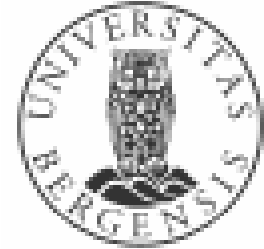




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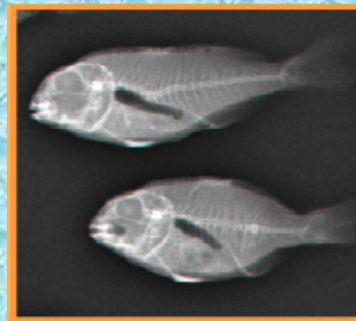
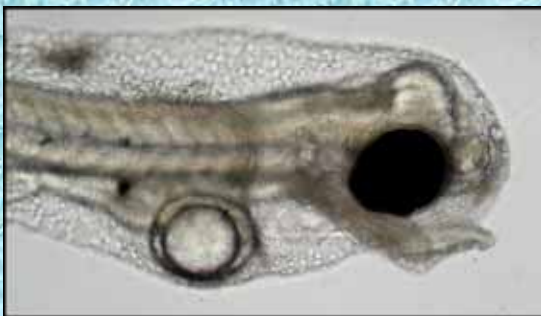
# The effect of larval rearing on juvenile quality in finfish

**W. Koven<sup>1</sup>, G. Koumoundouros<sup>2</sup>, K. Pittman<sup>3</sup>, B. Ginzbourg<sup>1</sup>, E. Sandel<sup>1</sup>, S. Lutzky<sup>1</sup>, O. Nixon<sup>1</sup>, and A. Tandler<sup>1</sup>**

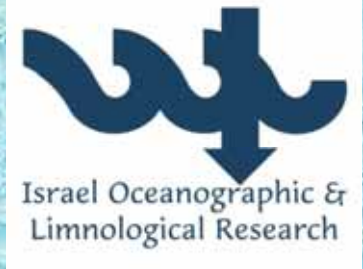
*<sup>1</sup>The National Center for Mariculture, IOLR, P.O.B. 1212, Eilat 88112, Israel*

*<sup>2</sup>Biology Dep., University of Patras, 26110 Patras, Rio, Greece*

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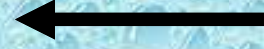
Larval Rearing



Major source of mortality

Metamorphosis

Level of Production





## Larval Rearing

Abiotic factors - temperature, salinity,  
current speed

Diet components – vitamin A, iodine, EFA, lipid class

## Metamorphosis

Juvenile quality

= loss to  
industry

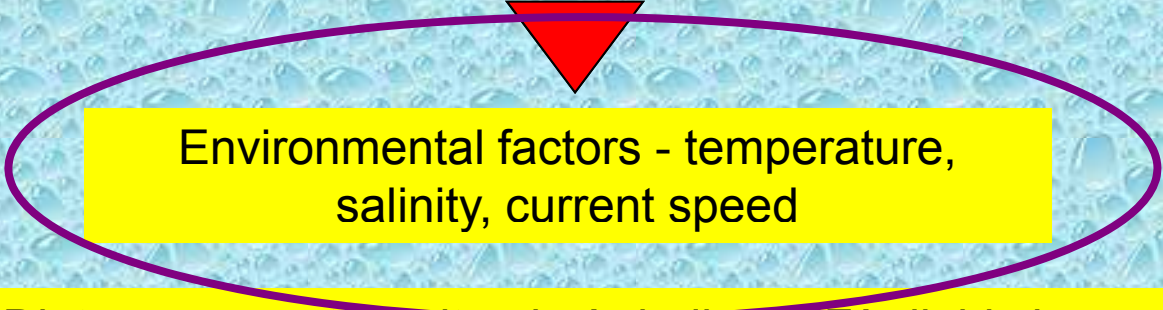
% deformity

Metamorphic success

Sex ratio

Level and quality of Production

# Larval Rearing



# Metamorphosis

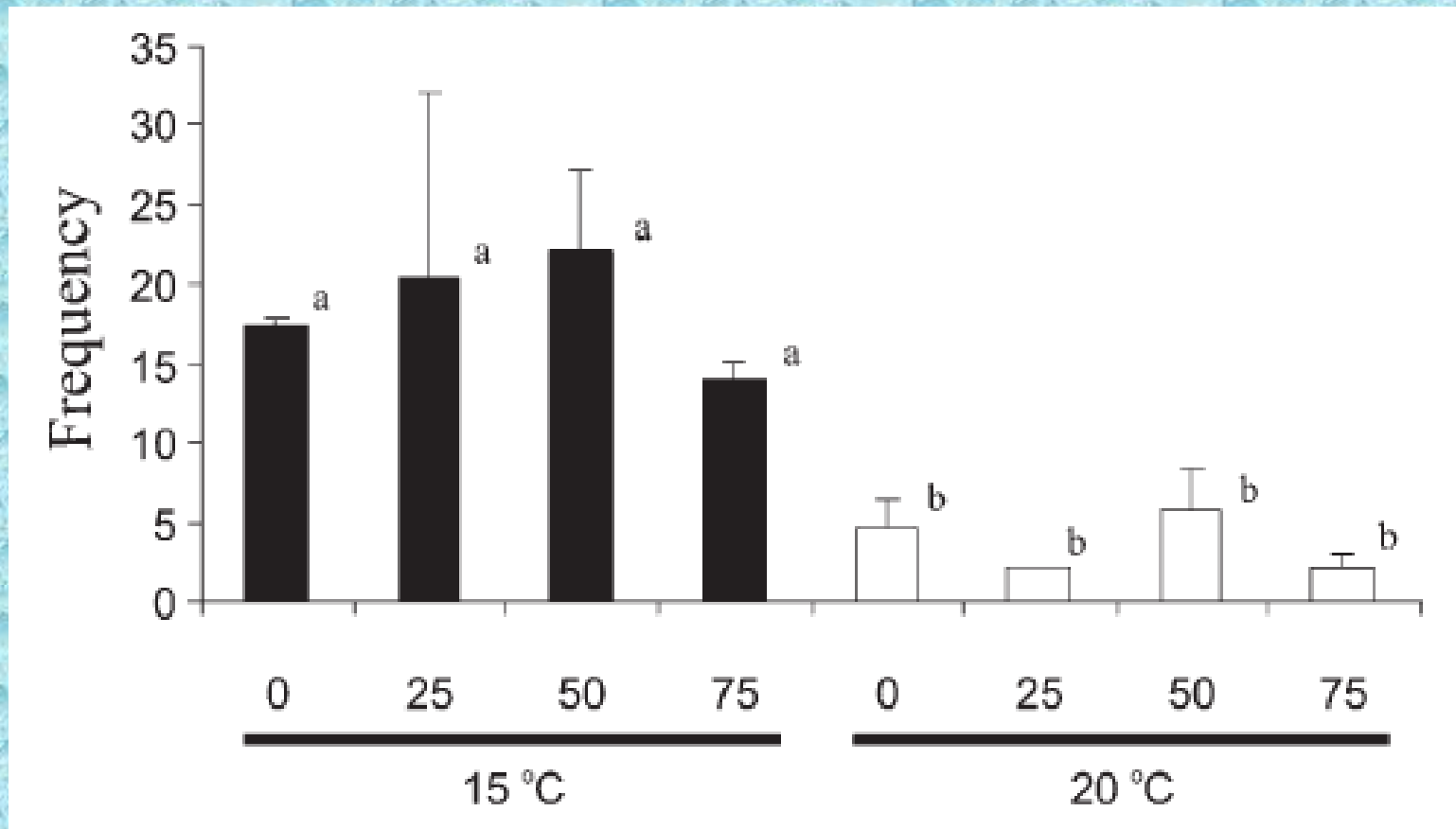
# Juvenile quality

= loss to industry



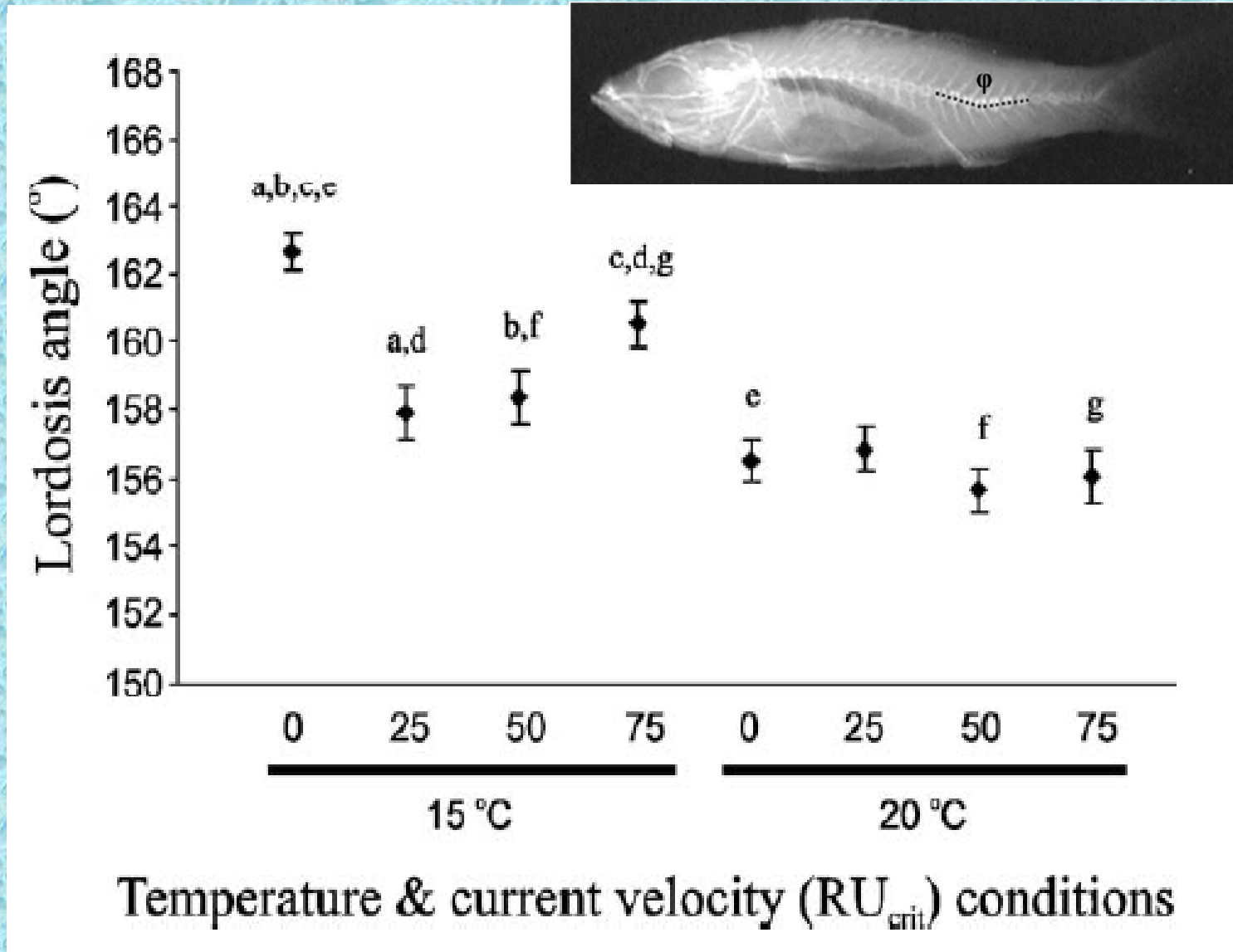
# Level and quality of Production

## Effect of developmental temperature at larval rearing on the sensitivity of *D. labrax* to the lordosis inducing factor of current speed at the juvenile stage

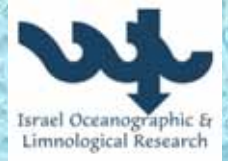




# Effect of temperature and current velocity on Lordosis severity

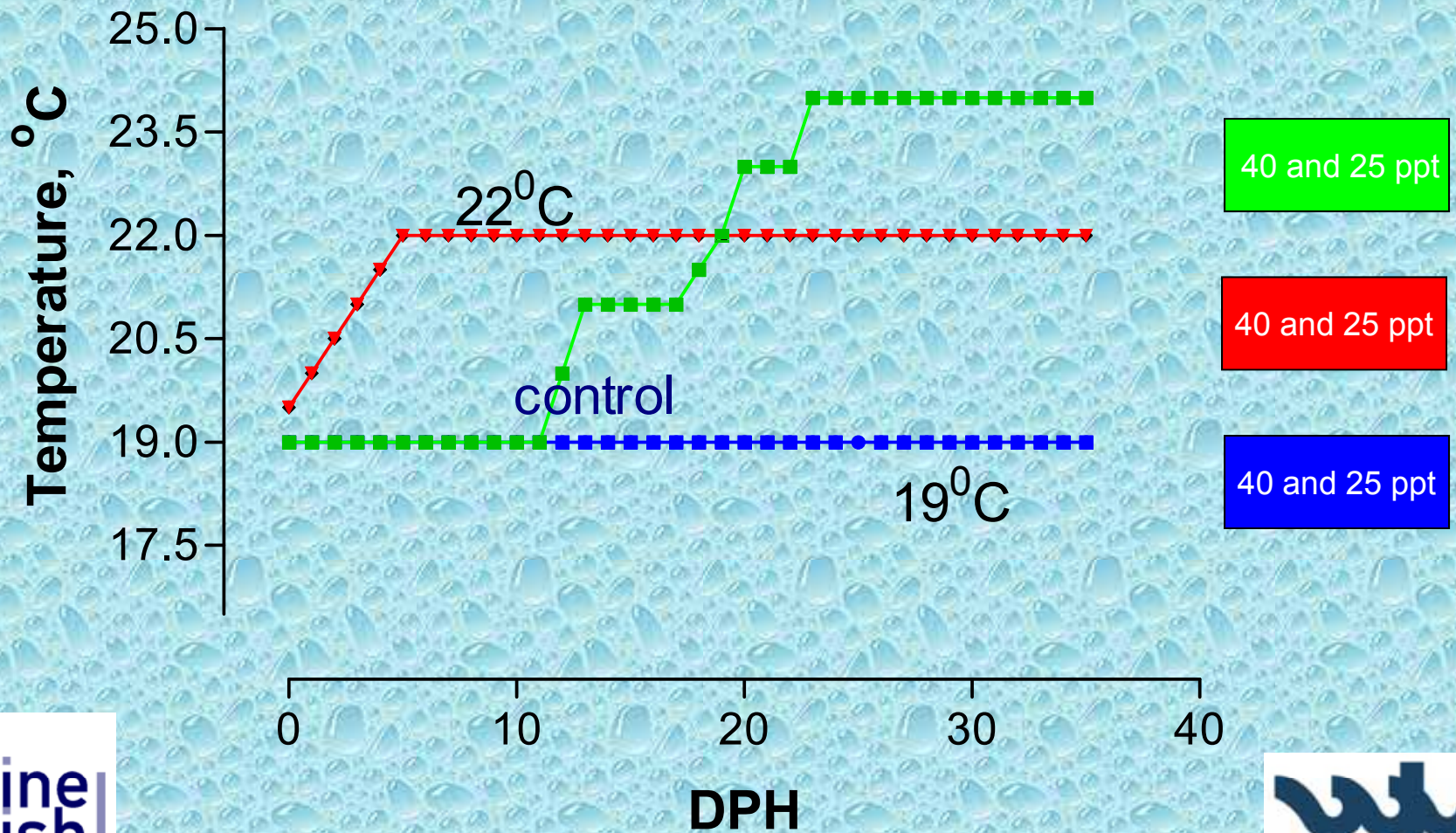


# Conclusions

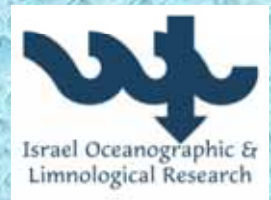


- Larval developmental temperature
  1. Due to ontogenetic plasticity of muscles and bones, temperature leads to abnormal development and skeletal deformity
  
- Juvenile swimming
  1. intensifies the severity of haemal lordosis.
  2. Kranenbarg et al. (2005) concluded that lordotic vertebrae are not deformed, but are just adapted to withstand the increased loads on the tail during swimming.

# Effect of salinity and temperature during larval rearing on the incidence of deformities in juvenile gilthead sea bream (*Sparus aurata*)

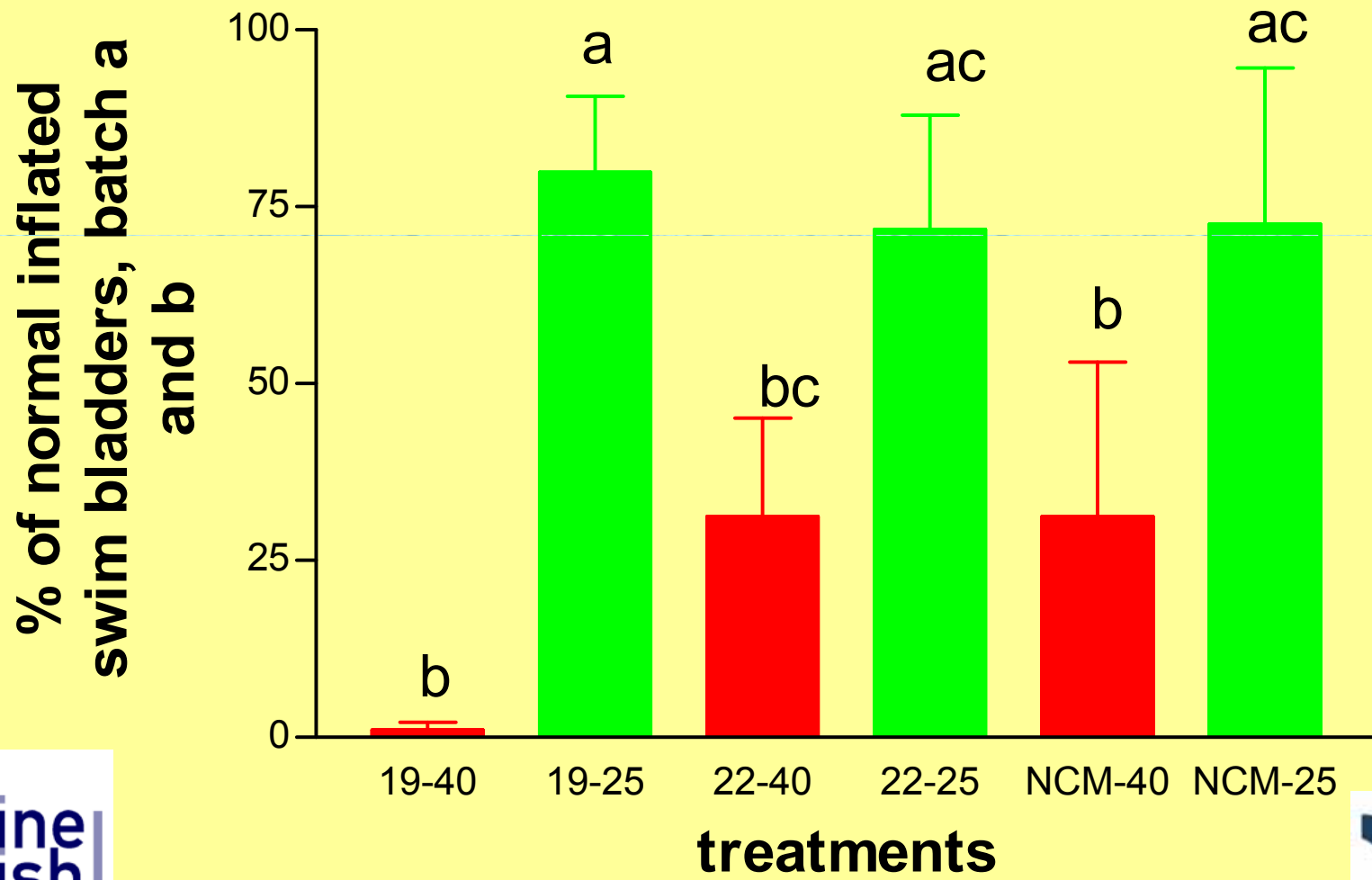


Koven et al. (in preparation)





# The effect of treatments on swim bladder inflation



# Percent of deformity type found in fish lacking swim bladders

deformity	19-40	19-25	22-40	22-25	NCM-40	NCM-25
Vertebral	70.2	75	75.2	0	96.3	43.3
Pughead.	27	0	27.6	5.1	23.3	0

# Conclusions

- High salinity (40‰) during larval rearing reduced % SB inflation, survival and increased skeletal deformities.
- Temperatures tested did not influence incidence of deformities.
- Skeletal deformities low in all treatments (1.7%±1.4).
- Incidence of deformities affecting juvenile quality may be genetically based. Possibly tied to brood stock selection from warmer seawater and the progeny exhibiting lower levels of abnormality.



# Larval Rearing

Environmental factors - temperature, salinity, current speed

Diet components – vitamin A, iodine, EFA, lipid class

## Metamorphosis

## Juvenile quality

= loss to industry

% deformity

Metamorphic success

Sex ratio

Level and quality of Production

# Effect of vitamin A level during larval rearing on juvenile deformities of gilthead sea bream

0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36

yolk sac

rotifers

Artemia

	4 – 19 DAH	20 – 34 DAH
<b>Exp 1</b>	Rotifers enriched with vitamin A	Artemia not enriched in vitamin A
<b>Exp 2</b>	Rotifers not enriched with vitamin A	Artemia enriched with vitamin A
<b>Exp 3</b>	Rotifers enriched with vitamin A	Artemia enriched with vitamin A

Larvae that were fed the vitamin A treatments were further grown until 120 DAH to determine the appearance of deformities in resultant fry

Ginsbourg et al. (in preparation)

- Increasing dose of dietary vitamin A significantly affected growth and the appearance of deformities in the seabream fry.
- A correlation was found between developmental stage and the effect of vitamin A dose on deformity type.
- ✓ First developmental period (4-20 DAH)-dose response effect between rotifer vitamin A level and cranium deformities.
- ✓ Second developmental period (20-34 DAH)-dose response effect between Artemia vitamin A level and skeletal deformities.

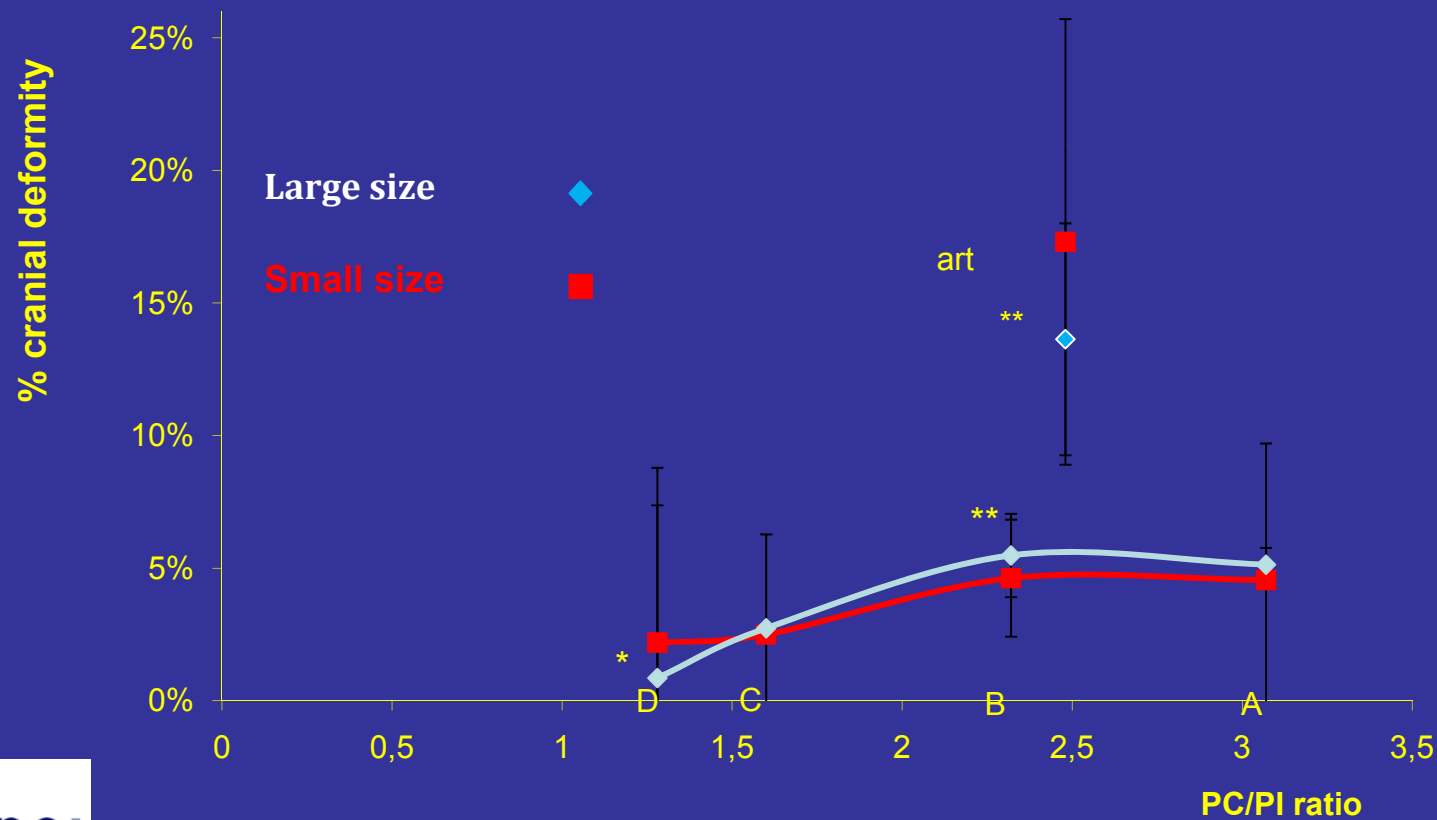


# Effect of PC:PI ratio fed during larval rearing on juvenile deformities in gilthead sea bream

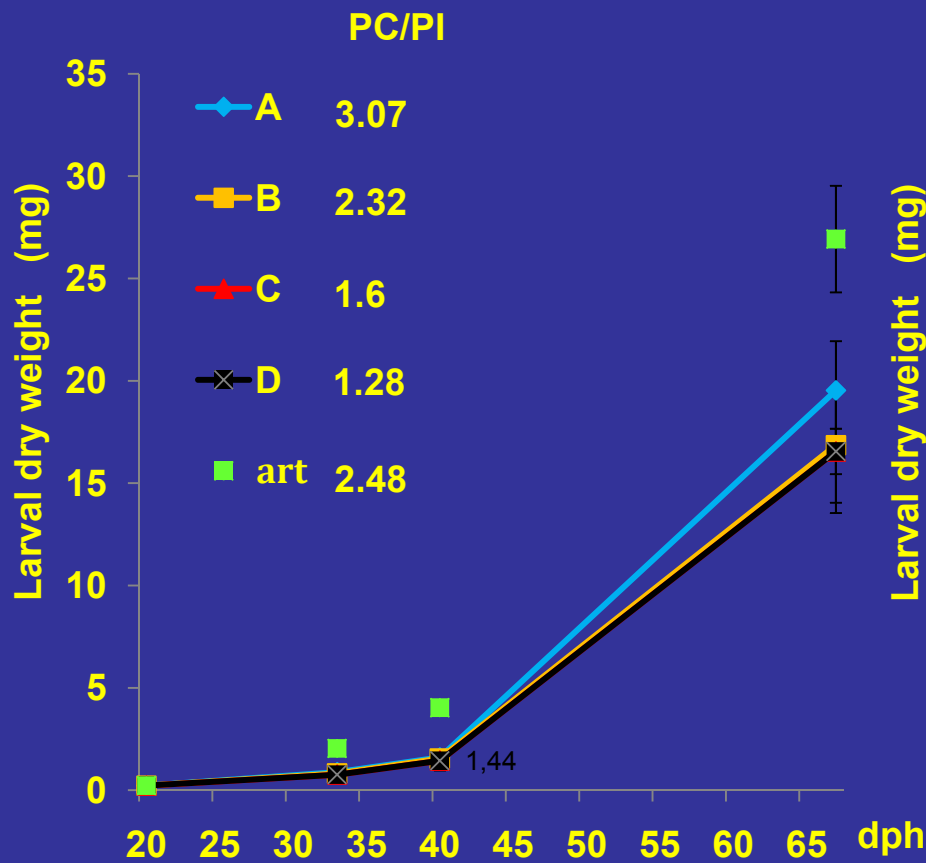
- 3-16 dph –rotifers (*Brachionus rotundiformis*).
- 16-22 dph –Artemia naupli.
- 22-35 dph – Feeding with 4 different PC/PI dietary ratios.
- 40 dph-divided into fast and slow growers in each of the treatments.
- 41-141 dph – treatments kept separate but reared on the same pelleted diet.

	A	B	C	D	art
	25% artemia +75% MD	25% artemia +75% MD	25% artemia +75% MD	25% artemia +75% MD	100% artemia
Phospholipids (g/100 g dry diet)					
Phosphatidylcholine	5.7	4.54	4.42	3.88	4.42
Phosphatidylinositol	1.86	1.95	2.76	3.04	1.78
PC/PI in total diet	3.07	2.32	1.6	1.28	2.48

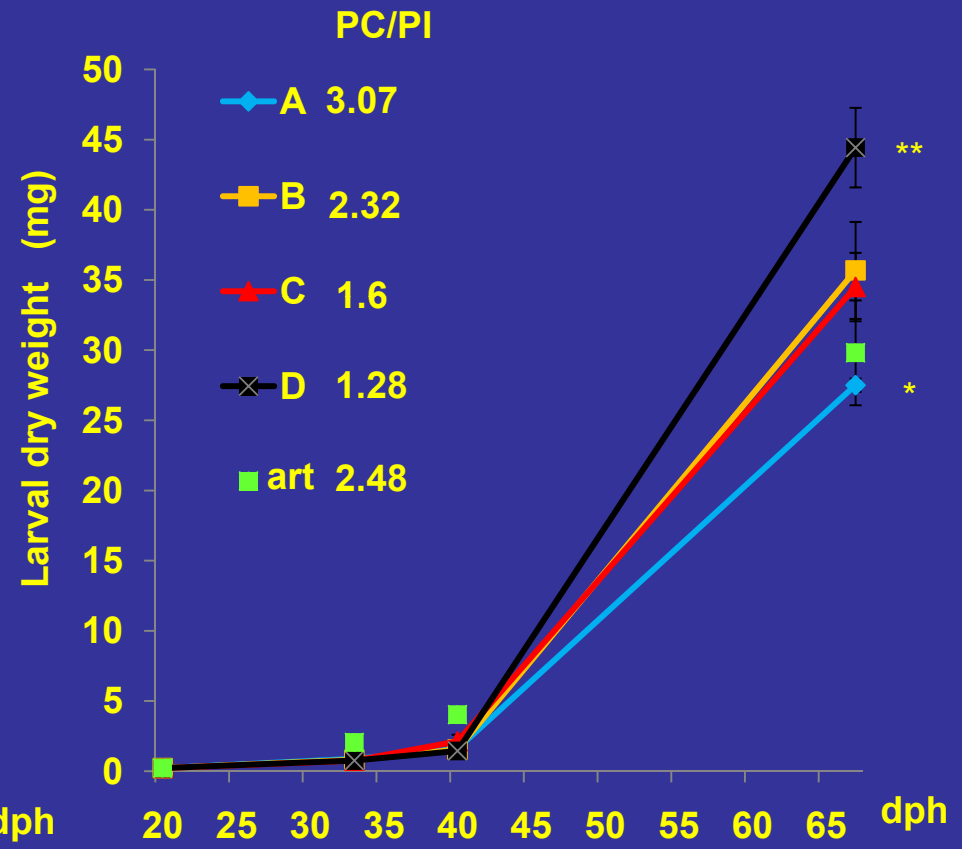
# Effect of PC/PI ratio during larval development on cranial deformities in 67 dph juveniles



# Effect of PC/PI ratio during larval development on dry wt in 67 dph juveniles



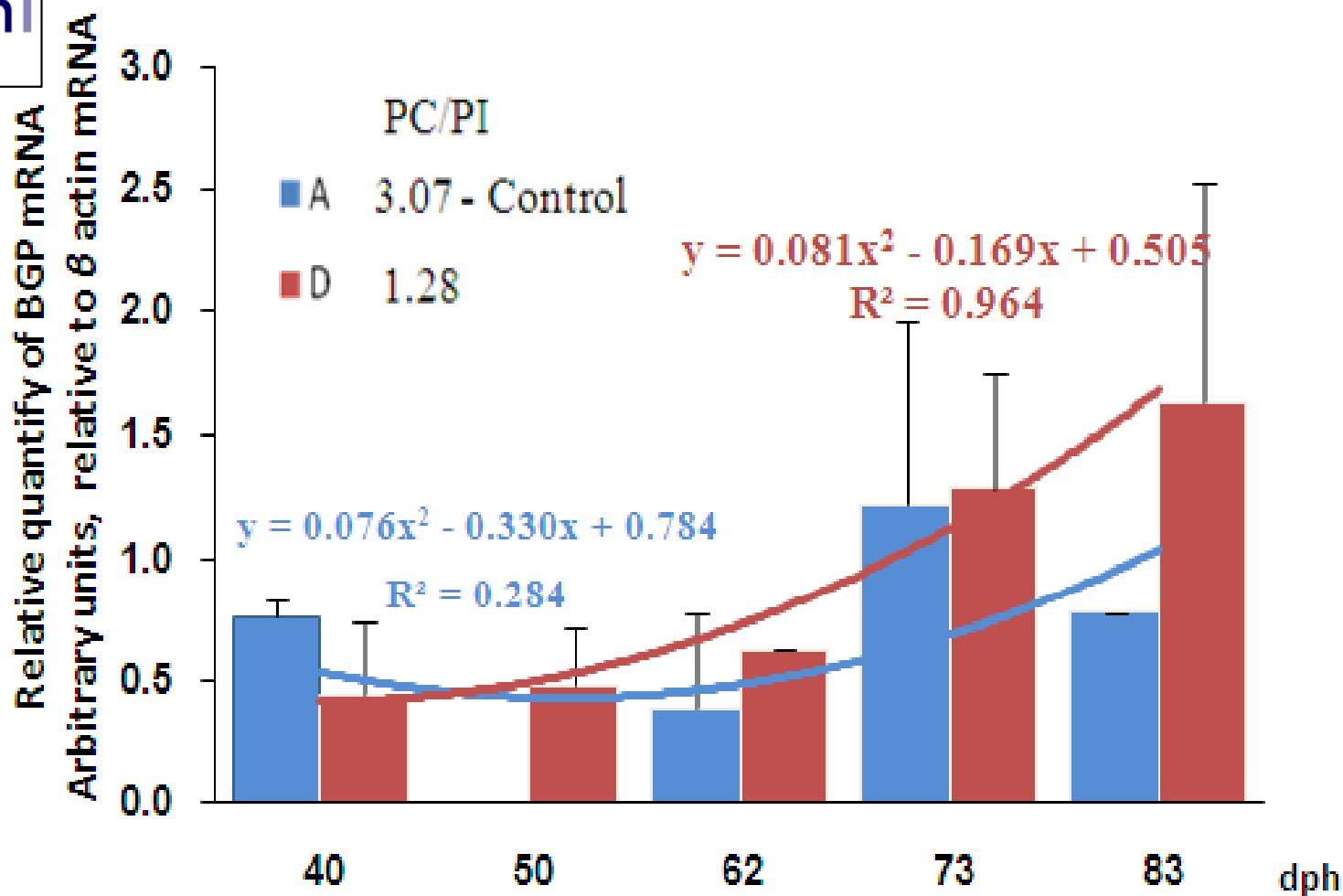
Small size (<1.3 mg dw larva<sup>-1</sup>)



Large size (>2.9 mg dw larva<sup>-1</sup>)



# Effect of high (3.07) and low (1.28) dietary PC/PI ratio fed during larval rearing on level of *spBGP* mRNA at different ages of juvenile gilthead sea bream



## Conclusions

- Decreasing dietary PC/PI ratio contributed to significantly better growth at larval and fry stages.
- Dietary effect was stronger in faster growing larvae.
- Decreasing dietary PC/PI ratio at the larval stage significantly reduced % cranial deformities and apparently affected feeding success on starter feed.

Larval Rearing

Environmental factors - temperature,  
salinity, current speed

Diet components – vitamin A, iodine, lipid class

Metamorphosis

Juvenile quality

= loss to  
industry

% deformity

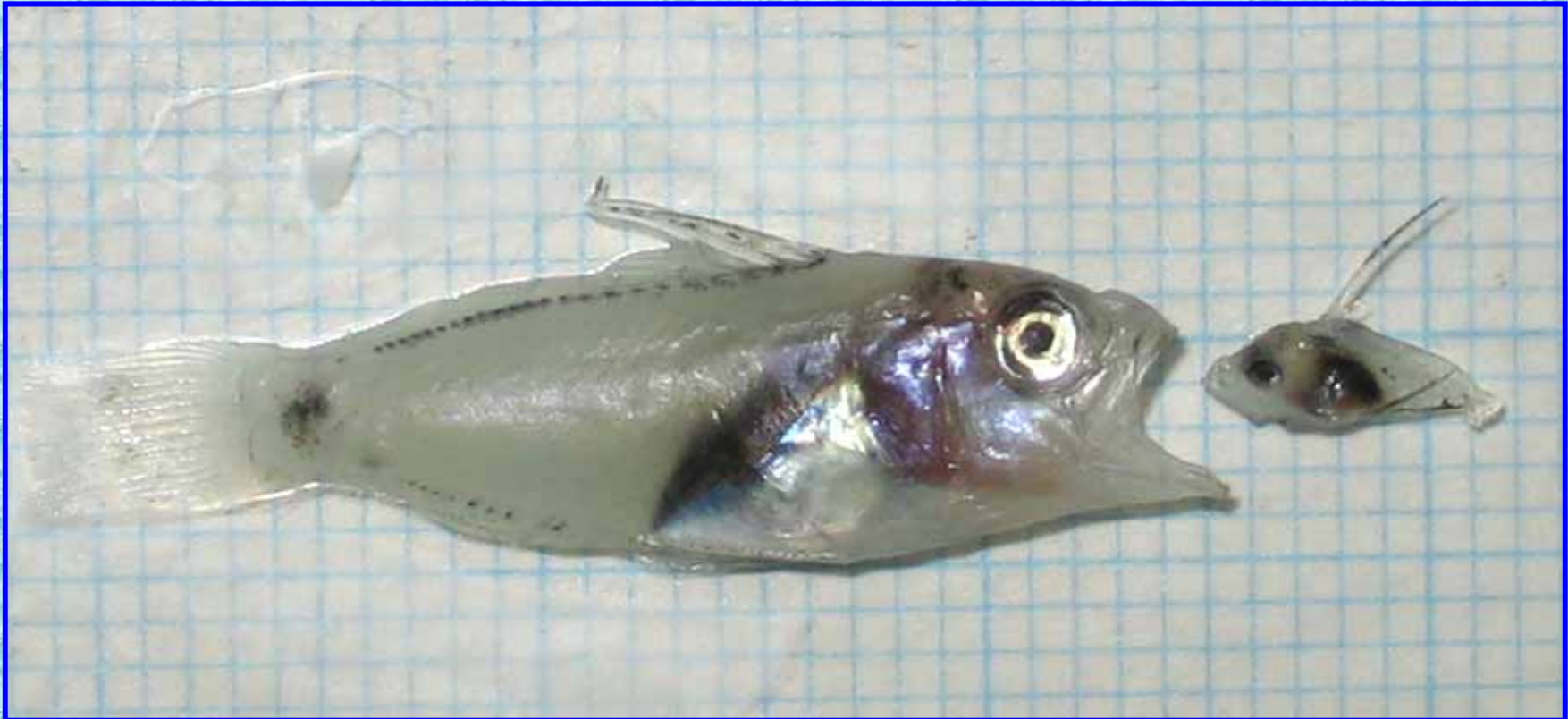
Metamorphic success

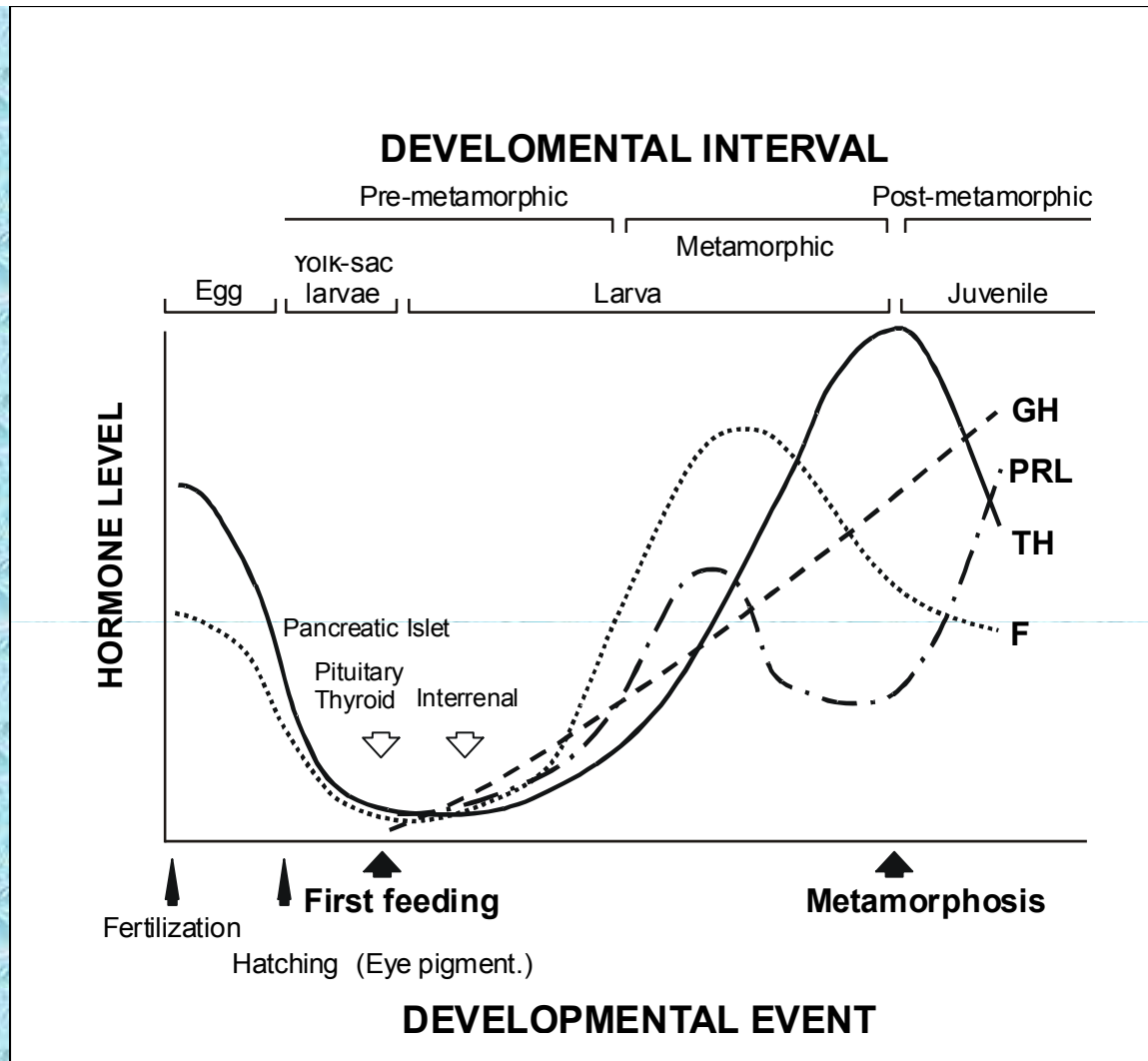
Sex ratio

Level and quality of Production



# Asynchronous and protracted metamorphosis



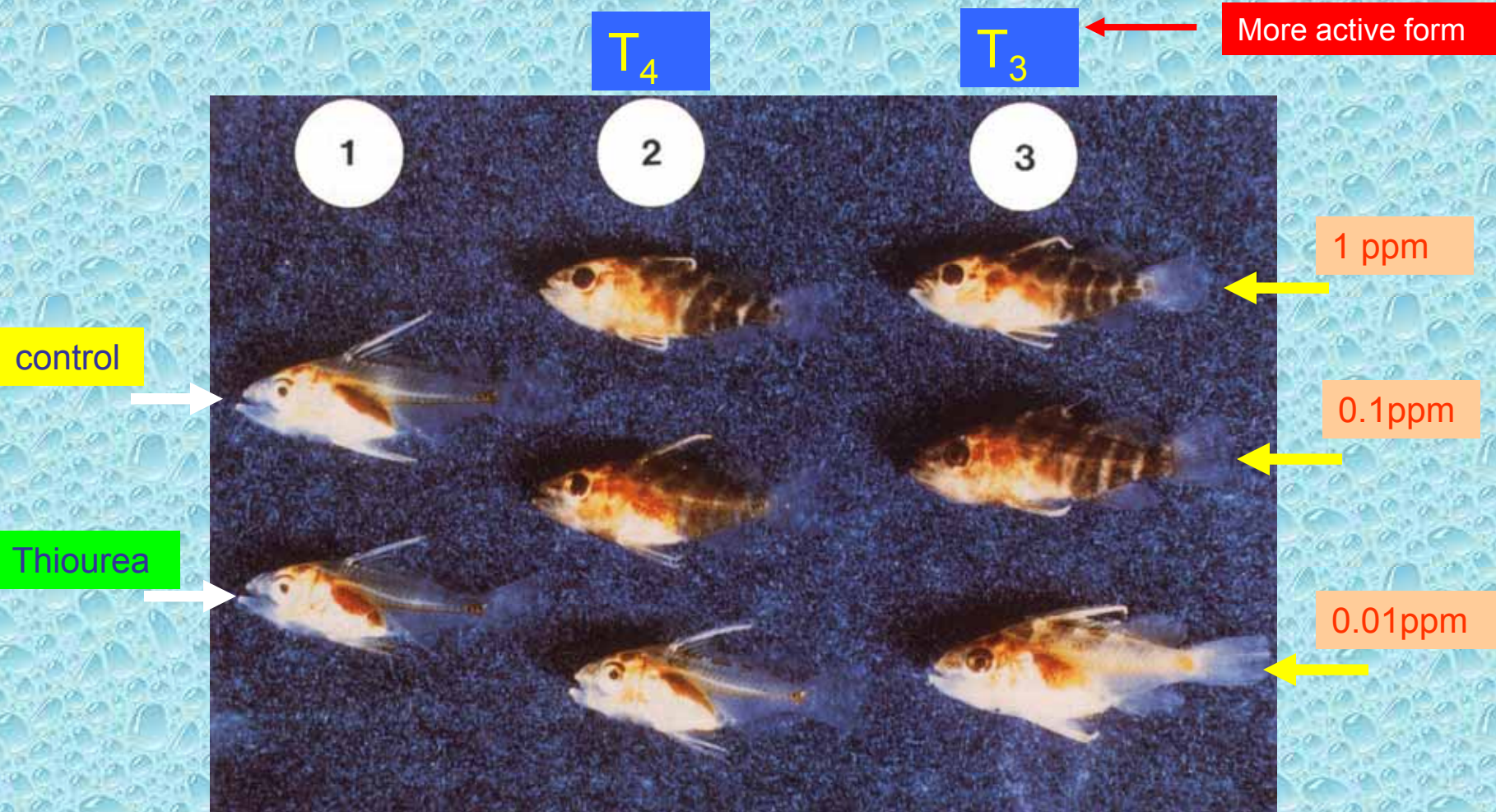


• **Cortisol and thyroid hormone levels synergistic effect on metamorphosis**

From Tanaka et al. 1995



# Effect of various doses of T4 or T3 on metamorphosis in 4 week old grouper



From De Jesus et al. 1998



# Unsuccessful metamorphosis in flat fish



Atlantic halibut

Abnormal pigmentation

Albinism



turbot

normal

abnormal pigmentation

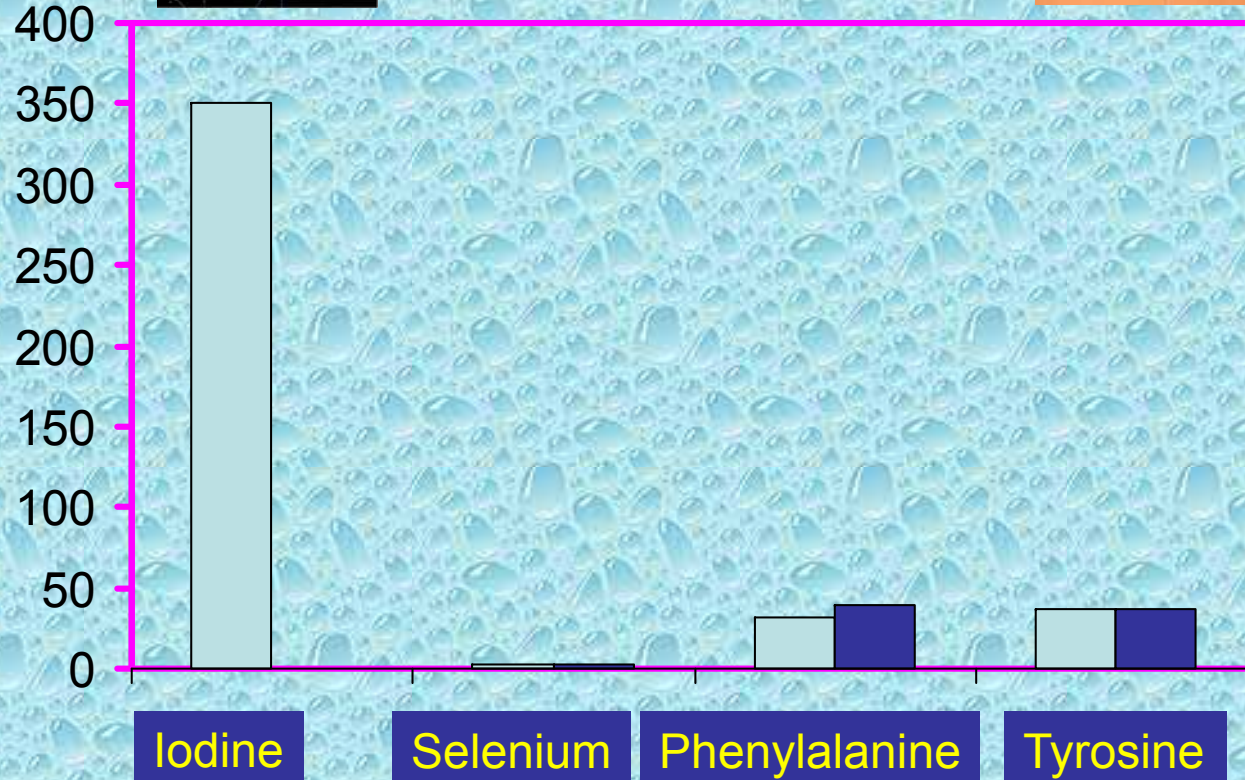
- Abnormal pigmentation unsuitable for market
- Growth generally independent of pigmentation

From Pittman, Solbakken and Hamre, 2001

# Total trace element and specific free amino acid Concentrations in prey fed to halibut



□ zooplankton ■ Artemia



From Solbakken et al. 2002



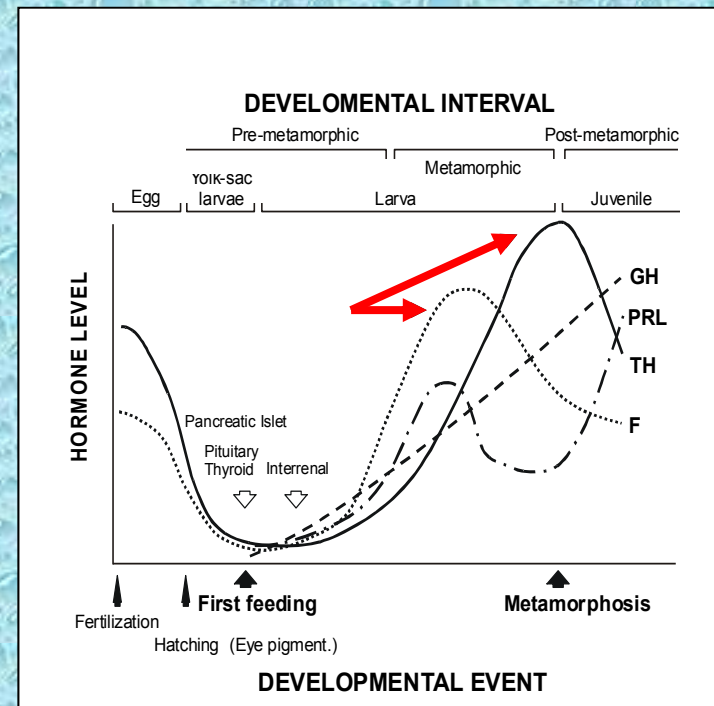
## Dietary factors affecting metamorphosis in flatfish include iodine as a precursor for thyroid

- Although copepod fed halibut shows better pigmentation and eye migration compared to Artemia fed halibut, iodine enriched Artemia did not markedly improve pigmentation or eye migration (Hamre et al. 2005)

- Possibly need iodine supplementation at first feeding

- Higher levels of cortisol which can be elevated with increased levels of arachidonic acid (20:4n-6)

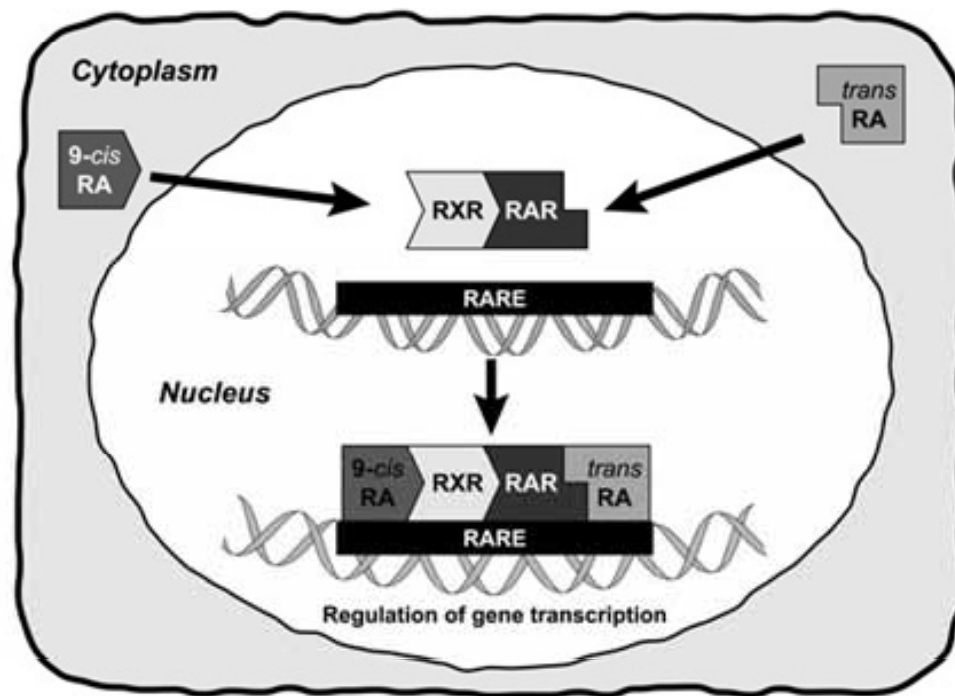
- Eicosanoids are known to modulate the response of thyroid tissue to thyroid stimulating hormone (TSH)





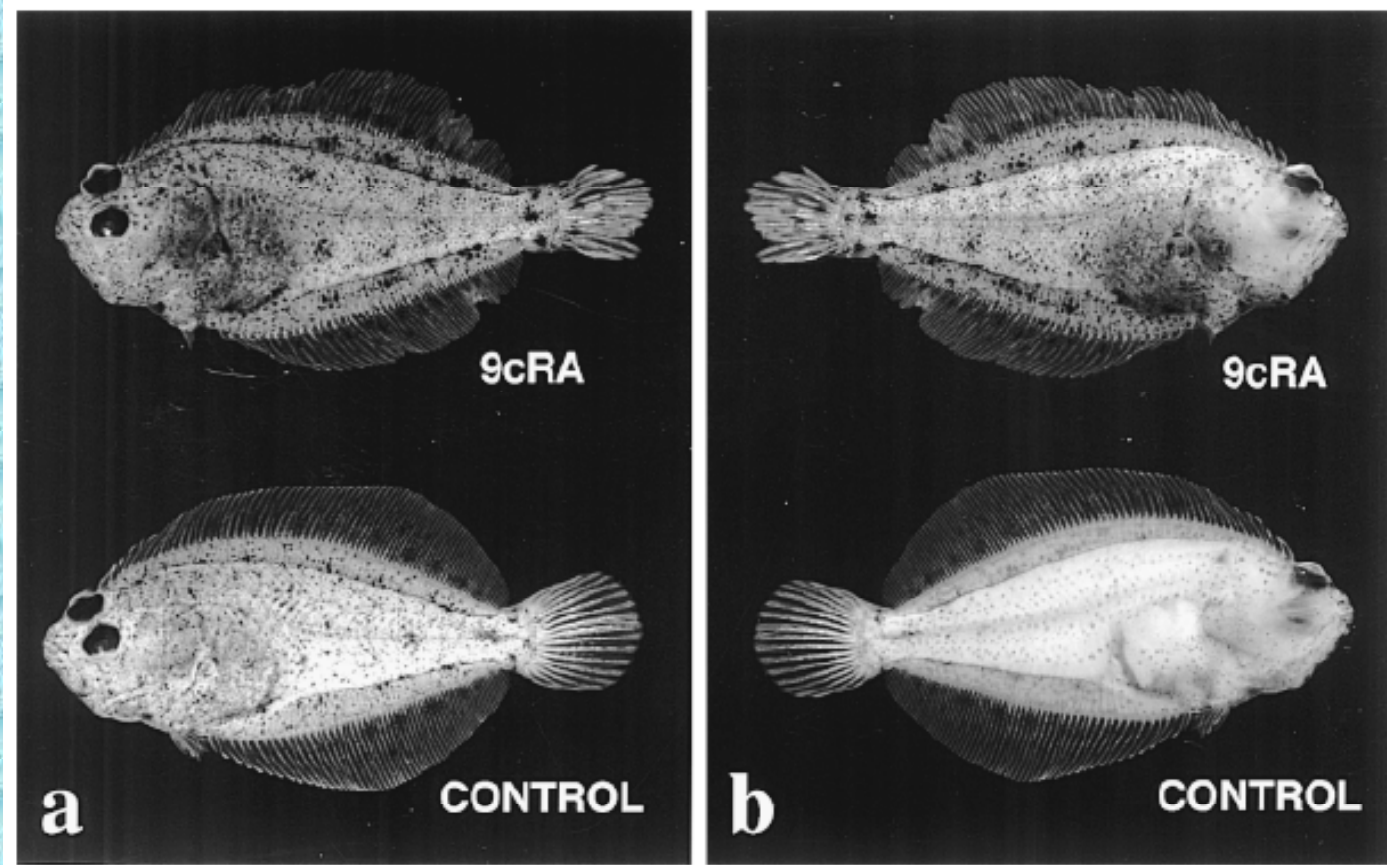
## Dietary factors affecting metamorphosis in flatfish include vitamin A (carotenoids in Artemia and copepods)

- Vitamin A-retinoic acid-important in embryonic development-modulates gene transcription → differentiation and proliferation.



- High levels produce deformity.

External appearances of metamorphosed juvenile flounder treated either with 25 nM of 9-cis retinoic acid (9cRA) or with Control.

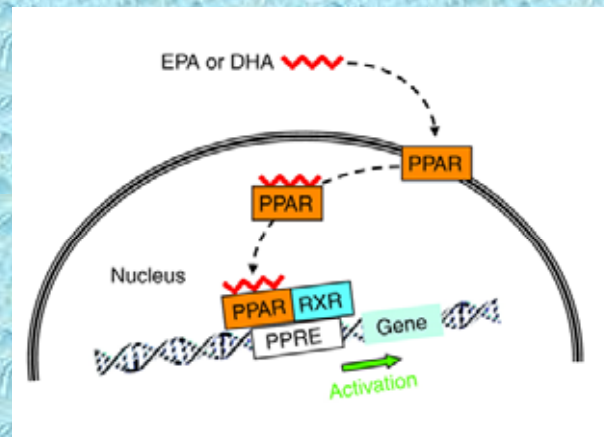


- Likely sufficient carotenoid levels in Artemia and copepods to meet vitamin requirement.



## Arachidonic acid affecting pigmentation during metamorphosis

- High levels of ArA during a pre-metamorphic pigmentation window results in malpigmentation in turbot, halibut, flounder and sole.
- ArA-derived prostanoids (in mammals) modify the production of tyrosinase, a key enzyme involved in the L-tyrosine to melanin pathway.
- Hamre et al. (2007)- high tissue ArA incorporation during pre-metamorphosis lowers concentrations of other LCPUFAs which ligand with PPAR and then dimerize with retinoic acid bound RXR.





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- Lower levels of these dimers would reduce the expression of key genes and consequently interfere with normal pigmentation.

# Larval Rearing

Environmental factors - temperature, salinity, current speed

Diet components – vitamin A, iodine, lipid class

## Metamorphosis

## Juvenile quality

= loss to industry

% deformity

Metamorphic success

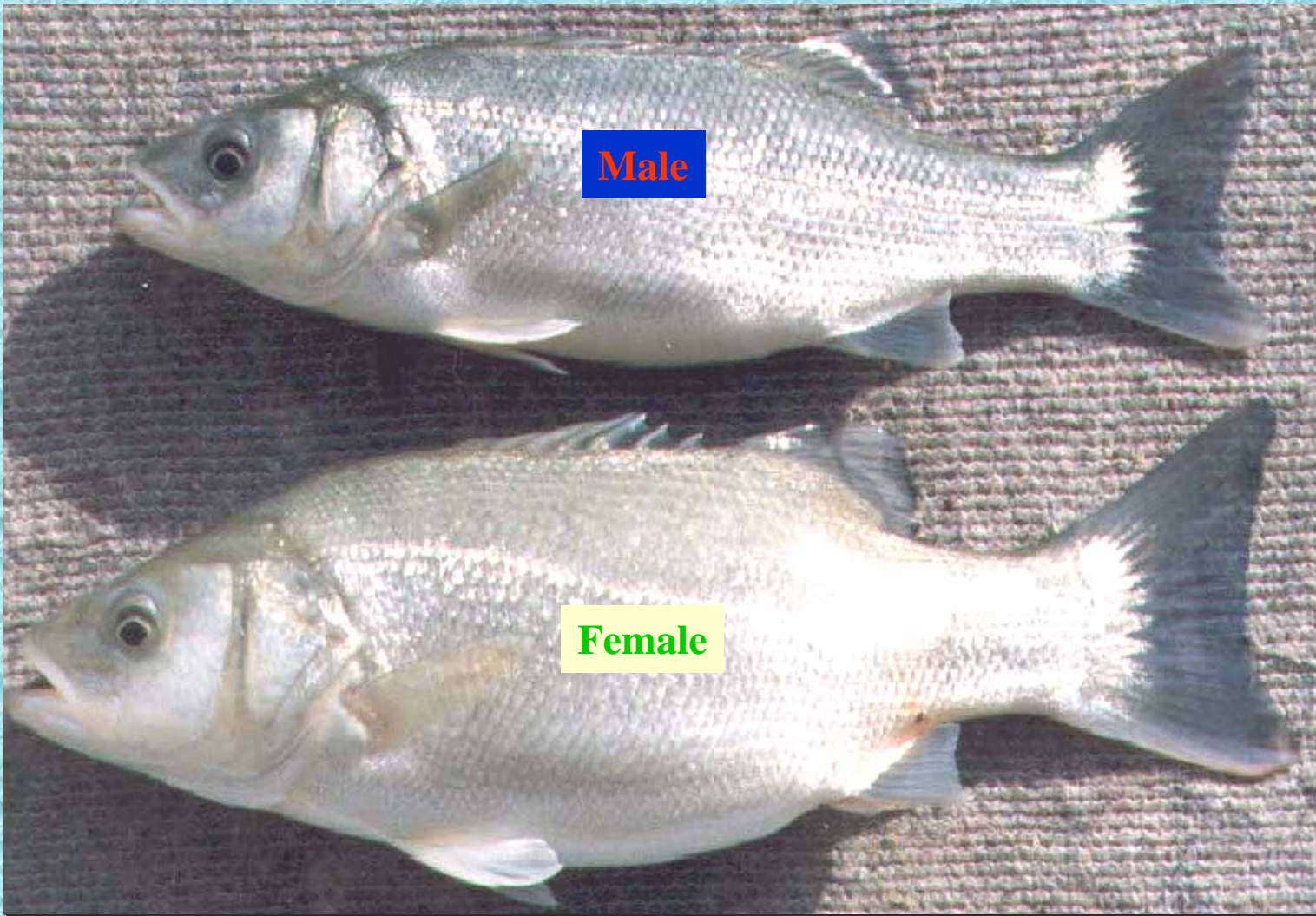
Sex ratio

Level and quality of Production





**In seabass females grow faster than males**



**Male**

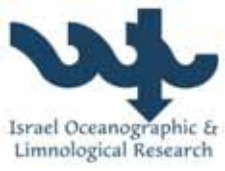
**Female**



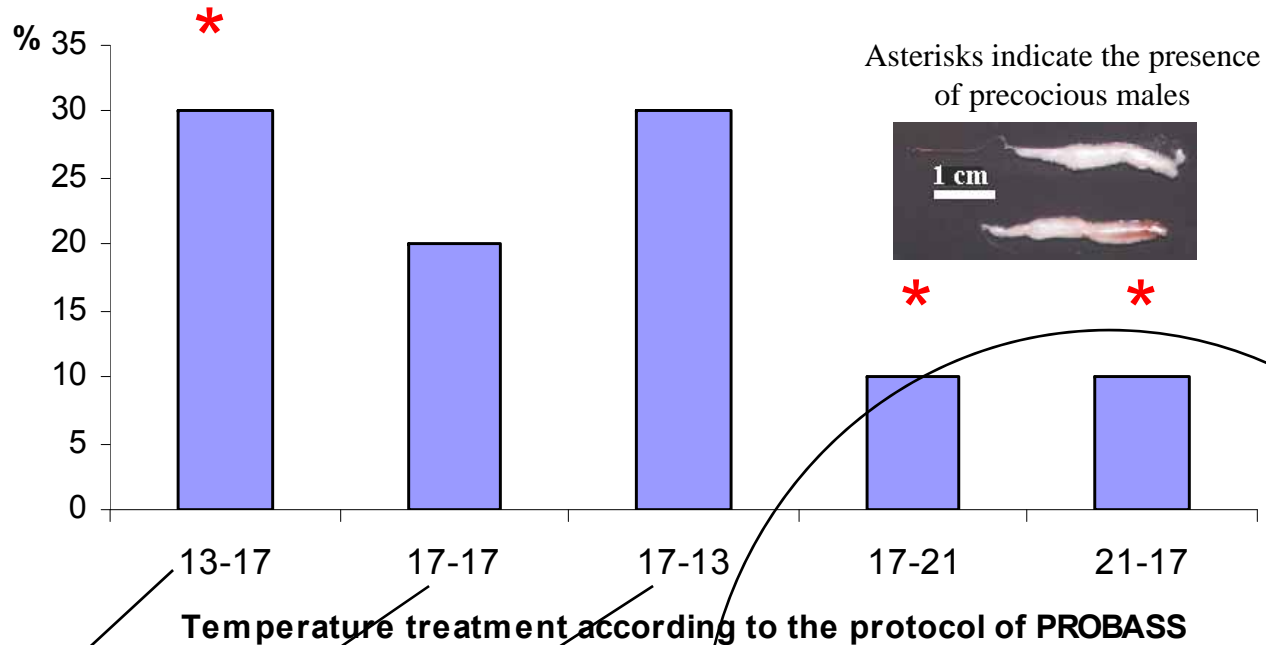
Influence of rearing temperature during the larval  
and nursery periods on sex differentiation in a  
Mediterranean strain of European Sea Bass  
*Dicentrarchus labrax*



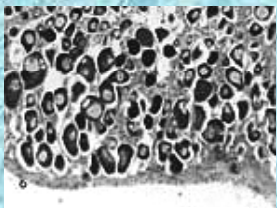
“ProBass” coordinator: C. Mylonas



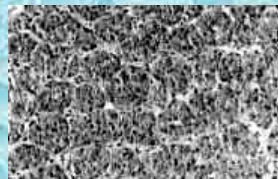
Proportion of females in the first sub-sampling (29 December 2001; 246 days posthatch) of seabass. Groups were exposed to different thermal treatments (PROBASS protocol). Subsamples represent 10 fingerlings from one replicate of each treated group.



The final sexual status of fish was confirmed by histological analysis of gonads



Female



Male

Female



Male

The sexual status could be correctly recognized macroscopically

## Summary of abiotic and biotic factors during larval rearing on juvenile quality

### Abiotic factors indirectly affect juvenile quality by causing developmental anomaly during early ontogeny

- High salinity during early ontogeny → poor swim bladder inflation → lordosis in juvenile and fry development.
- High temperature during early ontogeny → disproportionate muscle and bone development → lordosis in juvenile and fry → more severe by higher swimming speed.
- Abnormal temperatures during early ontogeny → sensitivity to sex differentiation (well before gonad differentiation) → skewed sex ratio and differential growth in adults.



## Summary of abiotic and biotic factors during larval rearing on juvenile quality

Biotic factors affect juvenile quality by directly changing developmental ontogeny through gene expression and hormone synthesis

- PI fed during Artemia feeding → osteocalcin expression and bone formation → to jaw deformity in juveniles
- Vitamin A → modulates gene expression but exposure during different developmental windows → deformity type
- Iodine (precursor for TH) and fed during specific developmental window → possibly affects metamorphic success.
- Arachidonic acid → pigmentation through gene expression and precursor for eicosanoids if fed during a specific developmental window.

**Thankyou for your attention**

