SAO PAULO STATE UNIVERSITY
AQUACULTURE CENTER

INFLUENCE OF INITIAL FEEDING ON MUSCLE GROWTH AND THE EXPRESSION OF MYOGENIC REGULATORY FACTORS IN PACU *Piaractus mesopotamicus* LARVAE

N. J. Leitão, M. Dal Pai-Silva, F. L. A. Almeida and *

M. C. Portella

* portella@caunesp.unesp.br

LARVI '09
5th FISH & SHELLFISH LARVICULTURE SYMPOSIUM

Pacu *Piaractus mesopotamicus* (Holmberg, 1887: Teleostei, Characidae, Serrasalminae)

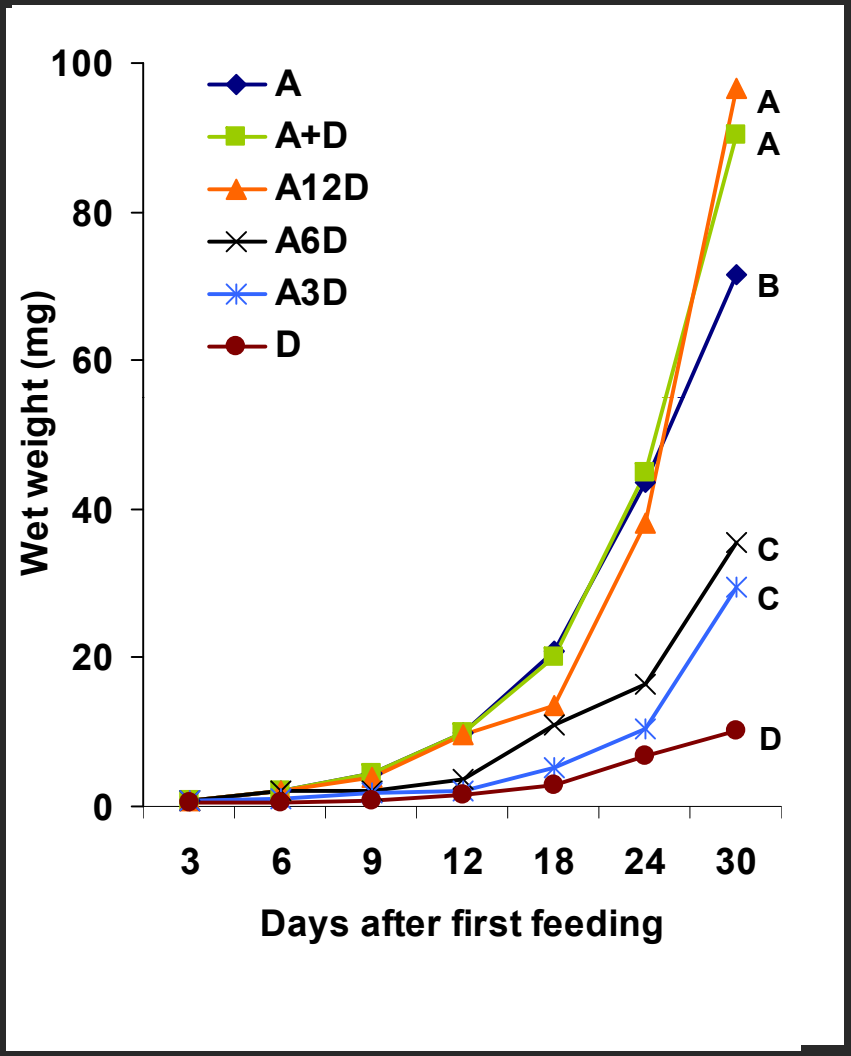
Omnivorours

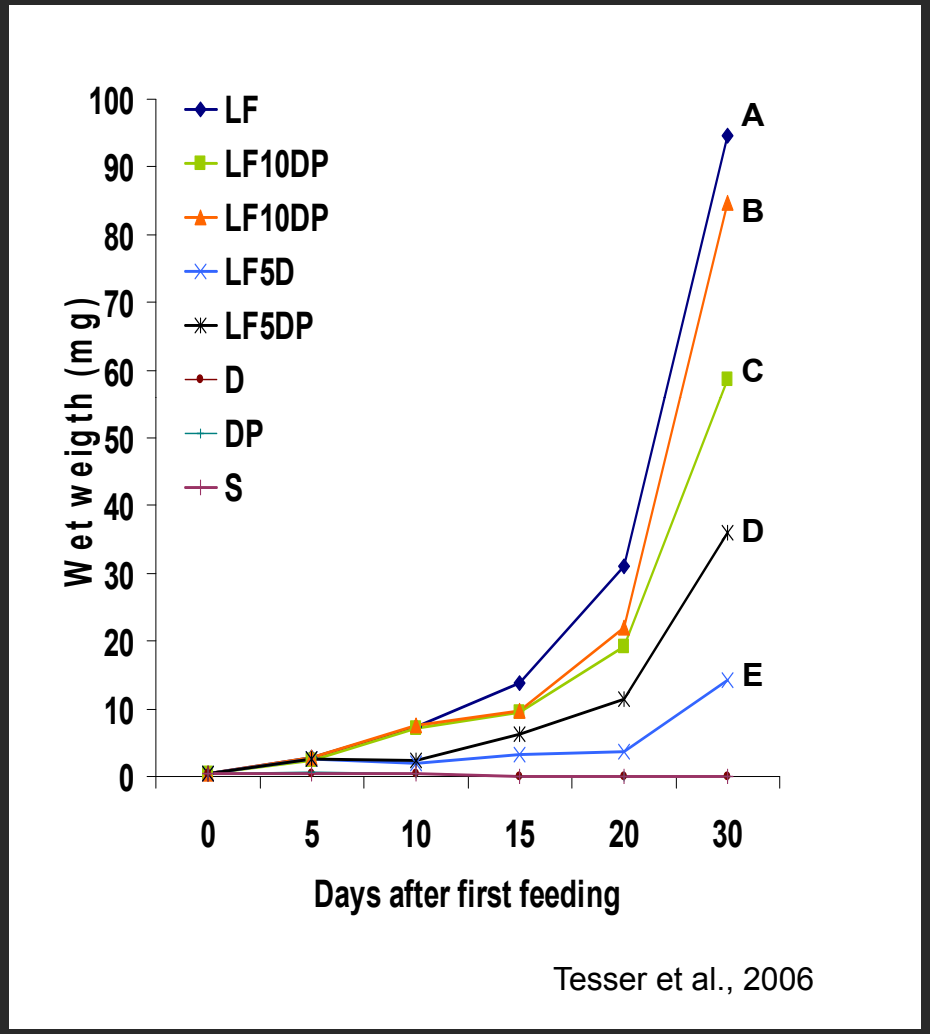
Fast
Growing
Fish

1.2 kg
1st year

20 kg
1 m

Pacu larvae initial feeding

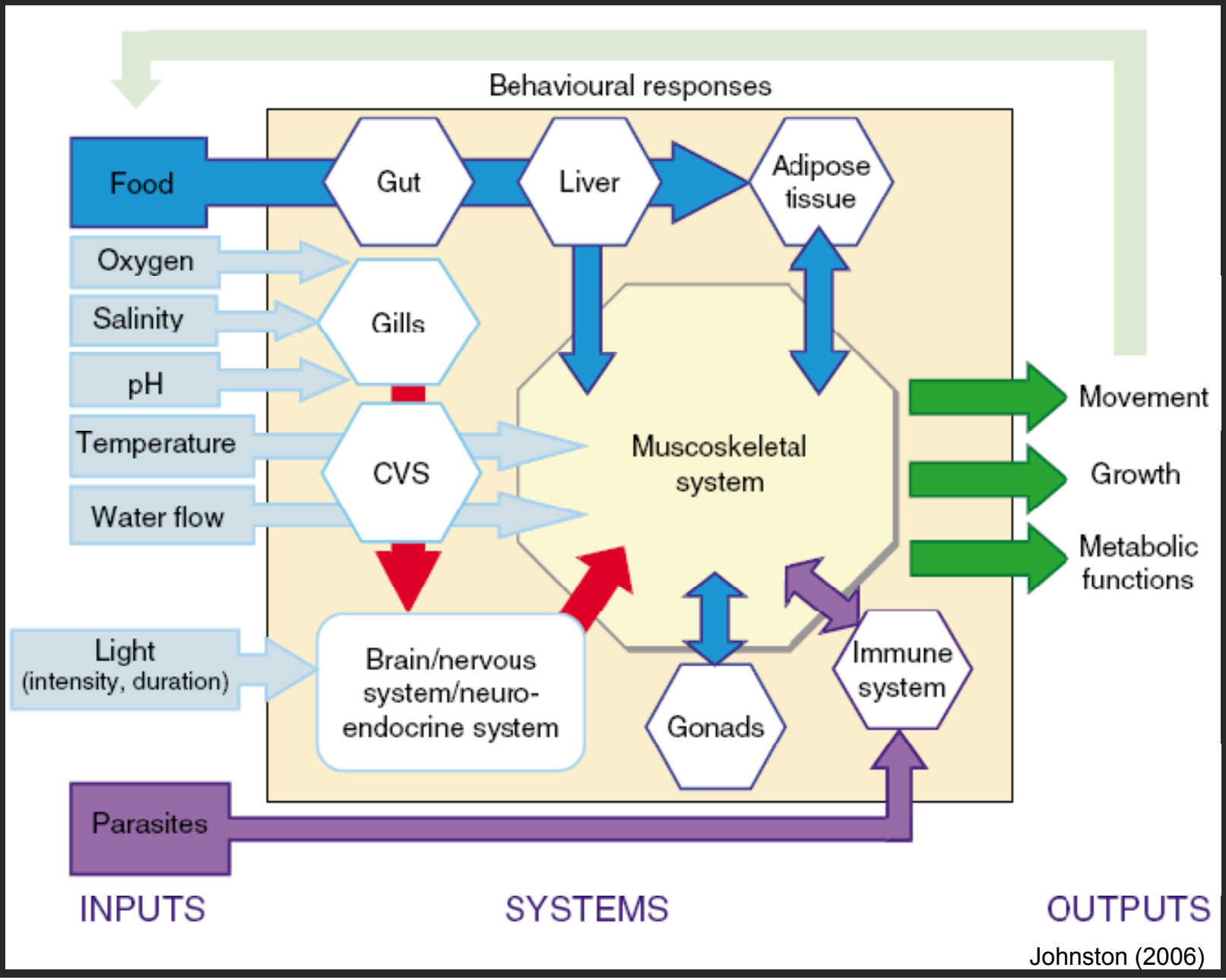




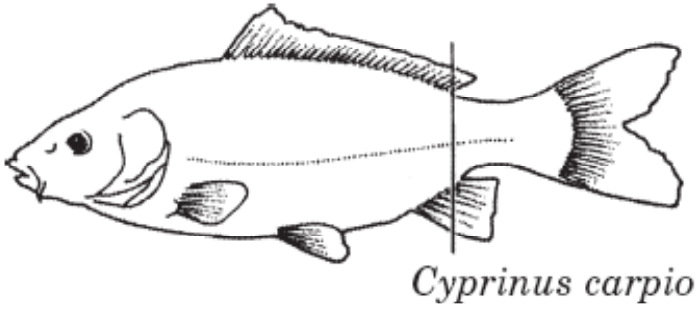
Tesser et al., 2006

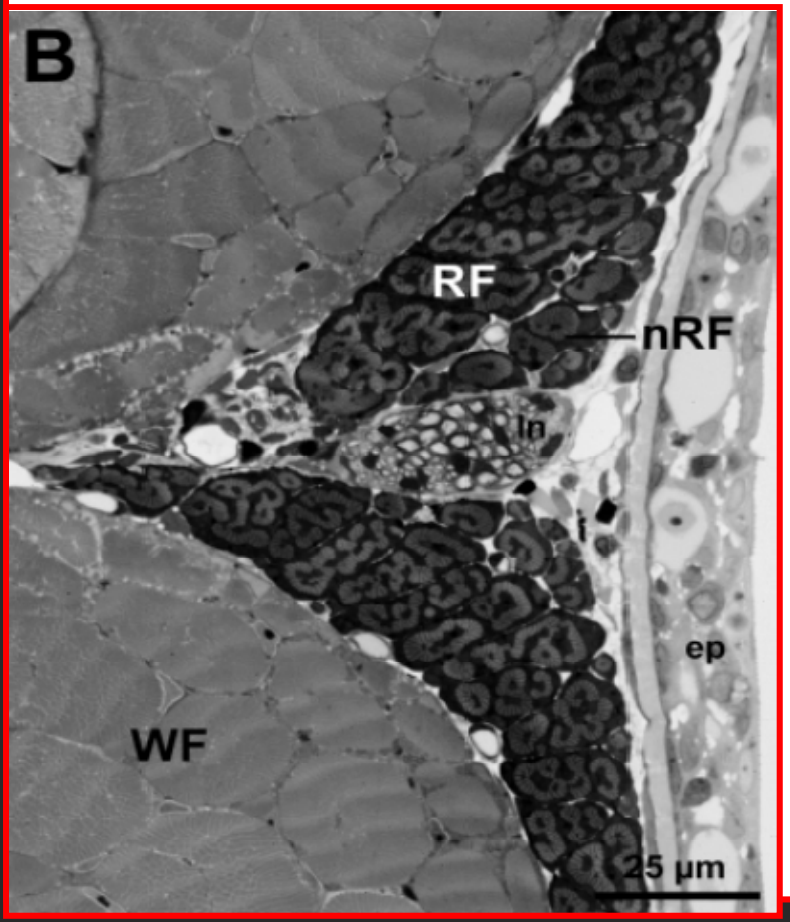
Effects of feeding schedule and diet quality on pacu larvae growth

The environmental inputs and physiological systems that affect the functional outputs of skeletal muscle in teleost fish

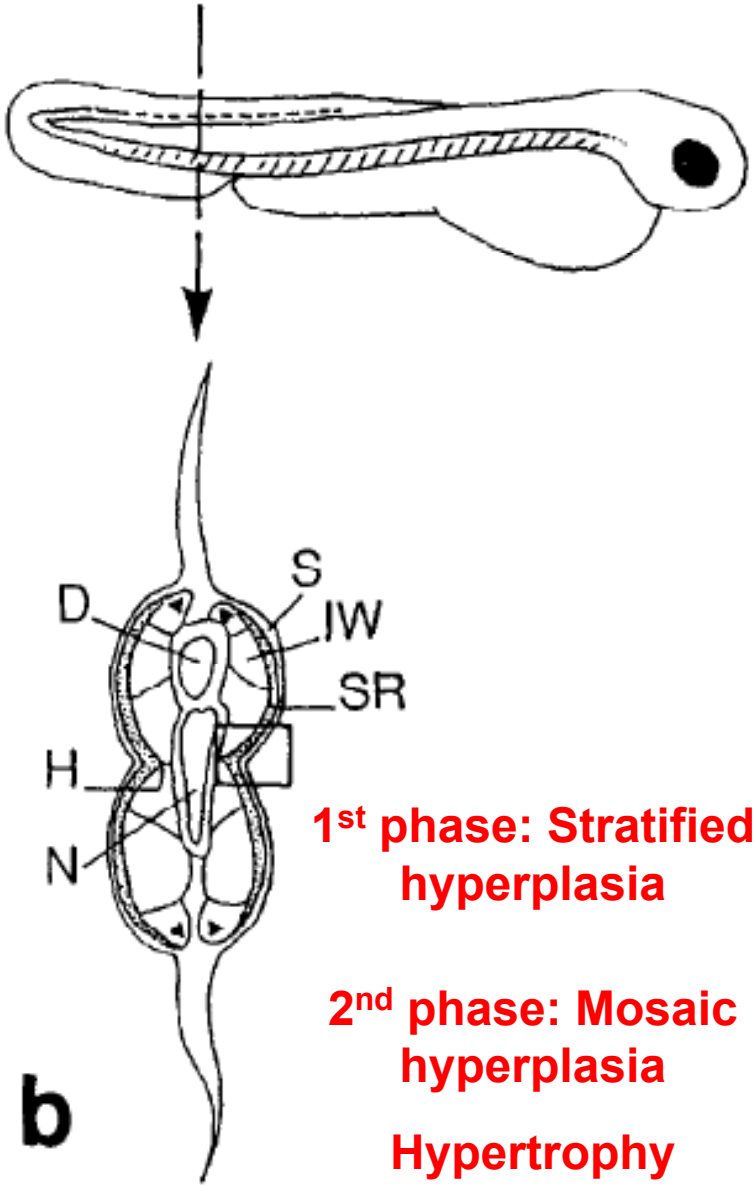


Johnston (2006)





Stoiber et al. (1999)



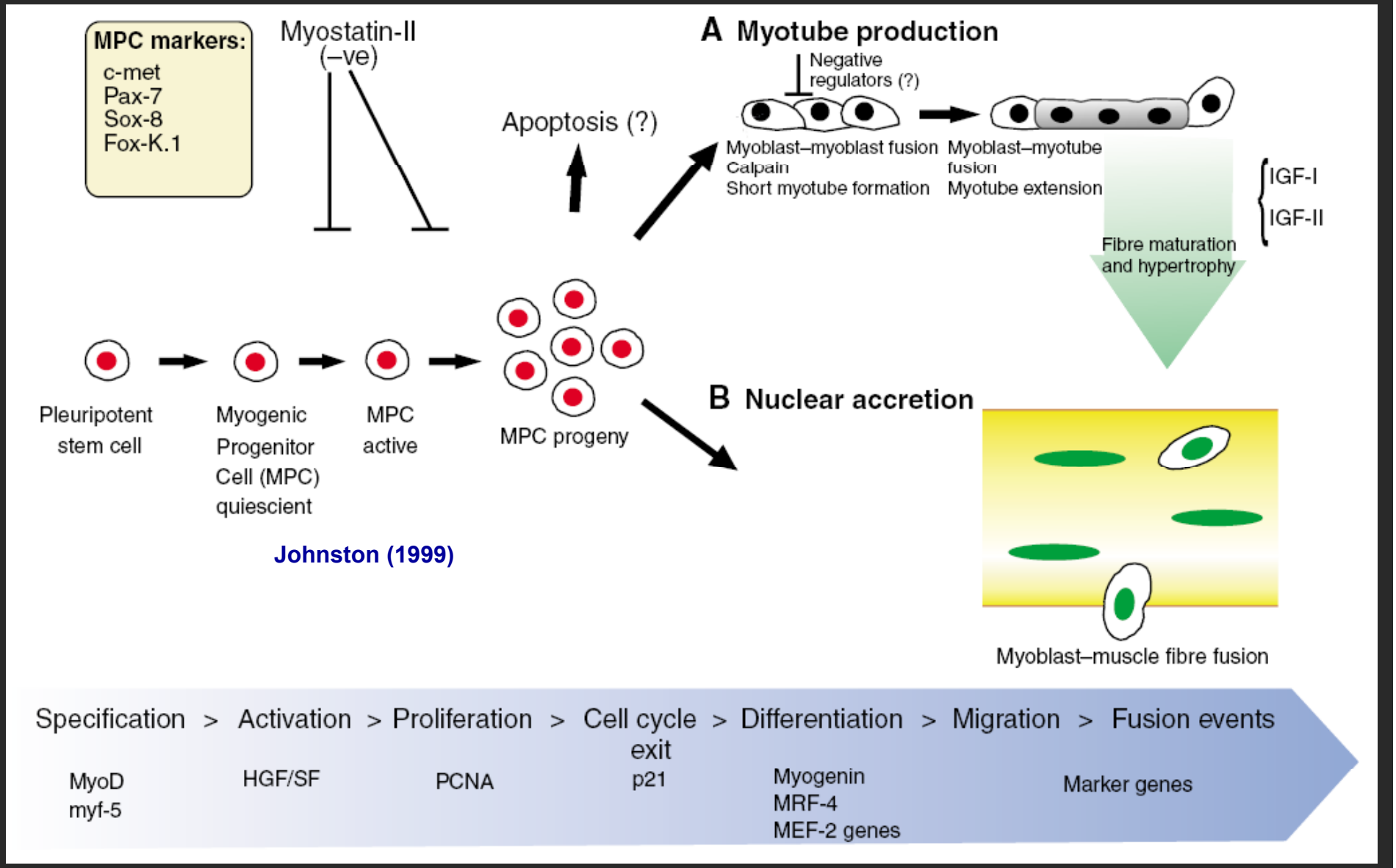
1st phase: Stratified hyperplasia

2nd phase: Mosaic hyperplasia

Hypertrophy

Koumans & Akster, 1995

Main Events of Myogenesis in Teleost Skeletal Muscle

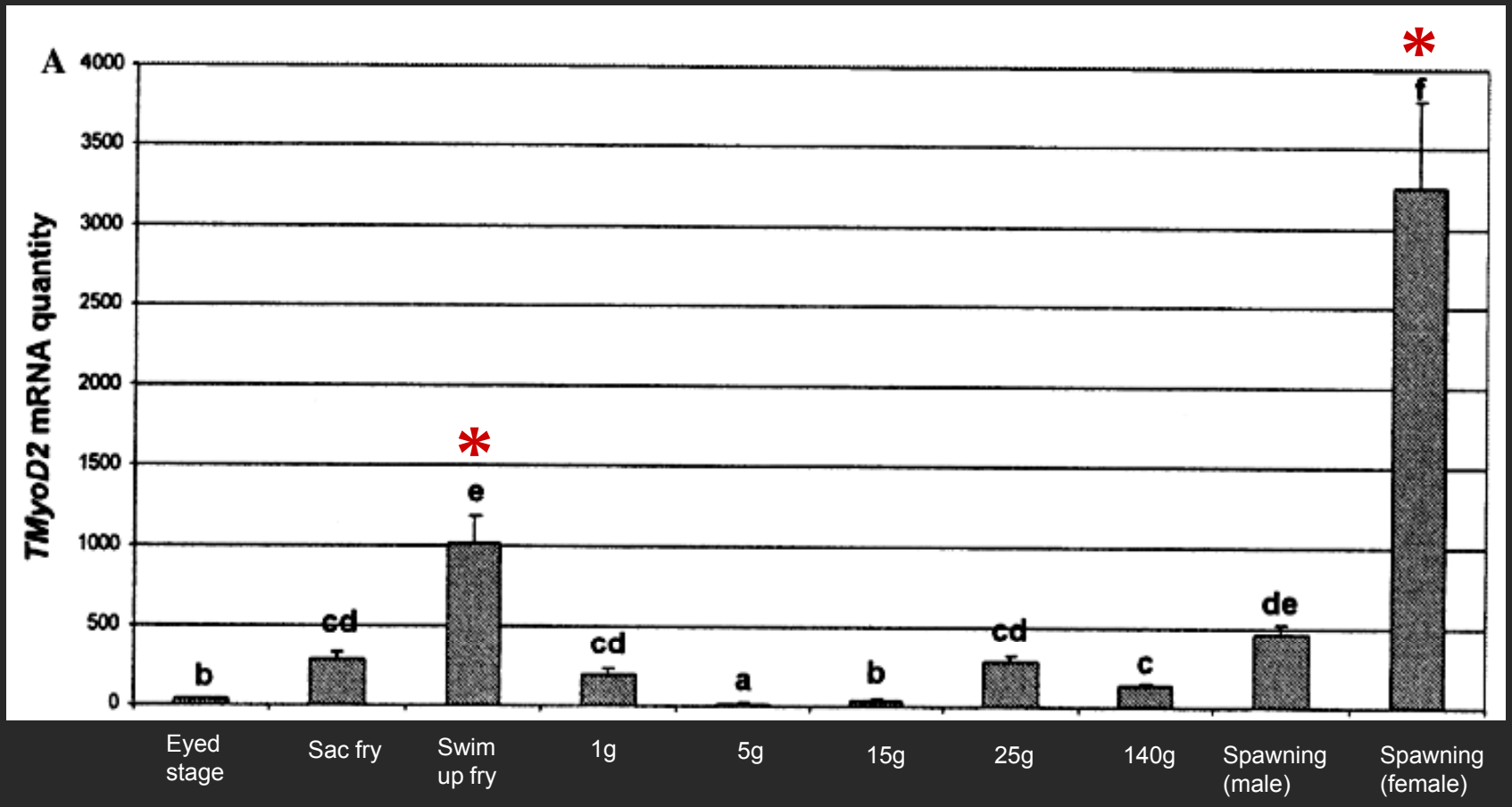


Johnston (1999)

Johnston (2006)

POST EMBRYONIC SKELETAL MUSCLE GROWTH

Quantitative expression analysis of genes affecting muscle growth during development of rainbow trout (*Oncorhynchus mykiss*). Expression levels of **TMyoD2**. Mean ± SE of mRNA quantity is shown.

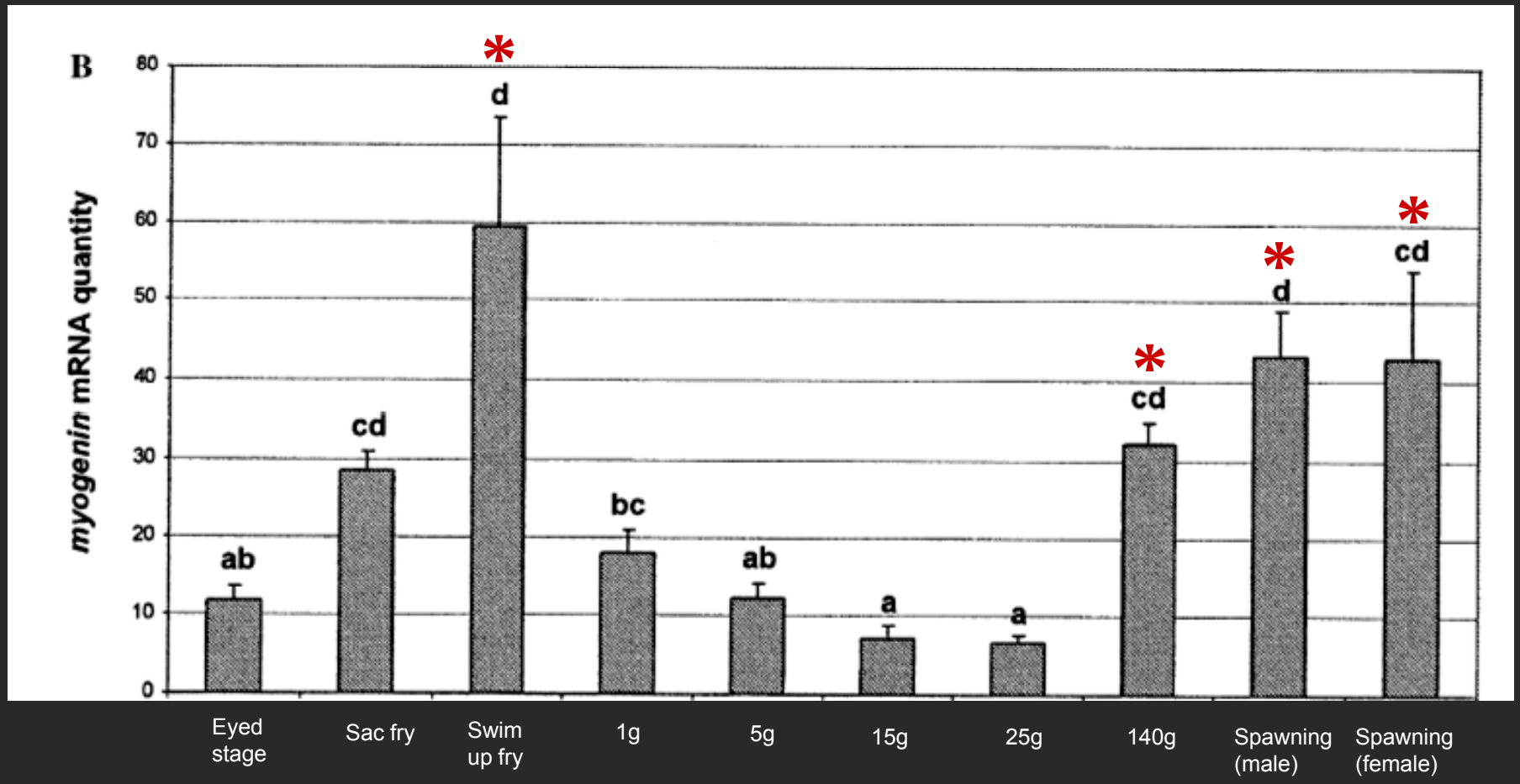


* **Myoblasts proliferation and hyperplasia**

Johansen & Overturf, 2005

POST EMBRYONIC SKELETAL MUSCLE GROWTH

Quantitative expression analysis of genes affecting muscle growth during development of rainbow trout (*Oncorhynchus mykiss*). Expression levels of **Myogenin**. Mean ± SE of mRNA quantity is shown.



* **Hypertrophy**

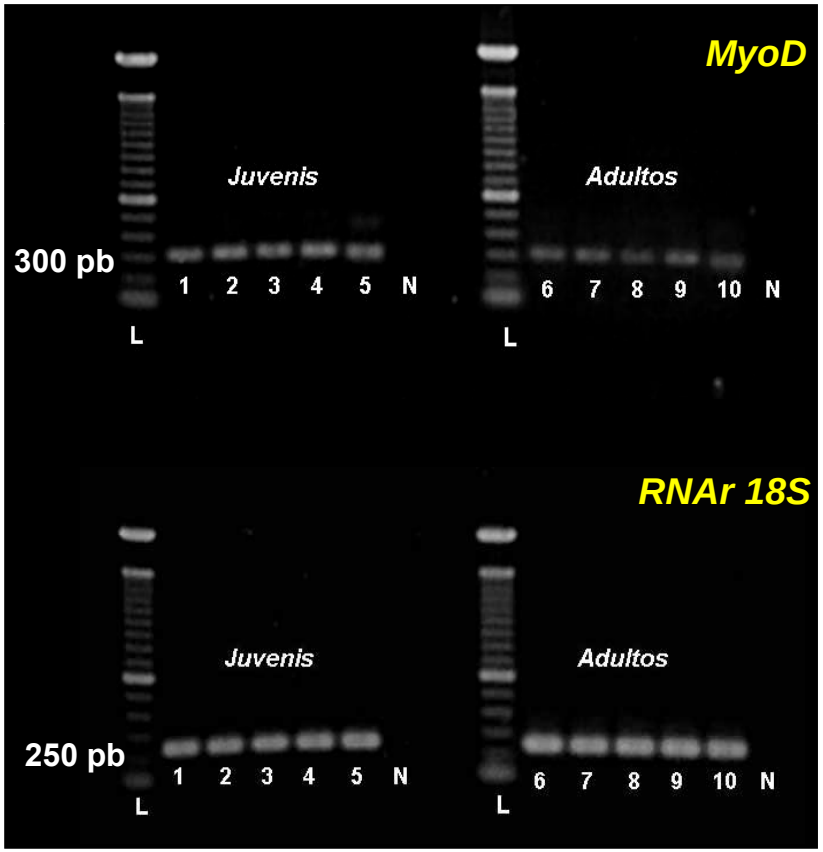
Johansen & Overturf, 2005

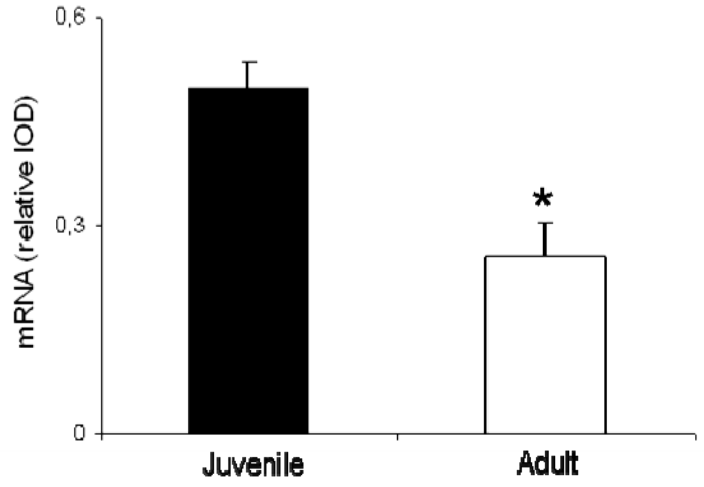
Differential expression of myogenic regulatory factor MyoD in pacu skeletal muscle (*Piaractus mesopotamicus* Holmberg 1887: Serrasalminae, Characidae, Teleostei) during juvenile and adult growth phases

Fernanda Losi Alves de Almeida [a,b], Robson Francisco Carvalho [a], Danillo Pinhal [a], Carlos Roberto Padovani [c], Cesar Martins [a], Maeli Dal Pai-Silva [a,*]

[a] UNESP, Institute of Biosciences, Department of Morphology, 18618-000 Botucatu, São Paulo, Brazil
[b] UNICAMP, Department of Cellular Biology, Institute of Biology, 13084-971 Campinas, São Paulo, Brazil
[c] UNESP, Department of Biostatistics, 18618-000 Botucatu, SP, Brazil

Micron, 2008





fibers <20µm **hyperplasia**

fibers >50µm **hypertrophy**

Skeletal Muscle Biology Research Group, UNESP, Botucatu-Brazil

Dr Maeli Dal Pai-Silva & Fernanda L. A. de Almeida

☐ 1: GQ337002 Reports

Piaractus mesopotamicus 18S ribosomal RNA gene, partial sequence
gi|254763238|gb|GQ337002.1|[254763238]

☐ 2: FJ810421 Reports

Piaractus mesopotamicus myogenin mRNA, partial cds
gi|226433051|gb|FJ810421.1|[226433051]

☐ 3: FJ686692 Reports

Piaractus mesopotamicus MyoD mRNA, partial cds
gi|225580684|gb|FJ686692.1|[225580684]

Dipartimento di Scienze Sperimentale Veterinarie, Università degli Studi di Padova, Padova-Itália

Dr. Marco Patruno, Dr. Lisa Maccatrozzo, Dr. Roberta Sacchetto, Dr Maeli Dal-Pai Silva and Fernanda L. Almeida

- **IGF-1 (mRNA) Sequence in *P. mesopotamicus***
  (deleted on request by author)

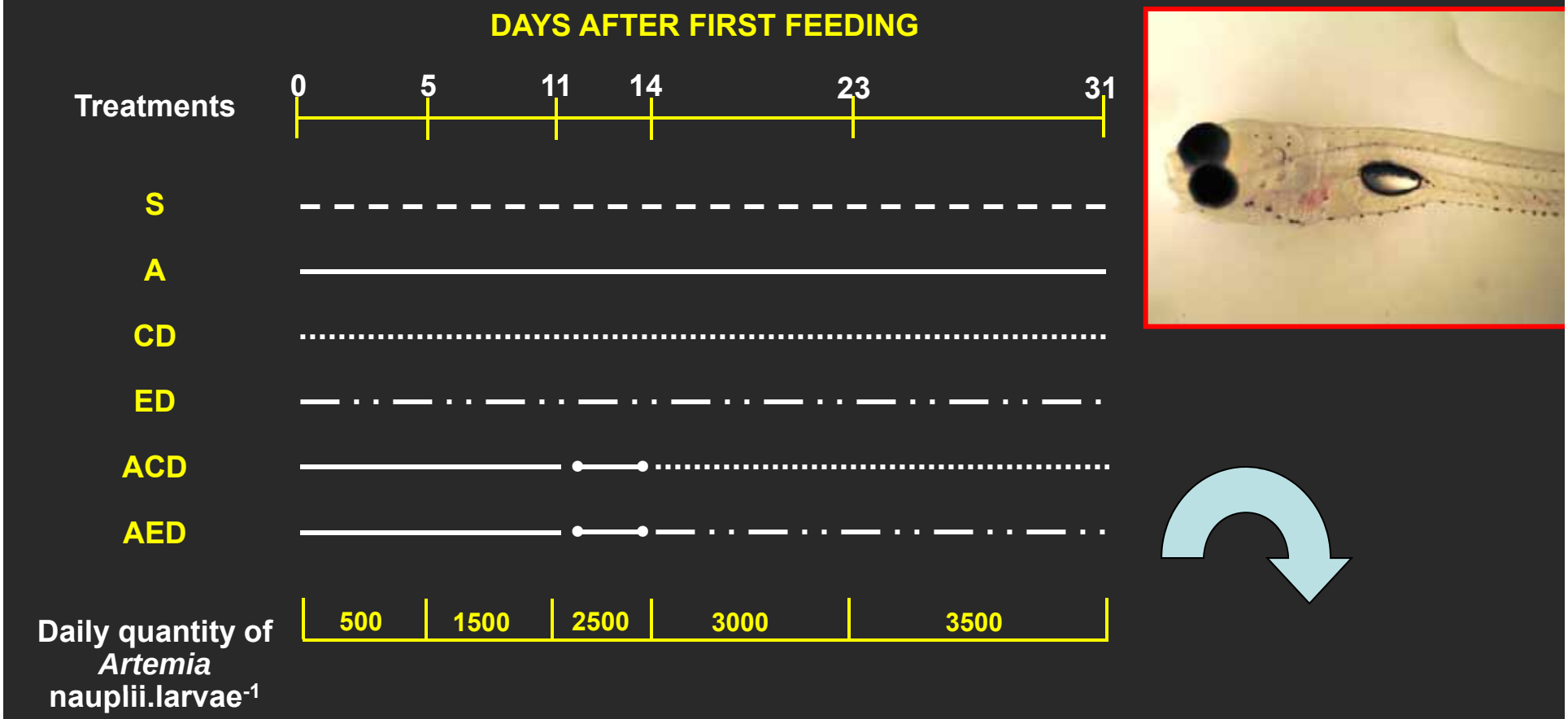
- **IGF-2 (mRNA) Sequence in *P. mesopotamicus***
  (deleted on request by author)

- **Beta-actina (mRNA) Sequence in *P. mesopotamicus* (5' – 3', 156 pb)**
  (deleted on request by author)

❖ Hypothesis

❖ The mechanisms of hyperplasic and hypertrophic growth of muscle fibers and the expression of MRFs (MyoD and Myogenin) in pacu *Piaractus mesopotamicus* larvae are influenced by feeding

Feeding protocols



evaluated the hypertrophic and hyperplasic growth of muscle fibers and the expression of MyoD and Myogenin

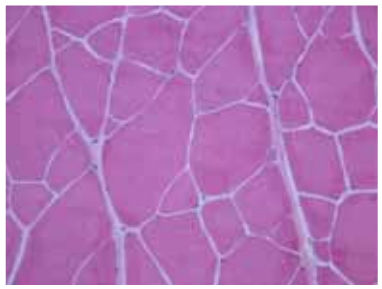
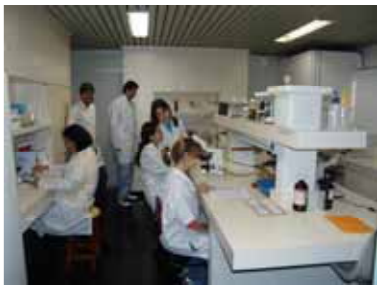
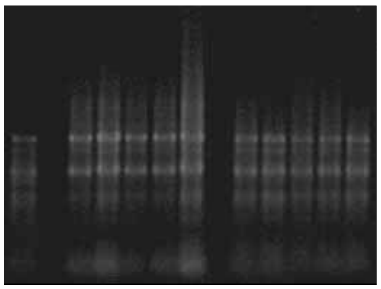
❖ Analyses

❖ **Performance** (weight, length, SGR and survival)

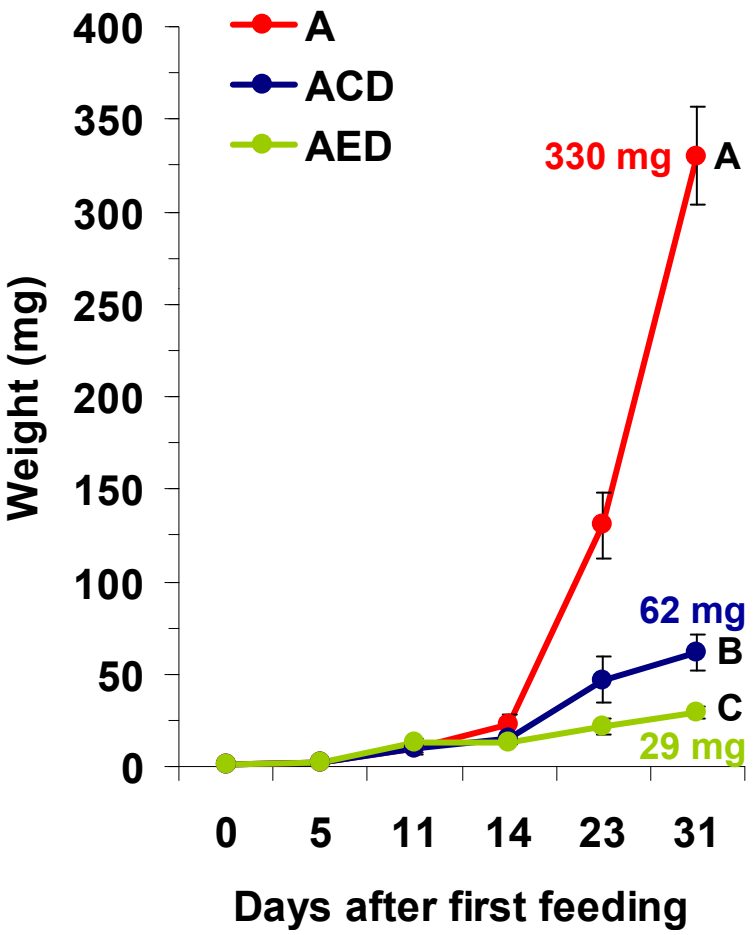
❖ **Morphology and Morphometry** (white muscle fibers were grouped into five diameter classes: ≤ 10, ≤ 20, ≤ 30, ≤ 40 and > 40 μm)

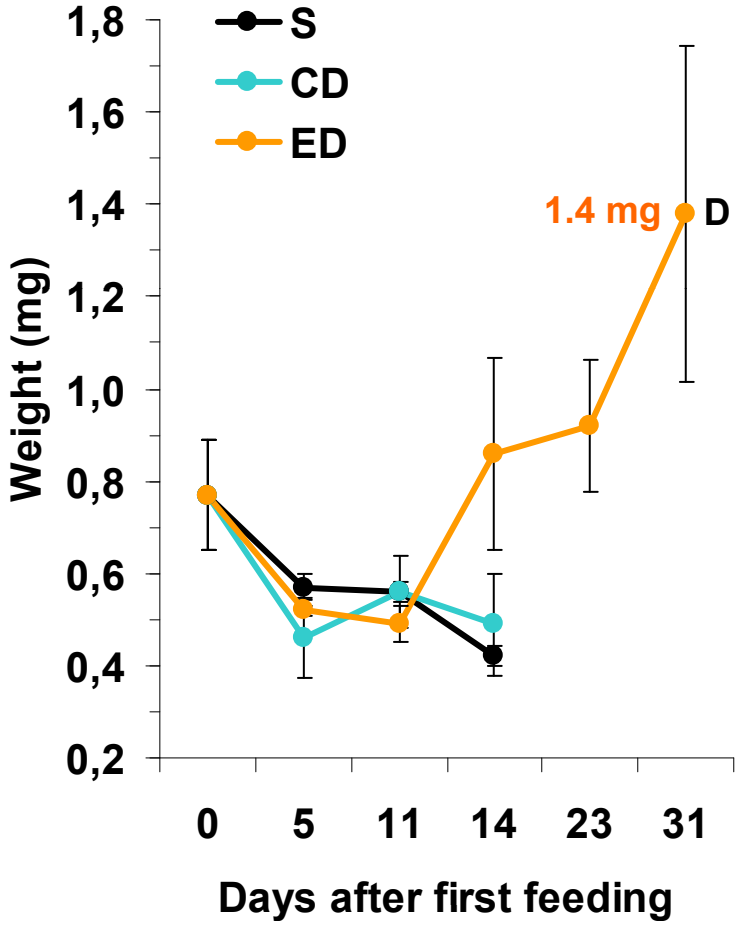
❖ **Expression of the MRFs MyoD and Myogenin by RT-PCR** (in the muscle of pacu larvae fed *Artemia* nauplii or formulated diets as a partial substitute for *Artemia* nauplii).

*the results of these analyses were compared with hyperplasic and hypertrophic muscle growth

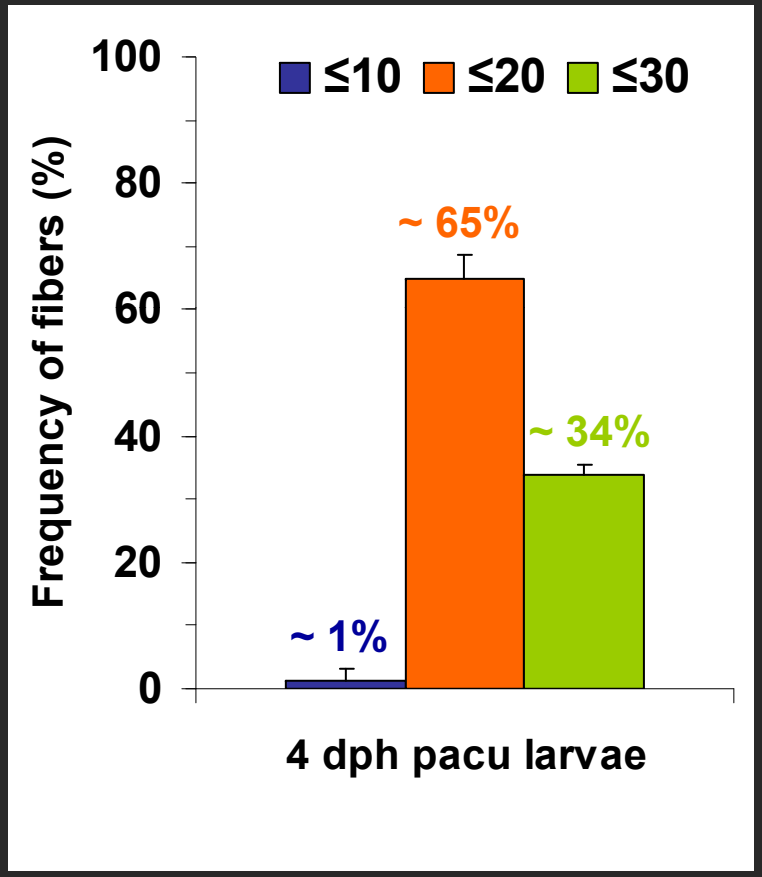




Growth

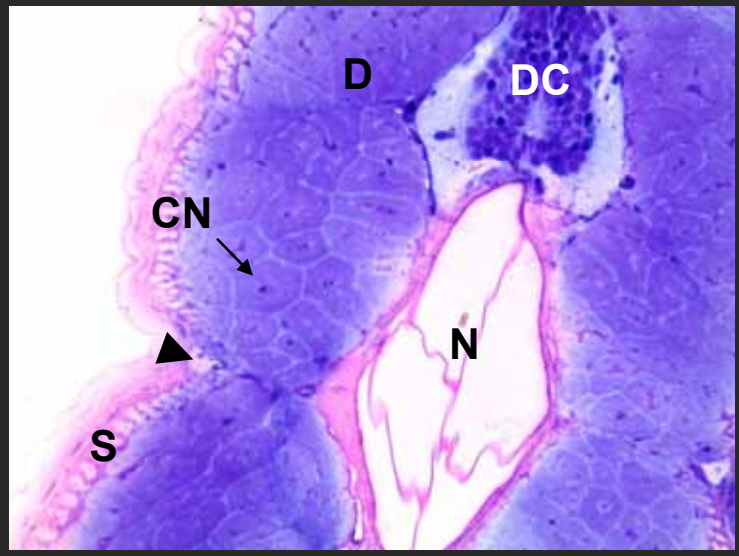

A
ACD
AED
330 mg
A
62 mg
B
C
29 mg
Weight (mg)
400
350
300
250
200
150
100
50
0
0
5
11
14
23
31
Days after first feeding


S
CD
ED
1.4 mg
D
Weight (mg)
1,8
1,6
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0
5
11
14
23
31
Days after first feeding

Morphology and morphometry



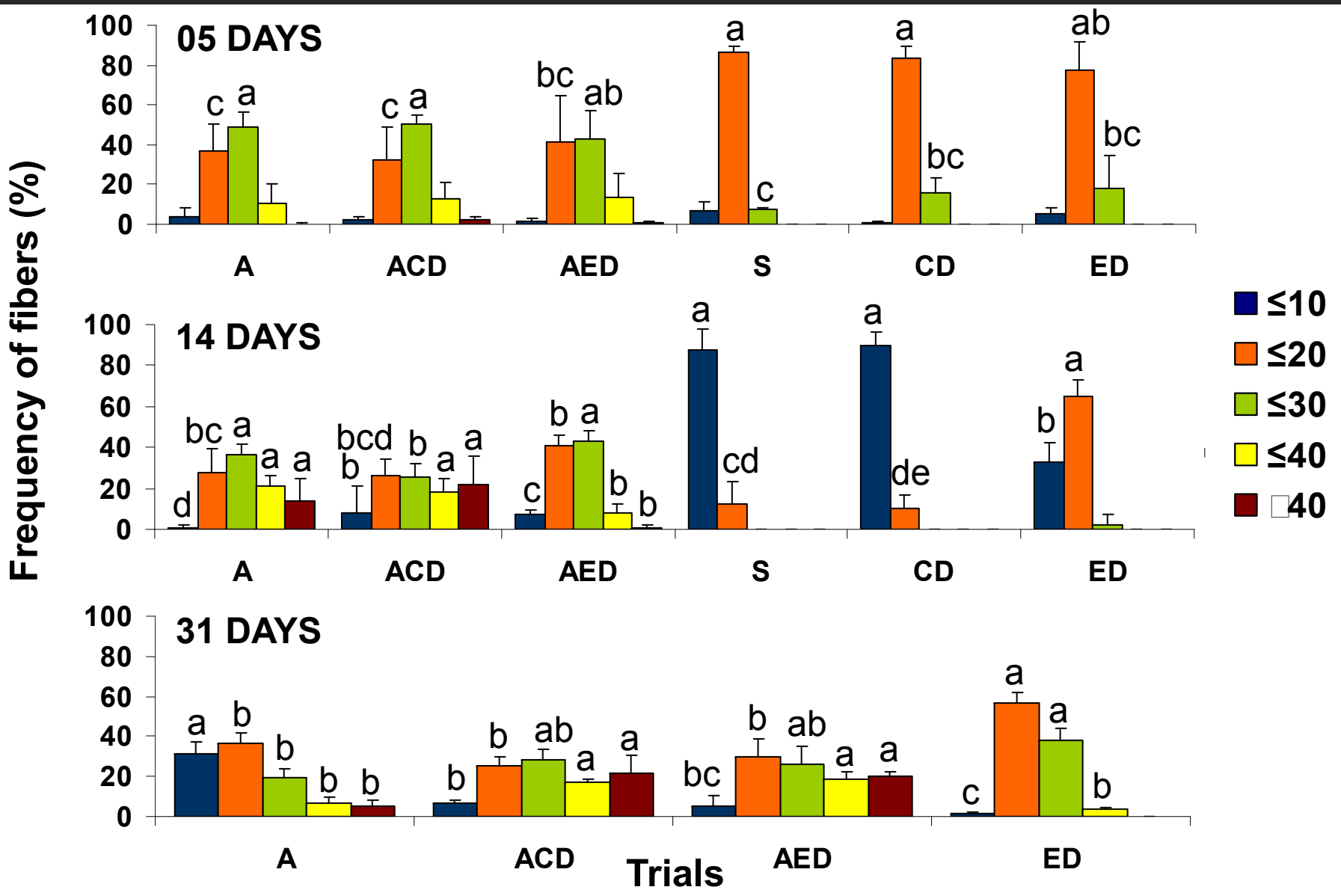
Muscular fiber diameter distribution (µm) in pacu larvae before first feeding



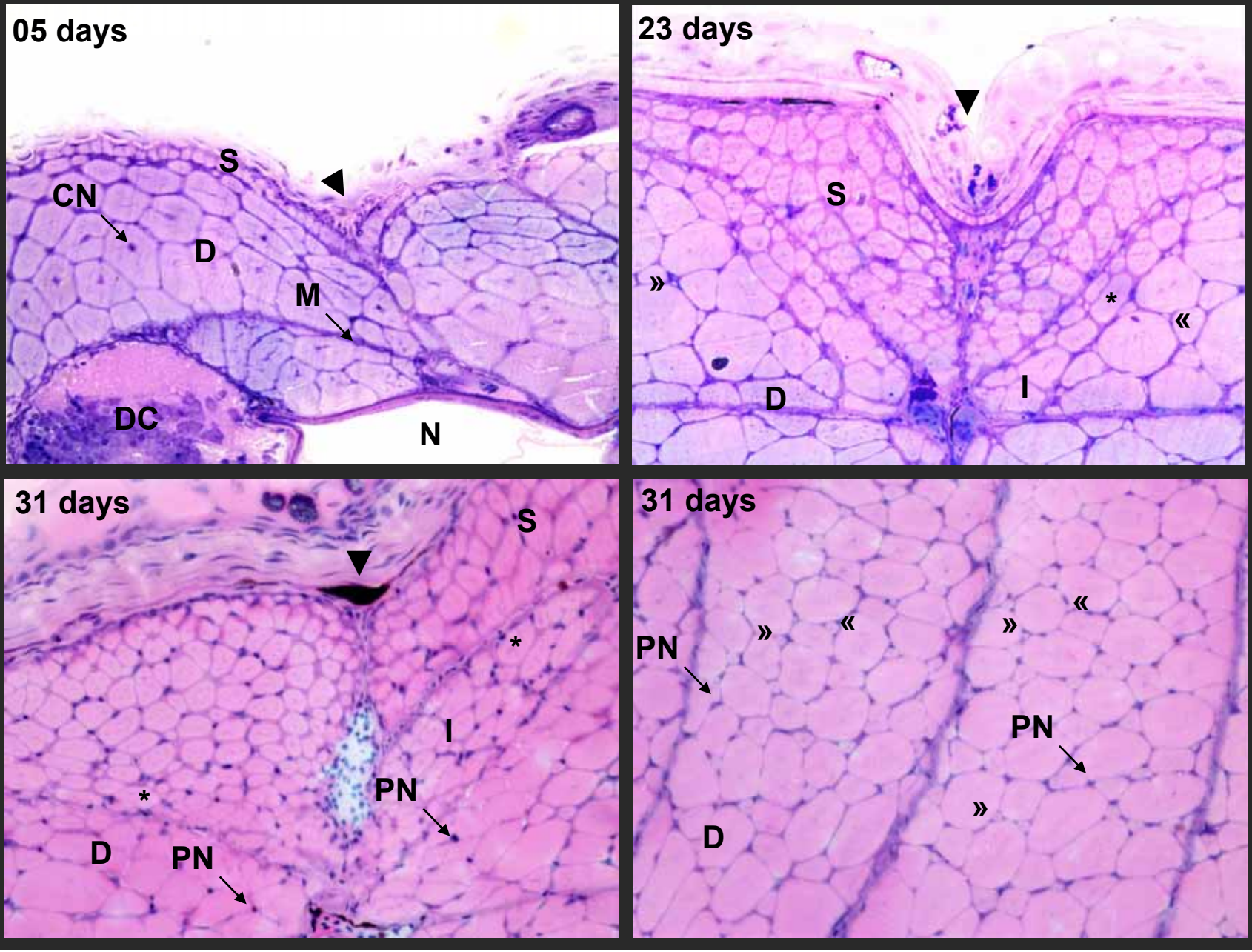
Transverse section of skeletal musculature in 4 dph pacu larvae. 400x.

Muscle fibers diameter distribution (µm) in pacu larvae

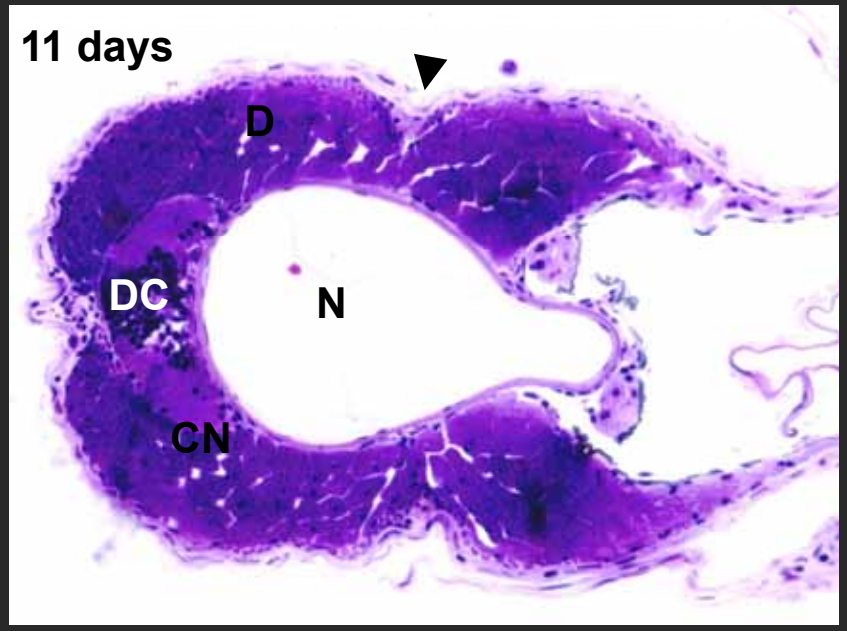
05, 11, 14, 23 and 31 DAFF

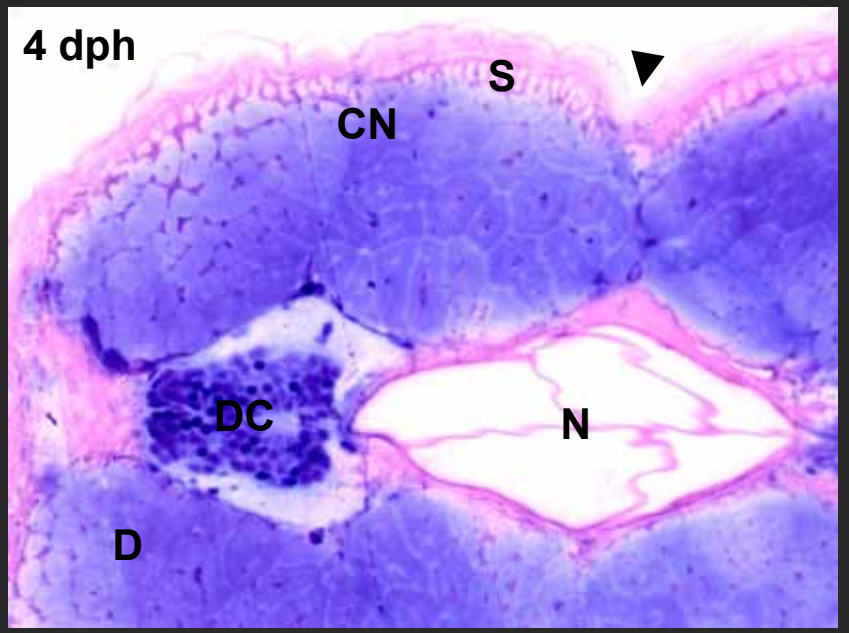


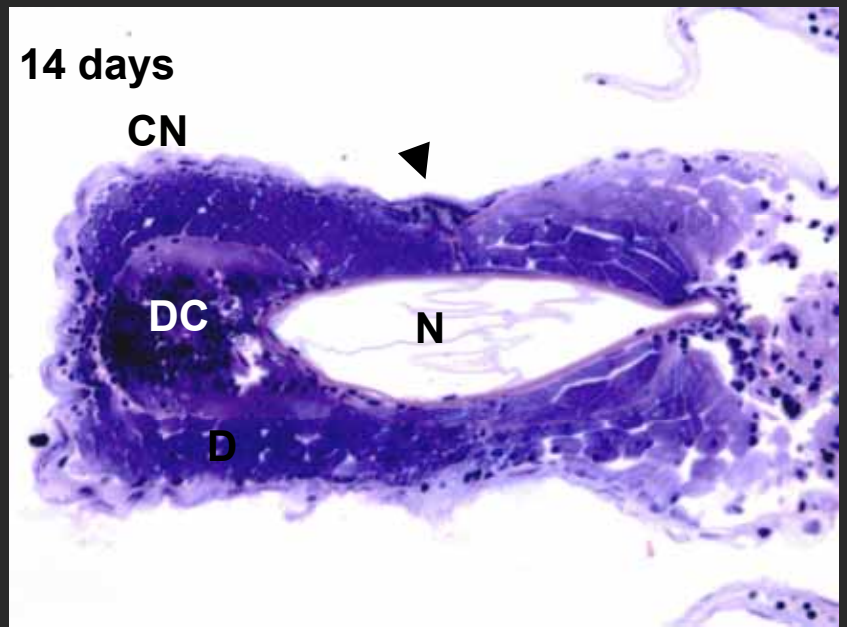
Transverse section of skeletal muscle in pacu larvae fed *Artemia* nauplii. 400X

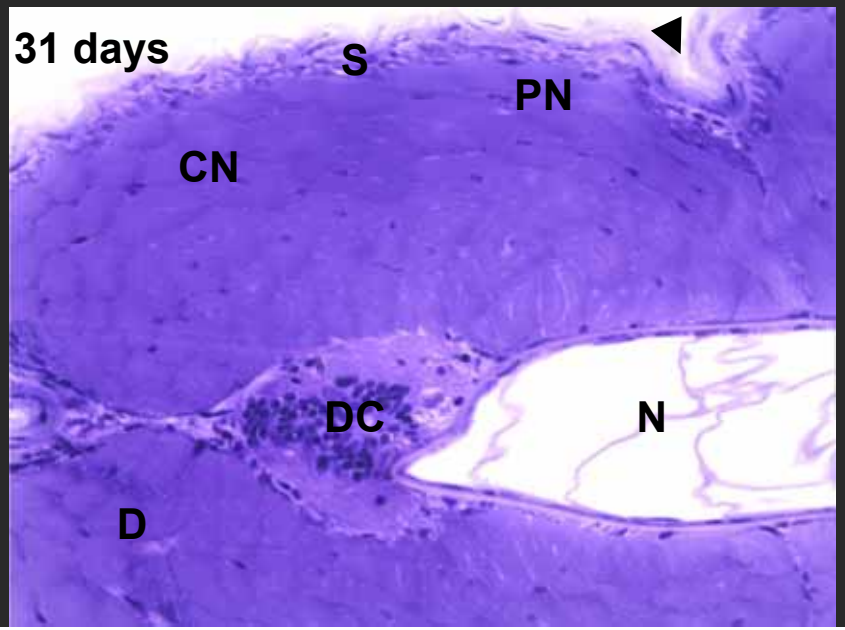


Transverse section of skeletal muscle in pacu larvae on starvation S (left) and fed experimental diet AED (right) . 400X.

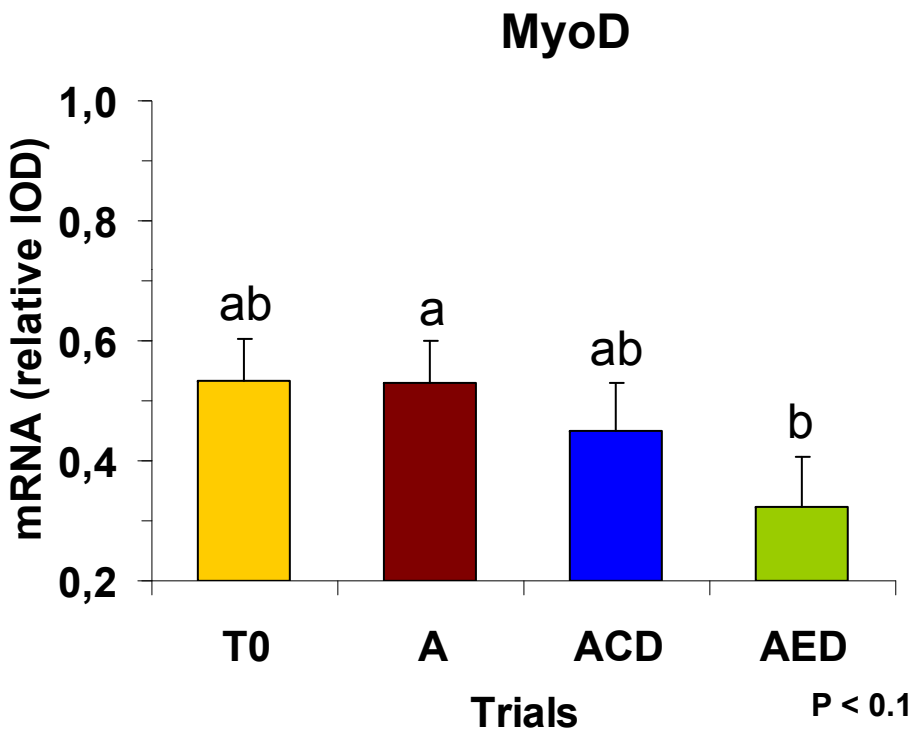


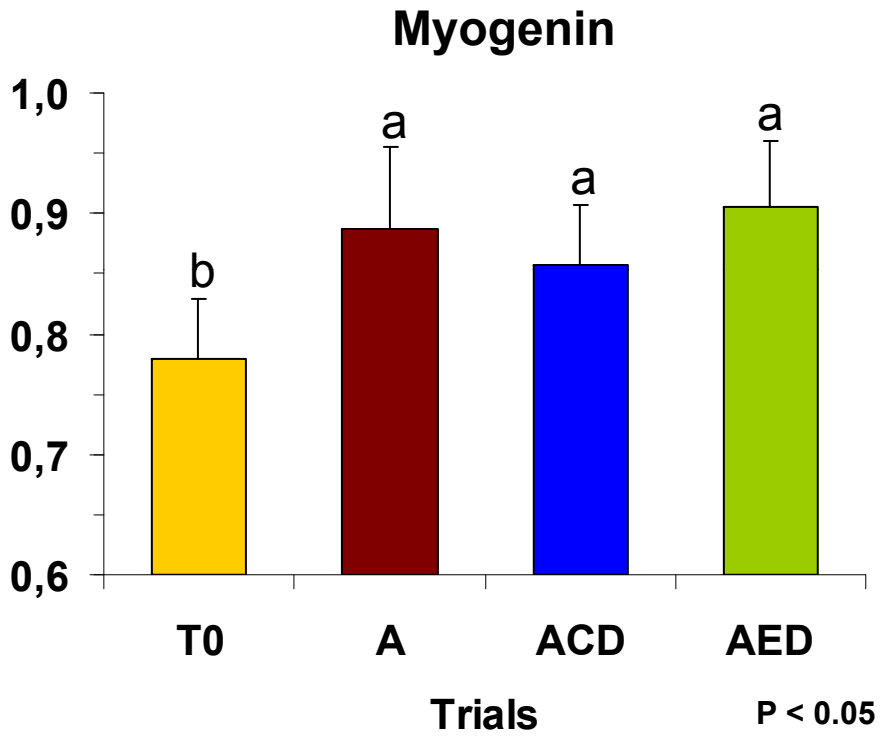


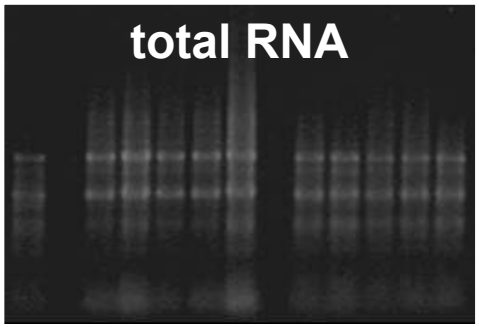


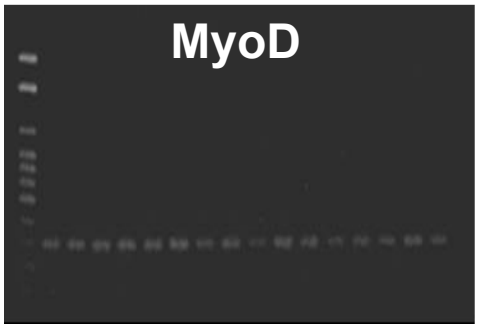


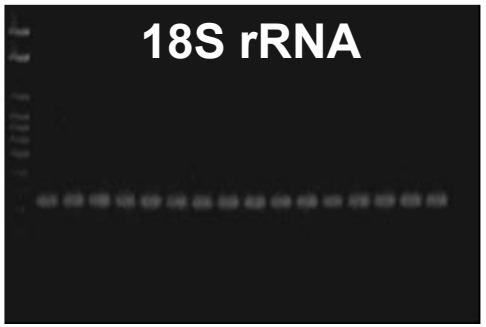
RNA content estimated by RT-PCR from skeletal muscle in pacu larvae











❖ Conclusions

❖ *Artemia* nauplii improved fish growth which resulted in larger fish at the end of the experiment. In addition, morphometric and gene expression results showed that hyperplasia affected muscle growth to a larger extent;

❖ These results suggest that myoblast proliferation phase maybe longer in fish well fed, thus causing increased recruitment of muscle cells;

❖ Fish that displayed delayed growth due to early weaning had a larger number of fibers with diameter > 40µm, a consequence of more intense hypertrophic activity;

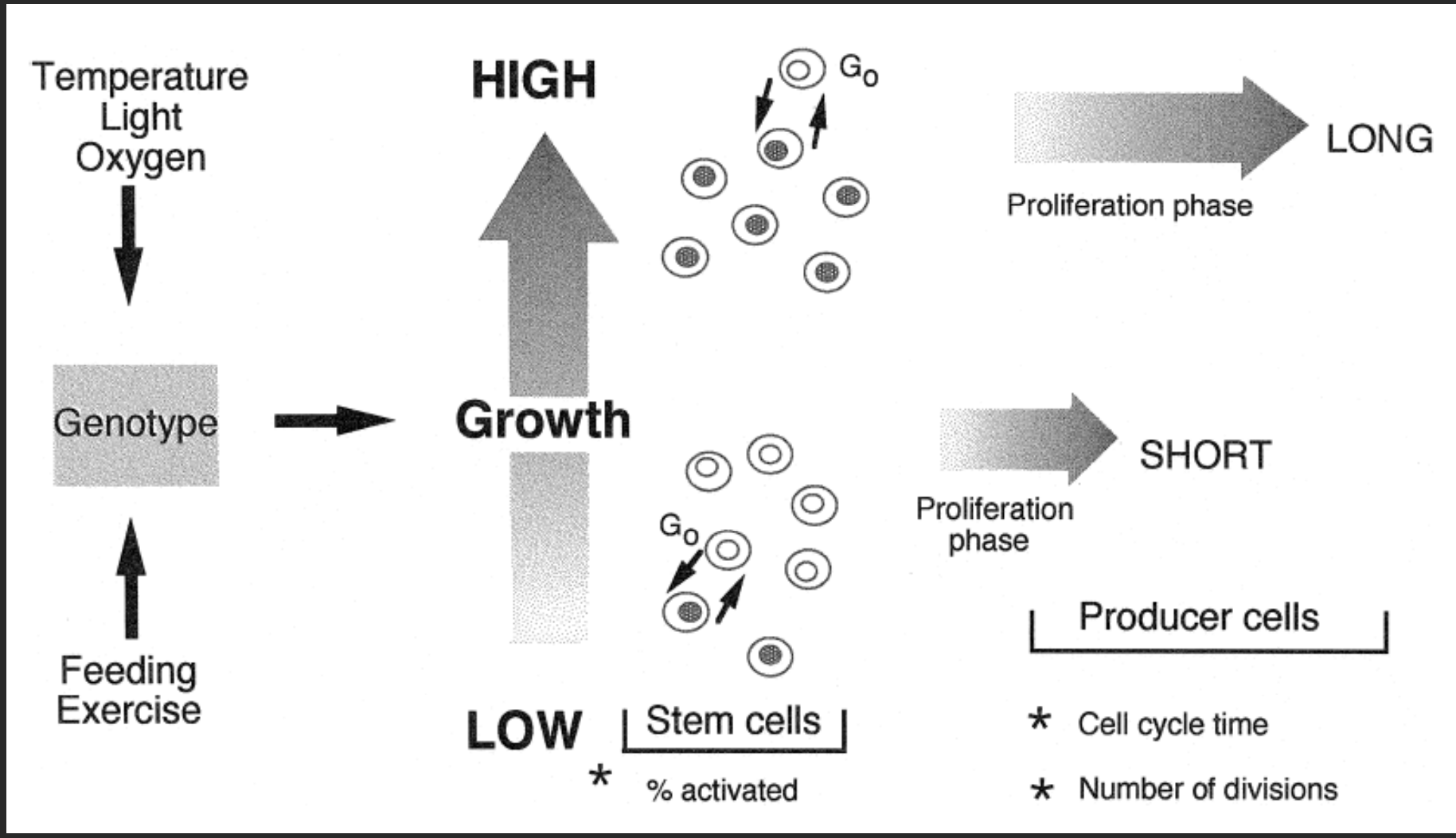
❖ These results open perspectives of investigation regarding the posterior growth and the use of more efficient and economic feeding strategies in fish commercial production

OBRIGADA
THANKS

The authors are grateful for the financial support received from the State of Sao Paulo Research Foundation

FAPESP

State of the art



Johnston (1999)