ONTOGENY OF PROTEOLYTIC DIGESTIVE ENZYME GENES EXPRESSION IN DEVELOPING SEABASS (LATES CALCARIFER) LARVAE

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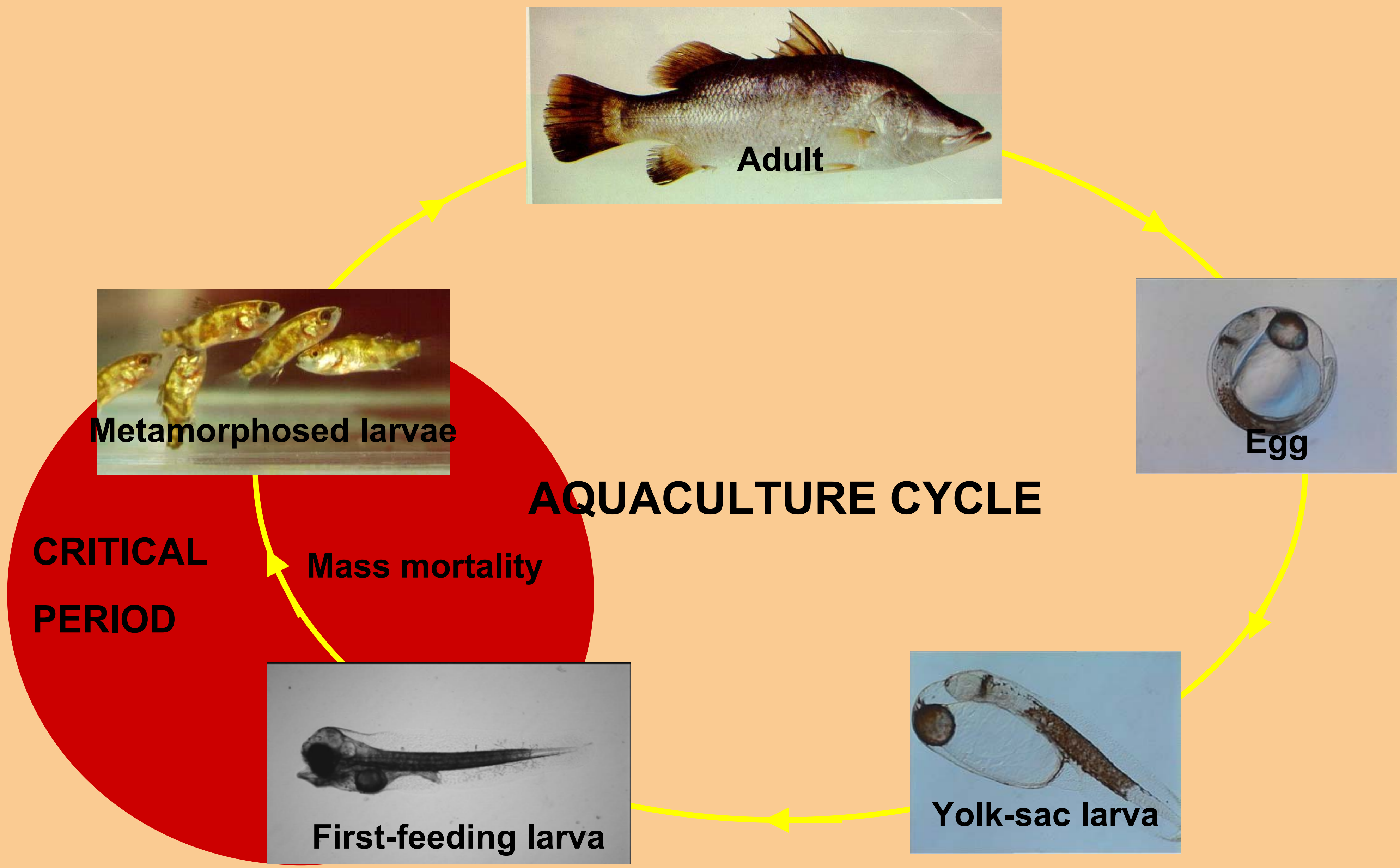
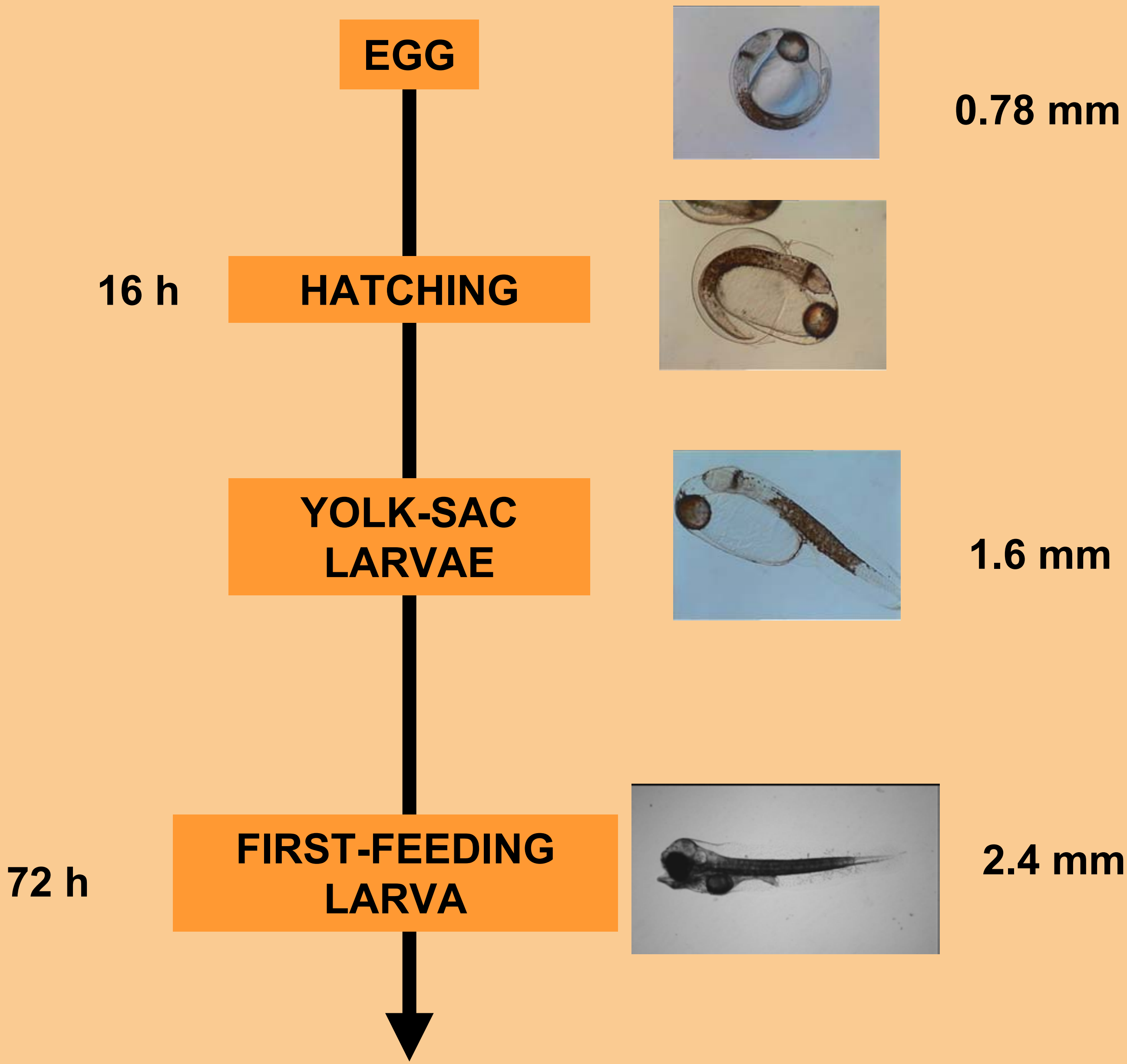
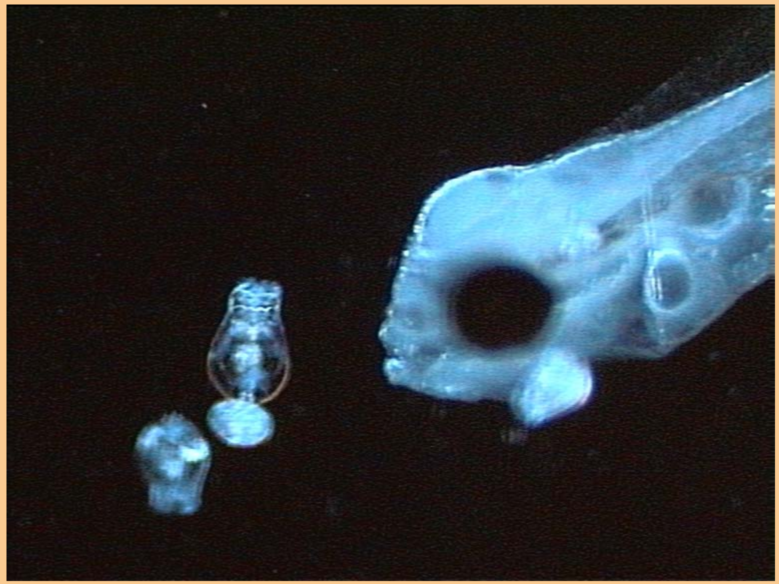

Adult
Egg
Yolk-sac larva
First-feeding larva
Metamorphosed larvae
AQUACULTURE CYCLE
CRITICAL
PERIOD
Mass mortality

EARLY DEVELOPMENTAL STAGES OF SEABASS

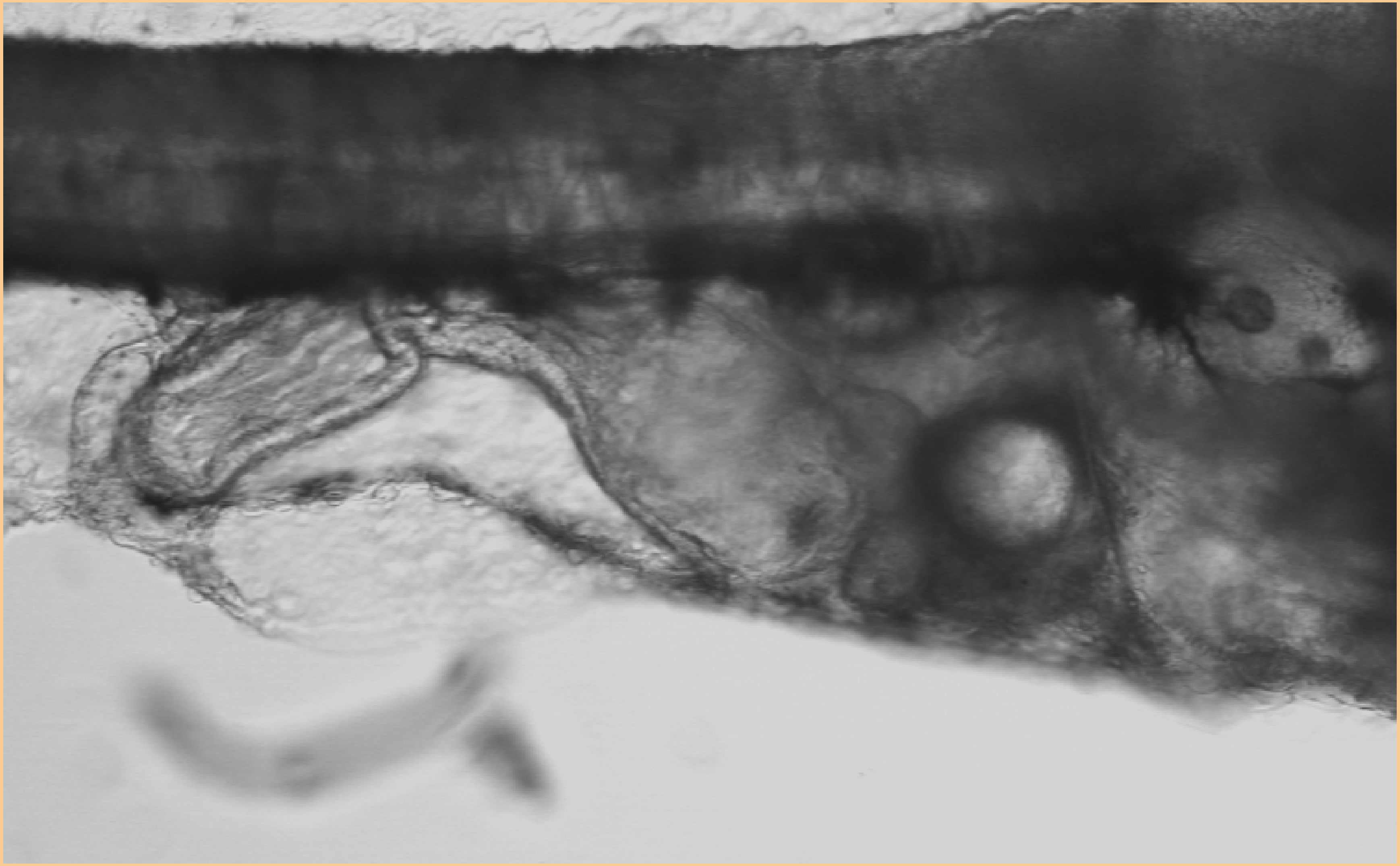


CRITICAL PERIOD

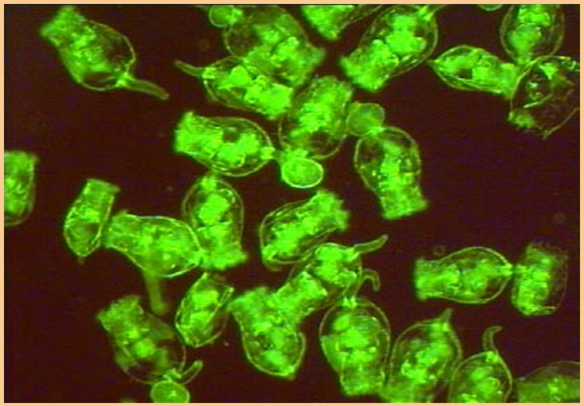


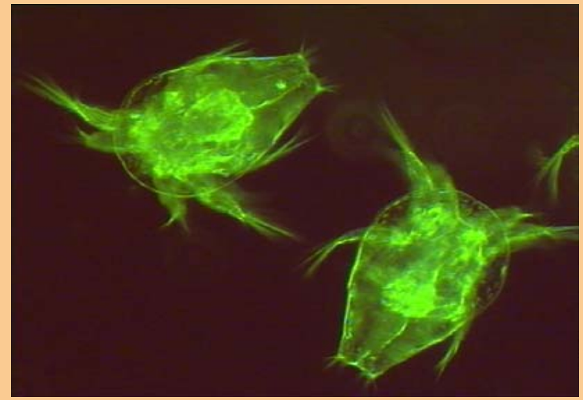
The transition from endogenous to exogenous feeding is a **CRITICAL PERIOD** during the early larval development

At first-feeding, larval gut is relatively simple and lacks a functional stomach



Larvae begin exogenous feeding on invertebrate zooplanktons - LIVE FEEDS

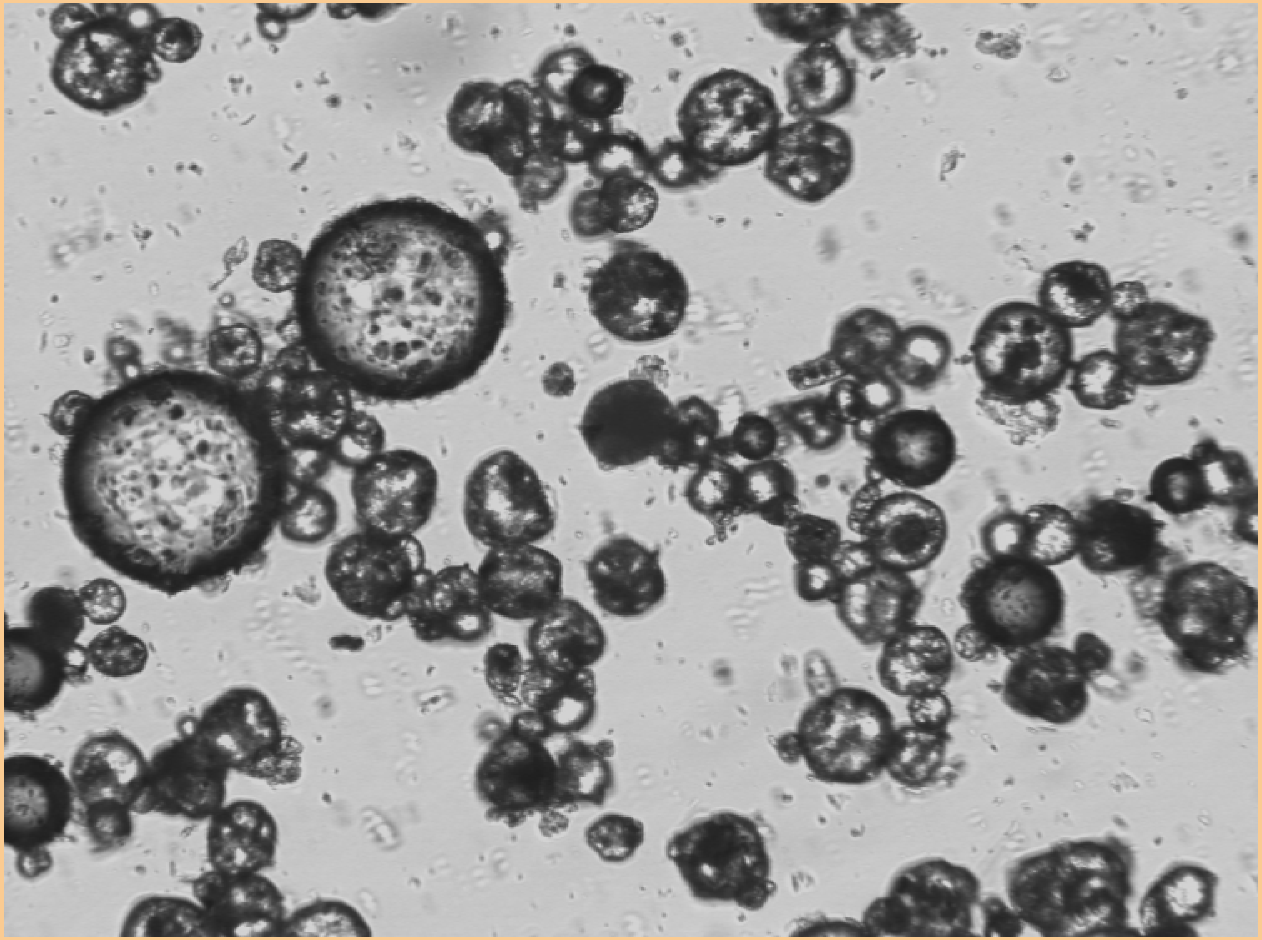




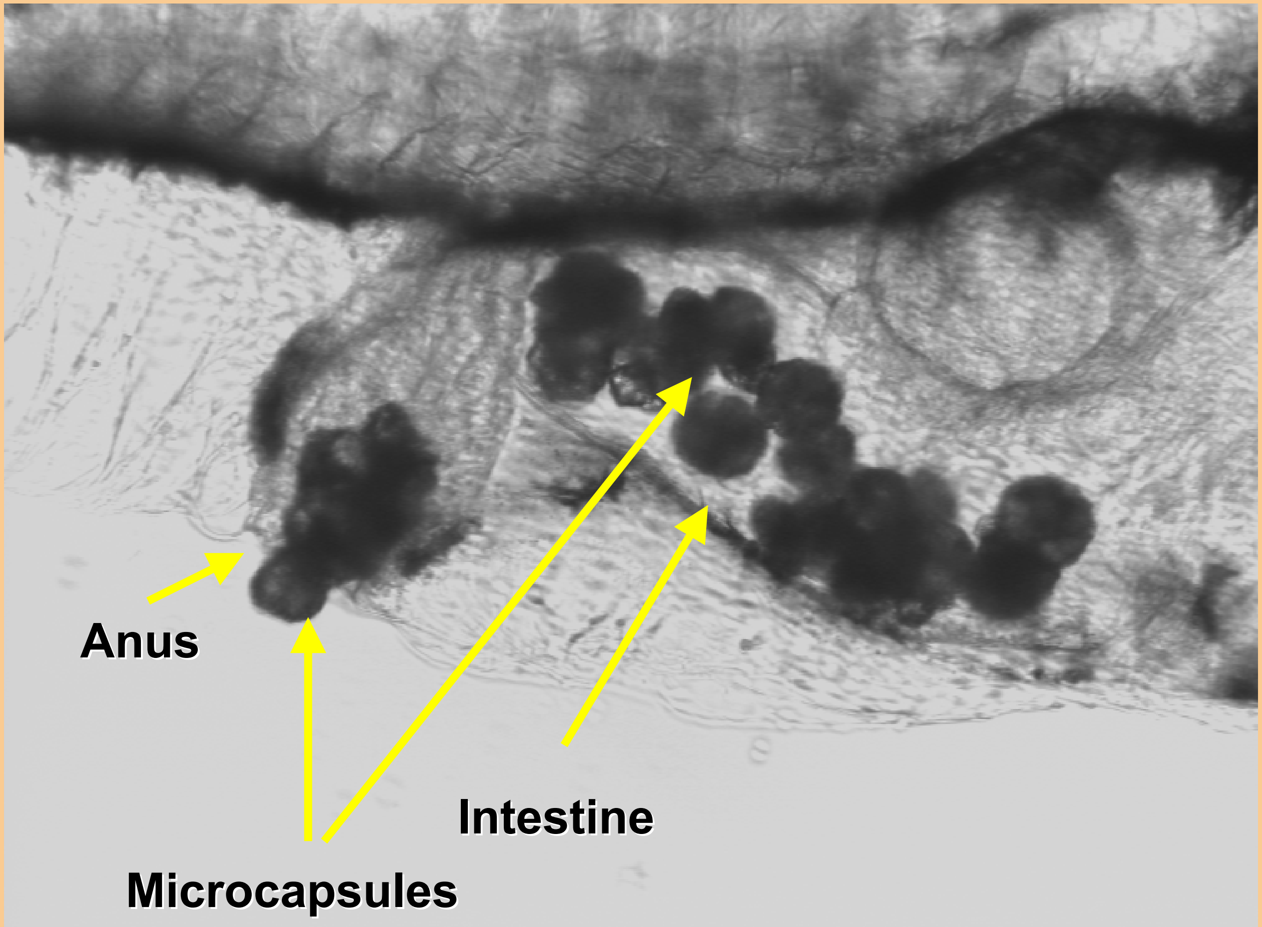
Limitations

- Expensive to produce
- Uncertain supply
- Deficiencies in certain nutrients

Formulated diets?



At first-feeding, the larvae cannot digest crosslinked – protein microencapsulated feed



- **What enzyme genes are expressed/not expressed at different stages of larval development?**
- **What turns on/off the secretion of the different enzymes?**
- **What, when and how to feed the larvae?**

Objective of the present study

Ontogenic changes of proteolytic digestive enzymes gene expression in developing seabass larvae

Trypsinogen – Pancreas

Aminopeptidase N – Intestinal brush boarder

Pepsinogen - Stomach

Methodology

1. cDNA library – Constructed from whole gut of juvenile seabass (3 months old)
2. Cloning and sequencing of partial trypsinogen, aminopeptidase N and pepsinogen cDNA fragments

Gene	*Primers*	*Nucleotide positions*	*cDNA product size (bp)*
Trypsinogen	**LcTrypsin-F 5'-AACAATGACATCATGCTGATCAAG-3'**	**184 to 207**	**252**
	LcTrypsin-R 5'-CAGGTATCCAGCGCAGAACATG-3'	**435 to 414**	
Aminopeptidase N	**LcAmpN-F 5'-GAGAAGACTGGACCCATACT-3'**	**295 to 314**	**211**
	LcAmpN-R 5’-CATGGGAGATGACTGTTGC-3’	**505 to 487**	
Pepsinogen	**LcPepsin-F 5’-GTCCATCCAGTATGGCACTG-3’**	**163 to 182**	**312**
	LcPepsin-R 5’-GTGTAGTGGCTGCTGTCCAC-3’	**474 to 415**	

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GGCTACCACTTCTGCGGCGGCTCCCTGGTCAGCGAGAACTGGGTTGTGTCTGCTGCTCAC   60
G  Y  H  F  C  G  G  S  L  V  S  E  N  W  V  V  S  A  A  H     20
TGCTACAAGTCCCGTGTGGAGGTGCGTCTGGGCGAGCACAACATCAGGGTCACTGAGGGA  120
C  Y  K  S  R  V  E  V  R  L  G  E  H  N  I  R  V  T  E  G     40
ACCGAACAGTTCATCAGCTCCTCCCGTGTCATCCGCCACCCCAACTACAGCTCCTACAAC  180
T  E  Q  F  I  S  S  S  R  V  I  R  H  P  N  Y  S  S  Y  N     60
ATCAACAATGACATCATGCTGATCAAGCTGAGCAAGCCCGCCACCCTCAACCAGTACGTG  240
I  N  N  D  I  M  L  I  K  L  S  K  P  A  T  L  N  Q  Y  V     80
CAGCCCGTGGCTCTGCCCACCAGCTGTGCCCCCGCTGGCACCATGTGCAAAGTCTCTGGC  300
Q  P  V  A  L  P  T  S  C  A  P  A  G  T  M  C  K  V  S  G    100
TGGGGCAACACCATGAGCTCCACTGCTGACAGGAACAAGCTGCAGTGCCTGGACATCCCC  360
W  G  N  T  M  S  S  T  A  D  R  N  K  L  Q  C  L  D  I  P    120
ATCCTGTCCGACAGGGACTGTGATAACTCCTACCCTGGAATGATCACCCAGGCCATGTTC  420
I  L  S  D  R  D  C  D  N  S  Y  P  G  M  I  T  Q  A  M  F    140
TGCGCTGGATACCTGGAGGGAGGCAAGGACTCTTGCCAGGGTGACTCTGGTGGTCCCGTT  480
C  A  G  Y  L  E  G  G  K  D  S  C  Q  G  D  S  G  G  P  V    160
GTGTGCAATGGTGAGCTGCAGGGTGTTGTGTCCTGGGGTCACAGATGTGC            530
V  C  N  G  E  L  Q  G  V  V  S  W  G  H  R  C                176
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Fig.1: 530 bp *L. calcarifer* trypsinogen

TTCCCCTGTTACGACGAGCCGGCTATGAAAGCTGTCTTCCACATGACCCTCATTCACCCC 60
F P C Y D E P A M K A V F H M T L I H P 20
CAGGAGACTGTGGCTCTGTCCAATGCCATGAACTACGACCCTGTTAACATCACTATGGAT 120
Q E T V A L S N A M N Y D P V N I T M D 40
GGCCAGAAATTAATCCAGACAAGATTTGAACCAACAGAGATTATGTCAACGTACTTGCTG 180
G Q K L I Q T R F E P T E I M S T Y L L 60
GCTTTTGTTGTGTGTGATTTTGCCTACATTGGGACGAAACCAGGGGCAGAAGTTTTGATC 240
A F V V C D F A Y I G T K P G A E V L I 80
AGGATCTGGGCTCGCAGGAAGGCCATTGAAGAGGGCCAAGGAAACTATGCCCTT**GAGAAG 300**
R I W A R R K A I E E G Q G N Y A L E K 100
ACTGGACCCATACTGTCATTCTTTGAGAACTACTACAACTCTTCCTACCCCCTGACCAAA 360
T G P I L S F F E N Y Y N S S Y P L T K 120
TCAGACCAGATCGCTCTTCCTGACTTCAGTGCTGGAGCGATGGAGAACTGGGGCCTGATC 420
S D Q I A L P D F S A G A M E N W G L I 140
ACCTACAGAGAGACTGCCCTCTTATATAATCCTGACGTGTCGTCCAATGGAGACAAGGAA 480
T Y R E T A L L Y N P D V S S N G D K E 160
TGGGTG**GCAACAGTCATCTCCCATGAGCTCGCTCATATGTGGTTCGGCAACCTGGTTAC** 539
W V A T V I S H E L A H M W F G N L V 179

Fig.2: 539 bp *L. calcarifer* aminopeptidase N

ATCTCCATCGGGACCCCCCCCCAGTCCTTCAGCGTCATCTTTGACACCGGCTTCTTCC 58
S P S G P P P S P S A S S L T P A S S 19
AACCTCTGGATCCCCTCAGTCTACTGCTCCAGCCAGGCCTGTCAGAACCACAGGAGATTC 118
N L W I P S V Y C S S Q A C Q N H R R F 39
AACCCACAGCAGTCCTCCACCTTCCACTGGGGCAACCAGCCTCT**GTCCATCCAGTATGGC 178**
N P Q Q S S T F H W G N Q P L S I Q Y G 59
ACTGGCAGCATGACTGGATATCTGGCCAGTGACACTGTTGAGGTGGGCGGCCTCTCTGTG 238
T G S M T G Y L A S D T V E V G G L S V 79
GCCAACCAGGTGTTTGGAATTAGCCAGACAGAGGCTCCCTTCATGGCCCACATGCAGGCT 298
A N Q V F G I S Q T E A P F M A H M Q A 99
GACGGTATCCTGGGACTGGCCTTCCAGAGCATTGCTTCTGACAATGTCGTGCCTGTCTTT 358
D G I L G L A F Q S I A S D N V V P V F 119
GACAACATGATCAAGCAGGGACTTGTGTCCCAGCCCATGTTCTCCGTCTACCTGAGCGGC 418
D N M I K Q G L V S Q P M F S V Y L S G 139
AACAGTGAGCAGGGCAGTGAGGTTGTCTTCGGTGGT**GTGGACAGCAGCCACTACACTGGA 478**
N S E Q G S E V V F G G V D S S H Y T G 159
CAAGTCACCTGGATCCCTCTGACCTCTGCCACCTACTGGCAGATCAAAATGGACAGTGTT 538
Q V T W I P L T S A T Y W Q I K M D S V 179
ACCATCAATGGACAGACTGTGGCCTGCTCTGGTGGCTGCCAGGCCATCATTGACACTGGT 598
T I N G Q T V A C S G G C Q A I I D T G 199
ACCTCCCTGATTGGTGGTCCAACCAATGACATCAACAACATGAATGCCTGGGTTGGAGCC 658
T S L I G G P T N D I N N M N A W V G A 219
TCAACCAACCAGTACGGAGAGGCCACAGTGAACTGCCAGAACATCCAGAGCATGCCCGAC 718
S T N Q Y G E A T V N C Q N I Q S M P D 239
GTCACCTTCACTCTCAACGGACACGCCTTCACTGTCCCTGCATCTGCCTACGTCTCTCAG 778
V T F T L N G H A F T V P A S A Y V S Q 259
AACTACTACGGTTGCAACACTGGCTTTGGCCAGGGTGGCTCTGACCAGCTCTGGATCC 836
N Y Y G C N T G F G Q G G S D Q L W I 278

Fig.3: 836 bp *L. calcarifer* pepsinogen

Relative semi-quantitative gene expression analysis

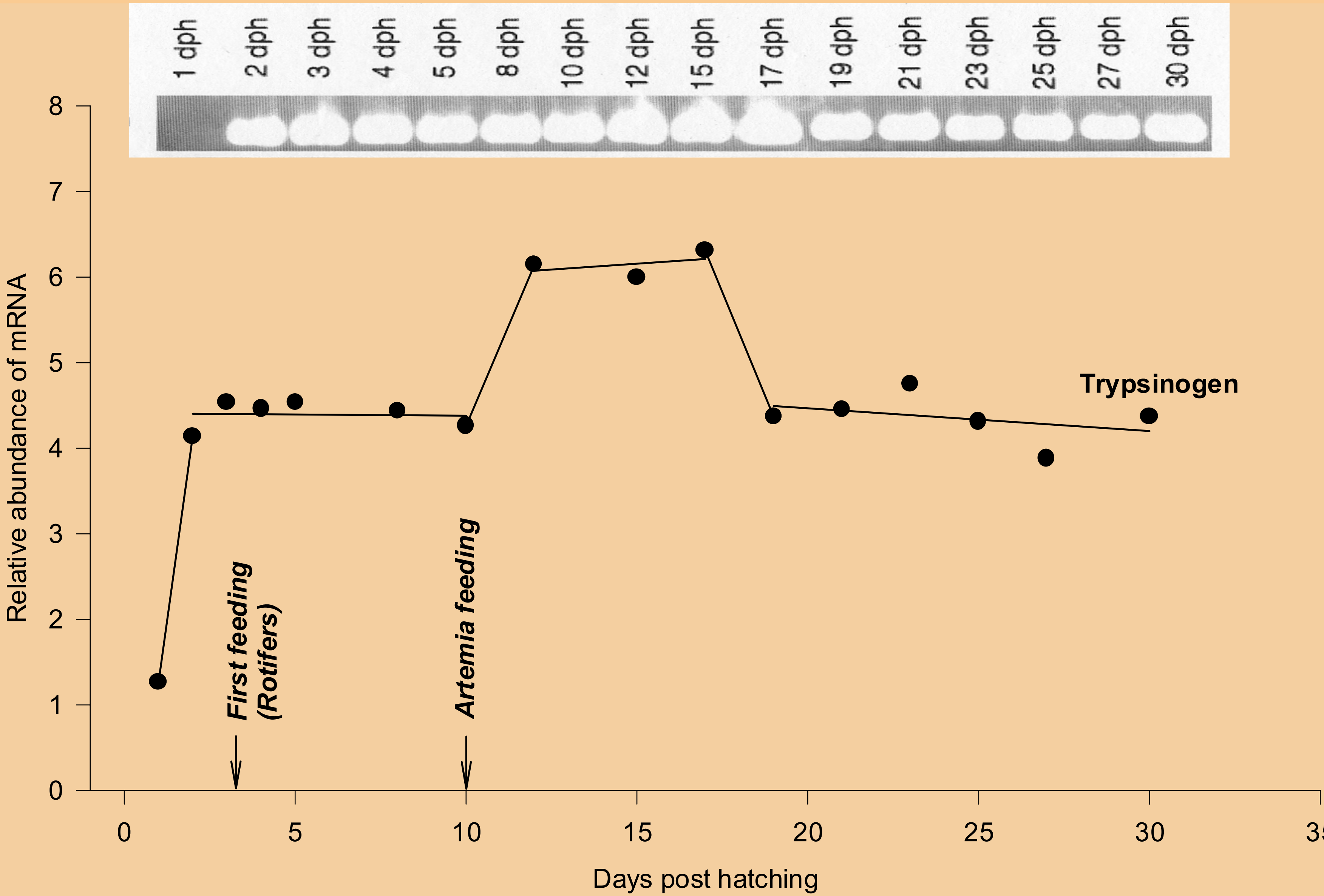
- Newly hatched seabass larvae were stocked in 300 l fiber-glass tanks
- Larvae were fed with rotifers from 3 days post hatching (dph)
- From 10 dph onwards larvae were fed with Artemia nauplii until 30 dph
- Larvae were sampled at frequent intervals for mRNA extraction

Total RNA from the larval samples was extracted using TRI Reagent and 1 mg was reverse transcribed in a total volume of 10 ml.

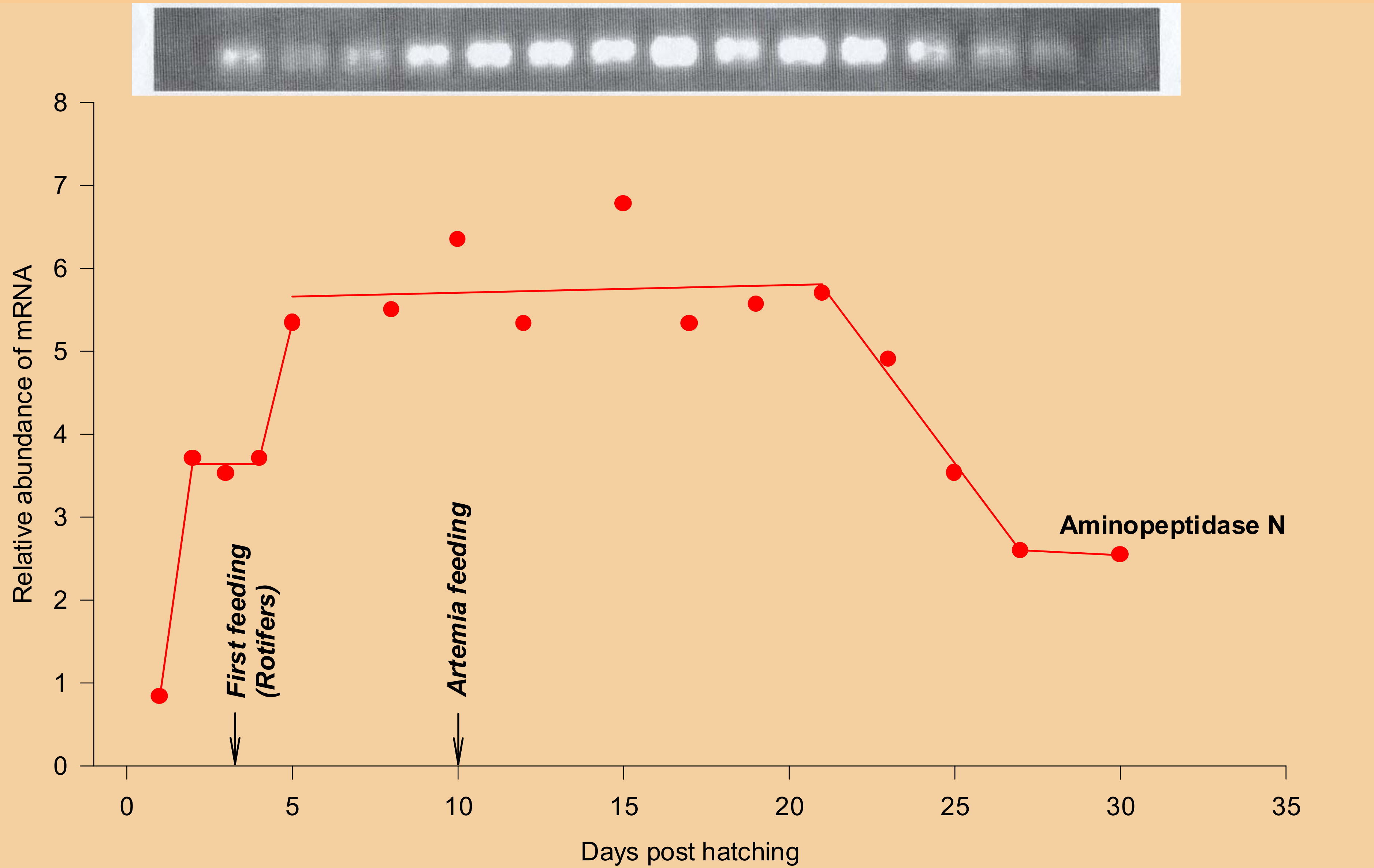
PCR amplification was performed on 0.5 ml cDNA using trypsinogen, aminopeptidase N, and pepsinogen specific intron-flanking oligonucleotide primers.

PCR products were run on ethidium bromide stained agarose gel and the band volume measured using a Gel-Doc2000 system and Quality one software (Biorad).

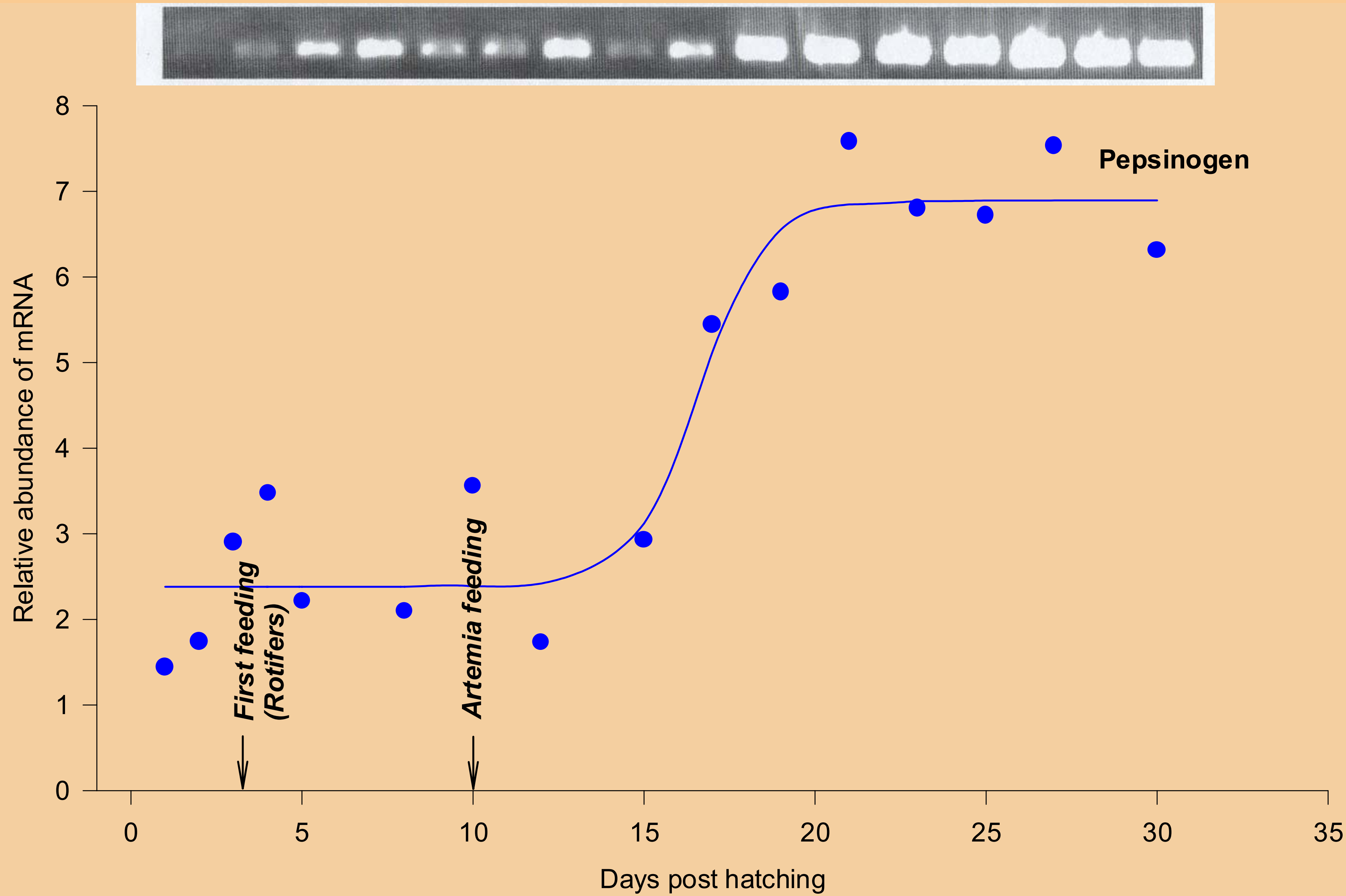
Trypsinogen



Aminopeptidase N



Pepsinogen



Pepsinogen
Relative abundance of mRNA
Days post hatching
First feeding (Rotifers)
Artemia feeding
0
1
2
3
4
5
6
7
8
0
5
10
15
20
25
30
35

Pepsinogen gene expression at different stages of gut development

❖ In 10 dph larvae gene expression was detected in the intestinal segment only.

❖ In 15 dph larvae it was detected in the anterior part of the intestinal segment but not in the posterior part.

❖ In 30 dph larvae it was only found in the stomach.

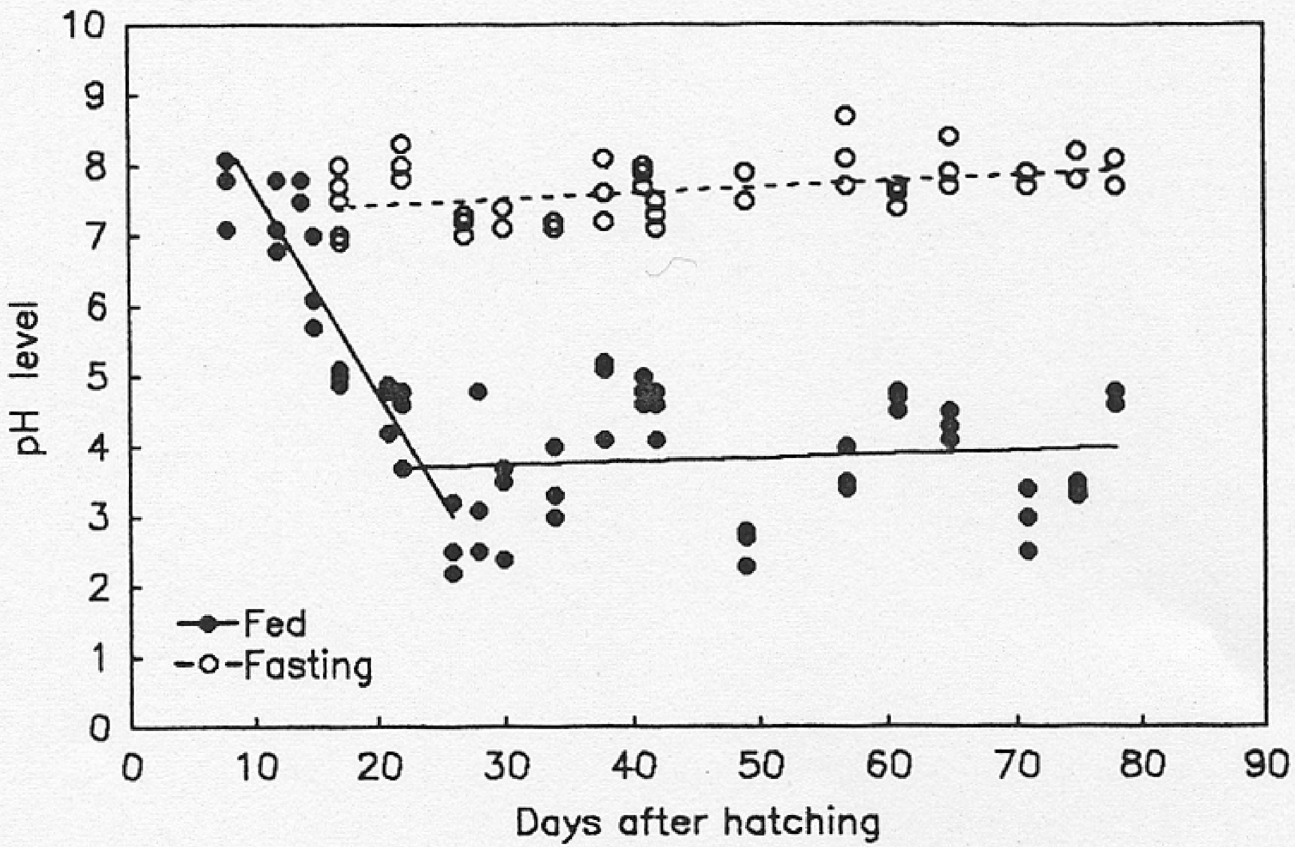


Fig. 8. The pH level in the anterior part of the digestive tube of fed and fastig seabass larvae and juveniles (measured in the presumptive stomach of early larvae before the stomach was formed and in the stomach of older larvae and juveniles).

Walford & Lam, 1993

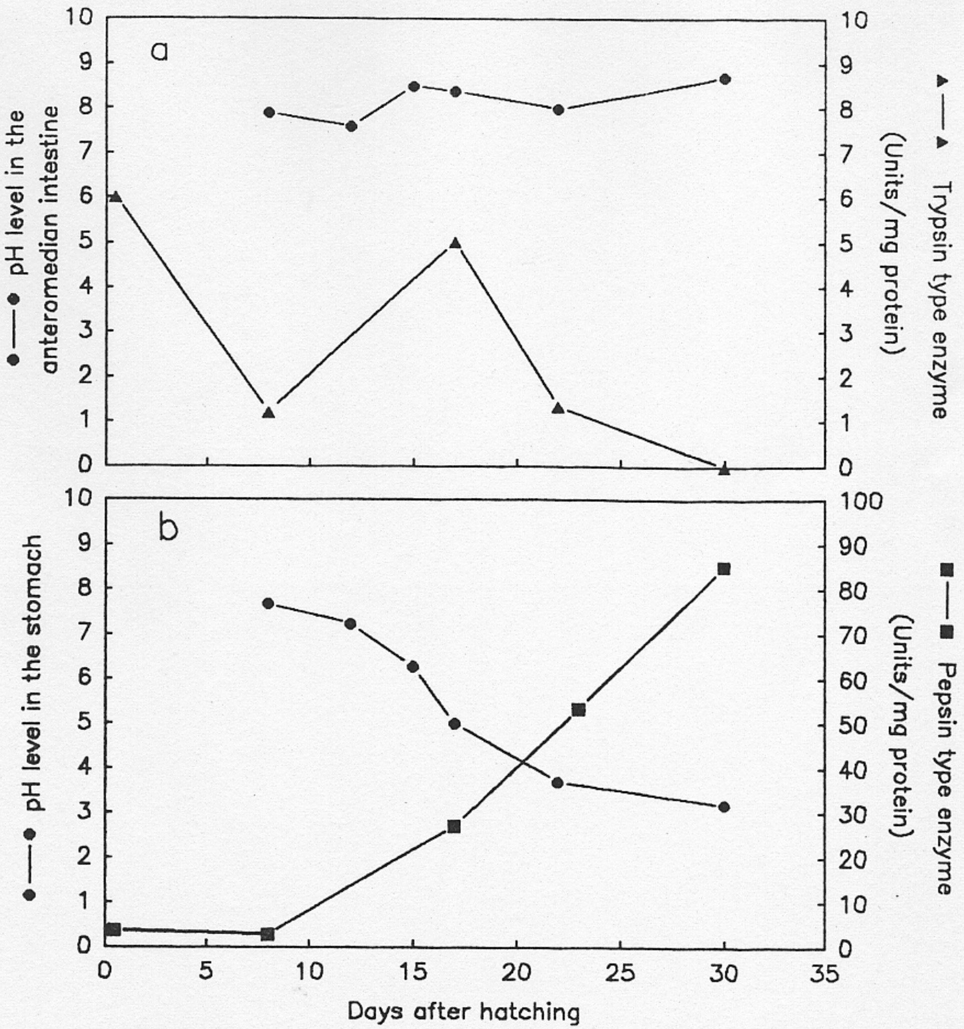
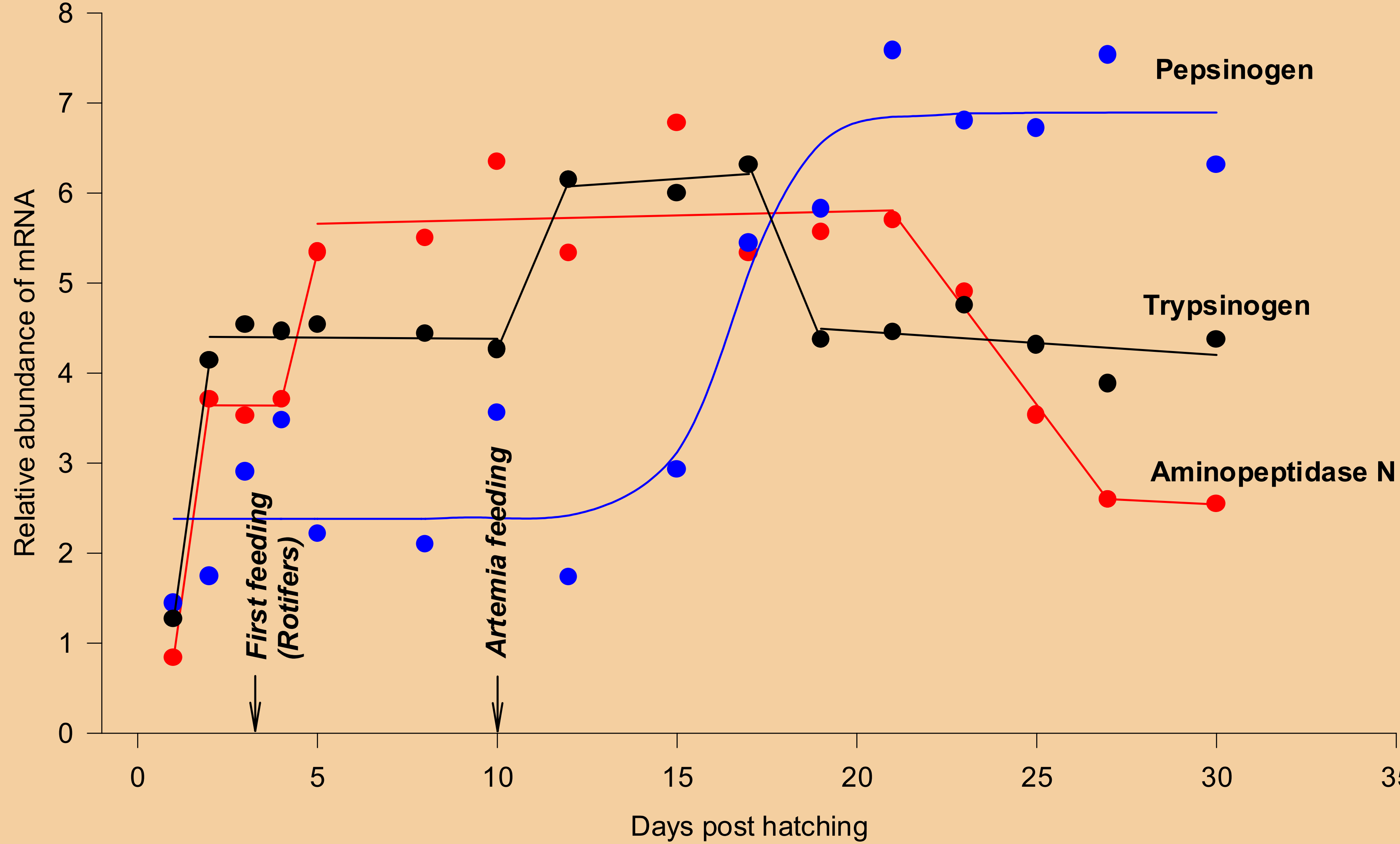


Fig. 11. Changes in the pH level in the gut of fed seabass larvae and juveniles and in the trypsin-type and pepsin-type enzyme activities in the homogenates of whole larvae and juveniles from just after hatching to 30 days after hatching: (a) pH level in the antero-median intestine and trypsin-type enzyme activity; (b) pH level in the stomach (or in the presumptive stomach before the stomach was formed) and pepsin-type enzyme activity (1 enzyme unit is equivalent to 1 μg tyrosine released in 1 min at 37°C).

Walford & Lam, 1993


Pepsinogen
Trypsinogen
Aminopeptidase N
First feeding (Rotifers)
Artemia feeding
Relative abundance of mRNA
Days post hatching
0
1
2
3
4
5
6
7
8
0
5
10
15
20
25
30
35

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