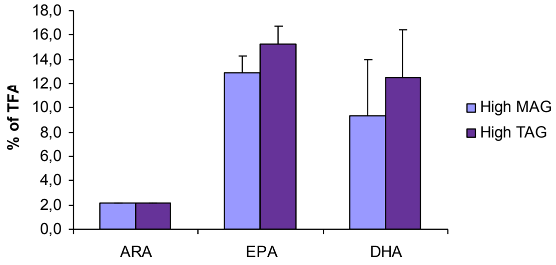


Larval eye migration

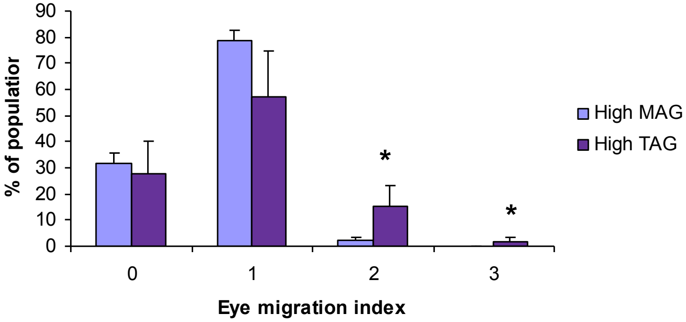


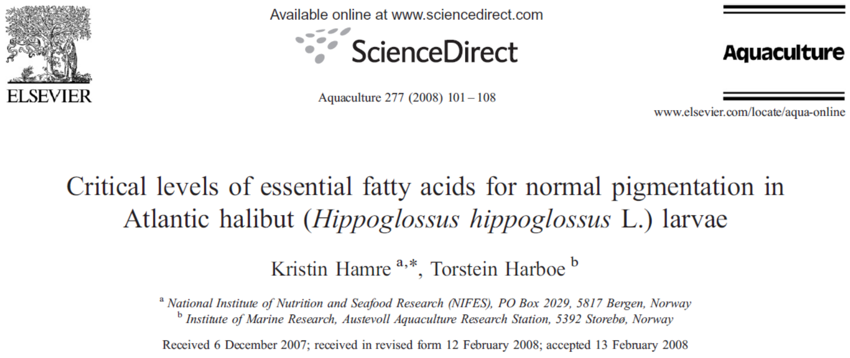
Effect of fatty acids on pigmentation and eye migration

Fatty acids in Artemia



Eye migration





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Aquaculture 277 (2008) 101 – 108

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Critical levels of essential fatty acids for normal pigmentation in Atlantic halibut (*Hippoglossus hippoglossus* L.) larvae

Kristin Hamre [a,*], Torstein Harboe [b]

[a] *National Institute of Nutrition and Seafood Research (NIFES), PO Box 2029, 5817 Bergen, Norway*
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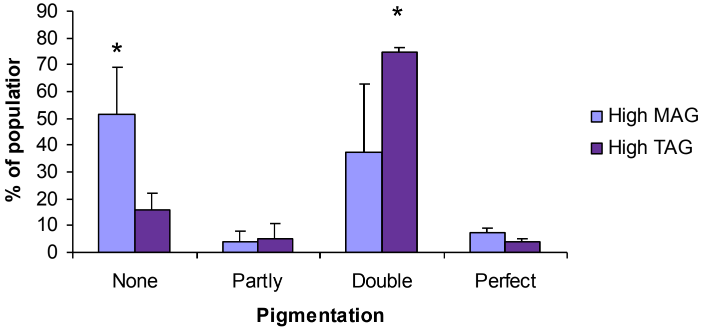
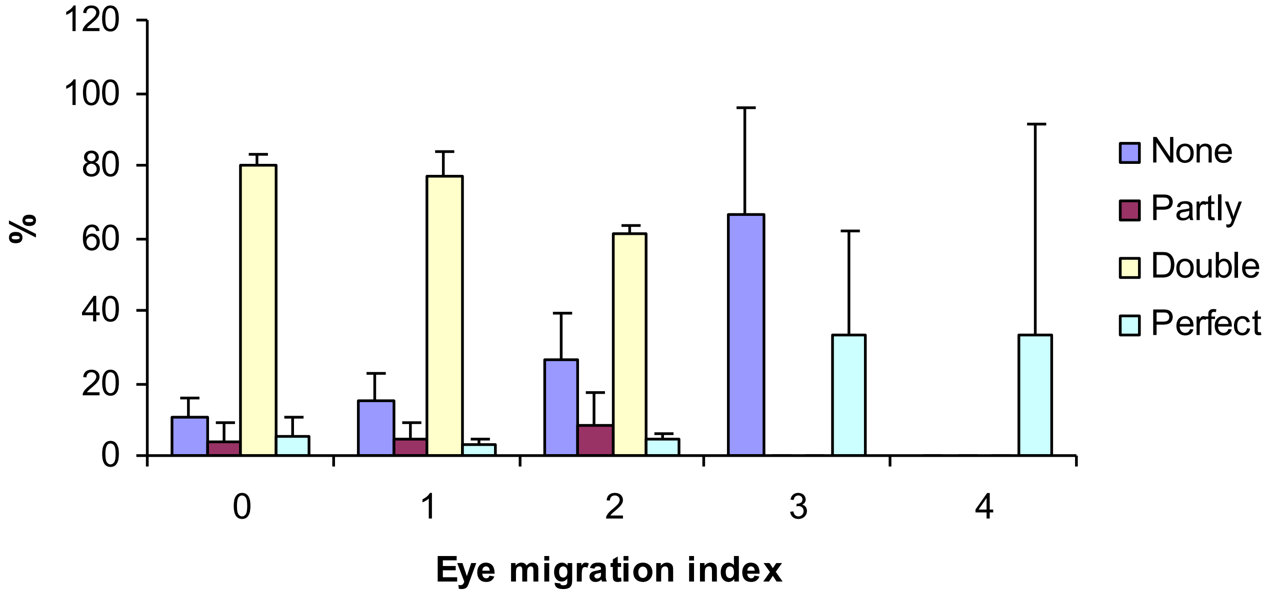
Received 6 December 2007; received in revised form 12 February 2008; accepted 13 February 2008

Pigmentation



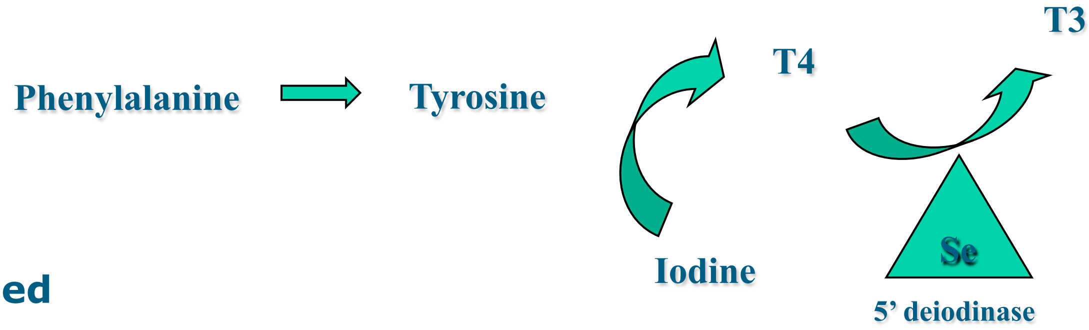
Correlation between eye migration and double pigmentation



Double pigmented fish are actually normally pigmented fish that lack eye migration

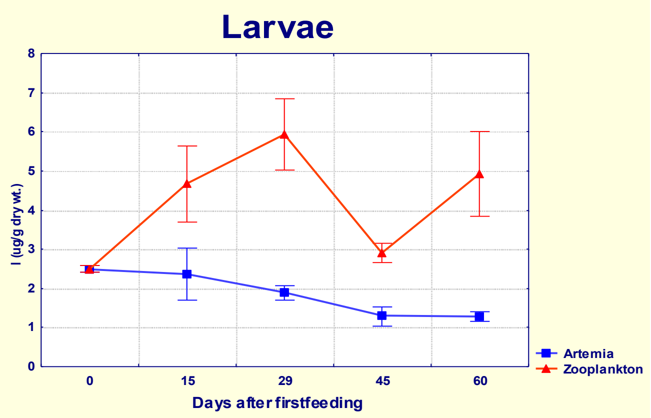
Normal pigmentation:
High TAG: 77±2
High MAG: 46±16%,

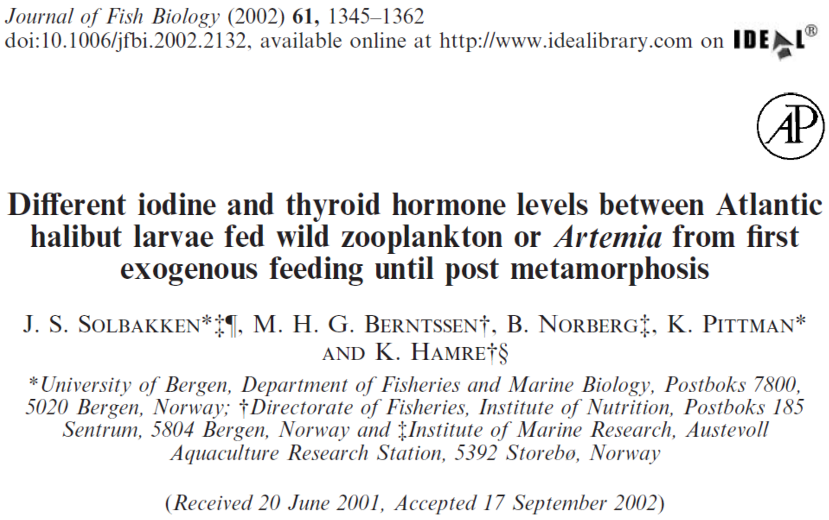
Nutritional input in synthesis of thyroid hormone



Feed

(µg/g dry wt)	Artemia	Copepods
Iodine	0,51±0.05^a	350±40^b




Journal of Fish Biology (2002) **61,** 1345–1362
doi:10.1006/jfbi.2002.2132, available online at http://www.idealibrary.com on IDEAL®

Different iodine and thyroid hormone levels between Atlantic halibut larvae fed wild zooplankton or *Artemia* from first exogenous feeding until post metamorphosis

J. S. Solbakken*‡¶, M. H. G. Berntssen†, B. Norberg‡, K. Pittman*
and K. Hamre†§

**University of Bergen, Department of Fisheries and Marine Biology, Postboks 7800, 5020 Bergen, Norway; †Directorate of Fisheries, Institute of Nutrition, Postboks 185 Sentrum, 5804 Bergen, Norway and ‡Institute of Marine Research, Austevoll Aquaculture Research Station, 5392 Storebø, Norway*

(*Received 20 June 2001, Accepted 17 September 2002*)

Iodine nutrition in flatfish

Aquaculture Nutrition

doi: 10.1111/j.1365-2095.2009.00740.x

Aquaculture Nutrition **2011 17;** 248–257

Iodine-enriched rotifers and *Artemia* prevent goitre in Senegalese sole (*Solea senegalensis*) larvae reared in a recirculation system

A.R.A. RIBEIRO[1], L. RIBEIRO[1], Ø. SÆLE[2], K. HAMRE[2], M.T. DINIS[1] & M. MOREN[2]

[1] *CCMAR, Universidade do Algarve, Campus de Gambelas, Faro, Portugal;* [2] *NIFES, National Institute of Nutrition and Seafood Research, Nordnes, Bergen, Norway*

Aquaculture Nutrition **2006 12;** 97–102

Iodine enrichment of *Artemia* and enhanced levels of iodine in Atlantic halibut larvae (*Hippoglossus hippoglossus* L.) fed the enriched *Artemia*

M. MOREN[1], I. OPSTAD[2], T. VAN DER MEEREN[2] & K. HAMRE[1]

[1] *National Institute of Nutrition and Seafood Research, Bergen;* [2] *Institute of Marine Research, Austevoll Aquaculture Research Station, Storebø, Norway*

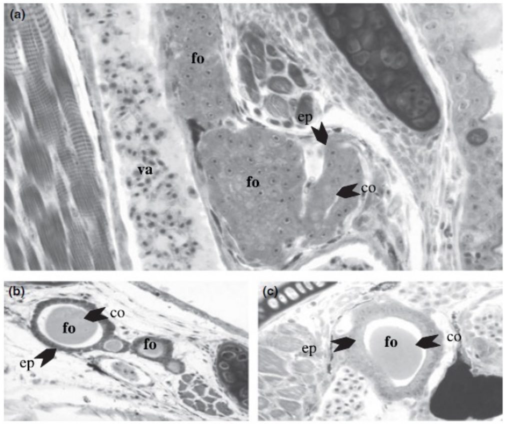


Figure 9 Thyroid follicles in Senegalese Sole. (a) Thyroid follicles (fo) from control treatment at 31 days after hatch (DAH). Thyroid tissue is very dense, characteristic of thyroid hyperplasia and hypertrophy (goitre); the proximity to ventral aorta (va) is evident. Follicle epithelial cells (ep – arrow) have lost the follicular type of morphology and colloid (co – arrow) is absent (magnification 400×); (b) Thyroid follicles (fo) from control treatment at 15 DAH; (c) Thyroid follicle (fo) from iodine treatment at 31 DAH. In both figures (b and c), the thyroid tissue is less dense compared to (a). Follicles are adjacent to the ventral aorta and bulbus arteriosus. Follicle epithelial cells are cuboidal and colloids are replete with thyroglobulin (magnification 640×).

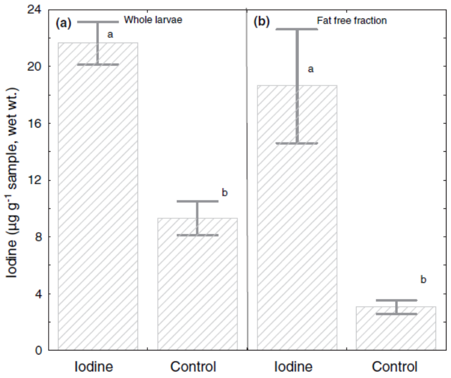
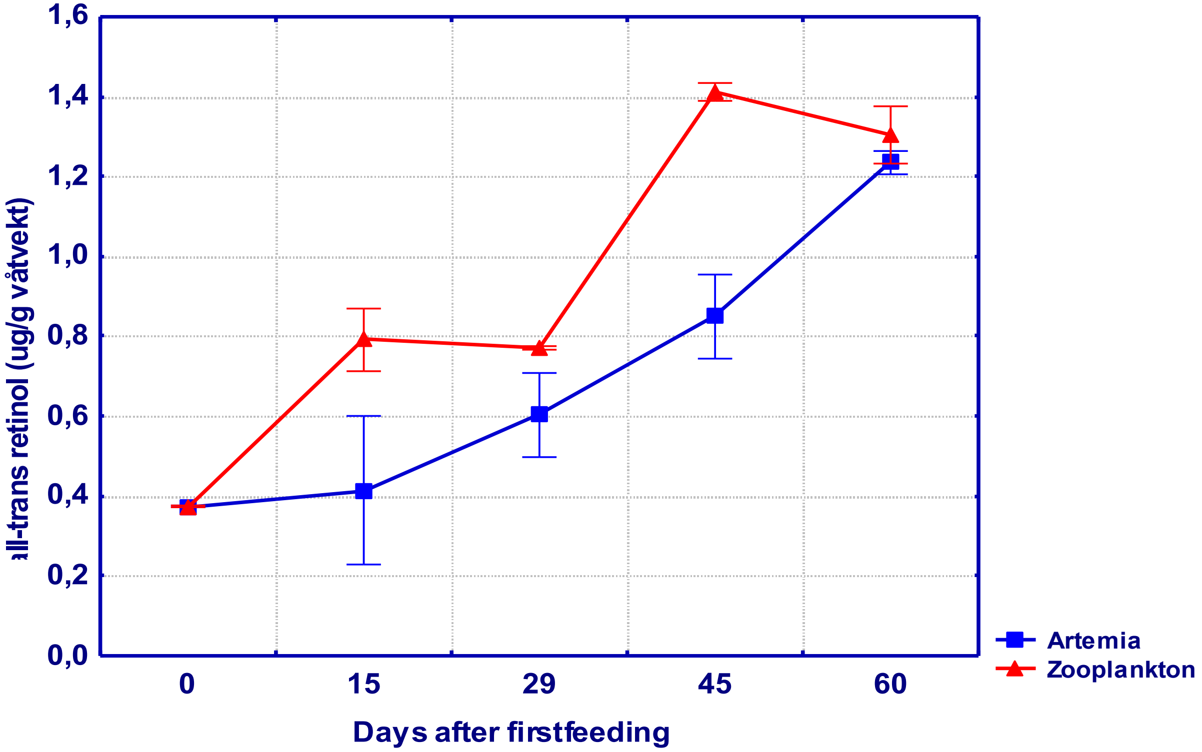


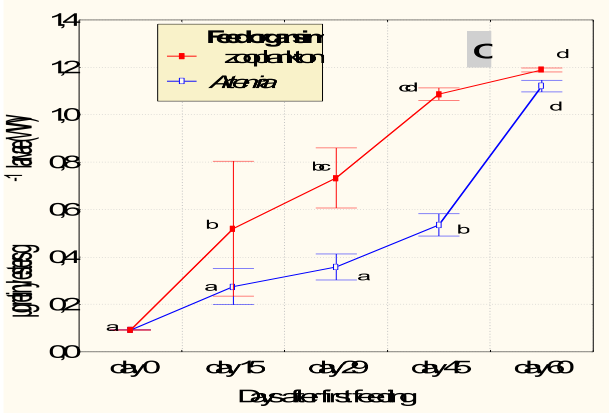
Figure 1 Concentration of iodine (μg g^{-1} wet weight) in Atlantic halibut larvae-fed *Artemia* with or without iodine supplementation from days 9 to 60 pff. (a) whole larvae (wet weight, $n = 6$), (b) the fat-free fraction of larvae (wet weight, $n = 3$). Analysis was conducted on samples containing five larvae from the end of the trial. Significant difference is indicated with different letters in the figure (one-way ANOVA, $P < 0.05$).

Total vitamin A in larvae fed *Artemia* or copepods

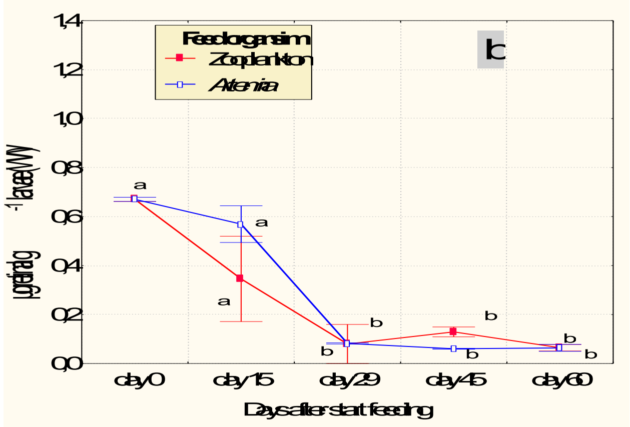


Physiological forms of vitamin A in larvae

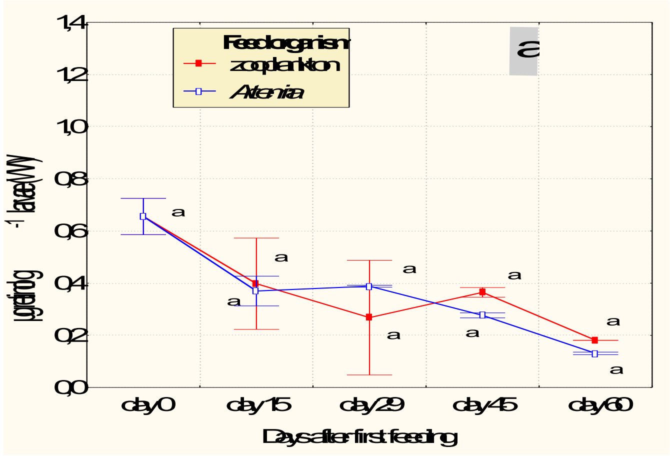
Retinyl esters

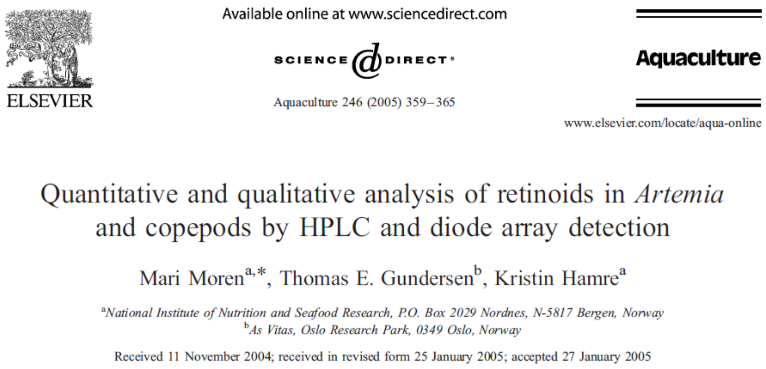


Retinal



Retinol





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Aquaculture 246 (2005) 359–365

Aquaculture

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Quantitative and qualitative analysis of retinoids in *Artemia* and copepods by HPLC and diode array detection

Mari Moren[a,*], Thomas E. Gundersen[b], Kristin Hamre[a]

[a]*National Institute of Nutrition and Seafood Research, P.O. Box 2029 Nordnes, N-5817 Bergen, Norway*
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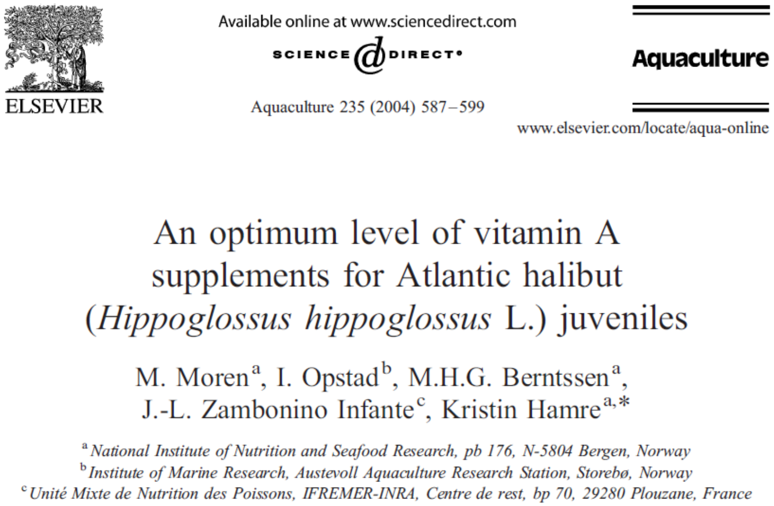
Vitamin A deficiency in halibut juveniles

Photo: Mari Moren



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Aquaculture 235 (2004) 587–599

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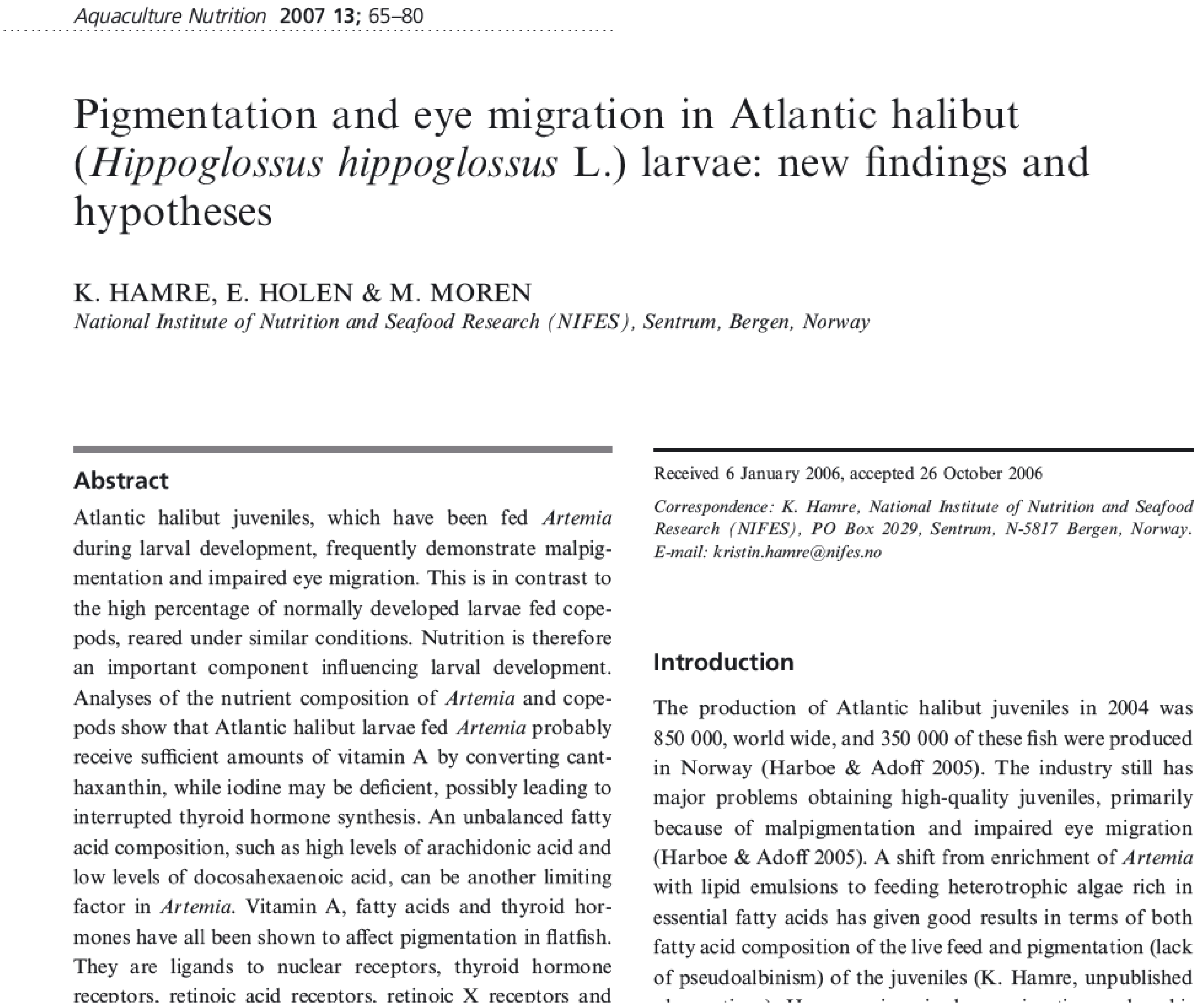
An optimum level of vitamin A supplements for Atlantic halibut (*Hippoglossus hippoglossus* L.) juveniles

M. Moren[a], I. Opstad[b], M.H.G. Berntssen[a],
J.-L. Zambonino Infante[c], Kristin Hamre[a,*]

[a] *National Institute of Nutrition and Seafood Research, pb 176, N-5804 Bergen, Norway*
[b] *Institute of Marine Research, Austevoll Aquaculture Research Station, Storebø, Norway*
[c] *Unité Mixte de Nutrition des Poissons, IFREMER-INRA, Centre de rest, bp 70, 29280 Plouzane, France*



Aquaculture Nutrition **2007 13**; 65–80

Pigmentation and eye migration in Atlantic halibut (*Hippoglossus hippoglossus* L.) larvae: new findings and hypotheses

K. HAMRE, E. HOLEN & M. MOREN
National Institute of Nutrition and Seafood Research (NIFES), Sentrum, Bergen, Norway

Abstract

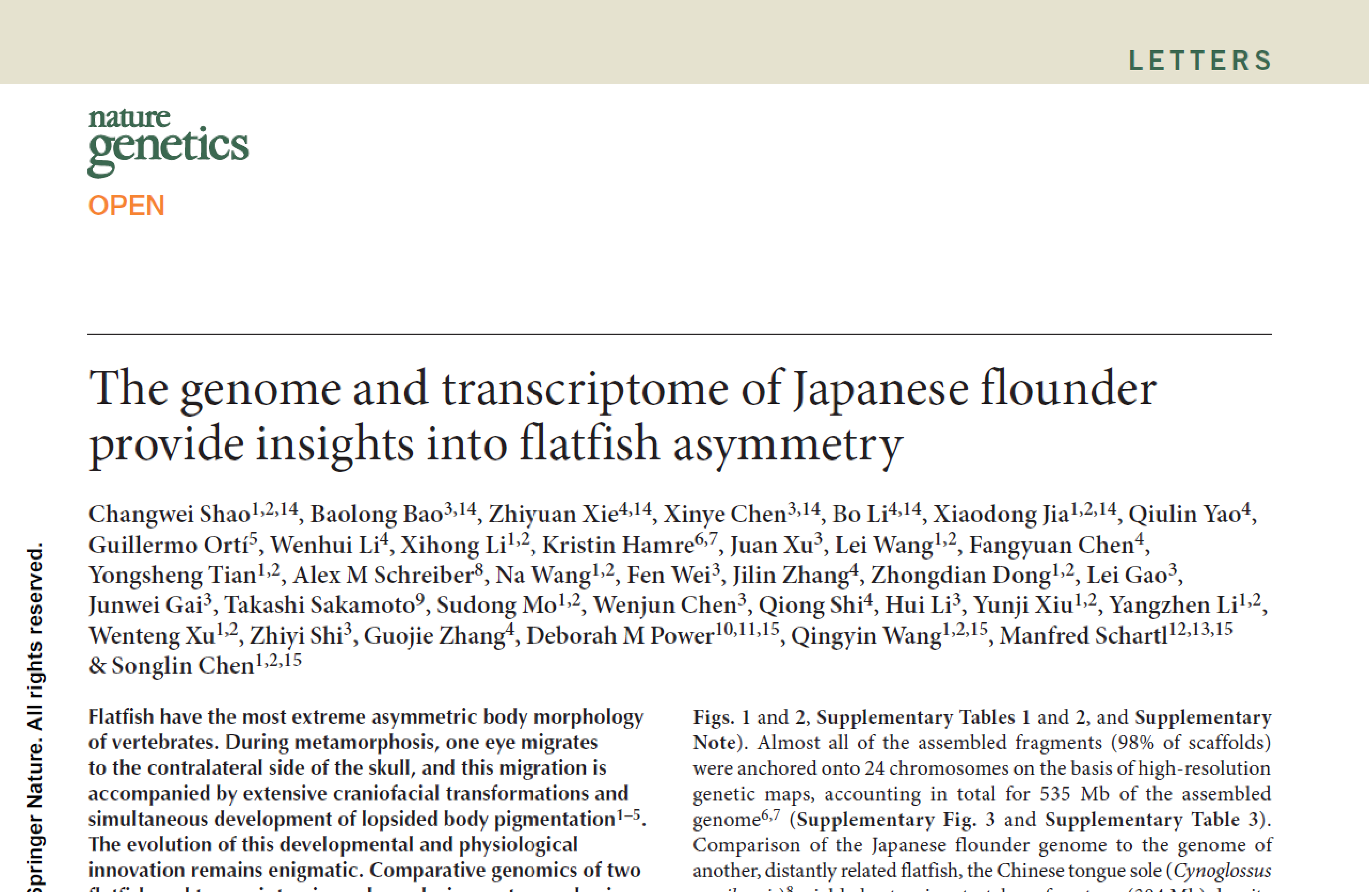
Atlantic halibut juveniles, which have been fed *Artemia* during larval development, frequently demonstrate malpigmentation and impaired eye migration. This is in contrast to the high percentage of normally developed larvae fed copepods, reared under similar conditions. Nutrition is therefore an important component influencing larval development. Analyses of the nutrient composition of *Artemia* and copepods show that Atlantic halibut larvae fed *Artemia* probably receive sufficient amounts of vitamin A by converting canthaxanthin, while iodine may be deficient, possibly leading to interrupted thyroid hormone synthesis. An unbalanced fatty acid composition, such as high levels of arachidonic acid and low levels of docosahexaenoic acid, can be another limiting factor in *Artemia*. Vitamin A, fatty acids and thyroid hormones have all been shown to affect pigmentation in flatfish. They are ligands to nuclear receptors, thyroid hormone receptors, retinoic acid receptors, retinoic X receptors and

Received 6 January 2006, accepted 26 October 2006

Correspondence: K. Hamre, National Institute of Nutrition and Seafood Research (NIFES), PO Box 2029, Sentrum, N-5817 Bergen, Norway. E-mail: kristin.hamre@nifes.no

Introduction

The production of Atlantic halibut juveniles in 2004 was 850 000, world wide, and 350 000 of these fish were produced in Norway (Harboe & Adoff 2005). The industry still has major problems obtaining high-quality juveniles, primarily because of malpigmentation and impaired eye migration (Harboe & Adoff 2005). A shift from enrichment of *Artemia* with lipid emulsions to feeding heterotrophic algae rich in essential fatty acids has given good results in terms of both fatty acid composition of the live feed and pigmentation (lack of pseudoalbinism) of the juveniles (K. Hamre, unpublished



LETTERS

nature genetics

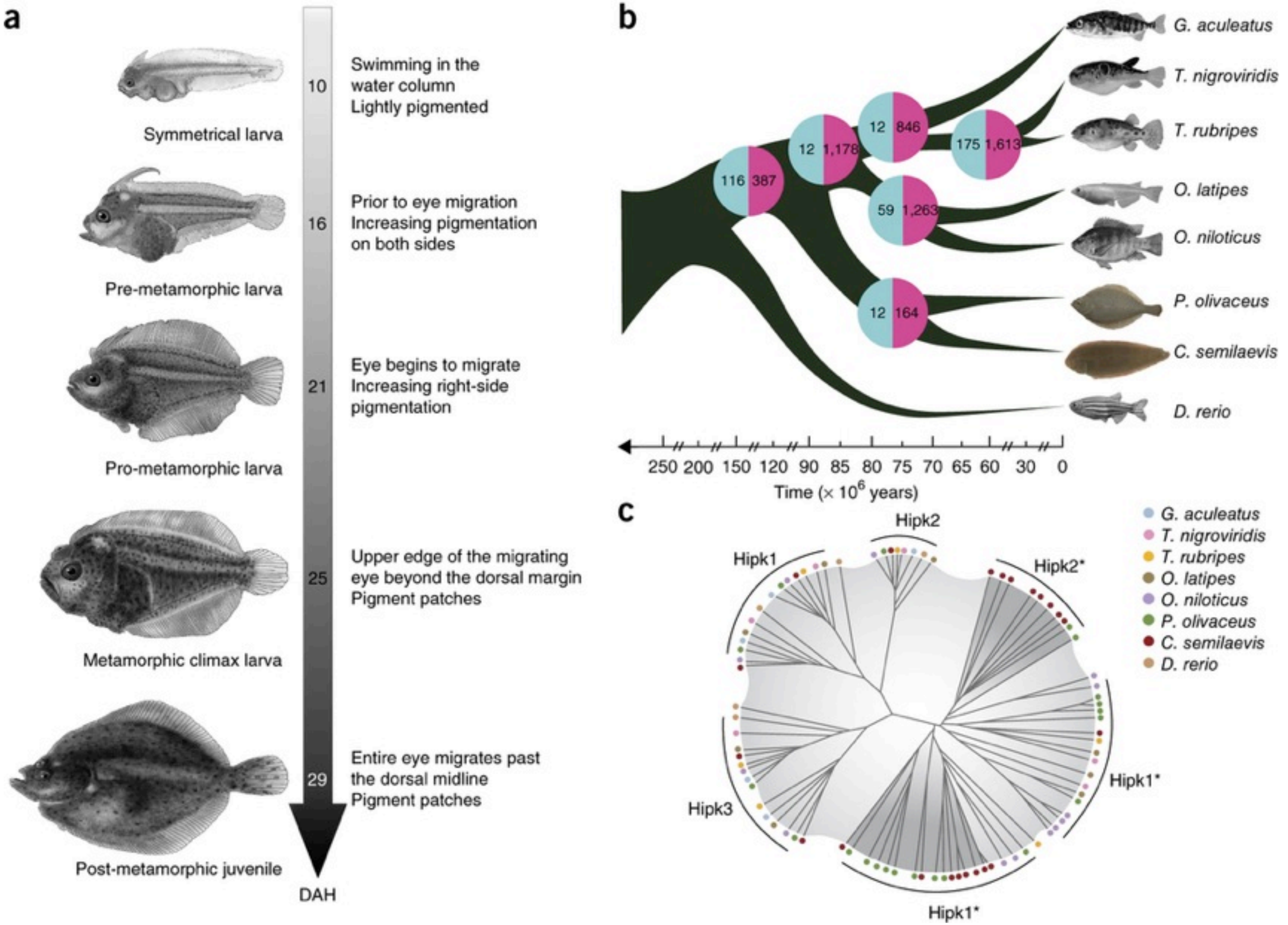
OPEN

The genome and transcriptome of Japanese flounder provide insights into flatfish asymmetry

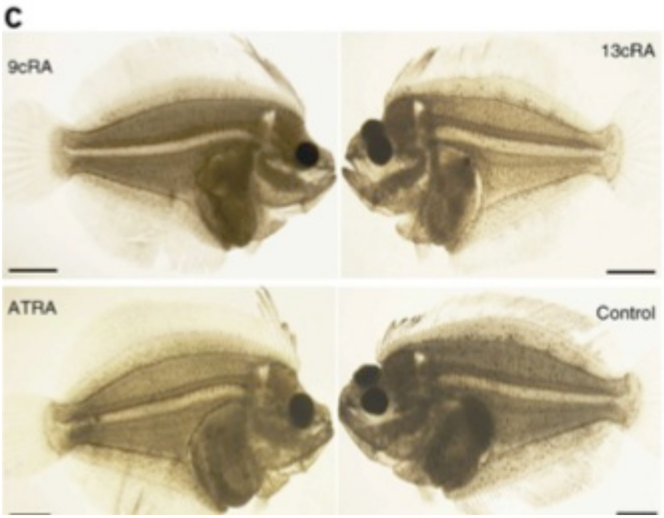
Changwei Shao[1,2,14], Baolong Bao[3,14], Zhiyuan Xie[4,14], Xinye Chen[3,14], Bo Li[4,14], Xiaodong Jia[1,2,14], Qiulin Yao[4], Guillermo Ortí[5], Wenhui Li[4], Xihong Li[1,2], Kristin Hamre[6,7], Juan Xu[3], Lei Wang[1,2], Fangyuan Chen[4], Yongsheng Tian[1,2], Alex M Schreiber[8], Na Wang[1,2], Fen Wei[3], Jilin Zhang[4], Zhongdian Dong[1,2], Lei Gao[3], Junwei Gai[3], Takashi Sakamoto[9], Sudong Mo[1,2], Wenjun Chen[3], Qiong Shi[4], Hui Li[3], Yunji Xiu[1,2], Yangzhen Li[1,2], Wenteng Xu[1,2], Zhiyi Shi[3], Guojie Zhang[4], Deborah M Power[10,11,15], Qingyin Wang[1,2,15], Manfred Schartl[12,13,15] & Songlin Chen[1,2,15]

Flatfish have the most extreme asymmetric body morphology of vertebrates. During metamorphosis, one eye migrates to the contralateral side of the skull, and this migration is accompanied by extensive craniofacial transformations and simultaneous development of lopsided body pigmentation[1–5]. The evolution of this developmental and physiological innovation remains enigmatic. Comparative genomics of two

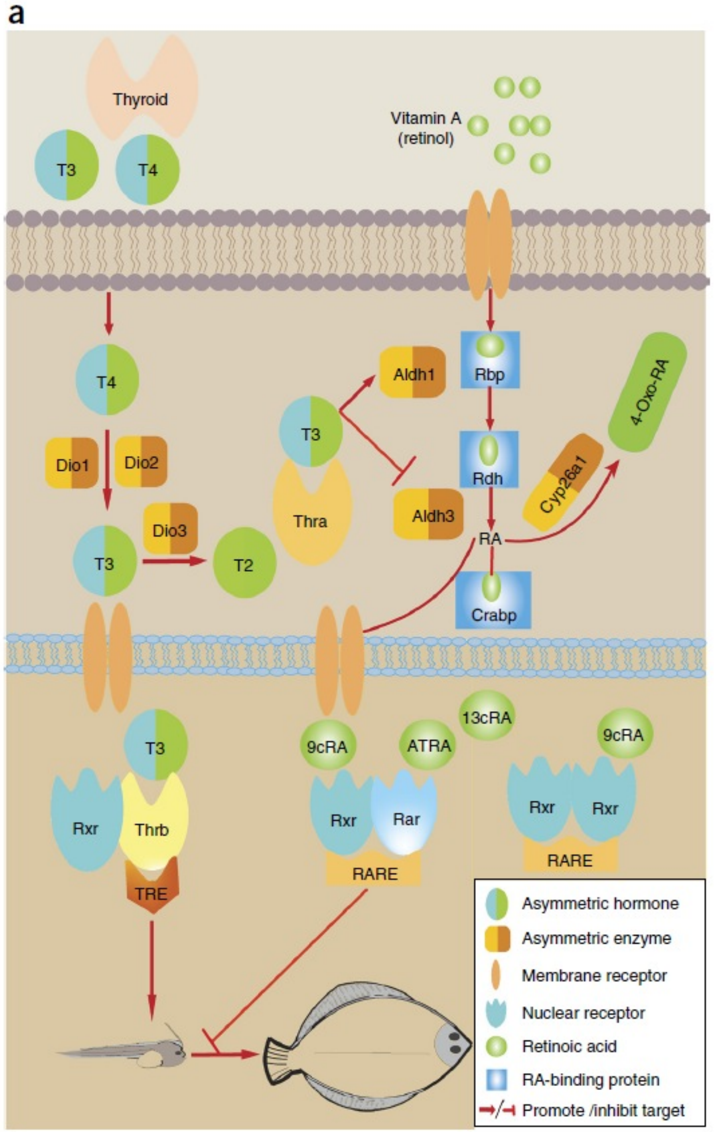
Figs. 1 and **2**, **Supplementary Tables 1** and **2**, and **Supplementary Note**). Almost all of the assembled fragments (98% of scaffolds) were anchored onto 24 chromosomes on the basis of high-resolution genetic maps, accounting in total for 535 Mb of the assembled genome[6,7] (**Supplementary Fig. 3** and **Supplementary Table 3**). Comparison of the Japanese flounder genome to the genome of another, distantly related flatfish, the Chinese tongue sole (*Cynoglossus*


a
Symmetrical larva
Pre-metamorphic larva
Pro-metamorphic larva
Metamorphic climax larva
Post-metamorphic juvenile
10
16
21
25
29
DAH
Swimming in the water column
Lightly pigmented
Prior to eye migration
Increasing pigmentation on both sides
Eye begins to migrate
Increasing right-side pigmentation
Upper edge of the migrating eye beyond the dorsal margin
Pigment patches
Entire eye migrates past the dorsal midline
Pigment patches
b
116 387
12 1,178
12 846
175 1,613
59 1,263
12 164
G. aculeatus
T. nigroviridis
T. rubripes
O. latipes
O. niloticus
P. olivaceus
C. semilaevis
D. rerio
250 200 150 120 90 85 80 75 70 65 60 30 0
Time (× 10^6 years)
c
Hipk2
Hipk1
Hipk2*
Hipk1*
Hipk3
Hipk1*
● G. aculeatus
● T. nigroviridis
● T. rubripes
● O. latipes
● O. niloticus
● P. olivaceus
● C. semilaevis
● D. rerio

Injection of retinoic acid by the blind side eye arrests eye migration and gives pigmentation on both sides



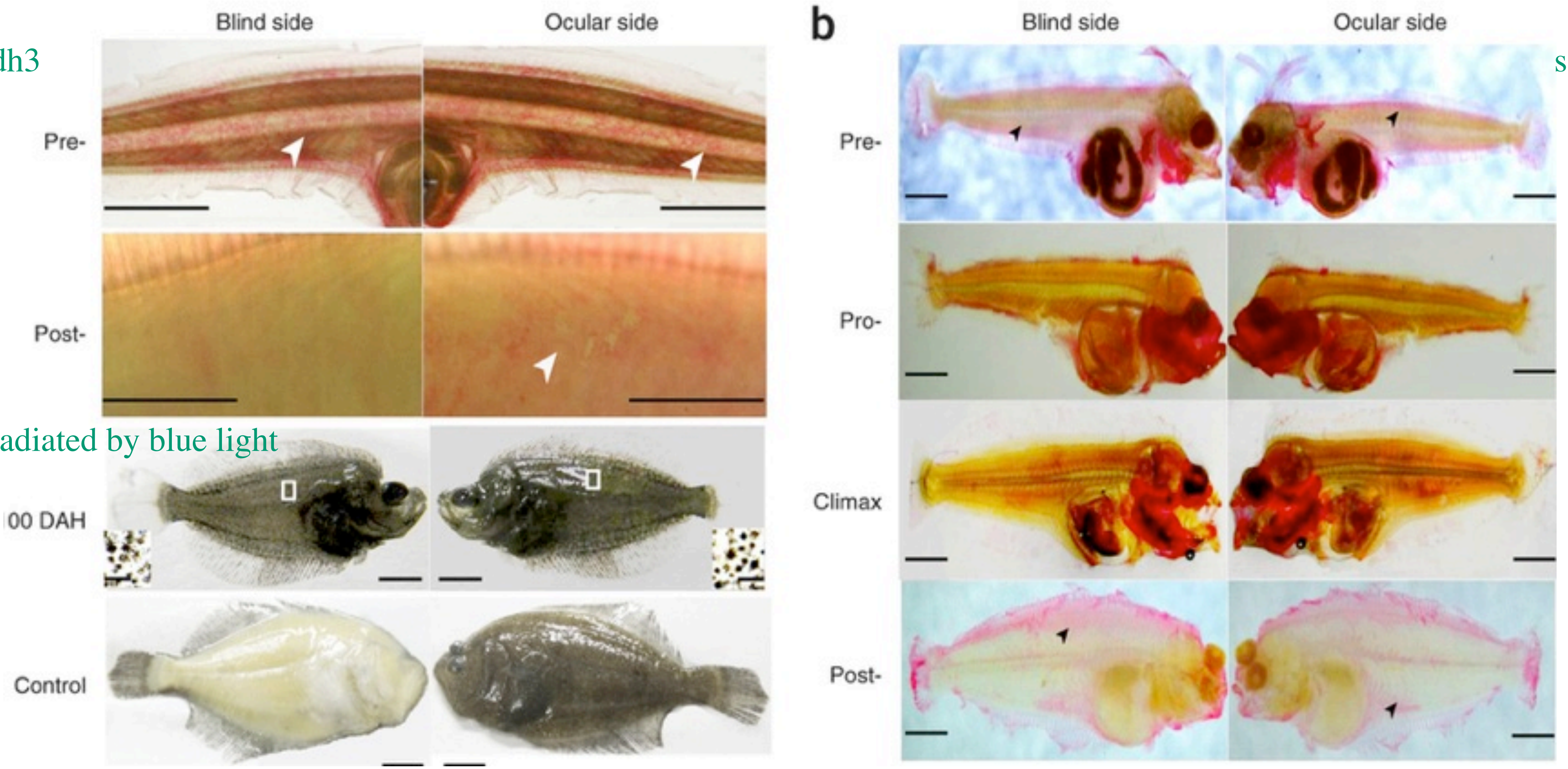
Retinoic acid gradient disrupted



Light sets up the retionic acid gradient

aldh3

sws2



Irradiated by blue light

Vitamin A deficiency in halibut juveniles

Photo: Mari Moren

Many things get their explanation due to this new knowledge

Aquaculture 290 (2009) 250–255

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Control of light condition affects the feeding regime and enables successful eye migration in Atlantic halibut juveniles

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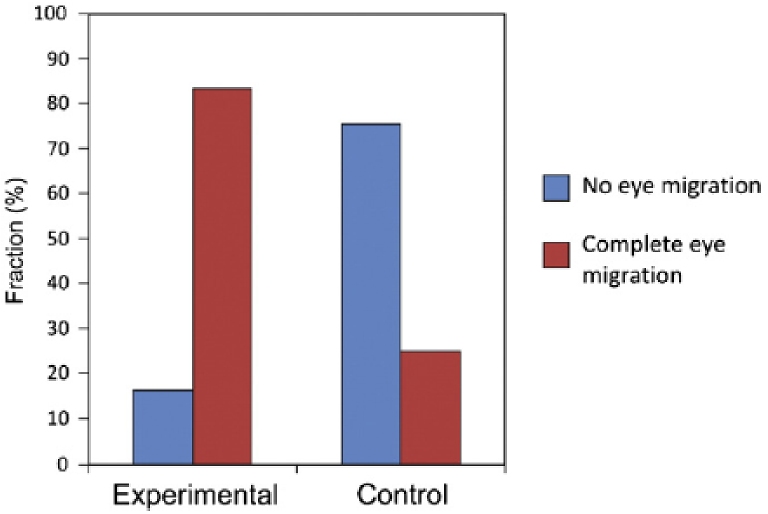
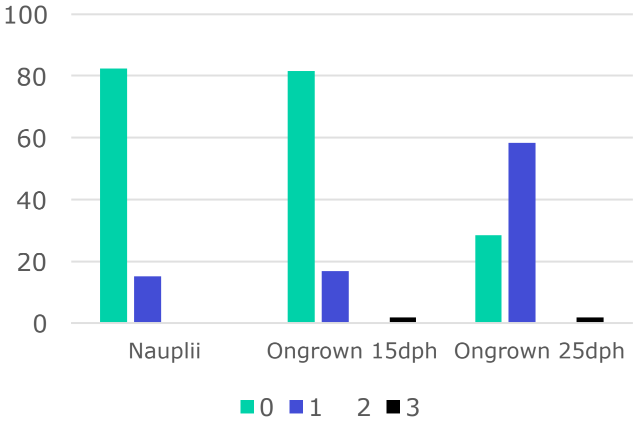
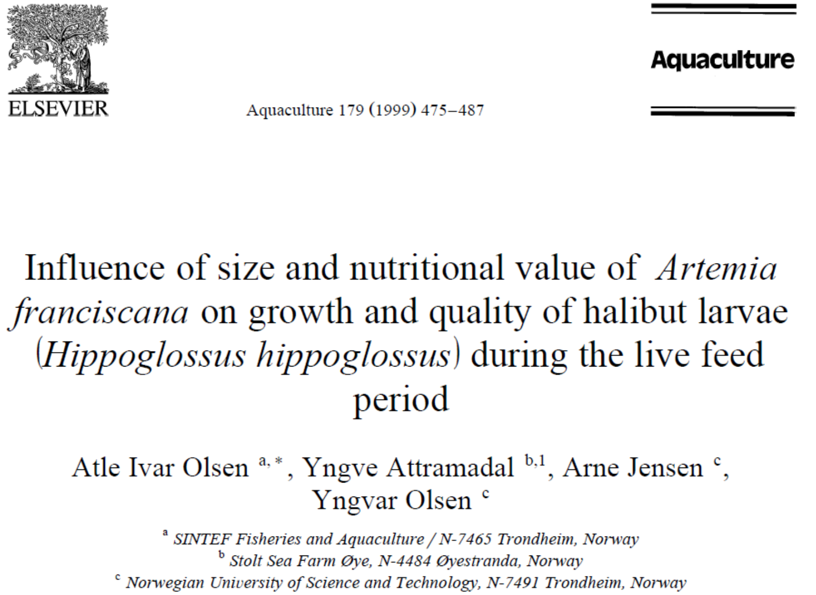


Fig. 1. Larvae with complete eye migration (red colour) and no eye migration (blue colour) for larvae reared with light and dark periods (experimental) and larvae reared under continuous light (control).

Effect of ongrown Artemia on eye migration in Atlantic halibut



Harboe and Hamre unpublished (2005)



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Influence of size and nutritional value of *Artemia franciscana* on growth and quality of halibut larvae (*Hippoglossus hippoglossus*) during the live feed period

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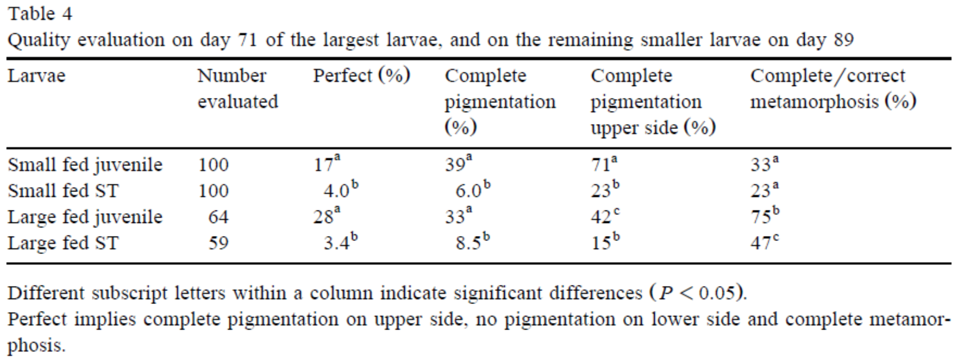

Table 4
Quality evaluation on day 71 of the largest larvae, and on the remaining smaller larvae on day 89

Larvae	Number evaluated	Perfect (%)	Complete pigmentation (%)	Complete pigmentation upper side (%)	Complete / correct metamorphosis (%)
Small fed juvenile	100	17^a	39^a	71^a	33^a
Small fed ST	100	4.0^b	6.0^b	23^b	23^a
Large fed juvenile	64	28^a	33^a	42^c	75^b
Large fed ST	59	3.4^b	8.5^b	15^b	47^c

Different subscript letters within a column indicate significant differences ($P < 0.05$).
Perfect implies complete pigmentation on upper side, no pigmentation on lower side and complete metamorphosis.

Task 11.2 and 11.3: Ongrown Artemia

Nutrient composition in nauplii and ongrown Artemia

On dry wt	Unit	Nauplii enriched	On-grown enriched	*P* Day 1-3
Protein Nx5.30	g 100g^{-1}	46±1	51±2	0.026
Free AA	g kg^{-1}	70±4	92±13	0.044
Taurine	g kg^{-1}	4.4±0.2	5.5±0.6	0.040
Glycogen	g kg^{-1}	25±3	7.1±3.2	0.002
Lipid	g 100g^{-1}	17±1	11±1	0.004
PL	% TL	24±3	34±3	0.013
ARA	% TFA	2.4±0.1	2.1±0.1	0.016
EPA	% TFA	4.1±0.2	6.0±0.7	0.010
DHA	% TFA	5.9±0.6	17±2	0.001
Thiamine	mg kg^{-1}	10.8±0.8	12.5±1.1	0.096
Vitamin C	mg kg^{-1}	1037±336	1401±166	0.168
Vitamin D3	mg kg^{-1}	0.12±0.01	0.24±0.01	0.000
Vitamin E	mg kg^{-1}	580±27	890±224	0.076
MK4	µg kg^{-1}	1040±137	102±37	0.000
Phylloquinone	µg kg^{-1}	13±1	281±131	0.024
MK6	µg kg^{-1}	nd	15±7	0.024
MK7	µg kg^{-1}	6.7±0.7	75±37	0.033
MK8	µg kg^{-1}	nd	242±111	0.020
MK9	µg kg^{-1}	nd	22±11	0.026
MK10	µg kg^{-1}	nd	41±22	0.031
Total vitamin K	µg kg^{-1}	1073±124	778±340	0.231
Iodine	mg kg^{-1}	5.2±0.5	8.2±0.5	0.002
Ca	g kg^{-1}	3.4±0.5	3.1±0.5	0.460
K	g kg^{-1}	15±1	14±0.1	0.152
Mg	g kg^{-1}	8.2±0.8	7.1±0.9	0.165
P	g kg^{-1}	11.1±0.9	10.9±0.4	0.420

Co-funded by the Seventh
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The control larvae fed nauplii had good eye migration and pigmentation

- No effect of feeding ongrown Artemia

Torstein’s tanks for halibut larvae

Thank you for your attention

Co-funded by the Seventh
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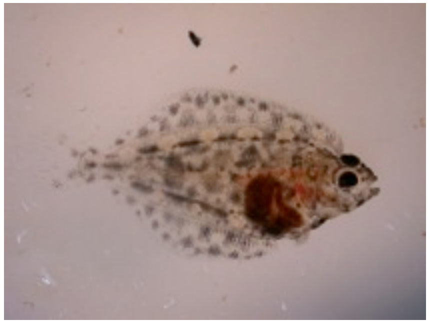
Malpigmented

No eye migration



Normal

Photos: Øystein Sæle