EFFECTS OF T3 & CORTISOL ON DIGESTIVE ENZYMES GENE EXPRESSION IN DEVELOPING SEABASS (*LATES CALCARIFER*) LARVAE

S H Tan[1], B Sivaloganathan[1], P K Reddy[1] & T J Lam[2]

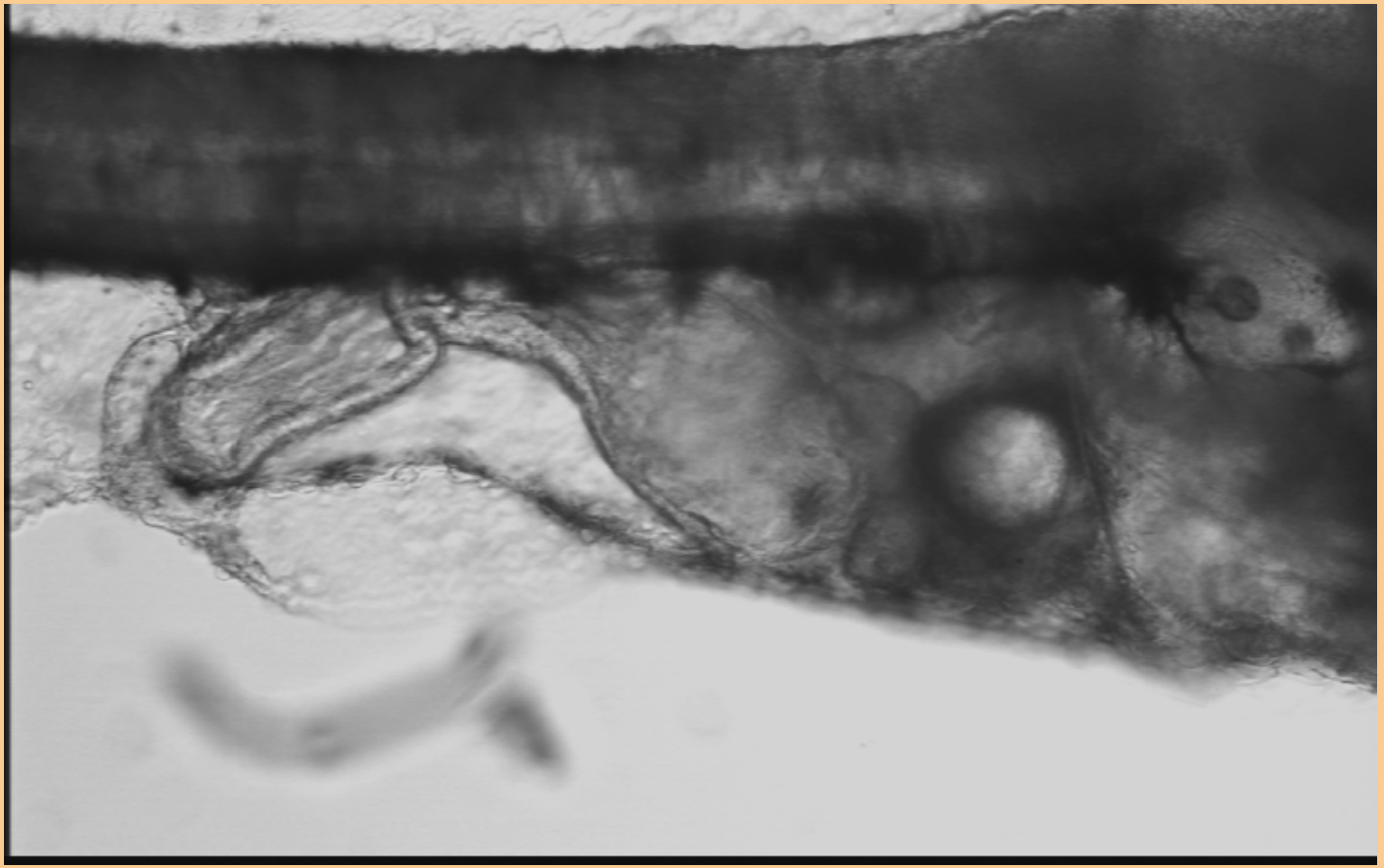
[1] Tropical Marine Science Institute & [2]Department of Biological Sciences, National University of Singapore, Singapore.

AQUACULTURE CYCLE

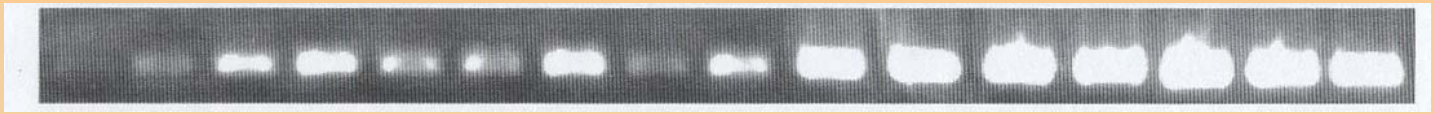
CRITICAL PERIOD

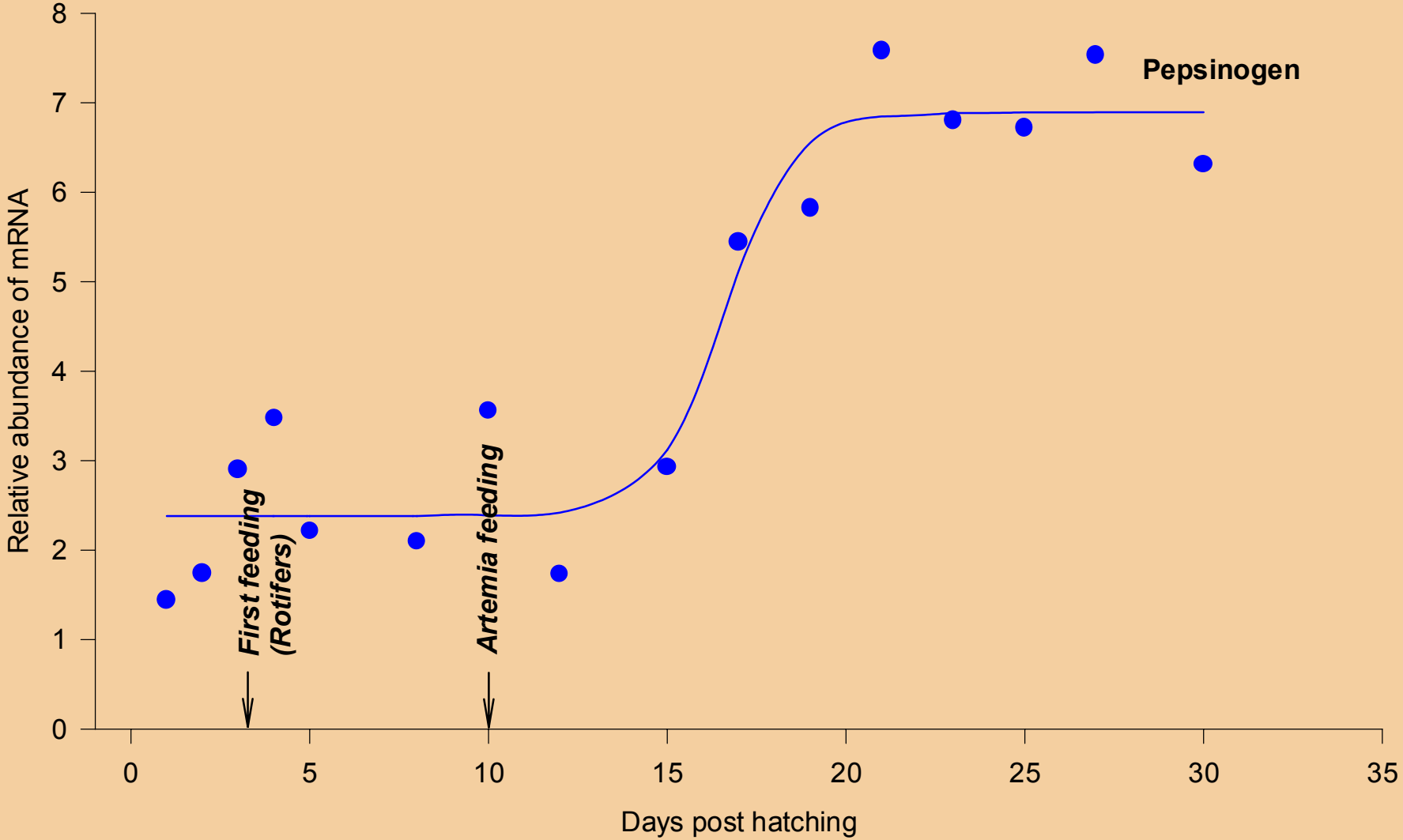
Mass mortality

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



- No acid secretion
- No acid protease secretion (pepsin)
- Unable to digest protein efficiently




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

Exogenous thyroid hormone and cortisol treatment has been shown to accelerate

- **Yolk absorption**
- **Morphogenesis**
- **Growth and survival**
- **Metamorphosis**

Thyroid hormone and cortisol treatment has also been shown to accelerate

- **Differentiation of stomach**
- **Formation of gastric glands**
- **Appearance of pepsinogen**

Objective of the present study

To evaluate the effects of T3 and cortisol on proteolytic digestive enzymes gene expression at two critical stages of larval development

- **First feeding**
- **Metamorphosis**

Materials and methods

Experiment I – First feeding

Newly hatched seabass larvae were distributed into 15, 20 l conical glass tanks.

The larvae were reared in seawater alone or in seawater containing T3 (5 nM and 10 nM) or cortisol (100 nM and 200 nM) with each treatment in triplicate.

The treatments were administered on day 1 post hatching (1 dph) and the media were not replaced until 3 dph.

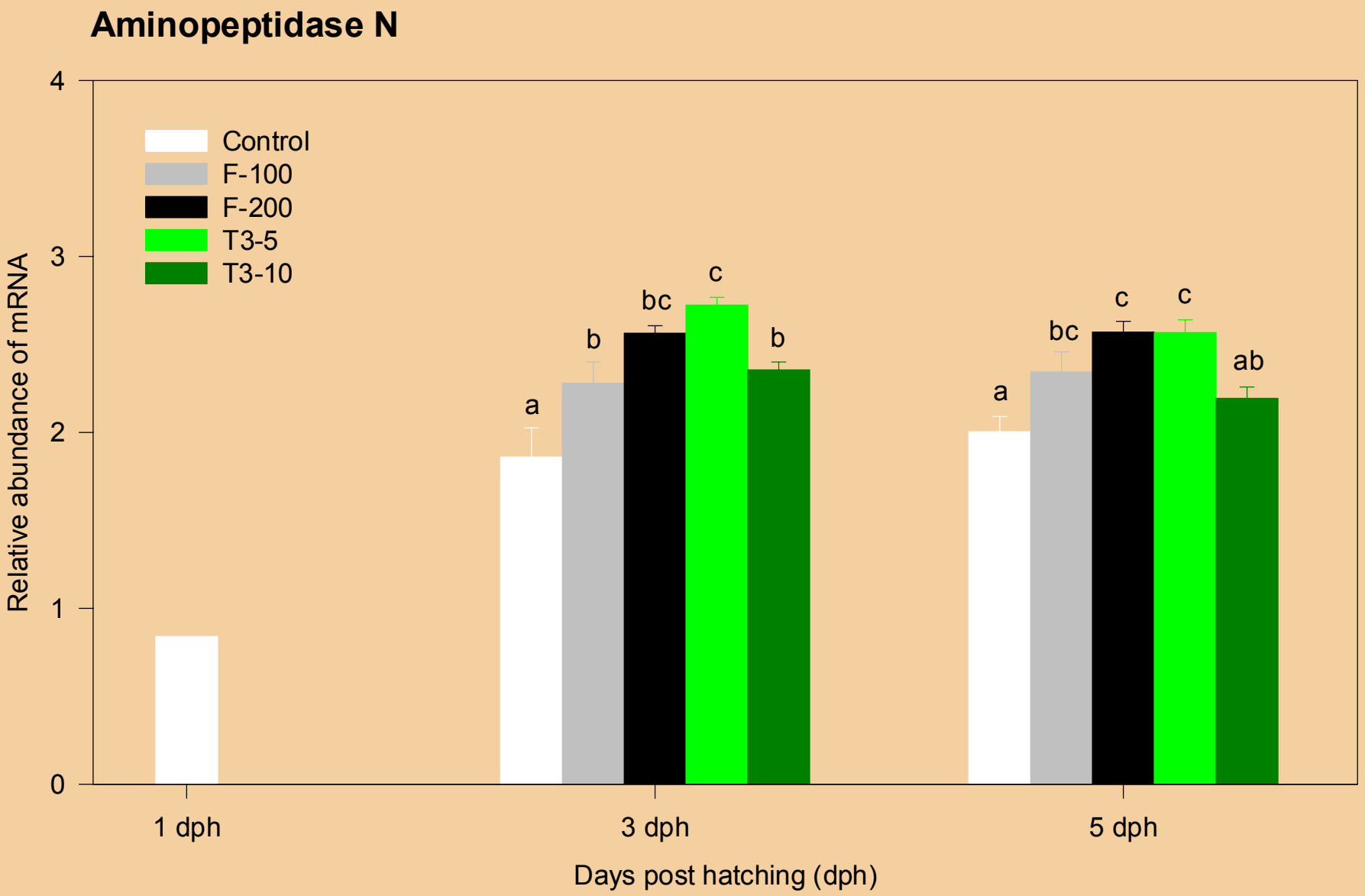
The larvae were fed with rotifers at a density of 15 ml^{-1} from 3 dph onwards.

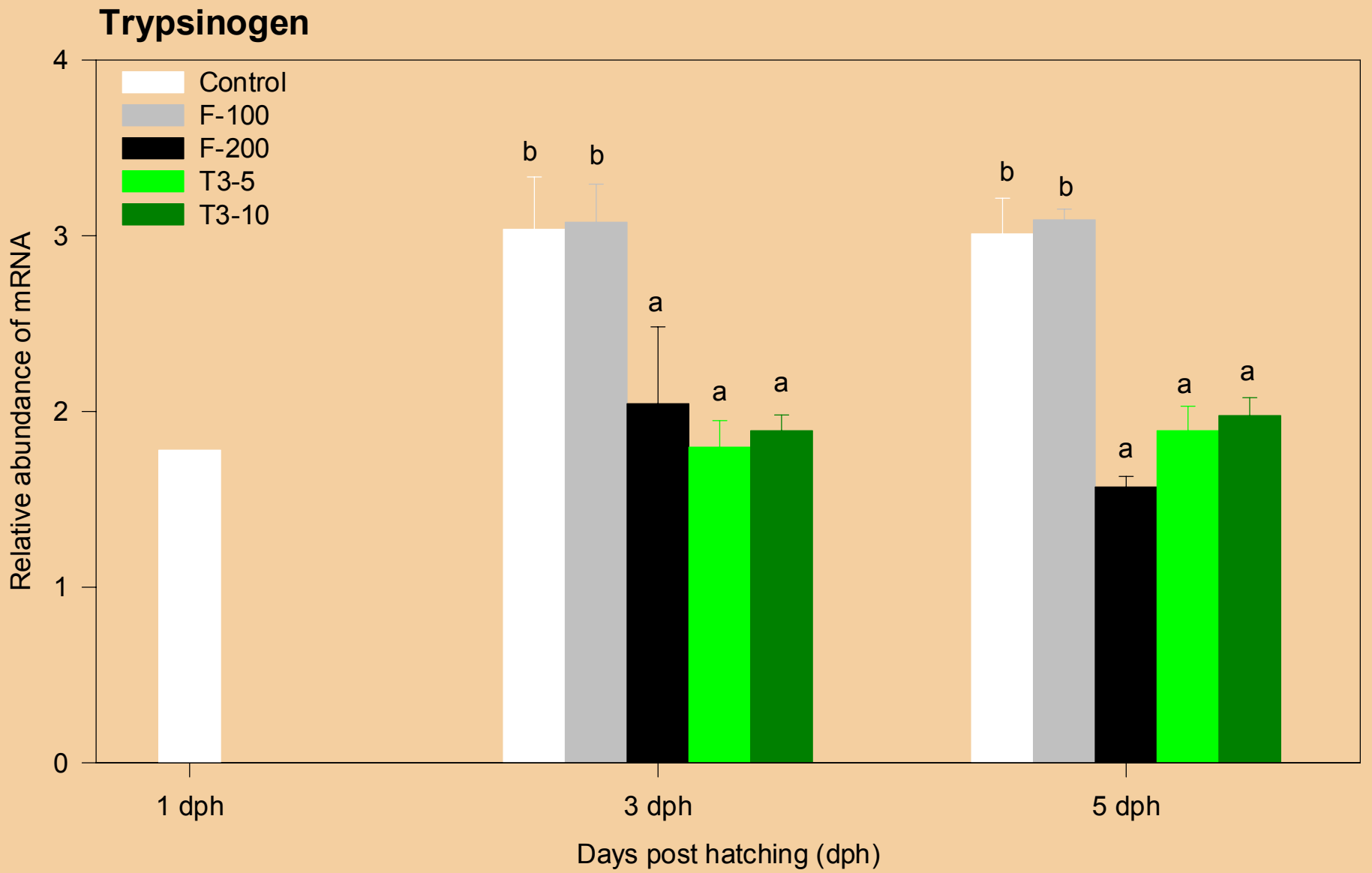
The larval samples were collected on 1,3, and 5 dph for RNA extraction.

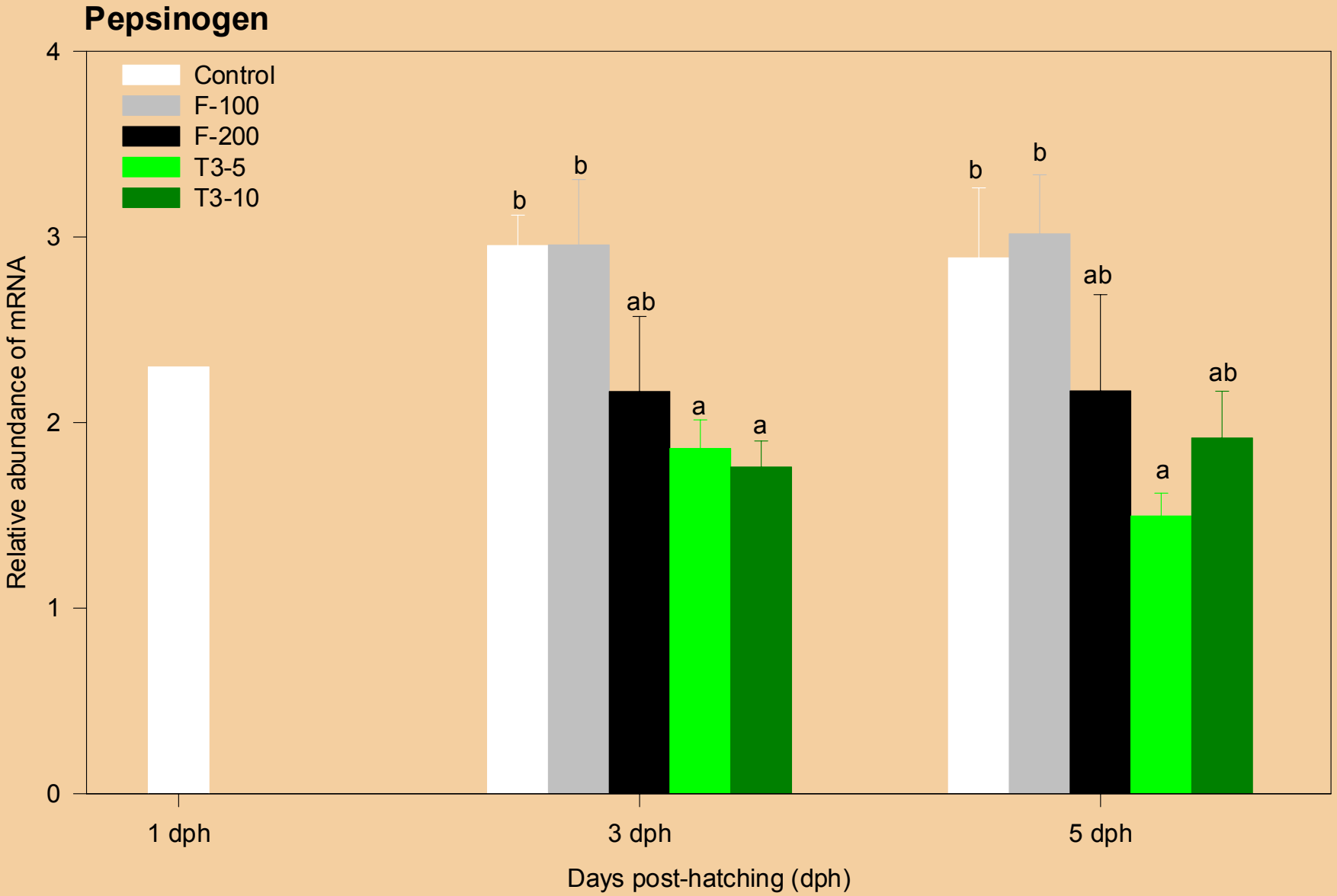
Total RNA from the larval samples was extracted using TRI Reagent and 1 mg was reverse transcribed in a total volume of 10 ml.

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).


Aminopeptidase N
Control
F-100
F-200
T3-5
T3-10
Relative abundance of mRNA
0
1
2
3
4
a
b
bc
c
b
a
bc
c
c
ab
1 dph
3 dph
5 dph
Days post hatching (dph)


Trypsinogen
Control
F-100
F-200
T3-5
T3-10
Relative abundance of mRNA
0
1
2
3
4
b
b
a
a
a
b
b
a
a
a
1 dph
3 dph
5 dph
Days post hatching (dph)


Pepsinogen
Control
F-100
F-200
T3-5
T3-10
Relative abundance of mRNA
0
1
2
3
4
b
b
ab
a
a
b
b
ab
a
ab
1 dph
3 dph
5 dph
Days post-hatching (dph)

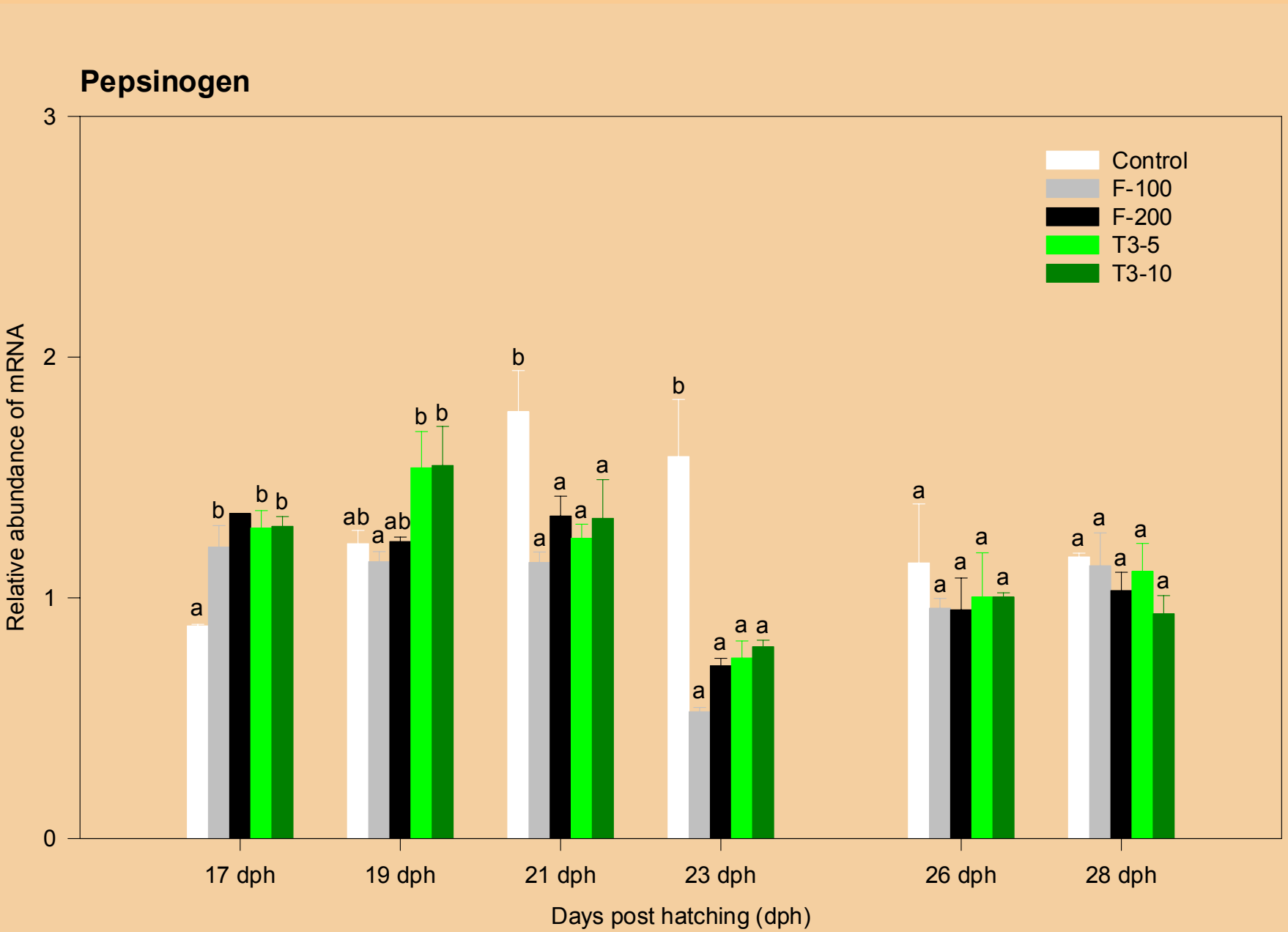
Experiment II – Metamorphosis

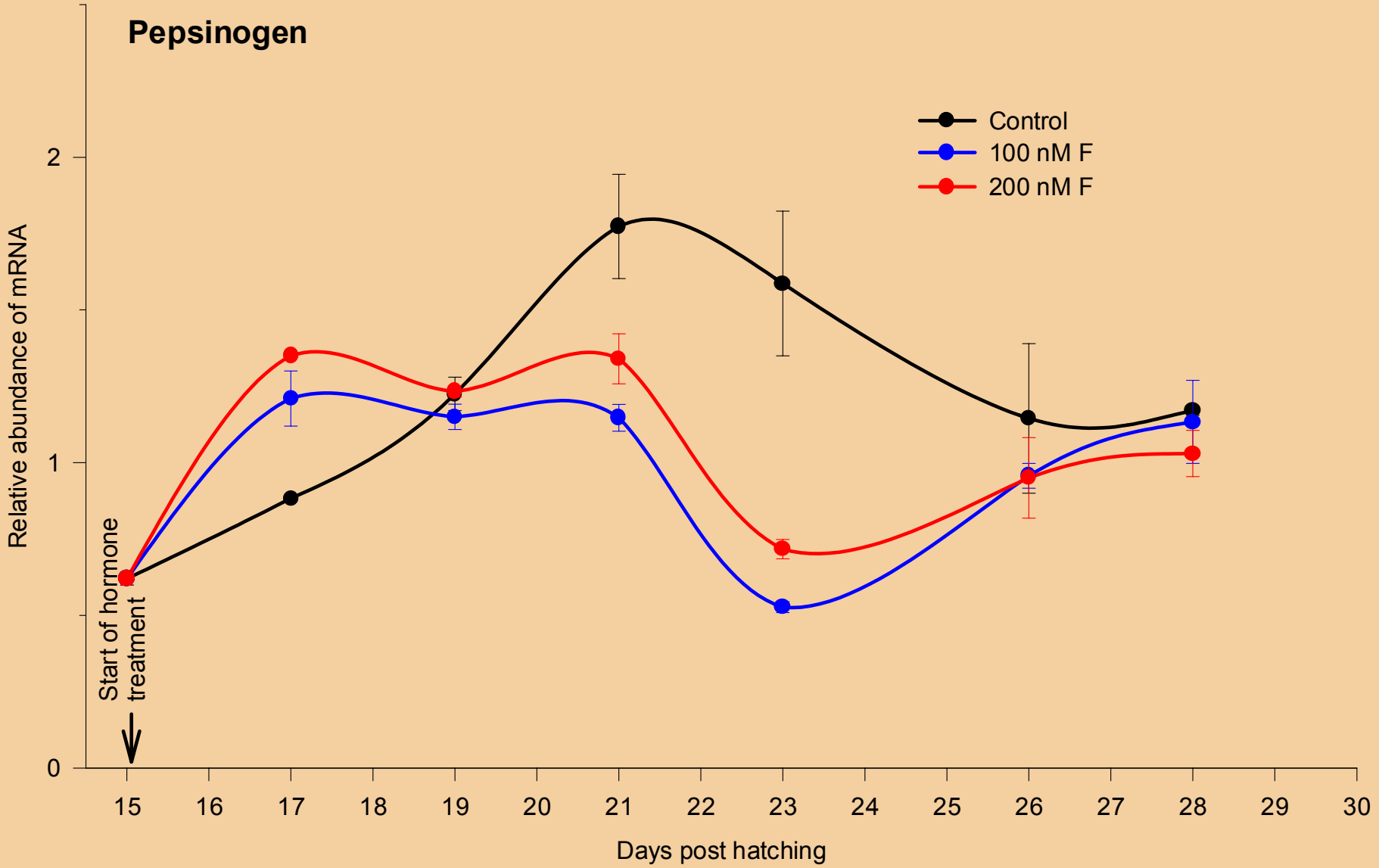
Fifteen-day-old seabass larvae were stocked in 20 l tanks at a density of 15 ml^{-1} and reared in the same treatments as in Experiment I.

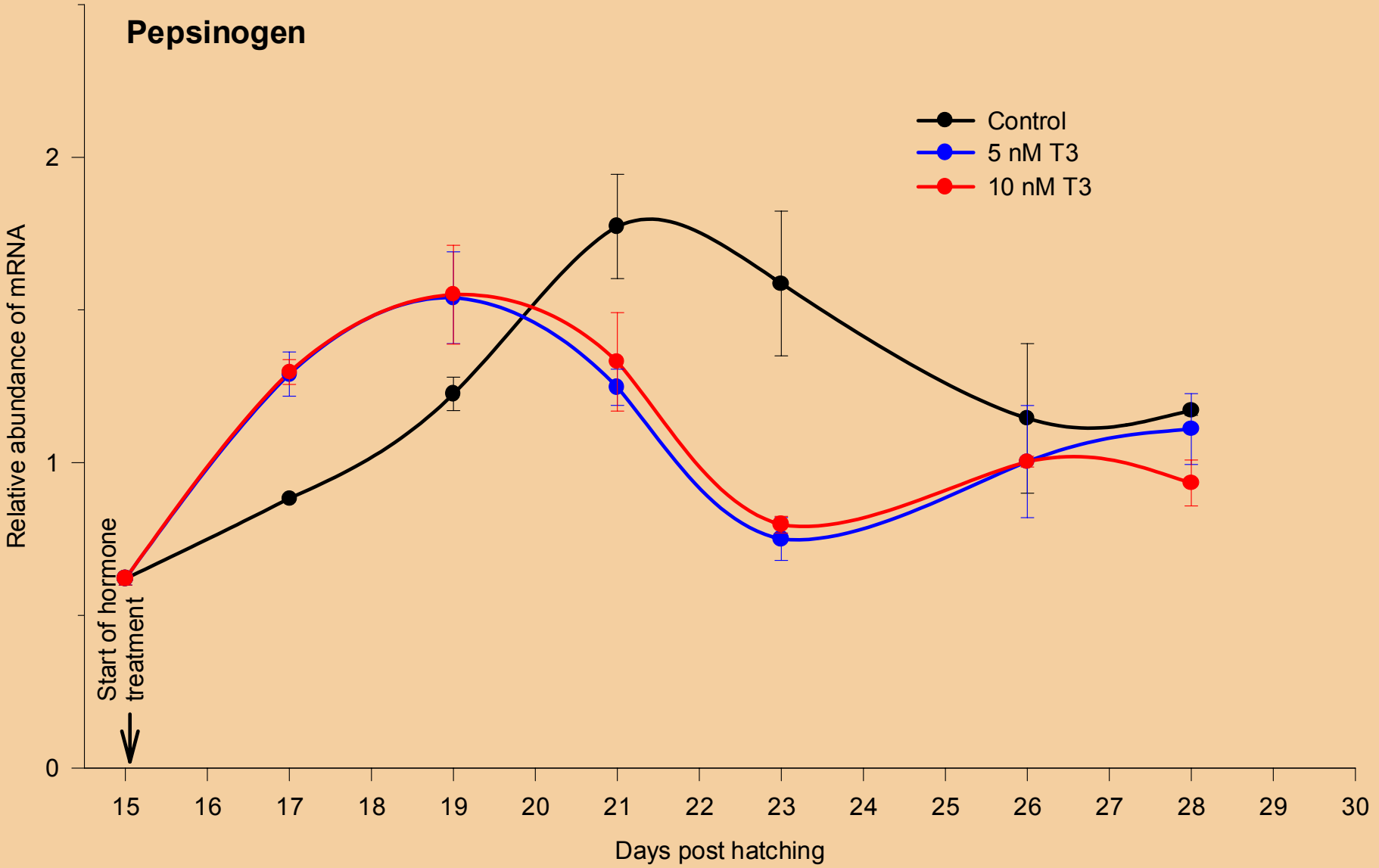
Each treatment was triplicated and the media were changed and replaced with fresh media daily.

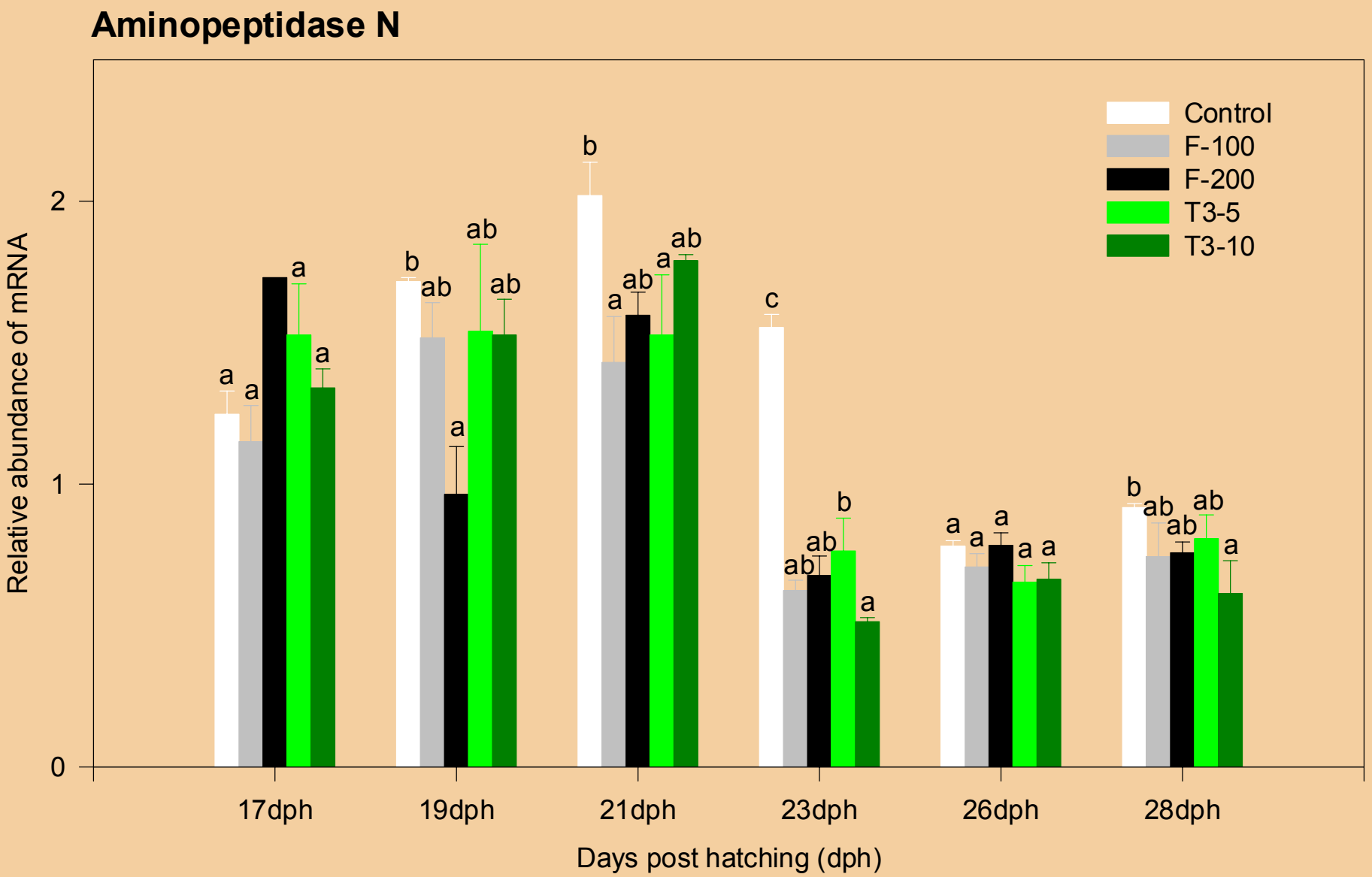
The larvae were fed with *Artemia* nauplii ad libitum daily.

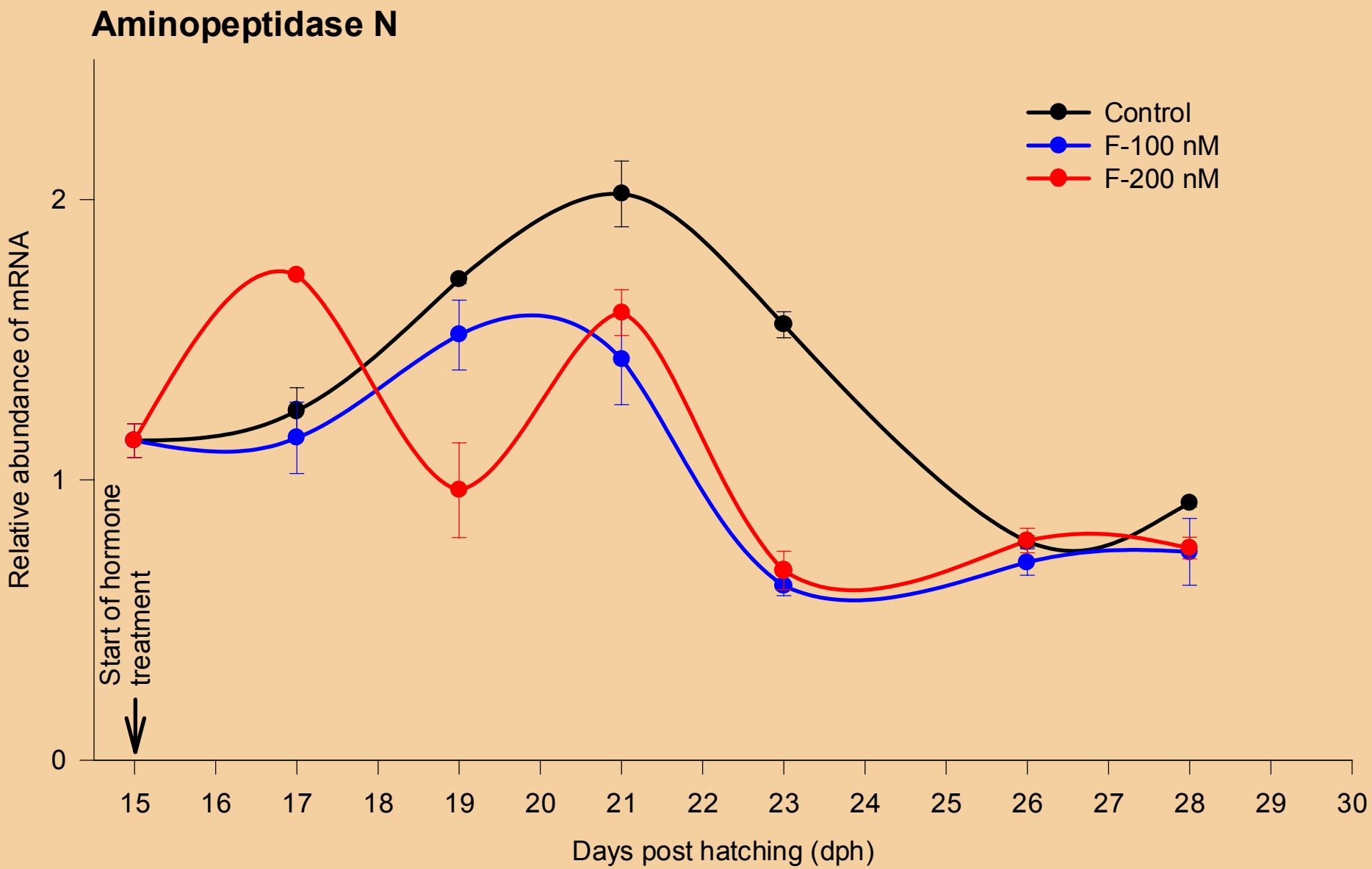
The larva samples for RNA were collected on 15, 17, 21, 23, 26, and 28 dph

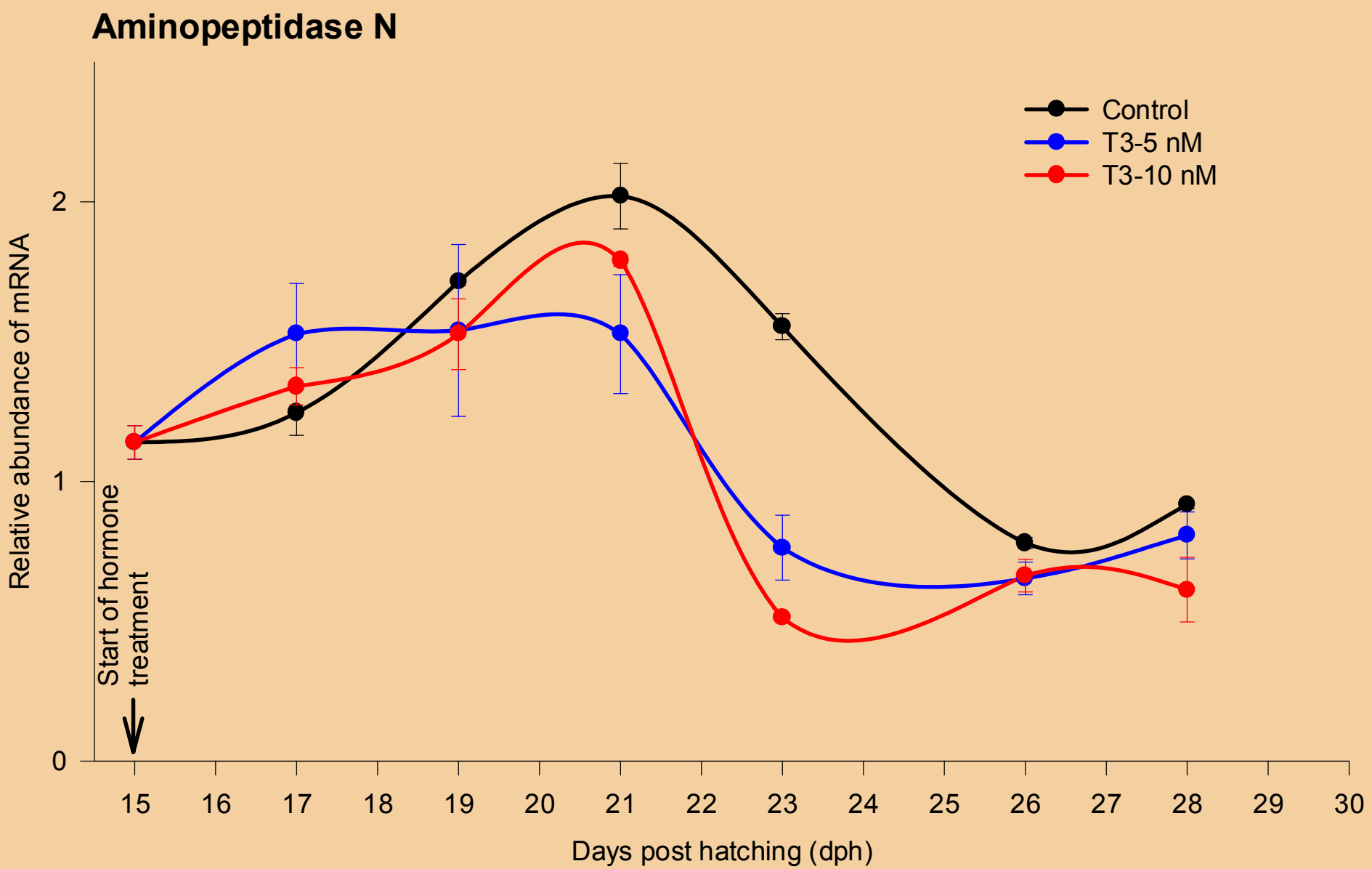

Pepsinogen
Relative abundance of mRNA
Days post hatching (dph)
Control
F-100
F-200
T3-5
T3-10
0
1
2
3
17 dph
19 dph
21 dph
23 dph
26 dph
28 dph
a
b
b
b
ab
a
ab
b
b
b
a
a
a
a
b
a
a
a
b
a
a
a
a
a
a
a
a
a
a
a
a
a
a
a

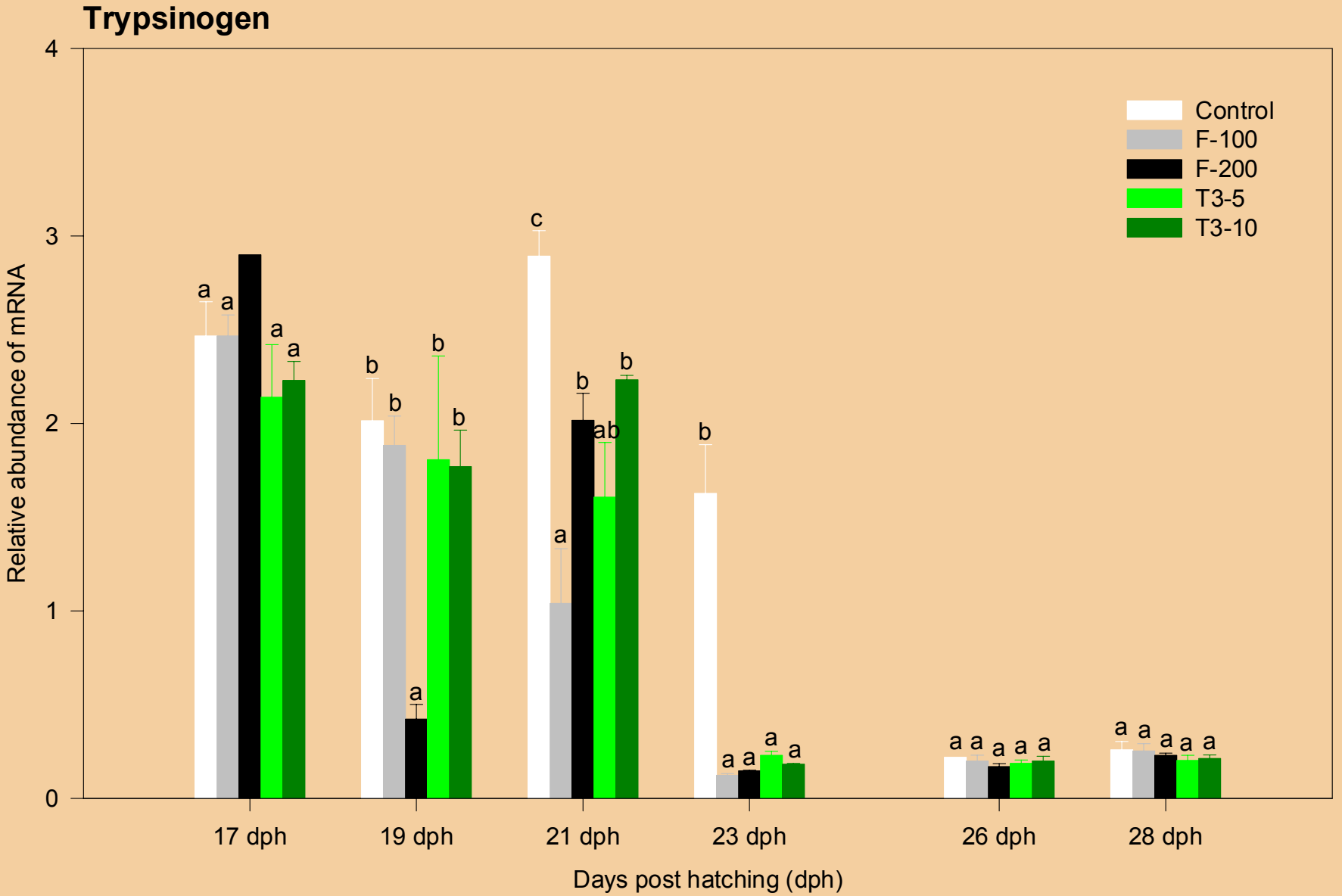

Pepsinogen
Control
100 nM F
200 nM F
Relative abundance of mRNA
0
1
2
Start of hormone treatment
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
Days post hatching

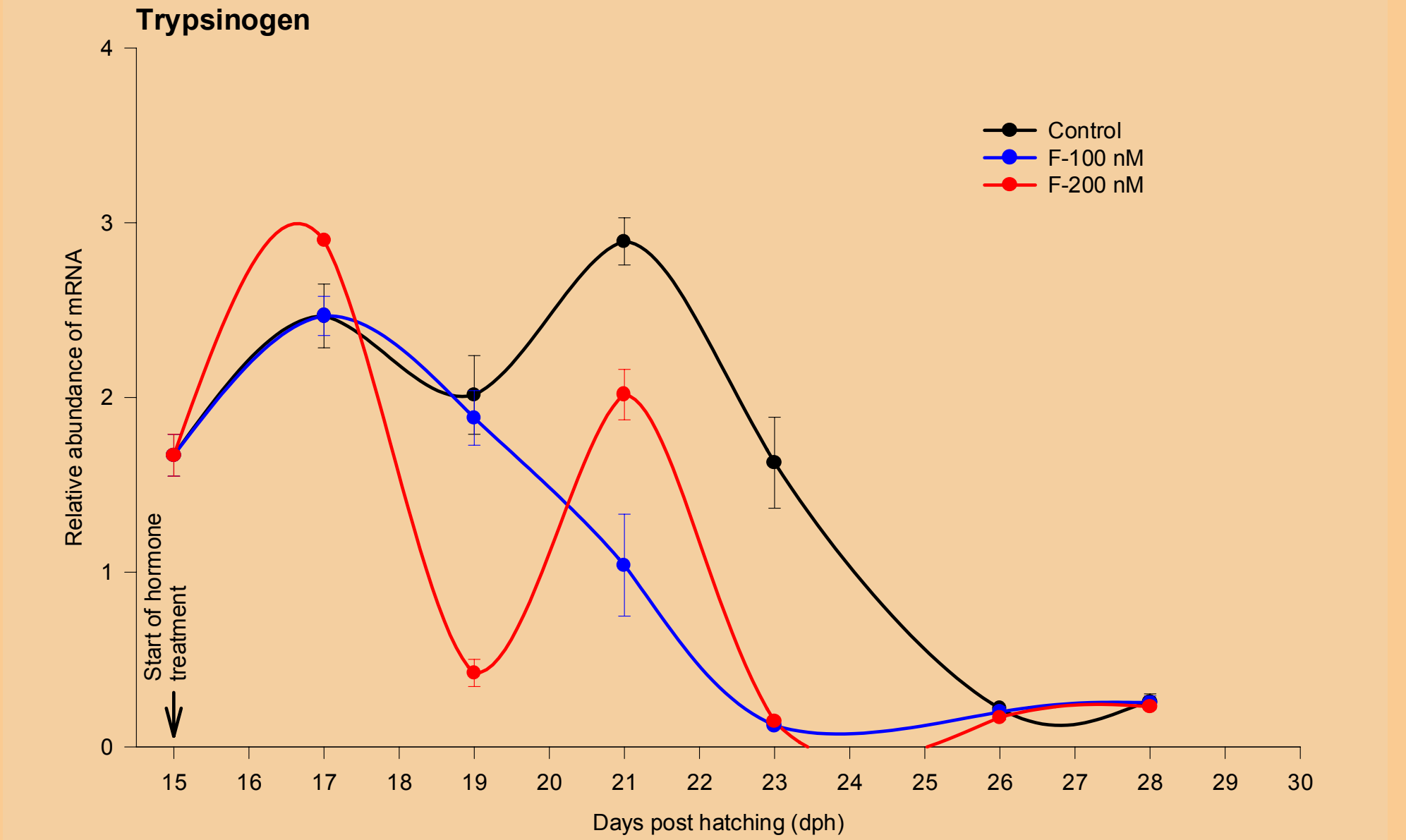

Pepsinogen
Control
5 nM T3
10 nM T3
Relative abundance of mRNA
0
1
2
Start of hormone treatment
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
Days post hatching

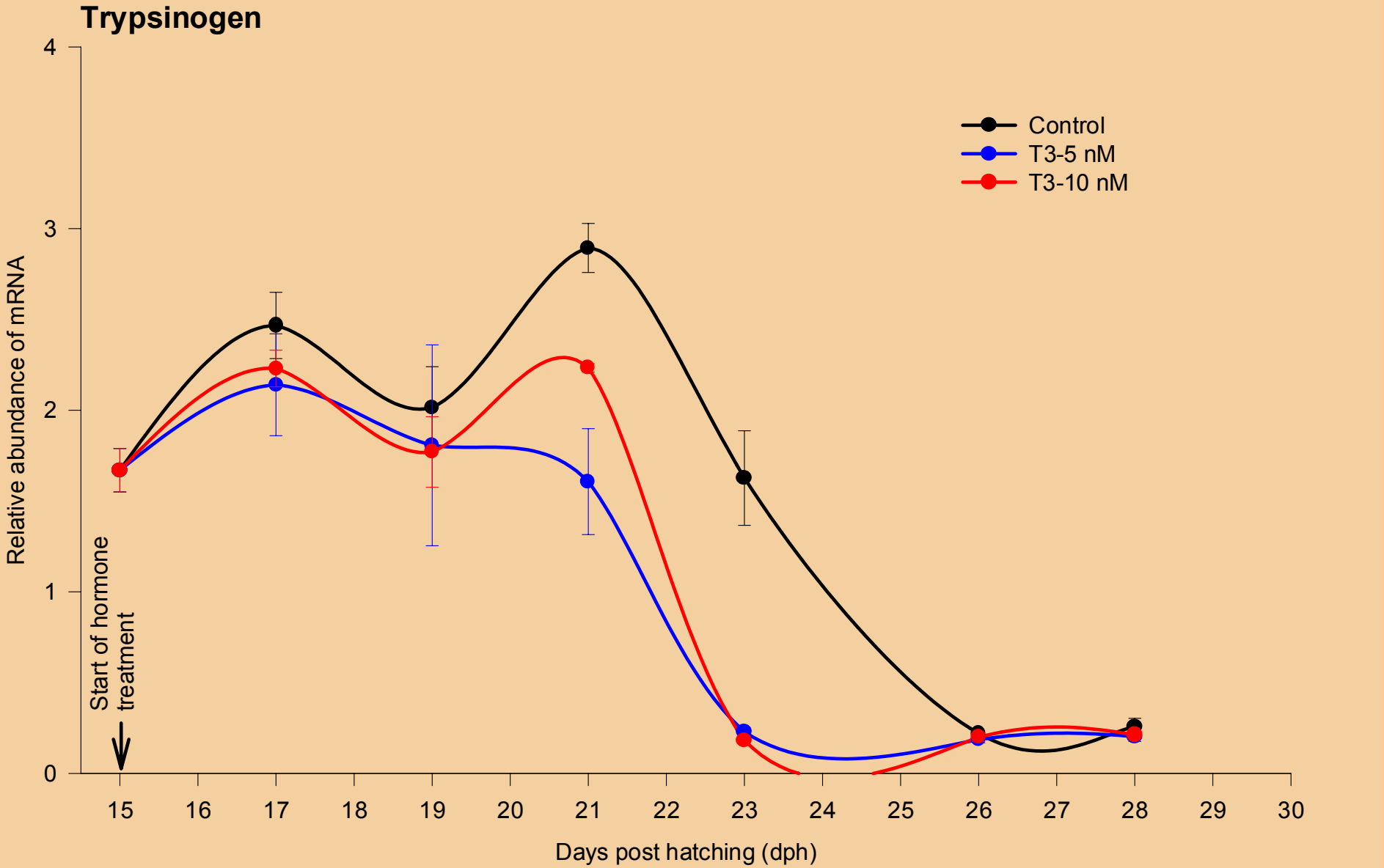

Aminopeptidase N
Relative abundance of mRNA
0
1
2
Control
F-100
F-200
T3-5
T3-10
a a a a
b ab a ab ab
b a ab a ab
c ab ab b a
a a a a a
b ab ab ab a
17dph
19dph
21dph
23dph
26dph
28dph
Days post hatching (dph)

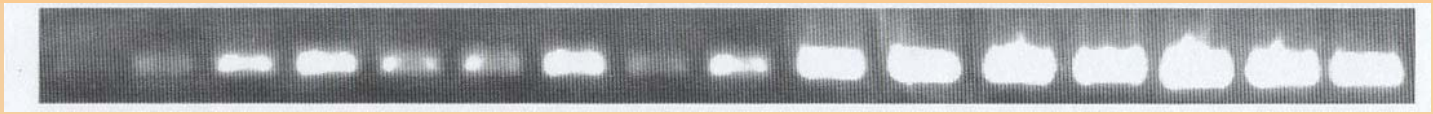

Aminopeptidase N
Control
F-100 nM
F-200 nM
Relative abundance of mRNA
0
1
2
Start of hormone treatment
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
Days post hatching (dph)

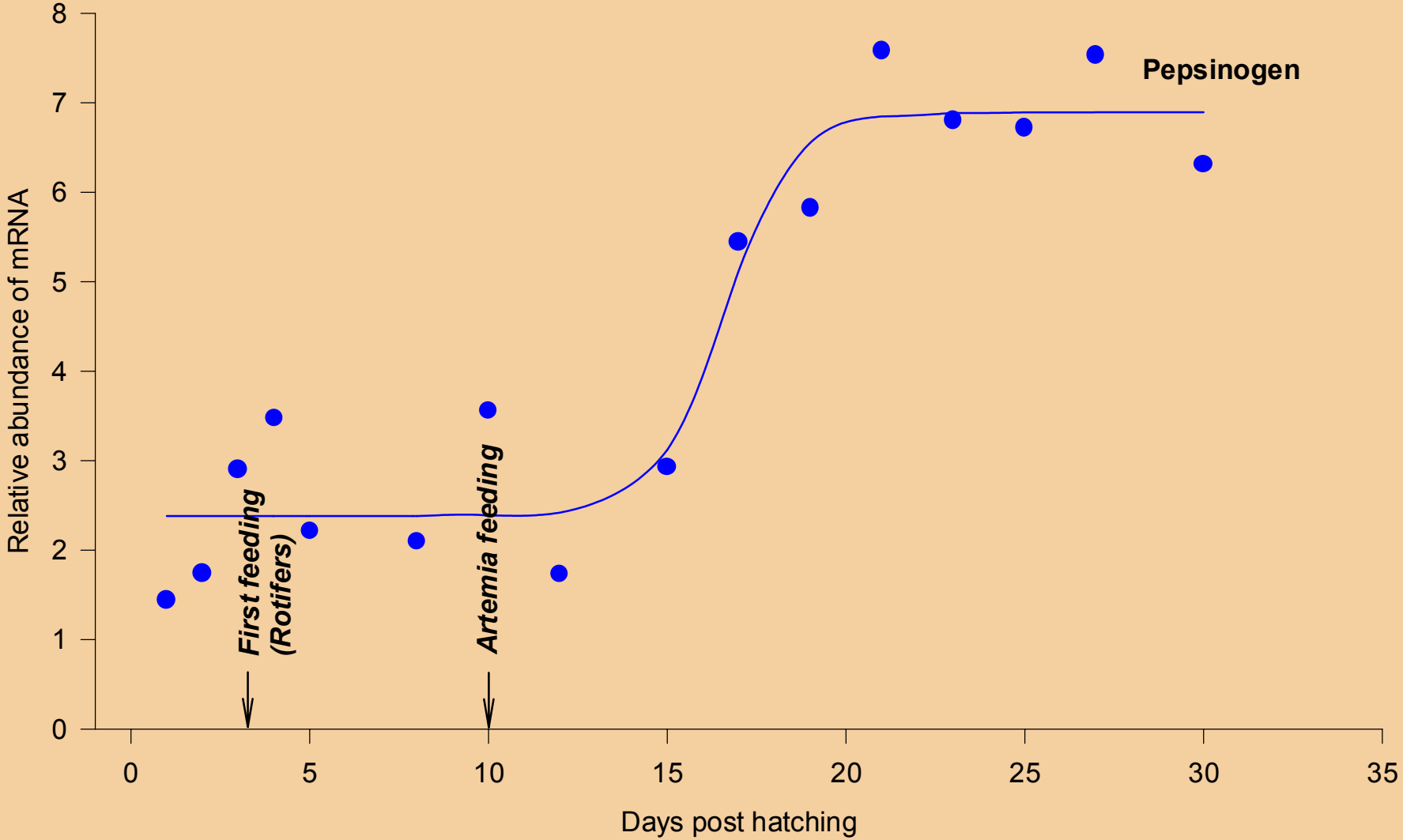

Aminopeptidase N
Control
T3-5 nM
T3-10 nM
Relative abundance of mRNA
0
1
2
Start of hormone treatment
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
Days post hatching (dph)

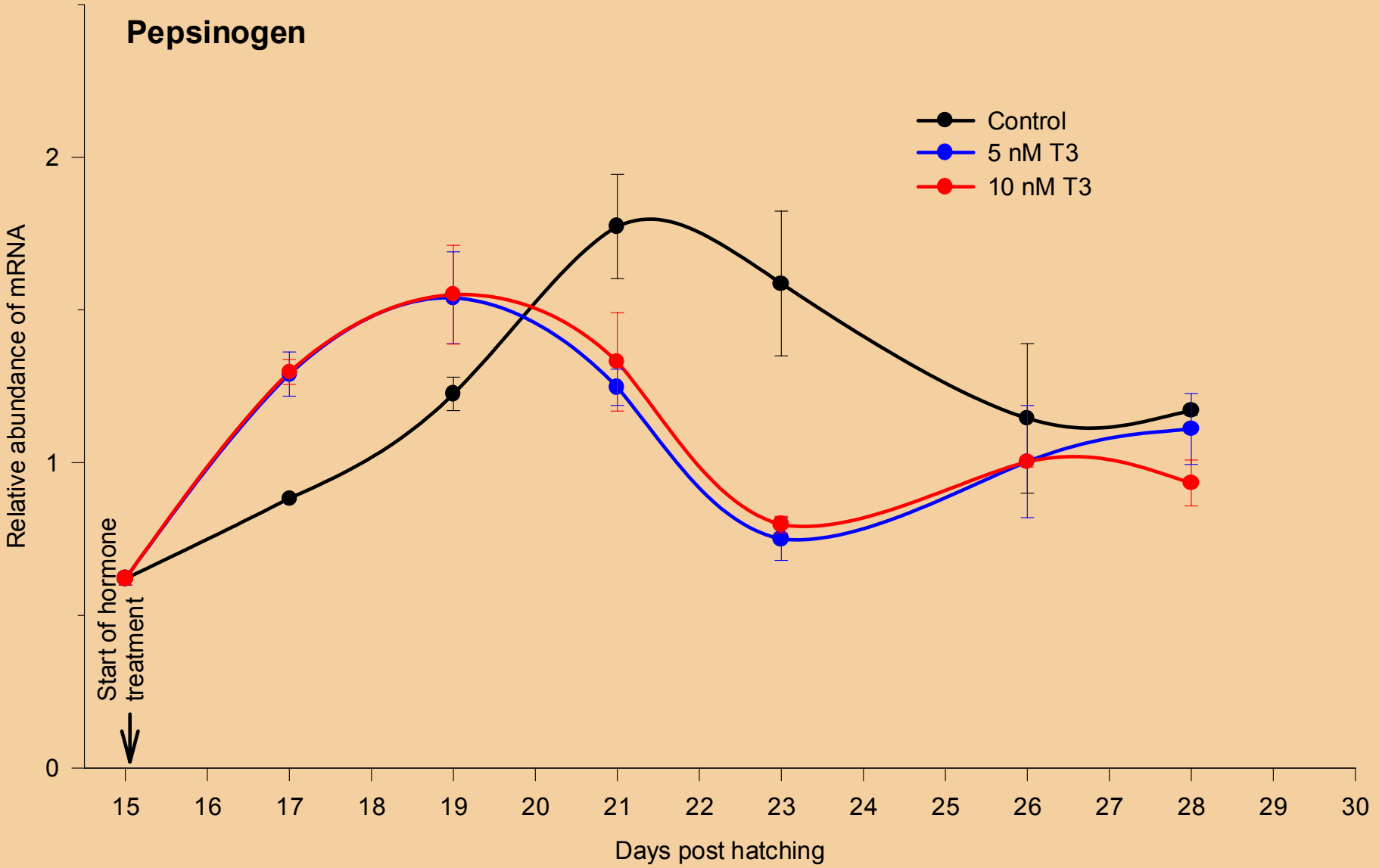

Trypsinogen
Relative abundance of mRNA
Days post hatching (dph)
Control
F-100
F-200
T3-5
T3-10
17 dph
19 dph
21 dph
23 dph
26 dph
28 dph
0
1
2
3
4


Trypsinogen
Control
F-100 nM
F-200 nM
Relative abundance of mRNA
Start of hormone treatment
Days post hatching (dph)
0
1
2
3
4
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30


Trypsinogen
Control
T3-5 nM
T3-10 nM
Relative abundance of mRNA
Start of hormone treatment
Days post hatching (dph)
0
1
2
3
4
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30




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
Control
5 nM T3
10 nM T3
Relative abundance of mRNA
0
1
2
Start of hormone treatment
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
Days post hatching

ACKNOWLEDGEMENT

Members of Marine Aquaculture Group, TMSI, NUS

Members of Fish Physiology Lab, DBS, NUS

National Science and Technology Board, Singapore