The Molcular Approach for feeding and nutrition studies in Aquaculture

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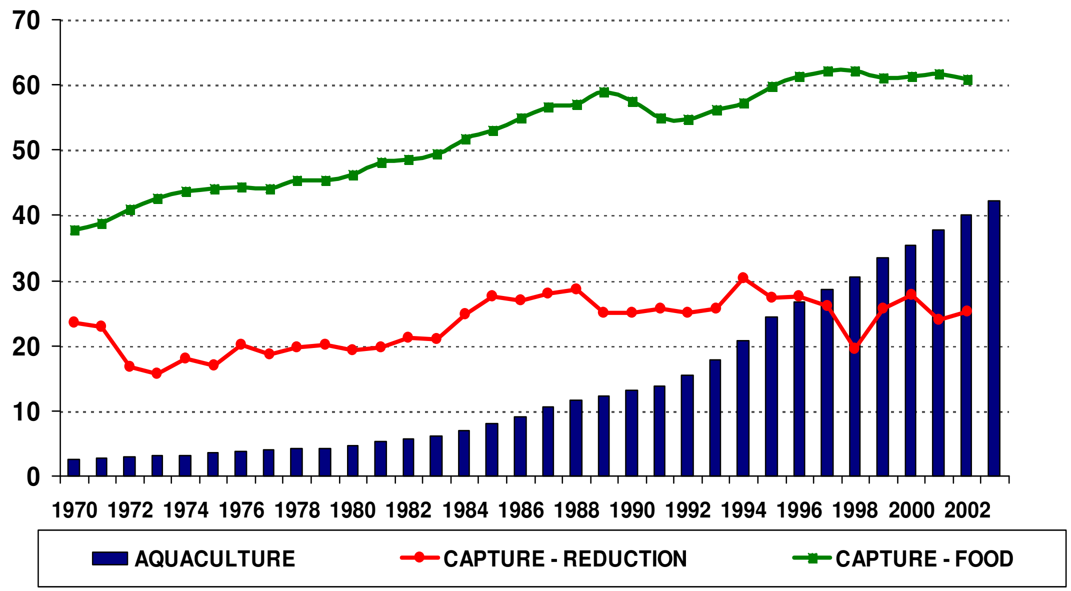
Fish farming demonstration near Orlando, Florida.

FUTURE FISH

The only way to meet the increasing demand for fish is through aquaculture. **Daniel Cressey** explores the challenges for fish farmers and what it means for dinner plates in 2030.

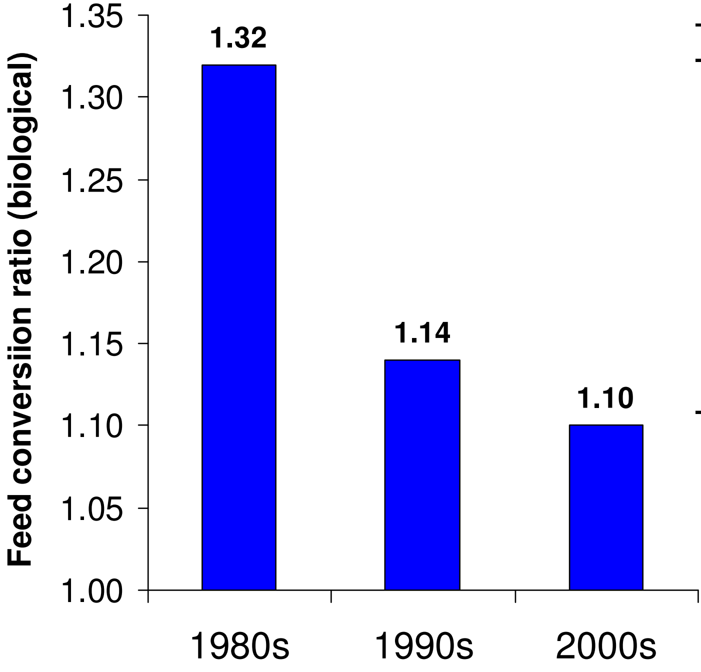
THE CHALLENGE AHEAD

- Fish meal production has not increased throughout the last 20 years
- The growth in aquaculture has partly been based on an increased share of marine raw materials allocated to aquaculture, and partly due to their replacement with other ingredients
- The challenge ahead is to allow further development by improving feed efficiency while reducing our dependence from marine raw materials



IMPROVING FEED EFFICIENCY

Impact of changes in dietary digestible energy (DE) content on the biological feed conversion ratio of Atlantic salmon in seawater – Progressive changes in the DE content of salmon feeds over 2 decades have reduced the FCR that can be achieved which, coupled with improvements in feeding technology and strategy, have resulted in substantially improved use of the feed resource.

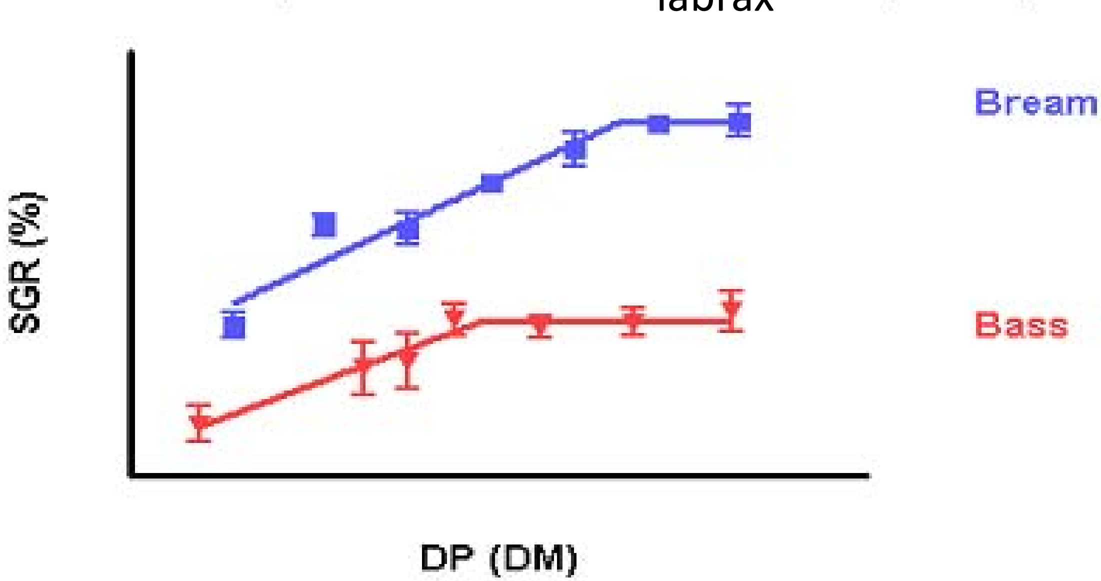


	1980s	1990s	2000s
Crude protein (%)	45	42	36
Crude fat (%)	18	32	38
Ash (%)	9	7	6
Crude fibre (%)	2	1	1
NFE (%)	18	12	13
Moisture (%)	8	6	6
DE (MJ/kg)	17	21	22
FCR, biological	1.32	1.14	1.10

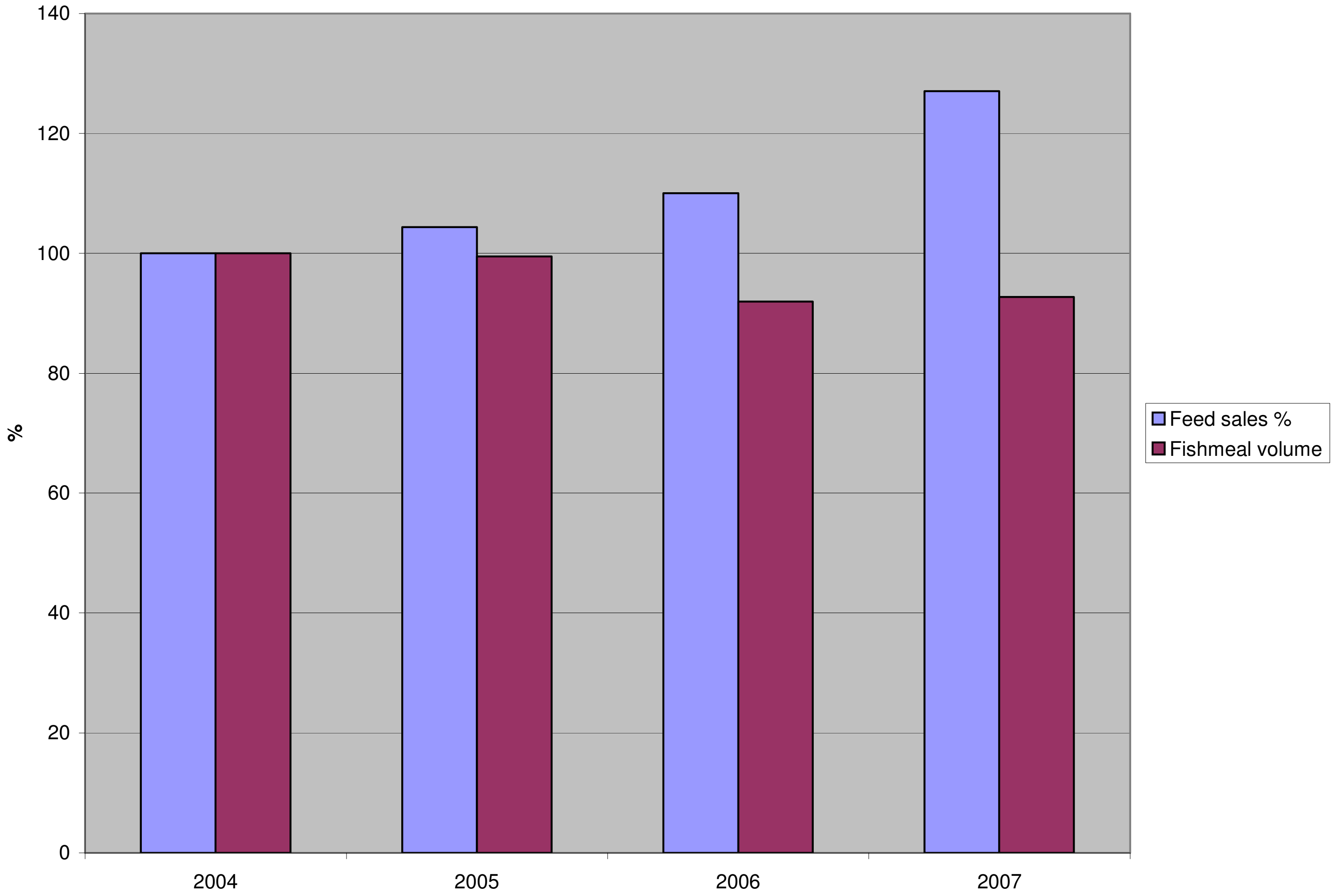
FABBISOGNI IN PROTEINA DIGERIBILE

- Due prove per ciascuna taglia e specie, condotte in parallelo in diversi centri di ricerca
- Spigola e orata hanno fabbisogni in proteina digeribile diversi

DP requirements of sea bream and sea bass (as SGR)



REDUCED MARINE RAW MATERIALS DEPENDENCY – FISH MEAL



Da: A. Allodi, 2008

Main Studies on FO and FM substitution

- RAFOA, www.rafoa.stir.ac.uk, established that salmonids could be cultured on diets where 100% of the FO is replaced by a single (or a blend of) VO, and that for bass and bream, replacement of up to 60% of FO showed no detrimental effects on growth.
- PEPPA www.st-pee.inra.fr/ici/stpee/nut/peppa/peppa.htm established that trout and bream can be cultured with 75% replacement of FM, by plant products, with no loss on growth performance.
- Other projects (AquaMax, for instance) take in consideration the quality of fish in human nutrition, after feeding with high percentage FO & FM substitutions .

Profilo degli acidi grassi POLINSATURI in trota bianca, trota salmonata e pangasio

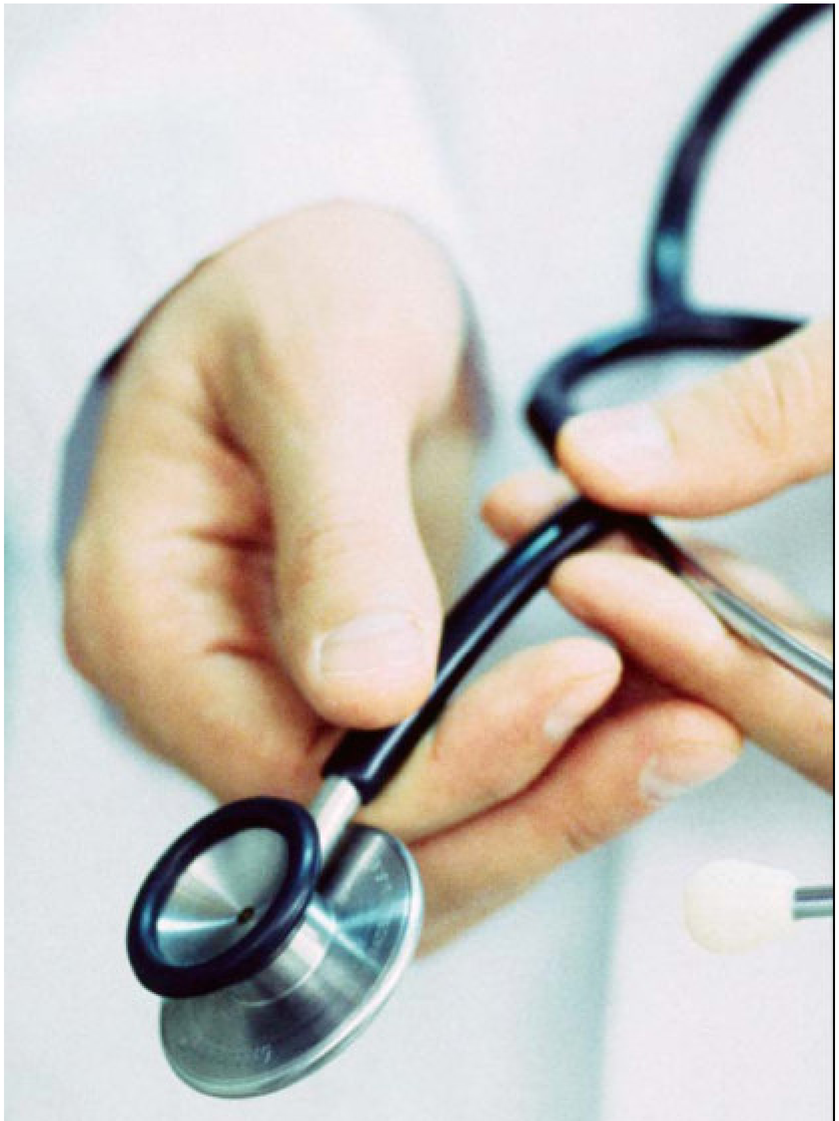
(% degli acidi grassi totali)

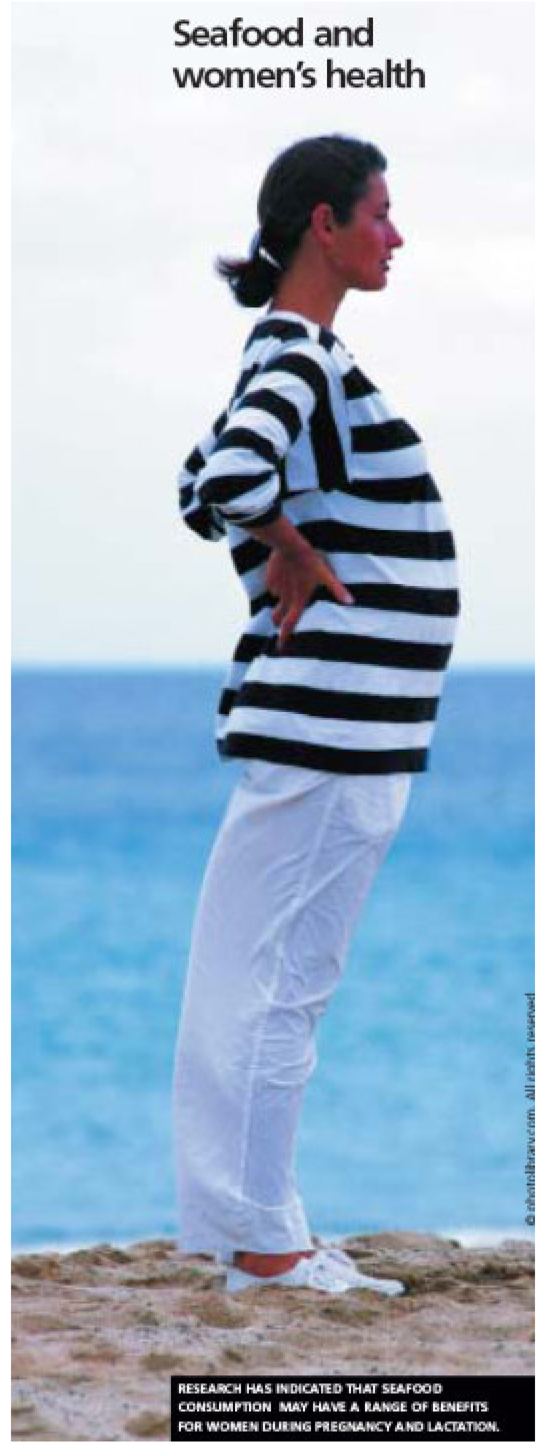
	Trota bianca	*Trota salmonata*	*Pangasio*
C18:2 n-6	5.25-7.36	4.63-8.09	7.16-8.19
C18:3 n-6	0.29-0.35	0.00-0.20	0.18-0.36
C18:3 n-3	1.16-1.71	1.32-2.29	0.28-0.57
C18:4 n-3	1.30-1.51	1.46-1.55	0.04-0.10
C20:2 n-6	0.33-0.44	0.36-0.54	0.42-0.59
C20:4 n-6	0.46-0.70	0.44-0.54	1.55-3.61
C20:5 n-3 (EPA)	4.83-6.20	5.62-5.86	0.19-1.31
C22:4 n-3	0.00-0.09	0.06-0.07	0.24-0.64
C22:5 n-3	1.55-1.98	1.68-1.82	0.34-1.06
C22:6 n-3 (DHA)	13.59-15.80	12.86-14.22	1.70-3.64
Polinsaturi totali	**30.55-35.73**	**30.27-33.32**	**12.48-18.76**
n-3 totali	**22.50-26.85**	**23.54-24.68**	**2.58-6.69**
n-6 totali	**6.28-8.88**	**5.60-9.33**	**9.89-13.38**
rapporto n-3/n-6	**2.74-3.89**	**2.57-4.41**	**0.26-0.64**

(Da: E. Orban, 2009)

High dietary VO inclusion result in reductions of flesh DHA & EPA (≈65% in salmon fed 100% VO or up to 50% in bass and bream fed 60% VO)

- However, flesh EPA and DHA can be restored to 70-100% in fish fed FO, by the use of FO-containing finishing diets in pre-harvest period (Bell et al., *Lipids,* 2004; Torstensen et al., *JAFC*, 2005; Mourente et al., *Aquac. Nutr*., 2005; Izquierdo et al., *Aquaculture*, 2005)




Seafood and
women's health
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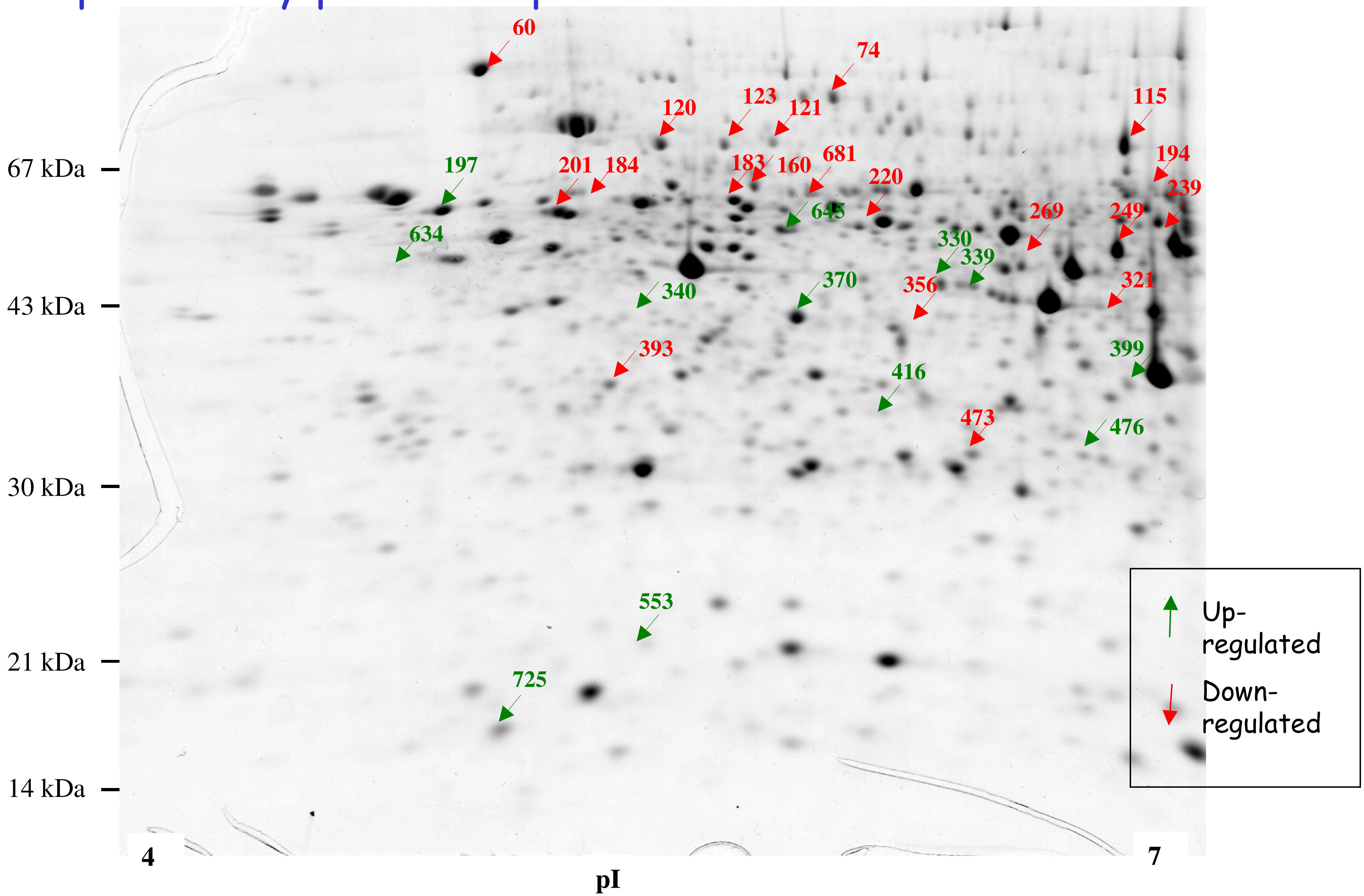
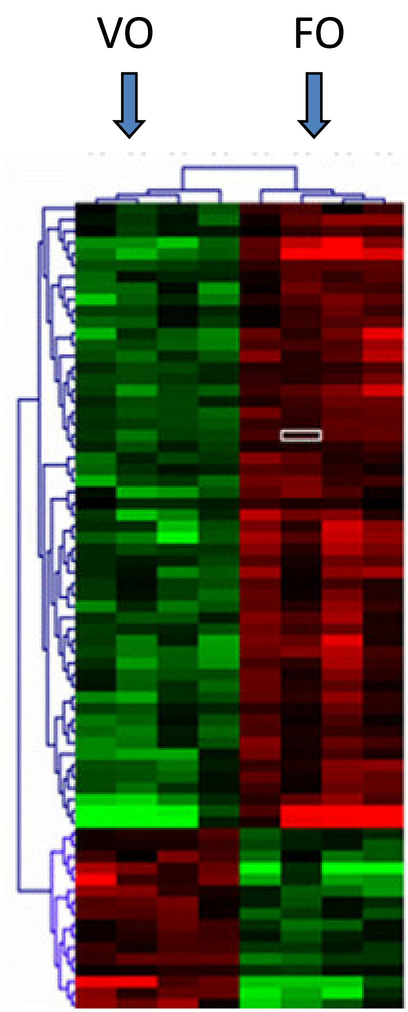
RESEARCH HAS INDICATED THAT SEAFOOD
CONSUMPTION MAY HAVE A RANGE OF BENEFITS
FOR WOMEN DURING PREGNANCY AND LACTATION.

Experiment 1: Proteins whose abundance is affected by partial soy protein replacement

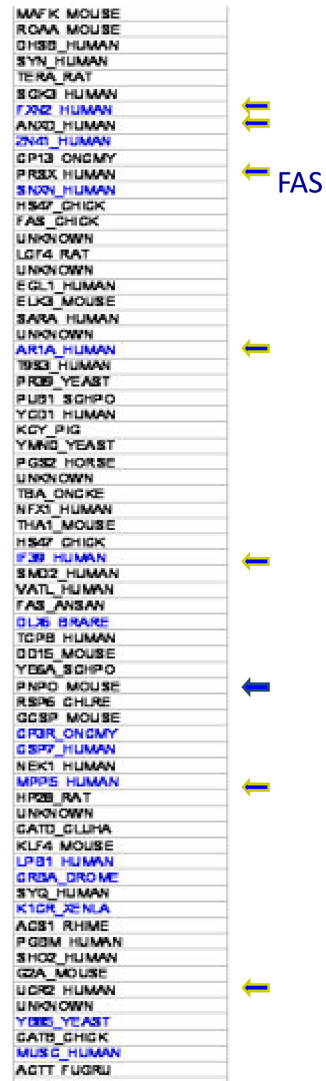


Hepatic transcriptome in fish FO / VO



71 genes differentially expressed
16 up-regulated in VO
55 down-regulated in VO

(From: S. Kaushik, 2009)



CYP1A
Proteolytic system : proteasome

Proteolytic system : proteasome

FAS

CYP3A

Proteolytic system : Lysosome (cathepsin)

Proteolytic system : Lysosome (cathepsin)

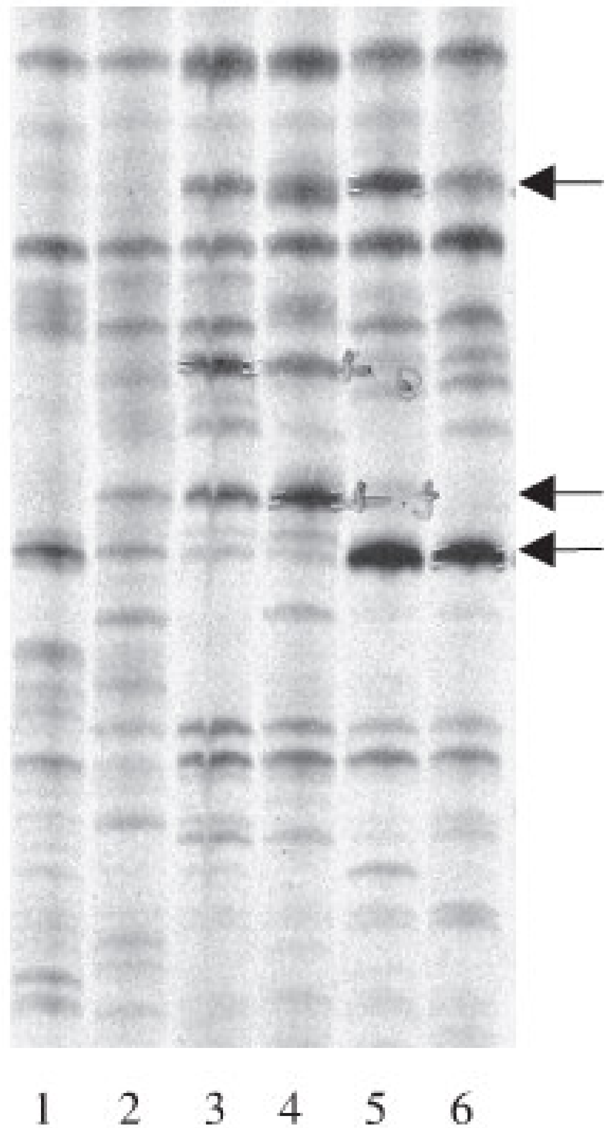
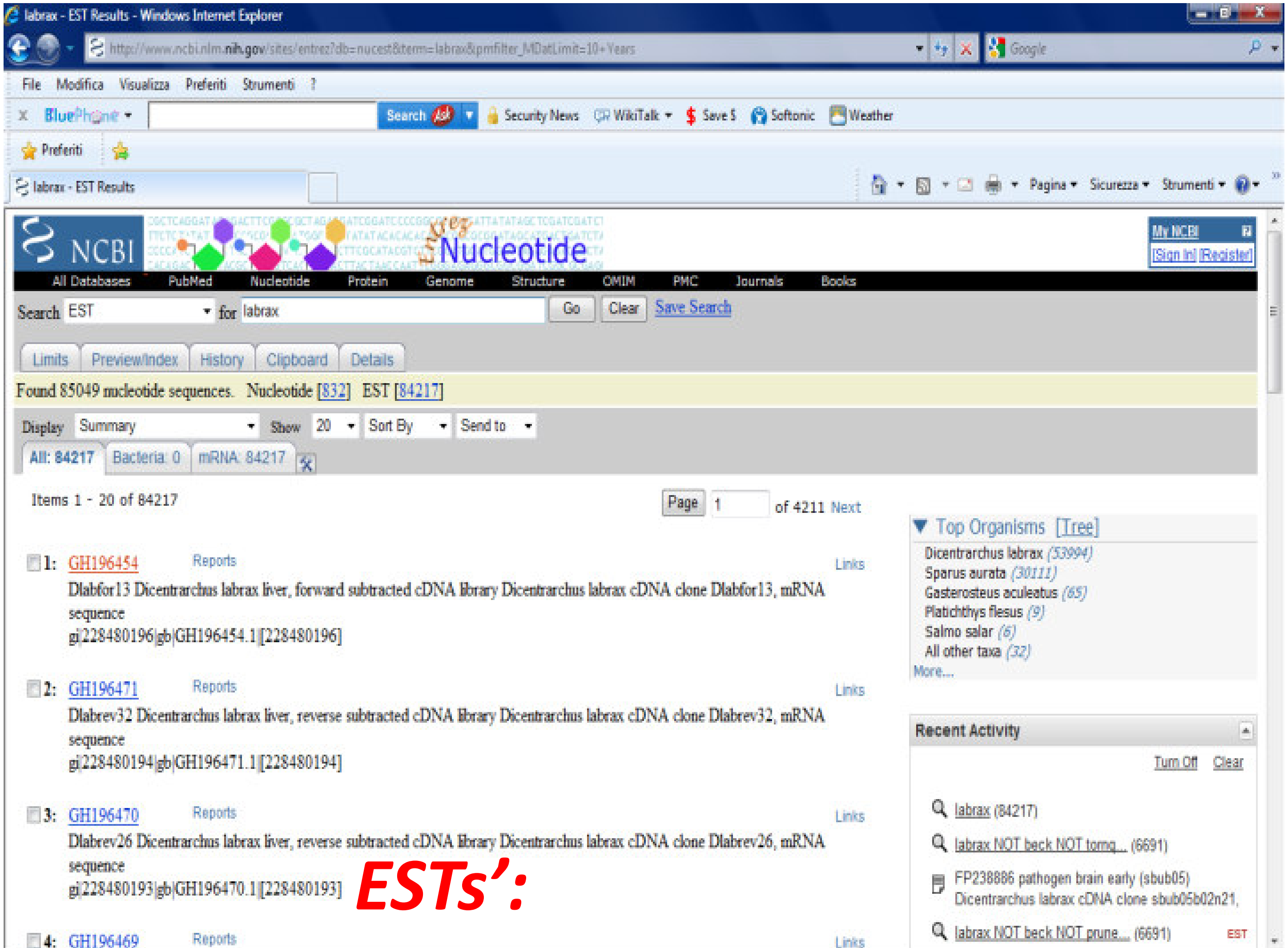


Fig. 1. Example of differential display on gills of *D. labrax*. Lanes 1 and 2, control; lanes 3 and 4, population density of 80 kg/m^3; lanes 5 and 6, population density of 100 kg/m^3.



ESTs':

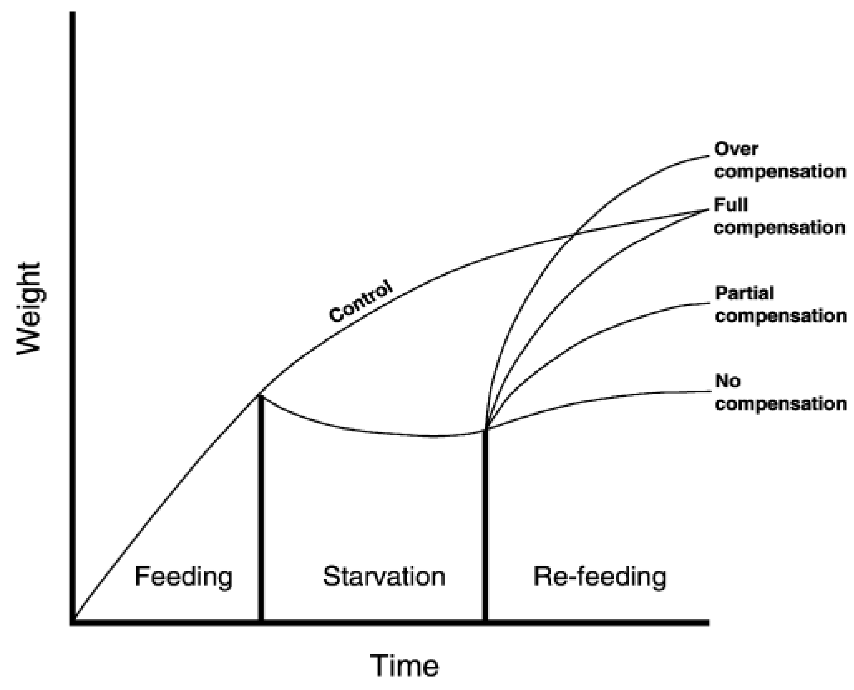
***D. labrax*: 85,049**

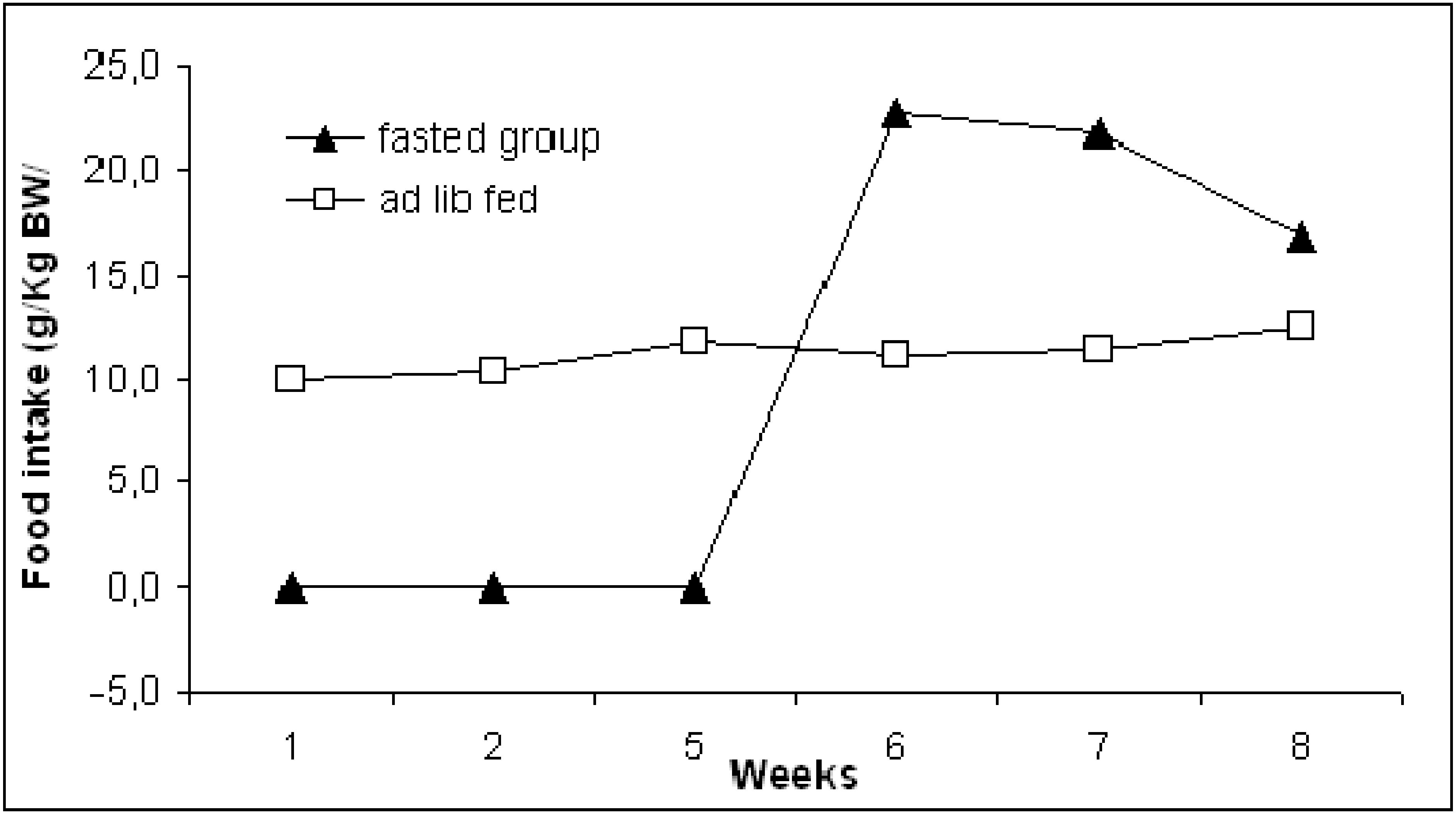
***S. aurata*: 103,320**

***O. mykiss*: 299,088**

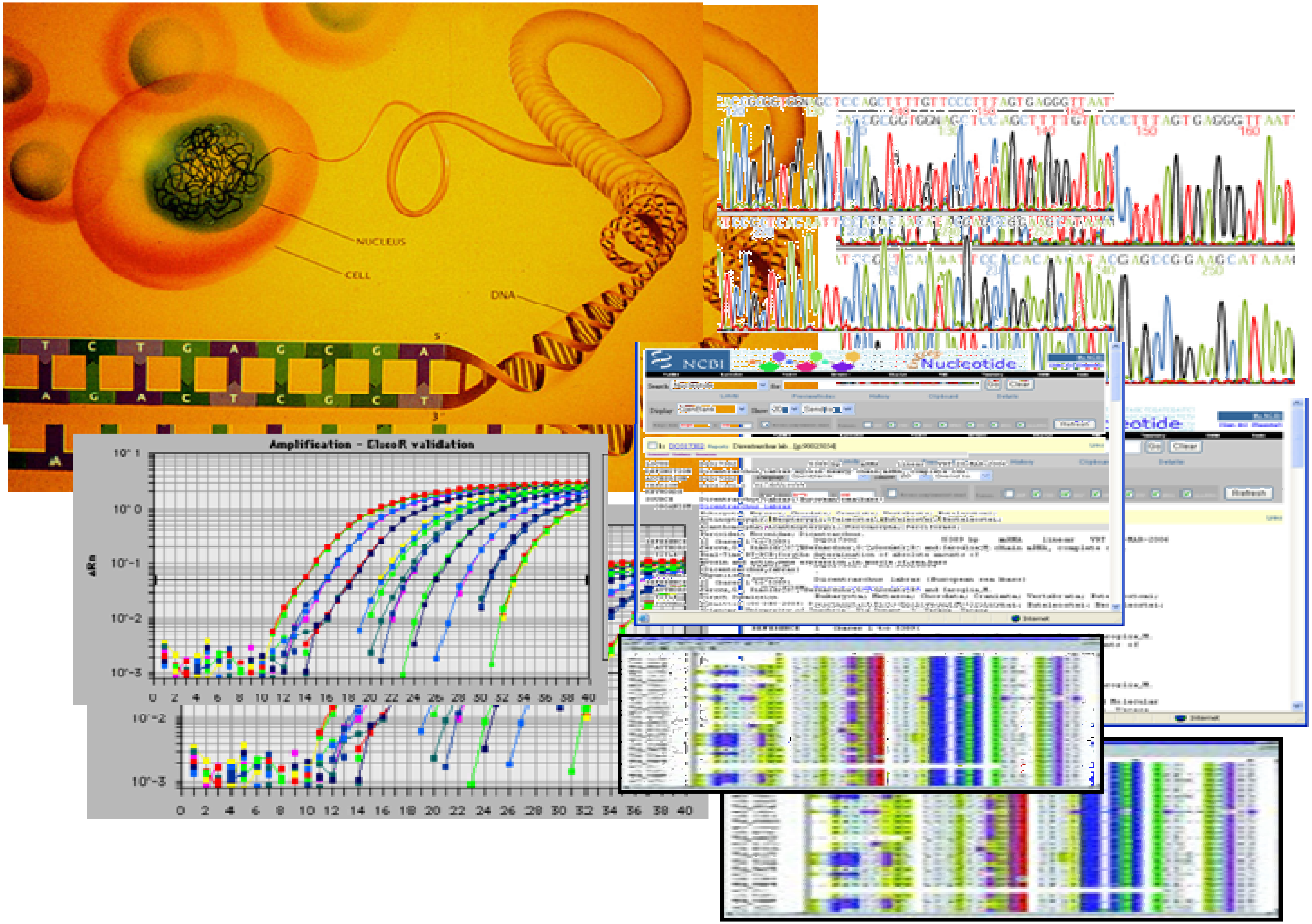
Compensatory growth

- The exceptionally fast growth that fish experience after periods of fasting has been called "compensatory growth"
- This phenomenon, reported in a wide range fish species, has been studied in intensive aquaculture as a means of enhancing feed conversion efficiency, but the mechanisms implicated are complex and not yet fully understood.



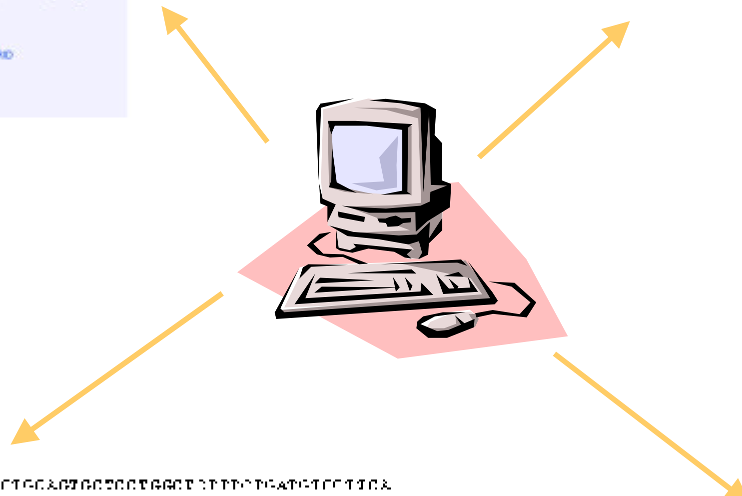


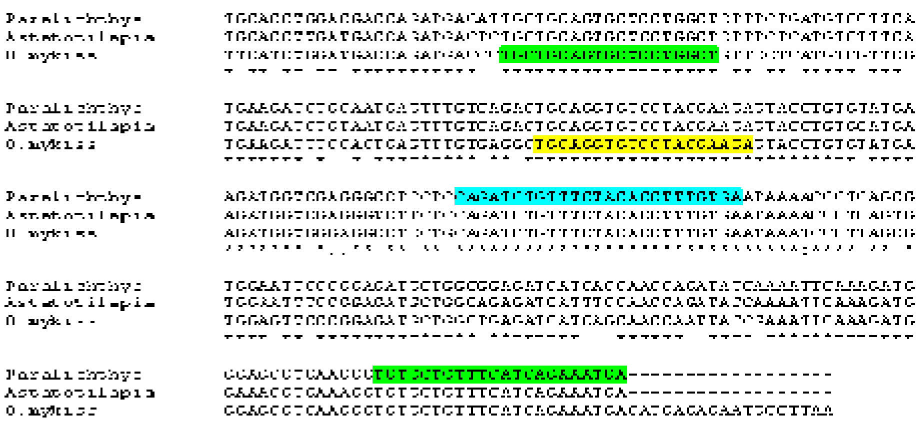
Food intake (g/Kg BW/
25,0
20,0
15,0
10,0
5,0
0,0
-5,0
fasted group
ad lib fed
1
2
5
6
7
8
Weeks


NUCLEUS
CELL
DNA
NCBI
Nucleotide

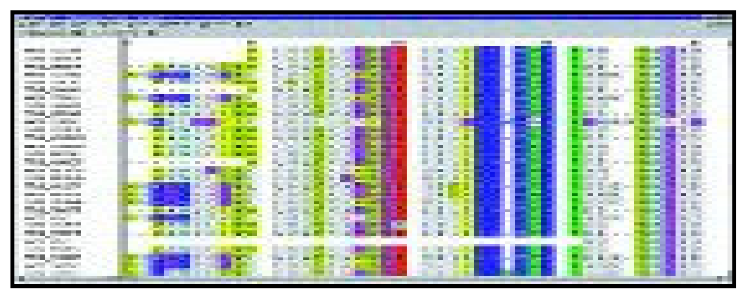
blast: **Basic Local Alignment Search Tool**

Sequences



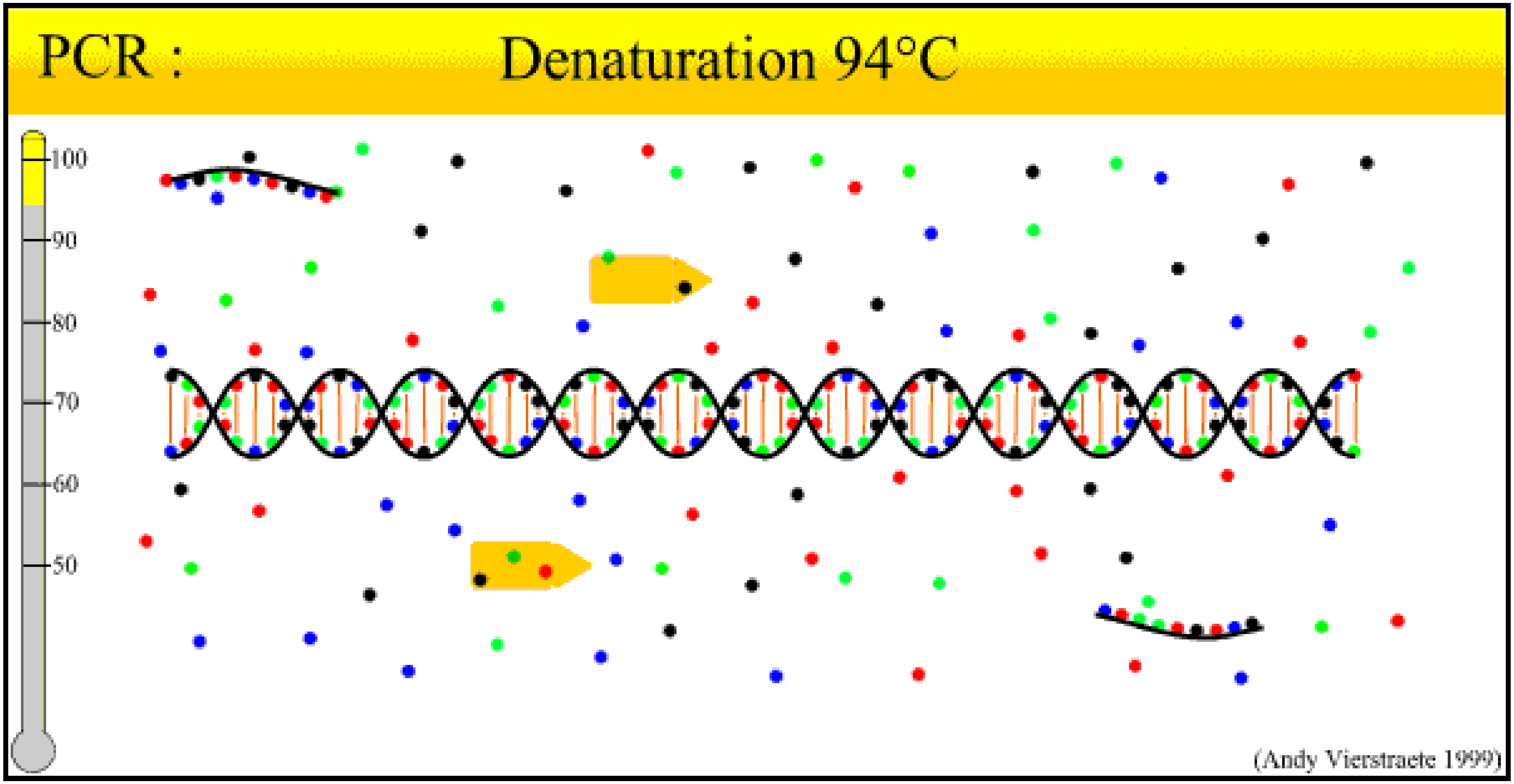


Primer design

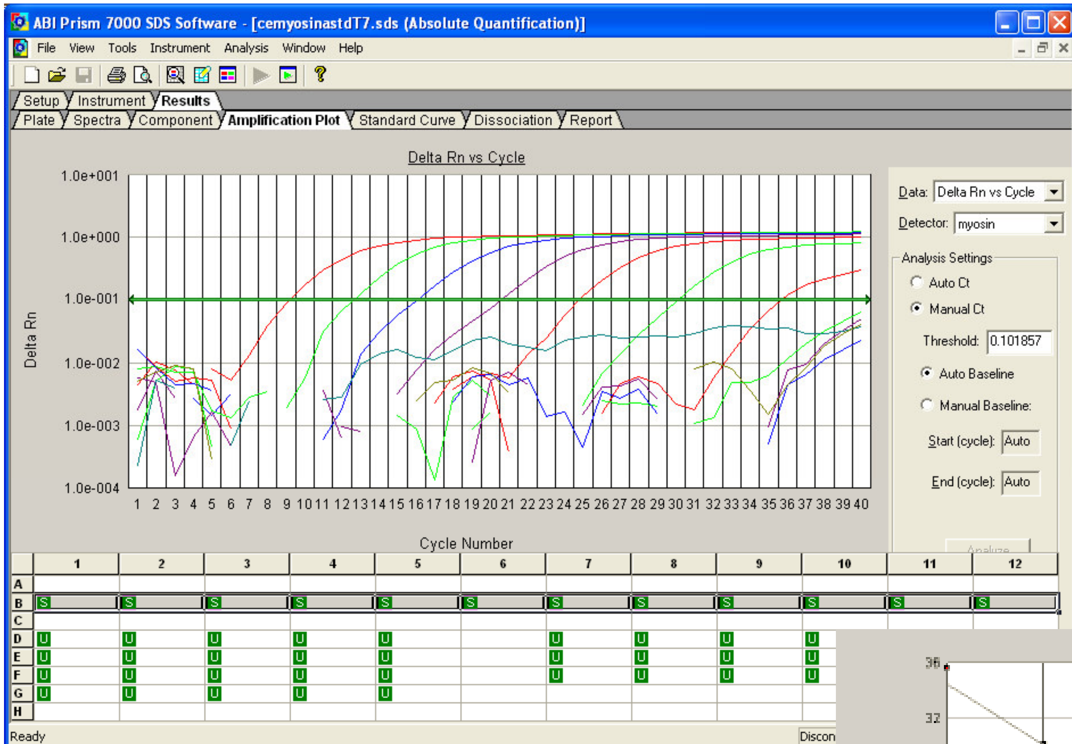


Alignments

PCR

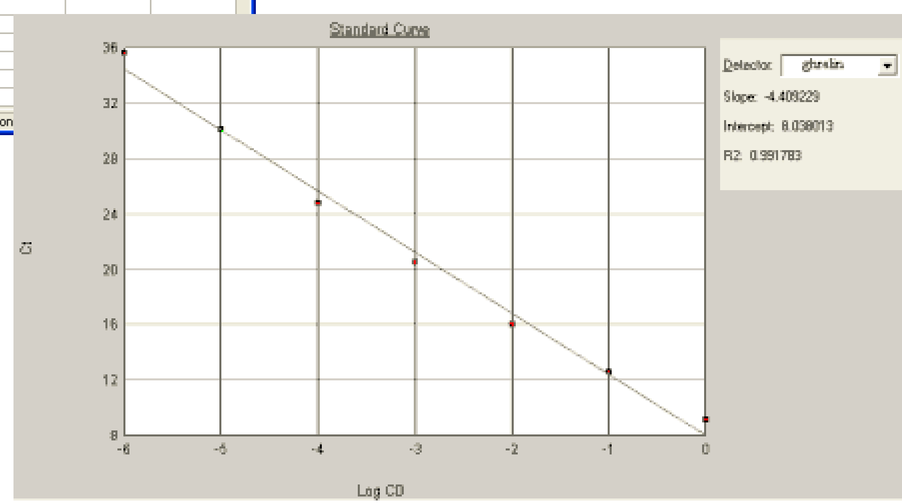

PCR :
Denaturation 94°C
100
90
80
70
60
50
(Andy Vierstraete 1999)

Absolute quantitative analyses with the Real Time PCR



Sequential dilution of the transcript, at known concentrations

One-step TaqMan EZ RT-PCR



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General and Comparative Endocrinology 155 (2008) 341–351
GENERAL AND COMPARATIVE ENDOCRINOLOGY
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Sea bass ghrelin: Molecular cloning and mRNA quantification during fasting and refeeding

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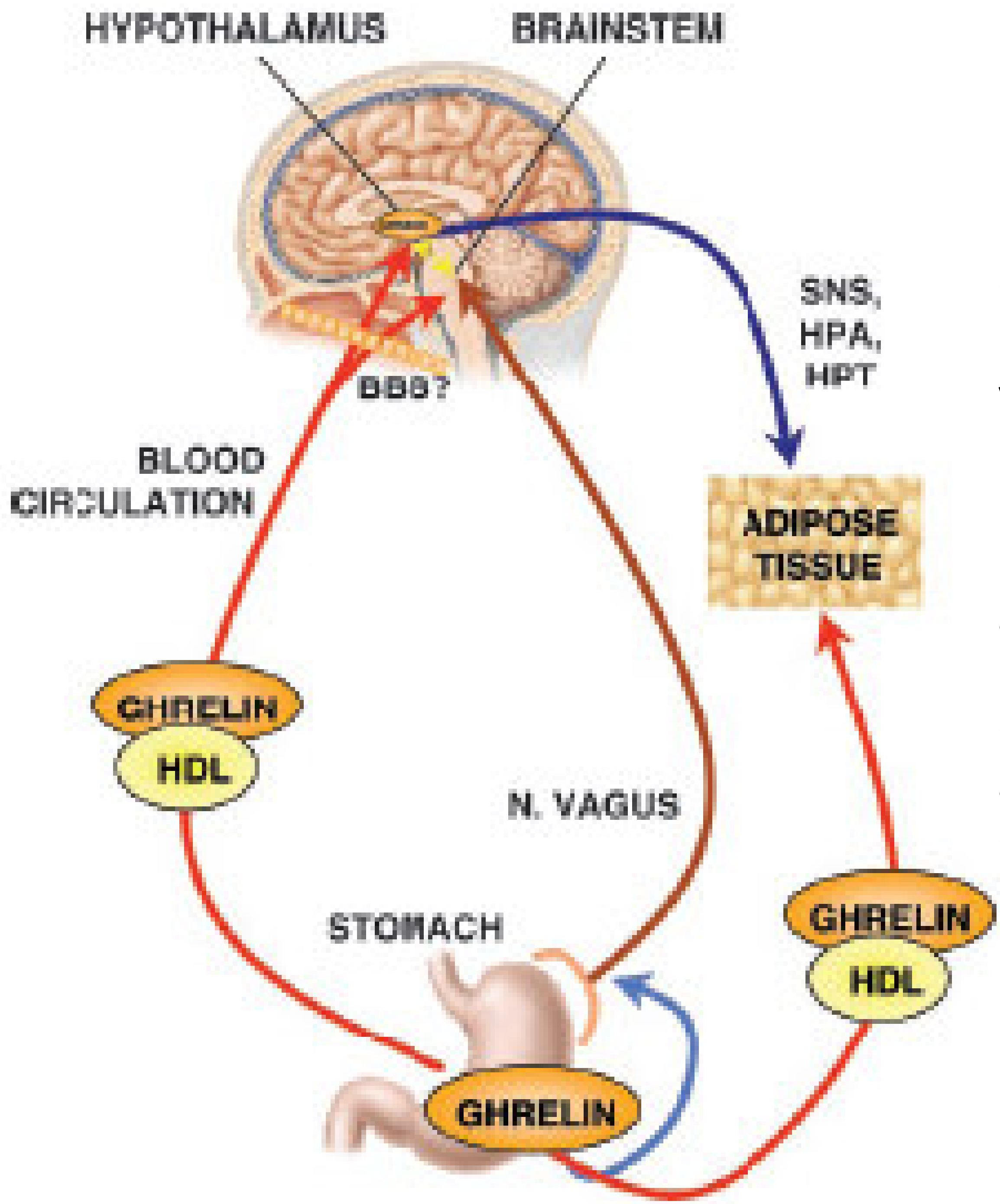
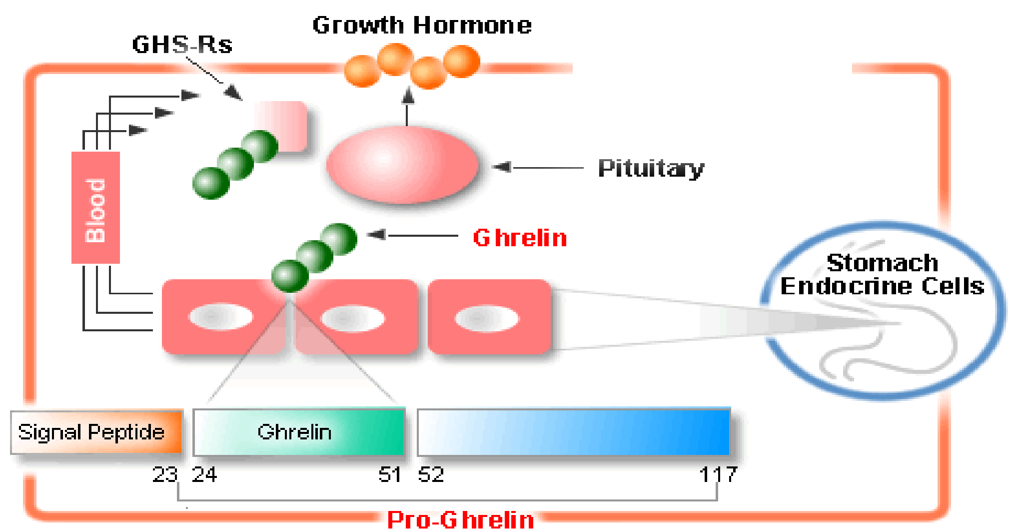


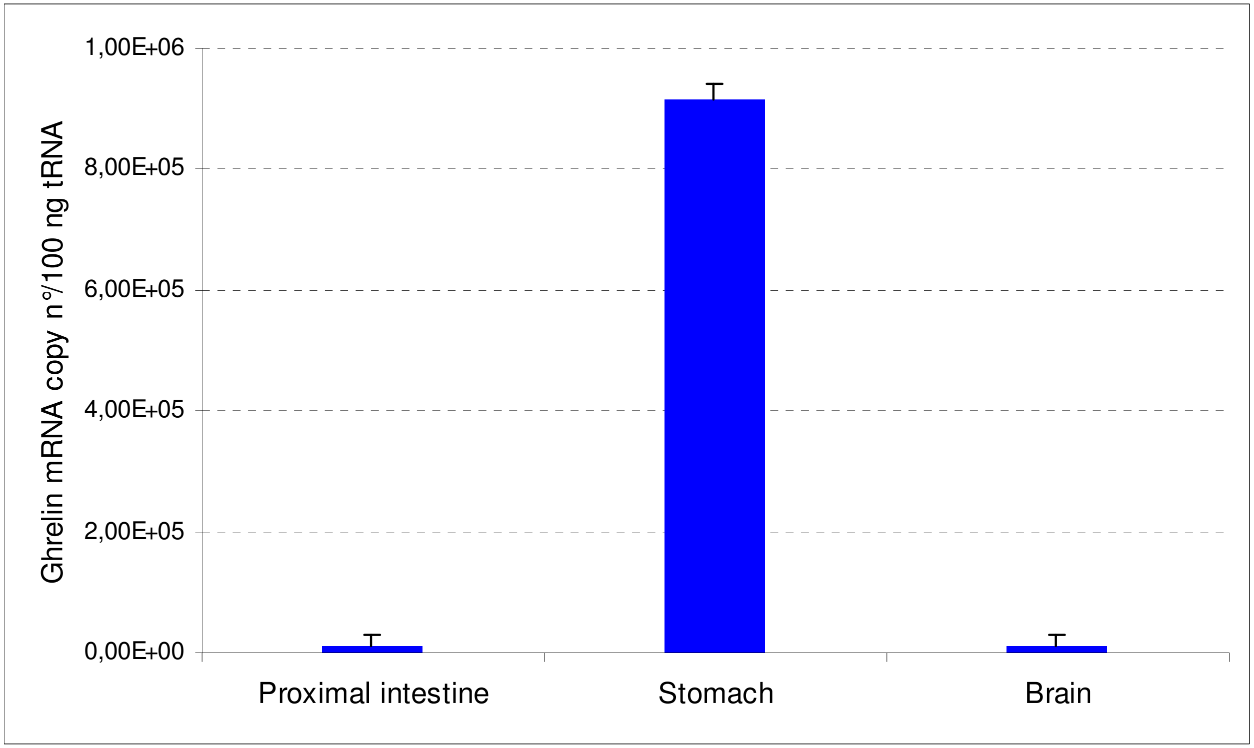
L’ormone grelina prodotto principalmente nello stomaco agisce sul cervello, interagendo con specifici recettori a livello delle fibre afferenti del nervo vago, o raggiungendo direttamente il cervello mediante la circolazione sanguigna. Questo peptide gastrointestinale è sintetizzato anche nel cervello e può per questo essere considerato allo stesso tempo un segnale sia periferico, sia centrale.

GRELINA nei PESCI

- La grelina è un peptide di 28 aa originariamente isolato dallo stomaco umano e murino.
- Trasmette le informazioni riguardanti la disponibilità di nutrienti
- Implicato nella secrezione di GH e nella modulazione del metabolismo energetico
- Recettori espressi principalmente nell'ipofisi e nell'ipotalamo, ma anche in altri tessuti
- Studi in teleostei a favore di un'azione oressigenica



Livelli tessutali di espressione di grelina in branzino



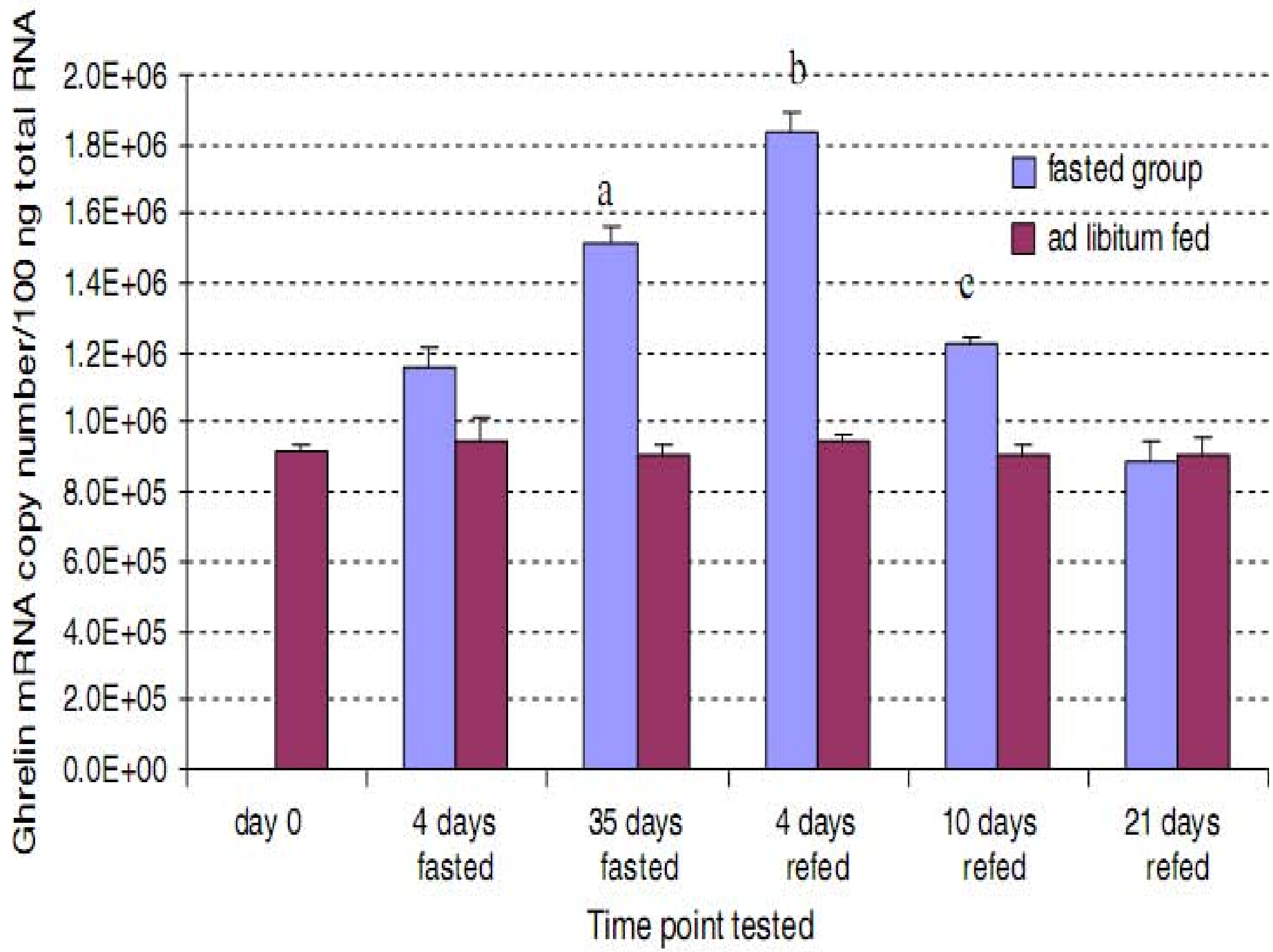


Fig. 8. Expression levels of ghrelin measured by real-time PCR in *D. labrax* stomach in the course of the experiment. Ghrelin mRNA copy number was normalized as a ratio to 100 ng total RNA. Fish were sampled before fasting (day 0), 4 days after fasting (4 days fasted), at the end of fasting (35 days fasted), and then sequentially at 4, 14 and 21 days following refeeding. The means of five animals in each group are shown. Bars indicate standard error of the mean. Differences were determined by one-way analysis of variance (ANOVA). Different letters indicate significantly different means from controls, for each time point tested ($P < 0.05$).

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Biochemical and Biophysical Research Communications 363 (2007) 591–596

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Regulation of progastricsin mRNA levels in sea bass (*Dicentrarchus labrax*) in response to fluctuations in food availability

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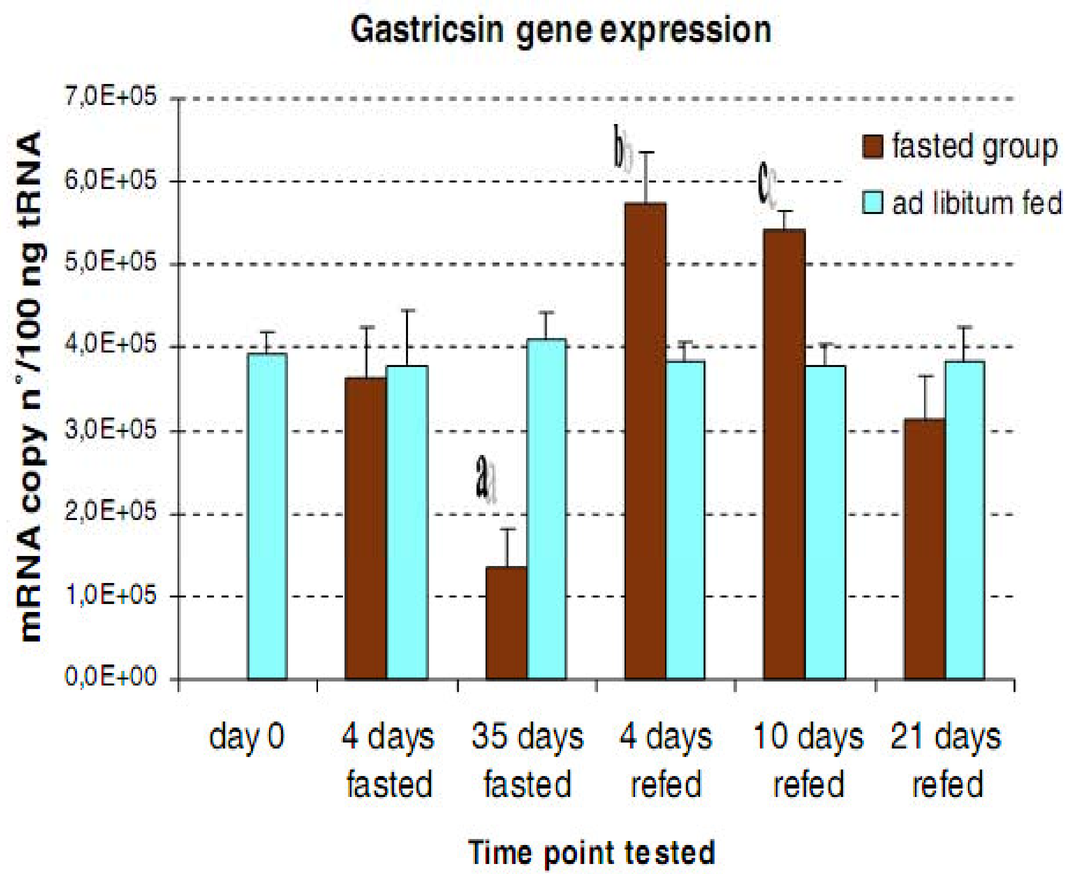


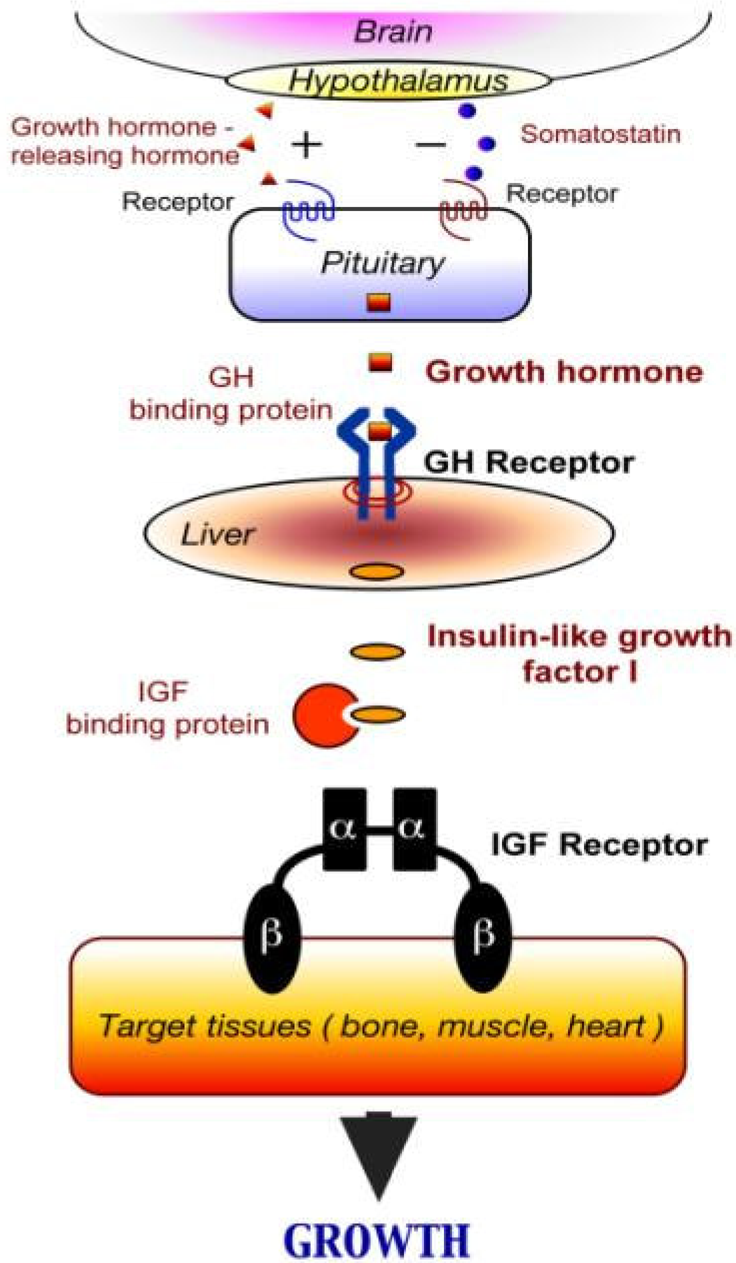
Fig. 3. Expression levels of gastricsin measured by real-time PCR in *D. labrax* stomach in the course of the experiment. Gastricsin mRNA copy number was normalized as a ratio to 100 ng total RNA. Fish were sampled before fasting (day 0), 4 days after fasting (4 days fasted), at the end of fasting (35 days fasted), and then sequentially at 4, 14 and 21 days following refeeding. The means of five animals in each group are shown. Bars indicate standard error of the mean. Differences were determined by one-way analysis of variance (ANOVA). Different letters indicate significantly different means from controls, for each time point tested ($P < 0.05$).

Journal of Fish Biology (2007) **70** (Supplement B), 219–233
doi:10.1111/j.1095-8649.2007.01402.x, available online at http://www.blackwell-synergy.com

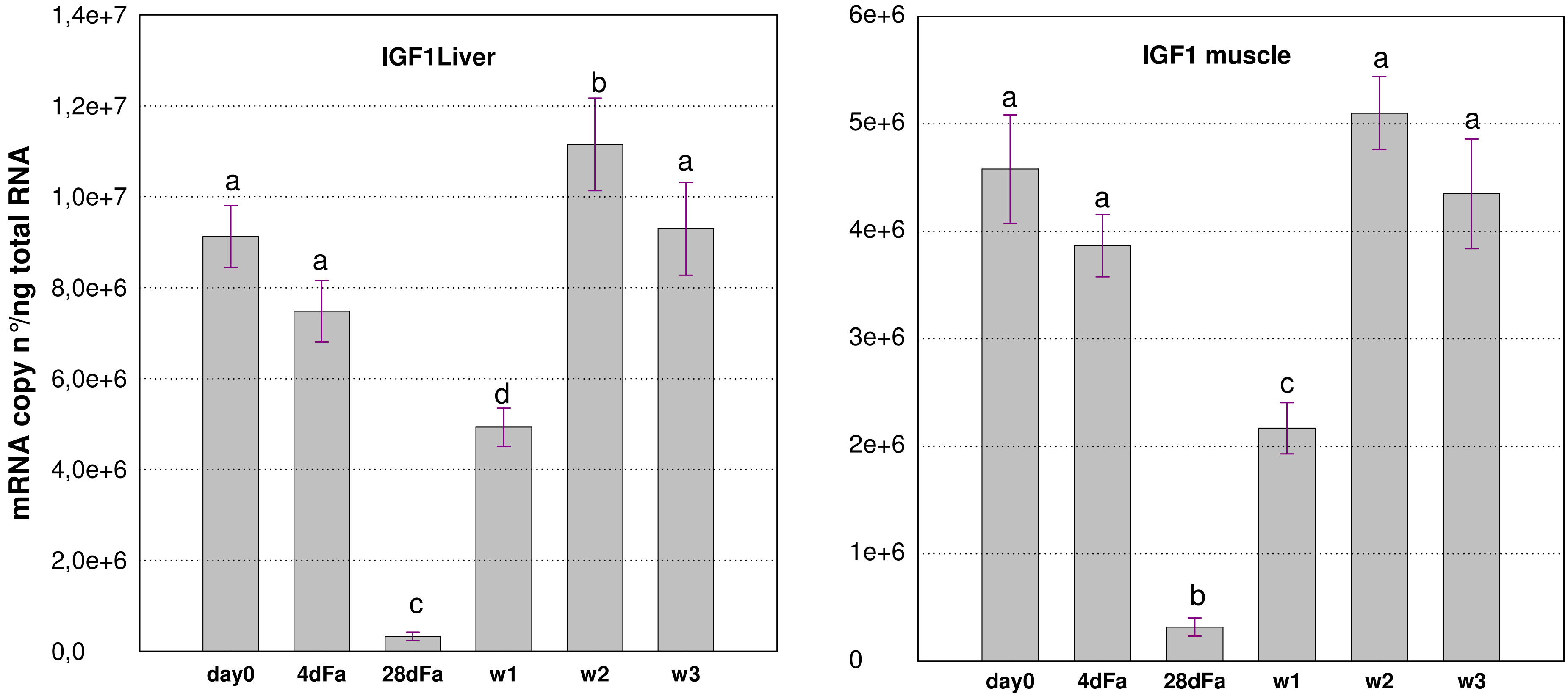
Cloning and expression analysis of insulin-like growth factor I and II in liver and muscle of sea bass (*Dicentrarchus labrax*, L.) during long-term fasting and refeeding

G. Terova*, S. Rimoldi, V. Chini, R. Gornati, G. Bernardini and M. Saroglia

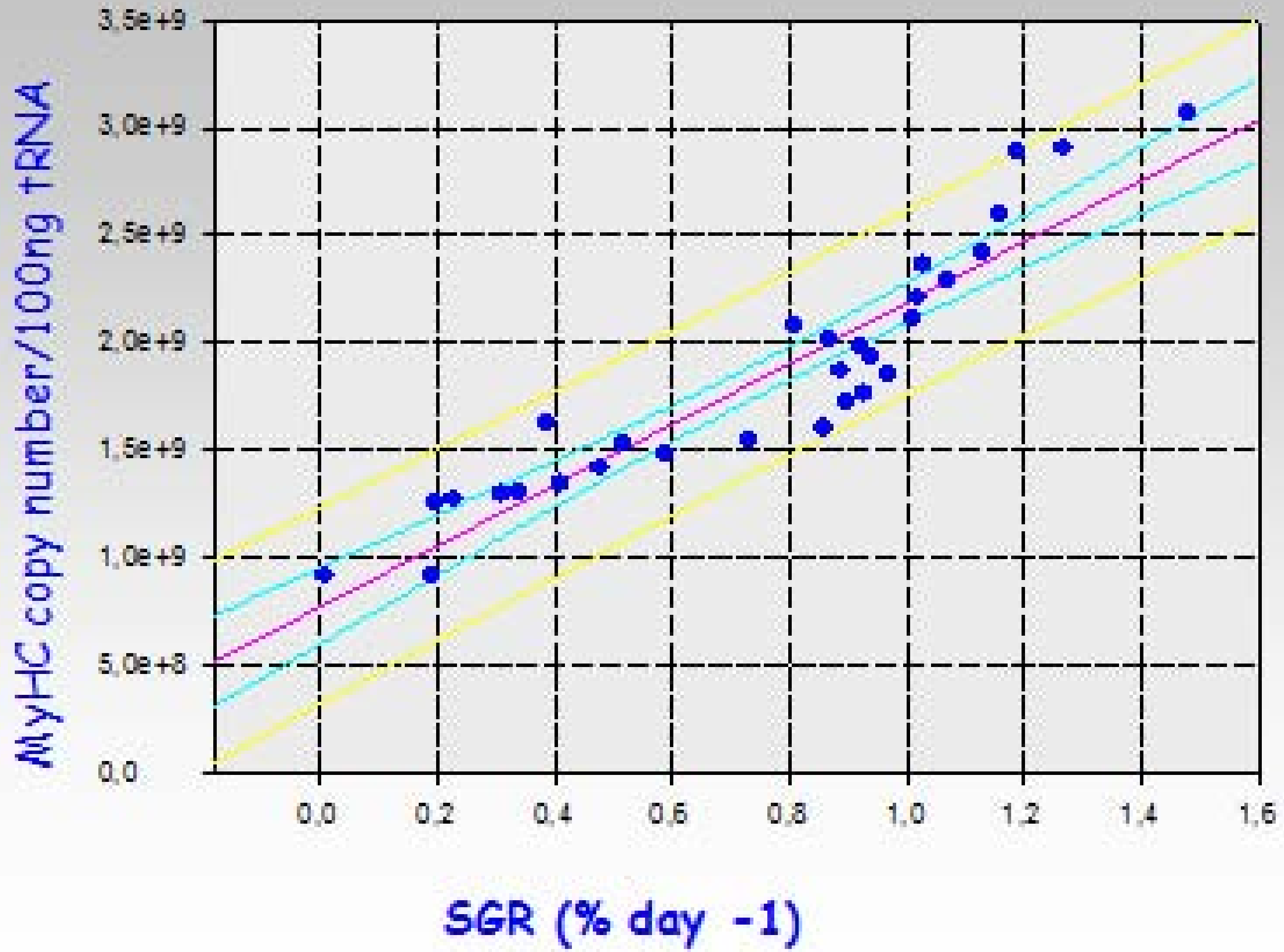
Growth hormone   Insulin-like growth factors (IGF) axis



Moriyama et al., 2000


IGF1Liver
mRNA copy n°/ng total RNA
1,4e+7
1,2e+7
1,0e+7
8,0e+6
6,0e+6
4,0e+6
2,0e+6
0,0
a
a
c
d
b
a
day0
4dFa
28dFa
w1
w2
w3
IGF1 muscle
6e+6
5e+6
4e+6
3e+6
2e+6
1e+6
0
a
a
b
c
a
a
day0
4dFa
28dFa
w1
w2
w3

Myosin gene expression levels correlated to specific growth rate in sea bass after 45 days of feeding



Terova et al., IJAS, 2007

Aquaculture 279 (2008) 150–159

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Acute and chronic hypoxia affects HIF-1α mRNA levels in sea bass (*Dicentrarchus labrax*)

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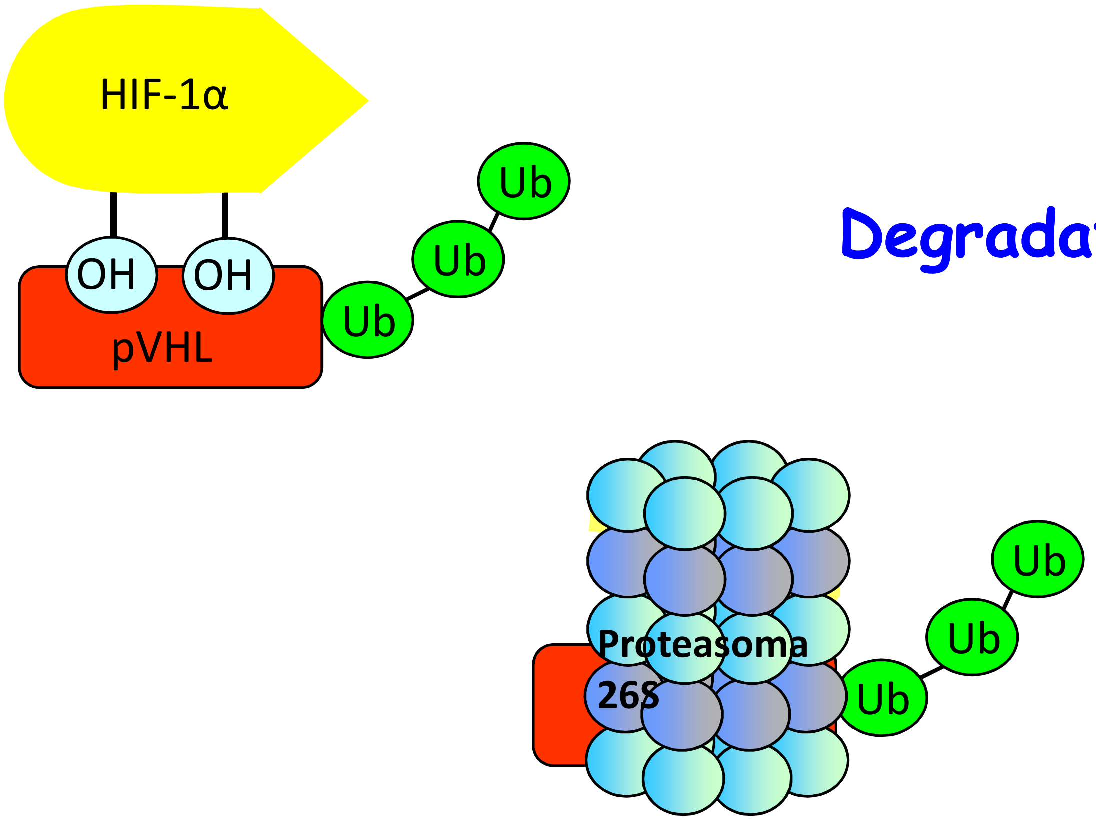
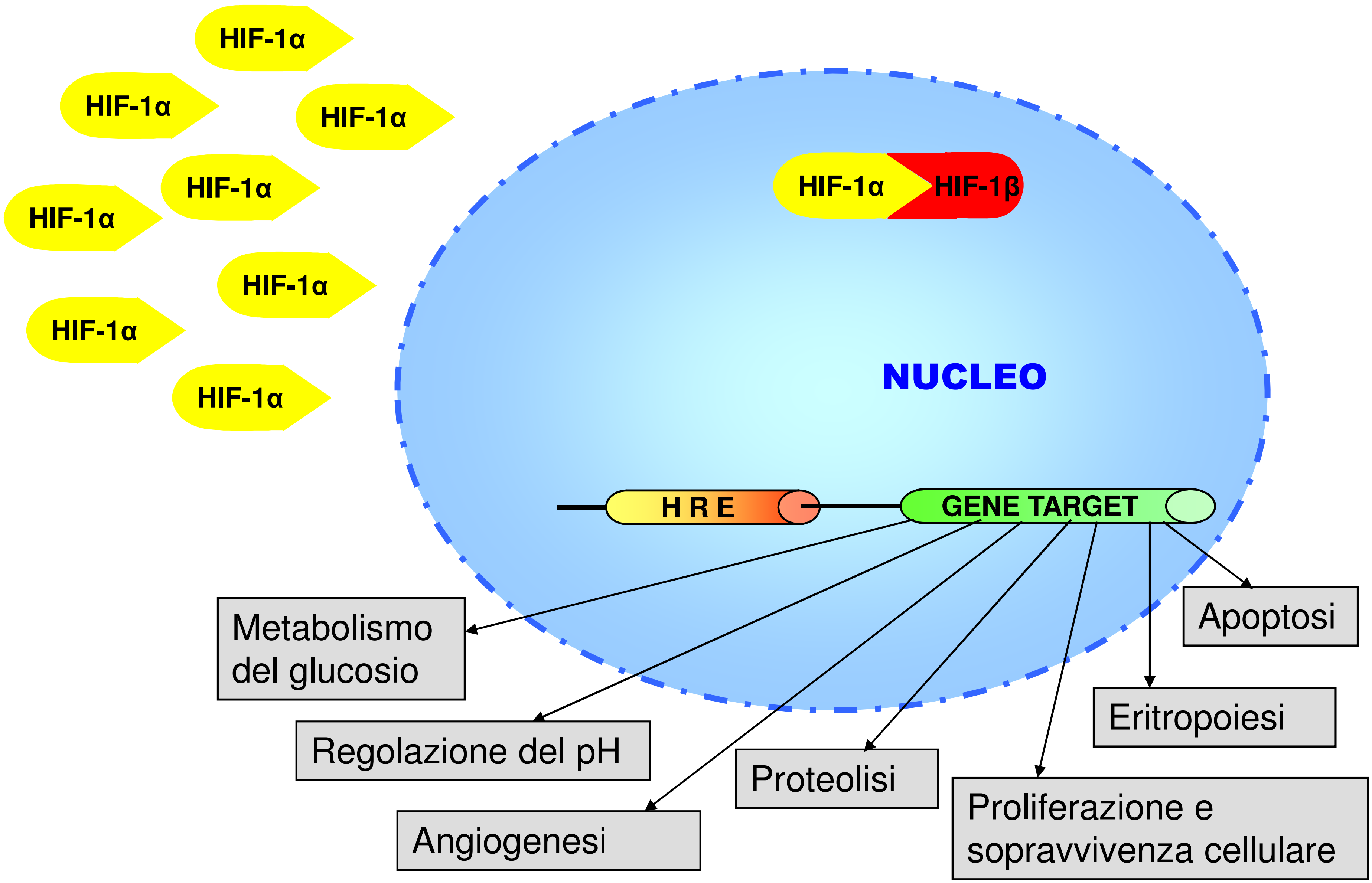
HIF-1α IN NORMOXIA

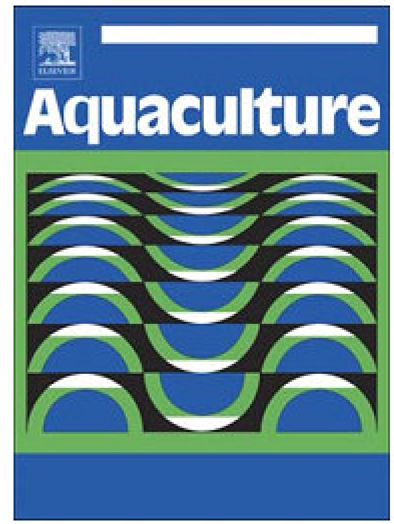


Degradation of HIF-1α

HIF-1 IN IPOSSIA



Accepted Manuscript



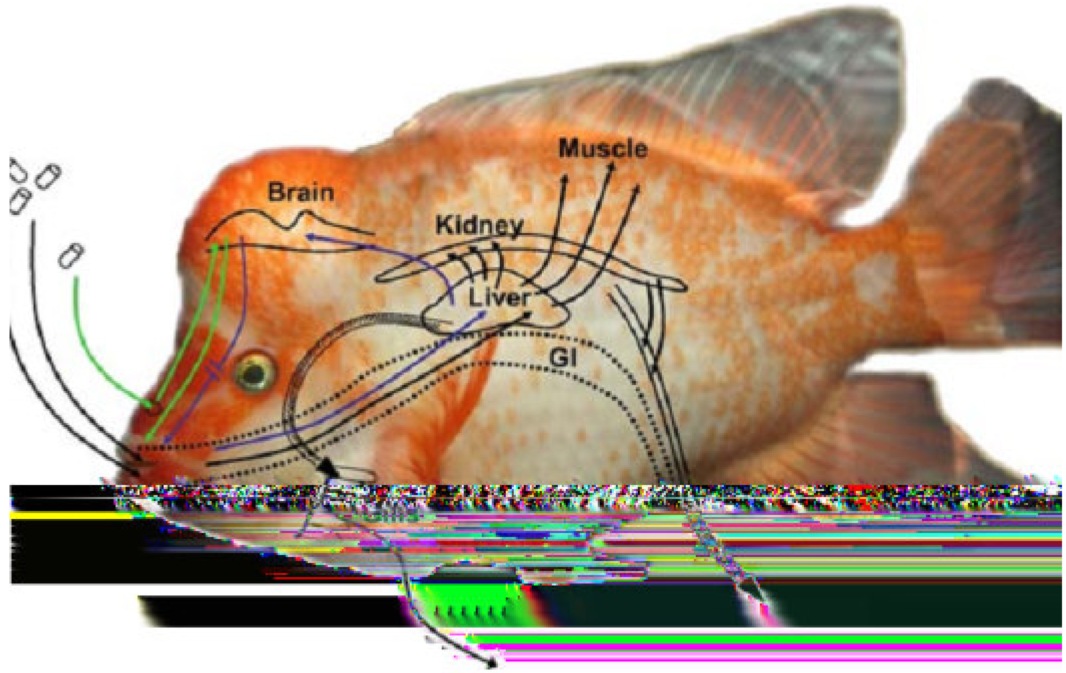
Impact of feed availability on PepT1 mRNA expression levels in sea bass (Dicentrarchus labrax)

Genciana Terova, Samuela Corà, Tiziano Verri, Simona Rimoldi, Giovanni Bernardini, Marco Saroglia

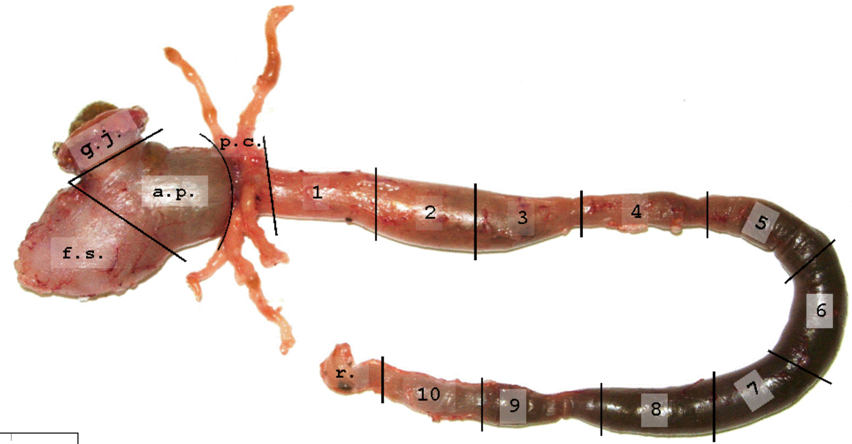
PII: S0044-8486(09)00518-3
DOI: doi: 10.1016/j.aquaculture.2009.06.014
Reference: AQUA 628792

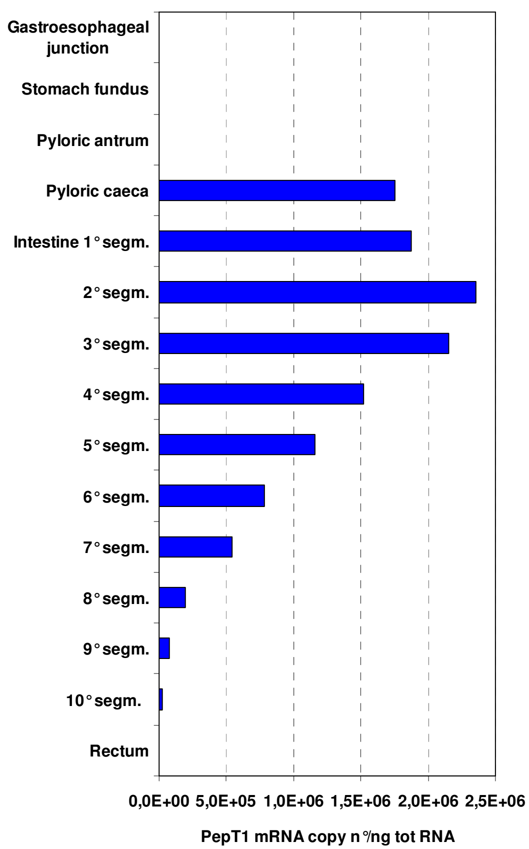
Impiego di idrolisati proteici nelle diete

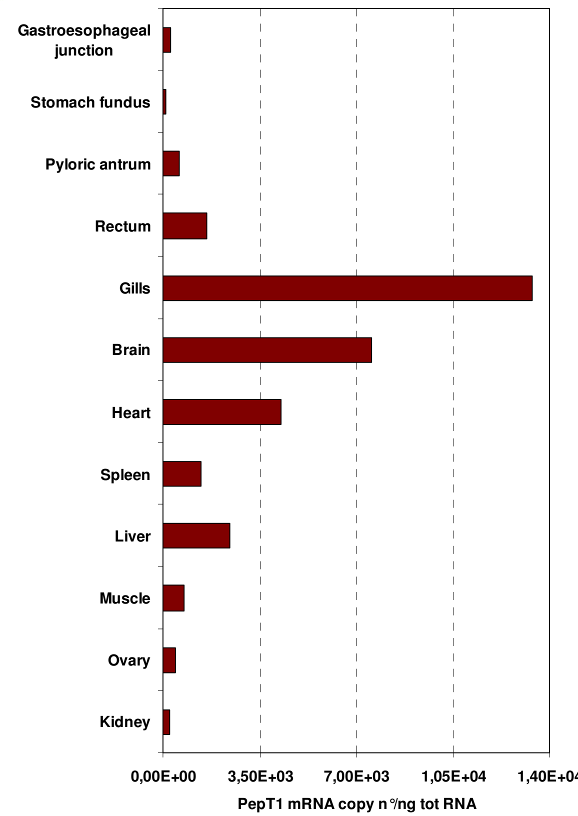
- Richiede compensazione con EAA in forma di dipeptidi (DP) o tripeptidi (TP).
- Ma il trasportatore intestinale Pept-1 non presenta la stessa affinità per tutte le possibili combinazioni di DP e TP



Attività del gene Pept-1 in diversi organi e parti dell'intestino







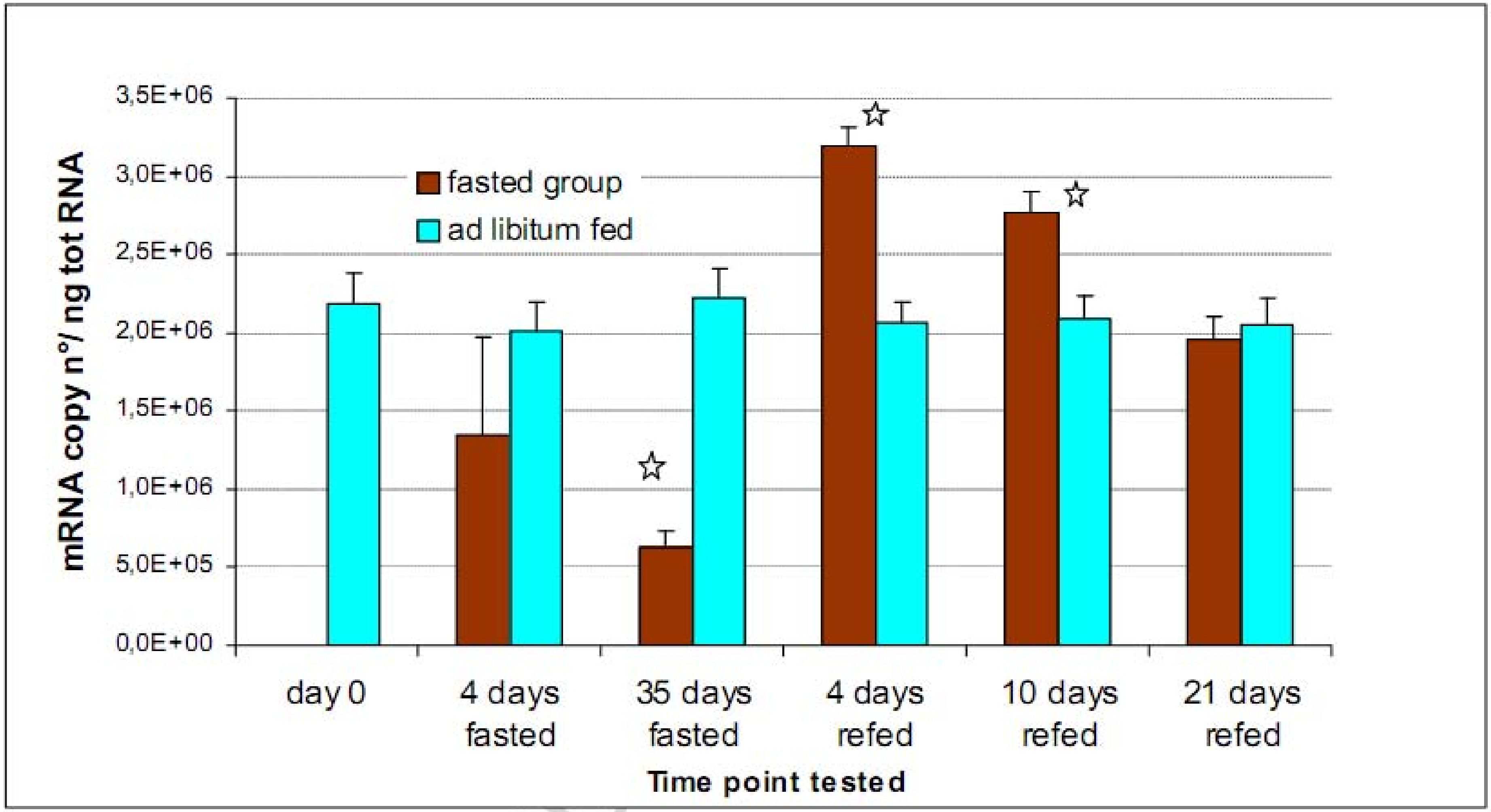


Figure 11. Expression levels of PepT1 measured by real-time PCR in *D. labrax* proximal intestine in the course of the experiment. PepT1 mRNA copy number was normalized as a ratio to 100 ng total RNA. Fish were sampled before fasting (day 0), 4 days after fasting (4days fasted), at the end of fasting (35 days fasted), and then sequentially at 4, 14 and 21 days following refeeding. The

A number of candidate genes are available for fish feeding and nutrition studies, and they may quantitatively describe:

- A response to FO and FM substitution
- The growth actitude
- Feeding interests
- A response of the digestive functions
- The response to specific environmental agents affecting appetite
- Nutrition strategies affecting the immunitary response
- Others……….

Molecular biology and fish feeding/nutrition: a winning combination

- A study on a larger number of candidate genes must be afforded on the most important farmed species
- And it should include genes that directly describe the CNS responses (brain-derived neurotrophic factor, BDNF?)
- In spite the quantitative real time RT-PCR already being a profitable approach, simplest "kits facilities" should be developed for larger "on farm" monitoring applications