



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

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LARVI '09

5<sup>th</sup> FISH & SHELLFISH LARVICULTURE SYMPOSIUM

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



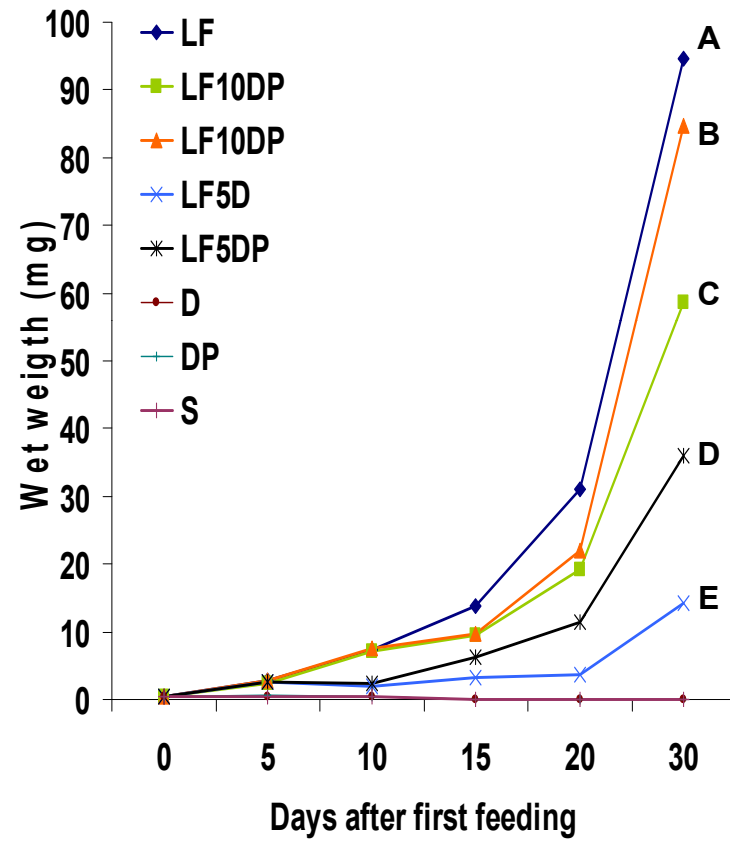
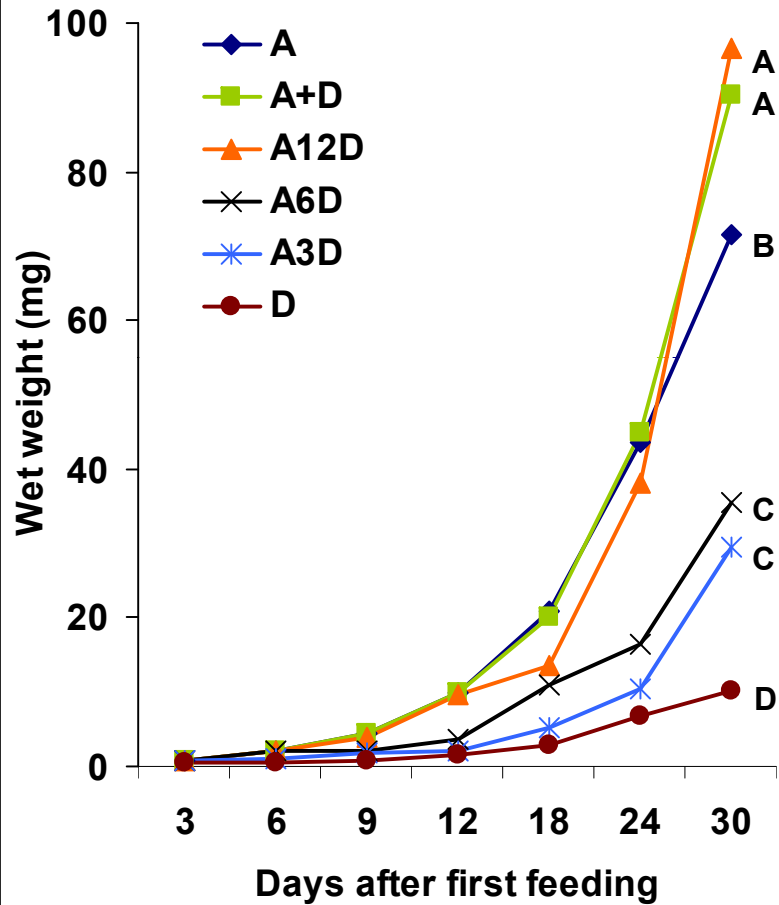
Omnivorours

Fast  
Growing  
Fish

1.2 kg  
1<sup>st</sup> year

20 kg  
1 m

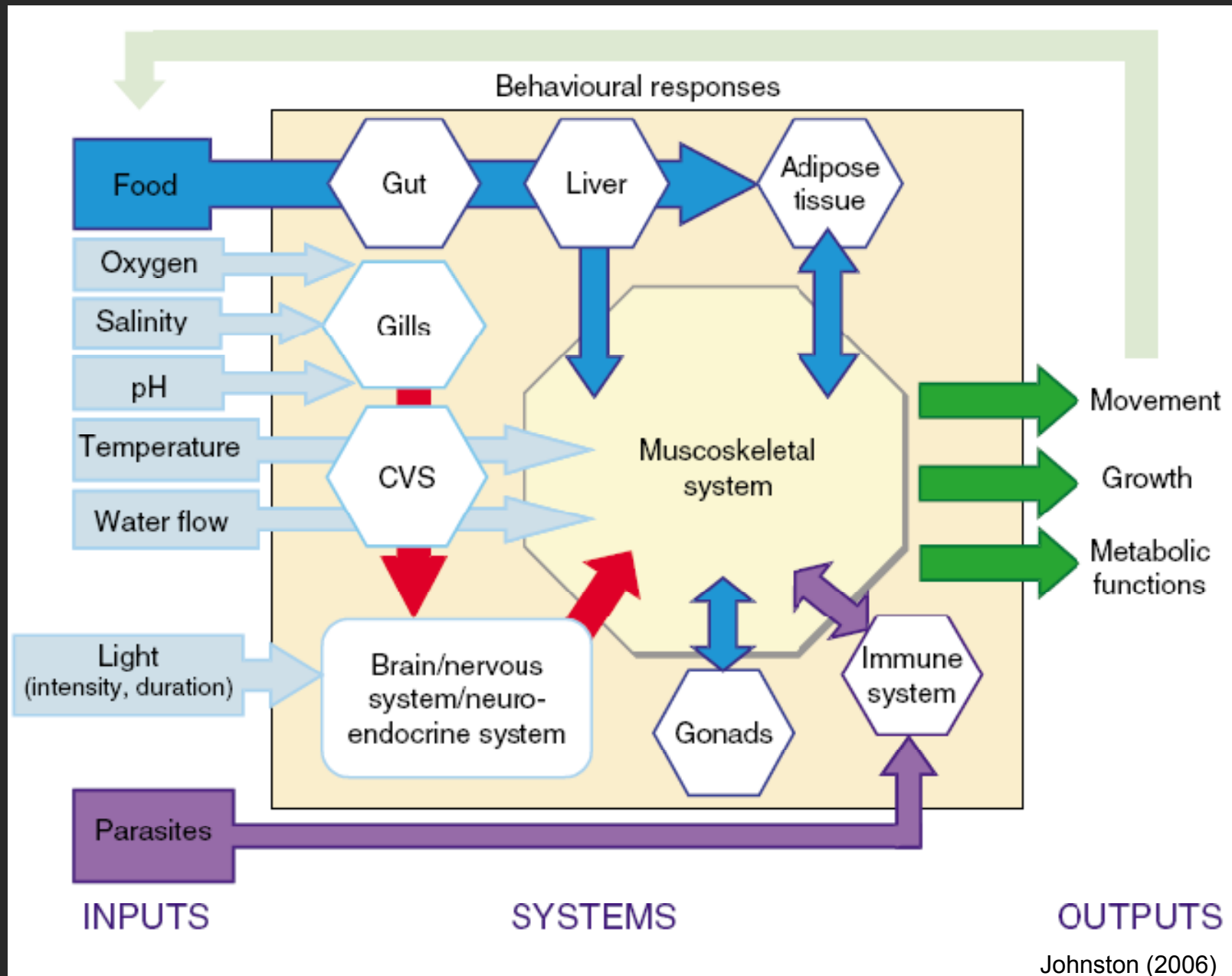
## Pacu larvae initial feeding

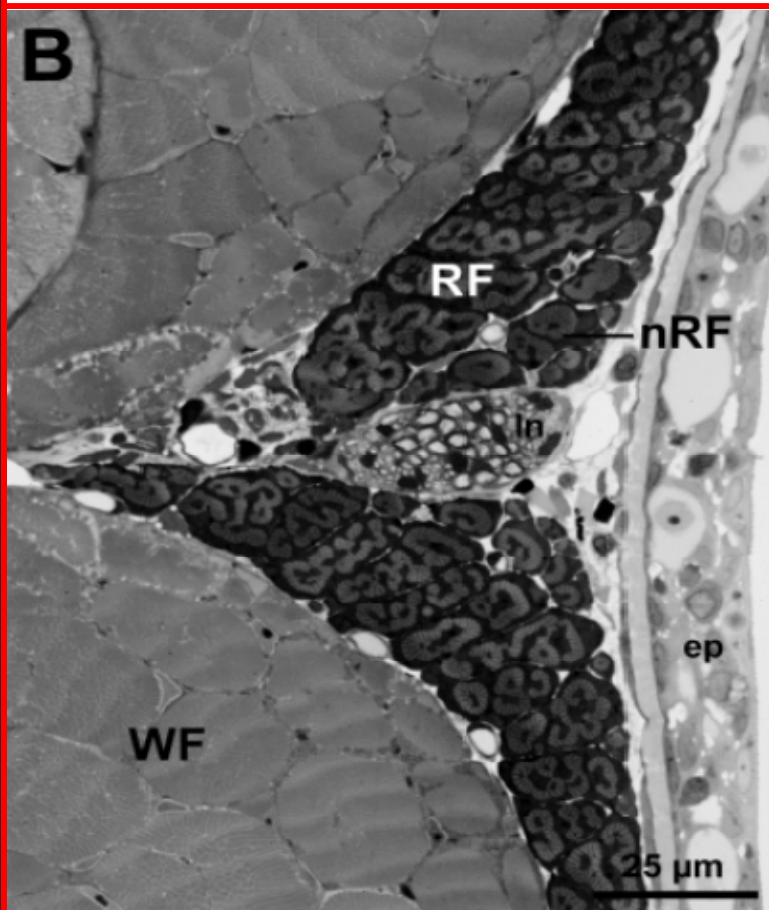
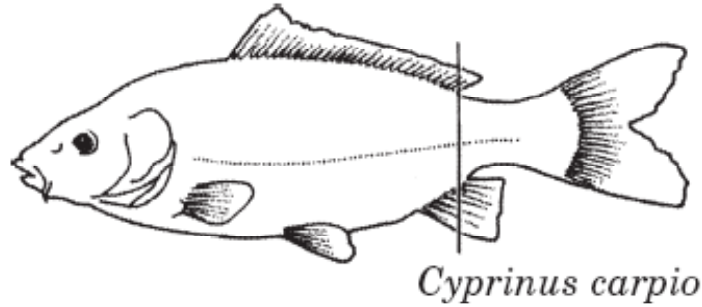


Tesser et al., 2006

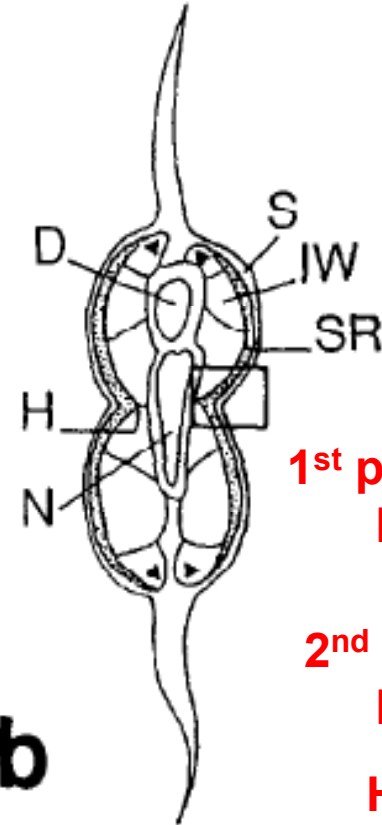
**Effects of feeding schedule and diet quality on pacu larvae growth**

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





Stoiber et al. (1999)



**1<sup>st</sup> phase: Stratified hyperplasia**

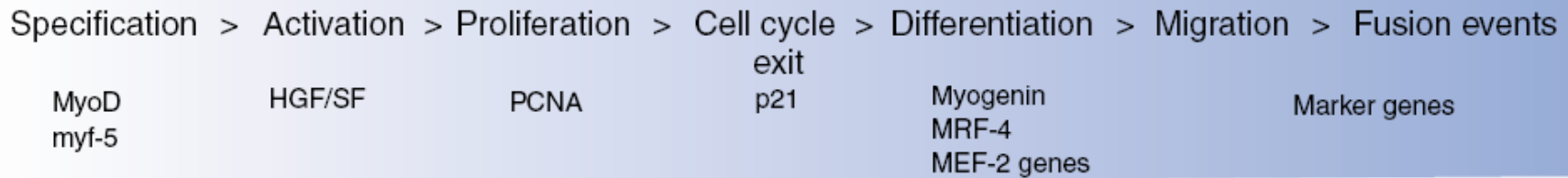
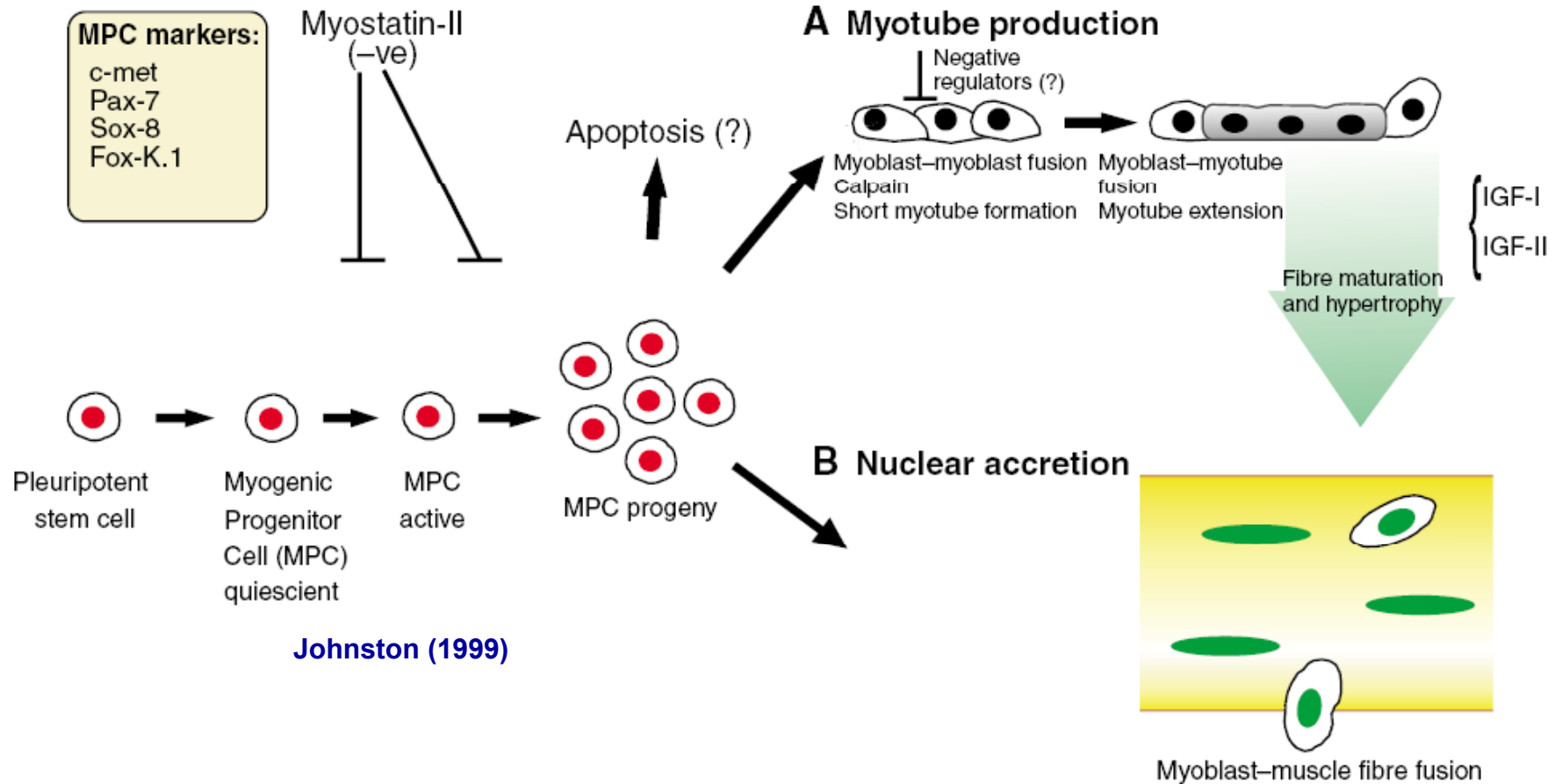
**2<sup>nd</sup> phase: Mosaic hyperplasia**

**Hypertrophy**

**b**

Koumans & Akster, 1995

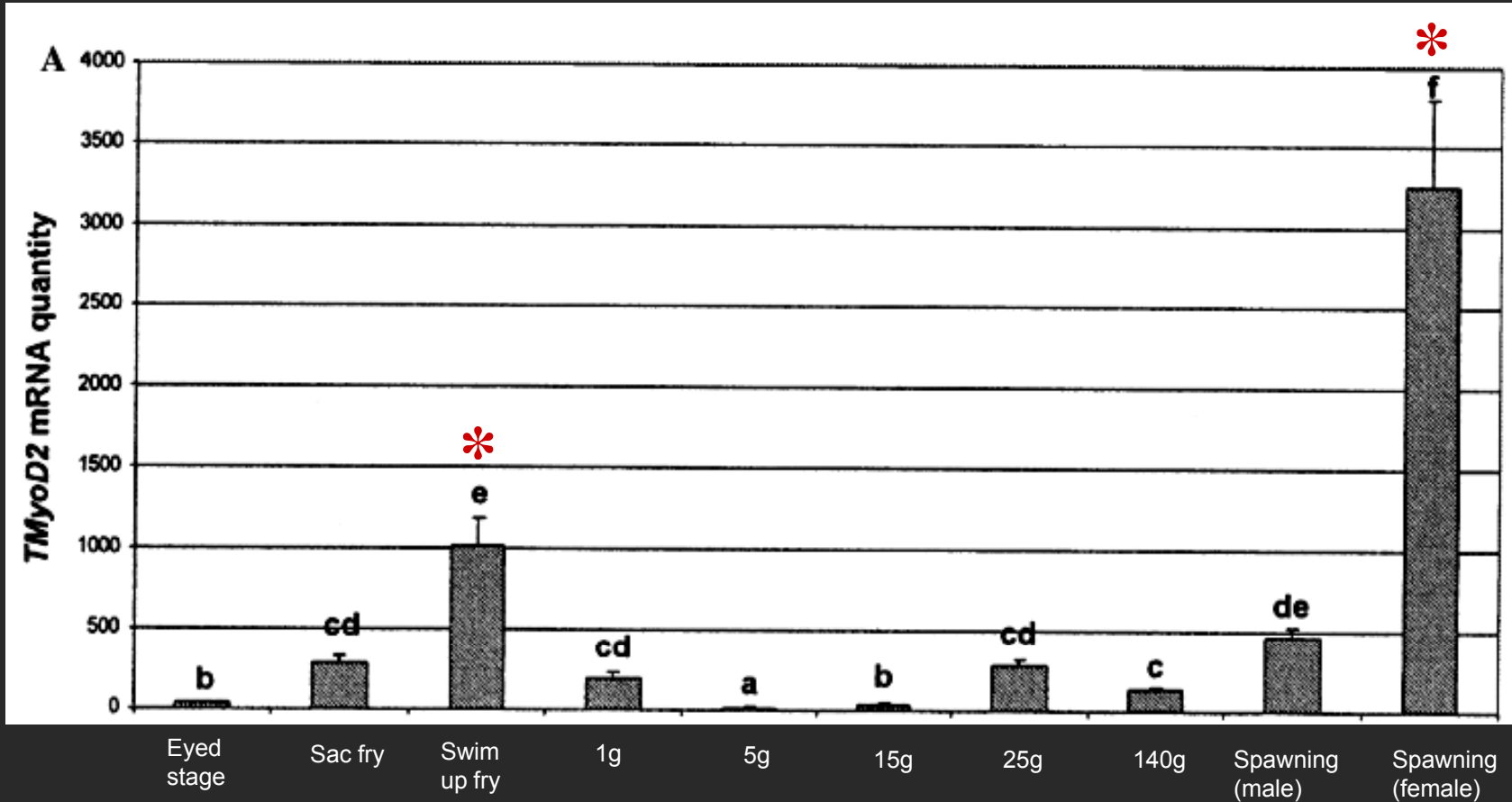
# Main Events of Myogenesis in Teleost Skeletal Muscle





# POST EMBRYONIC SKELETAL MUSCLE GROWTH

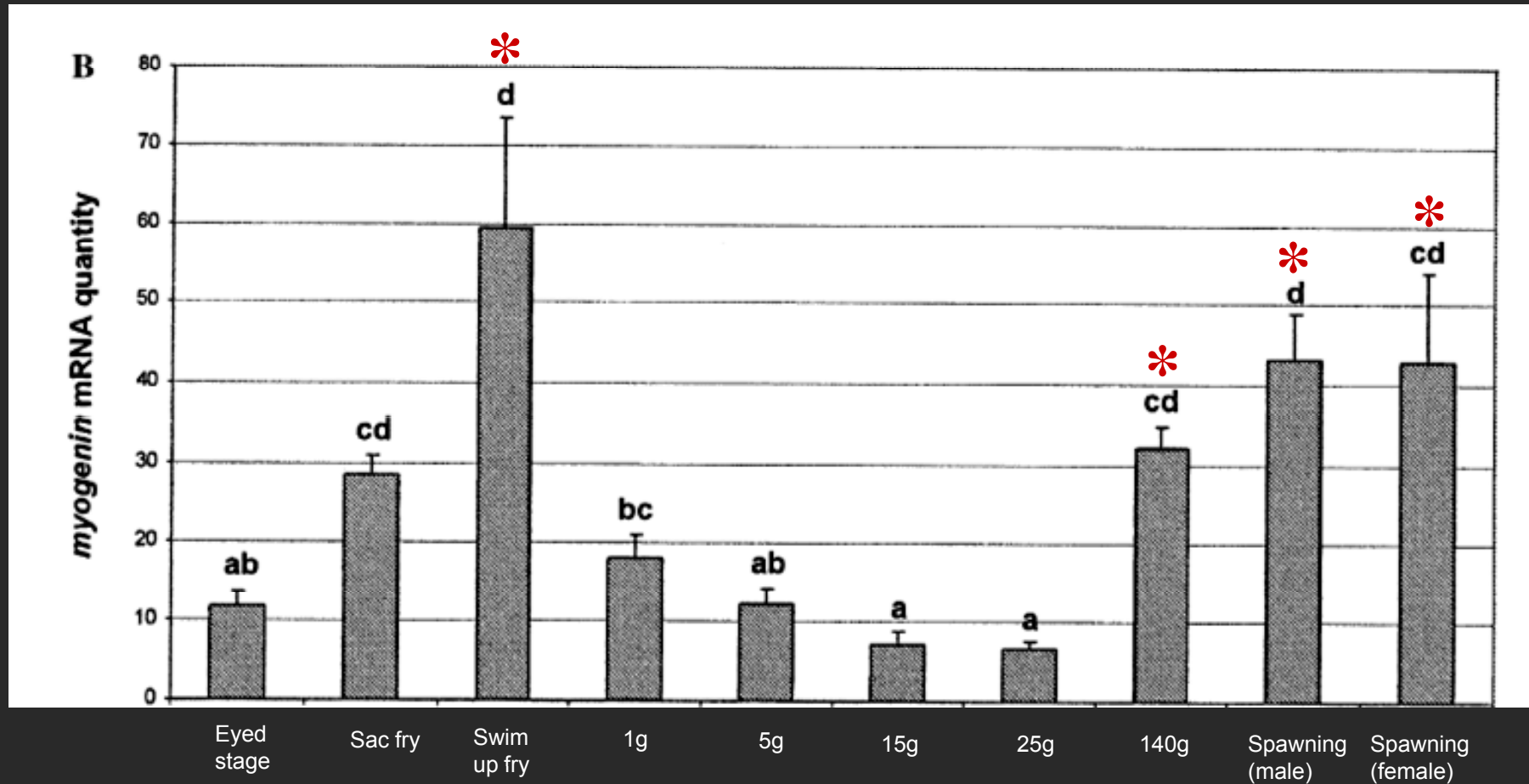
Quantitative expression analysis of genes affecting muscle growth during development of rainbow trout (*Oncorhynchus mykiss*). Expression levels of **TMyoD2**. Mean  $\pm$  SE of mRNA quantity is shown.



\* Myoblasts proliferation and hyperplasia

# 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  $\pm$  SE of mRNA quantity is shown.



\* Hypertrophy



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

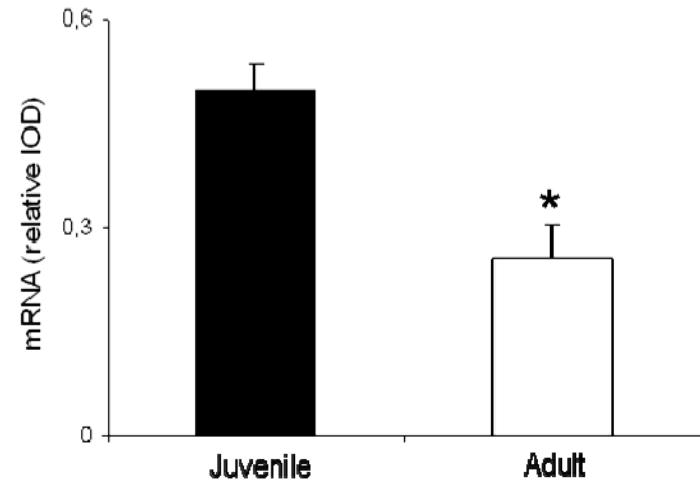
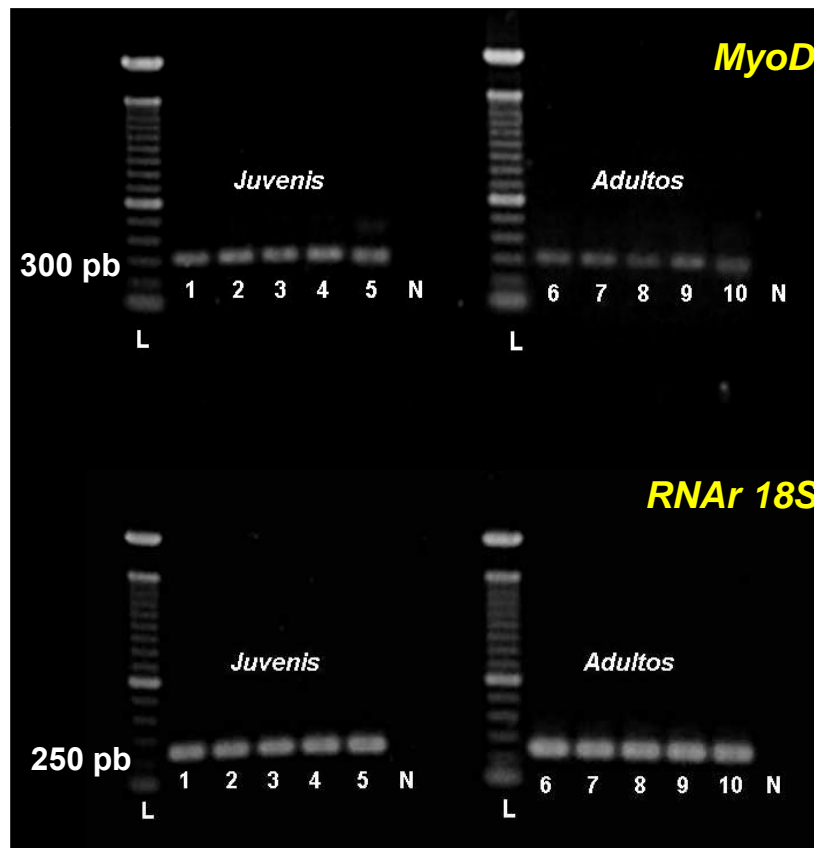
Fernanda Losi Alves de Almeida<sup>a,b</sup>, Robson Francisco Carvalho<sup>a</sup>, Danillo Pinhal<sup>a</sup>, Carlos Roberto Padovani<sup>c</sup>, Cesar Martins<sup>a</sup>, Maeli Dal Pai-Silva<sup>a,\*</sup>

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*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]

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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)

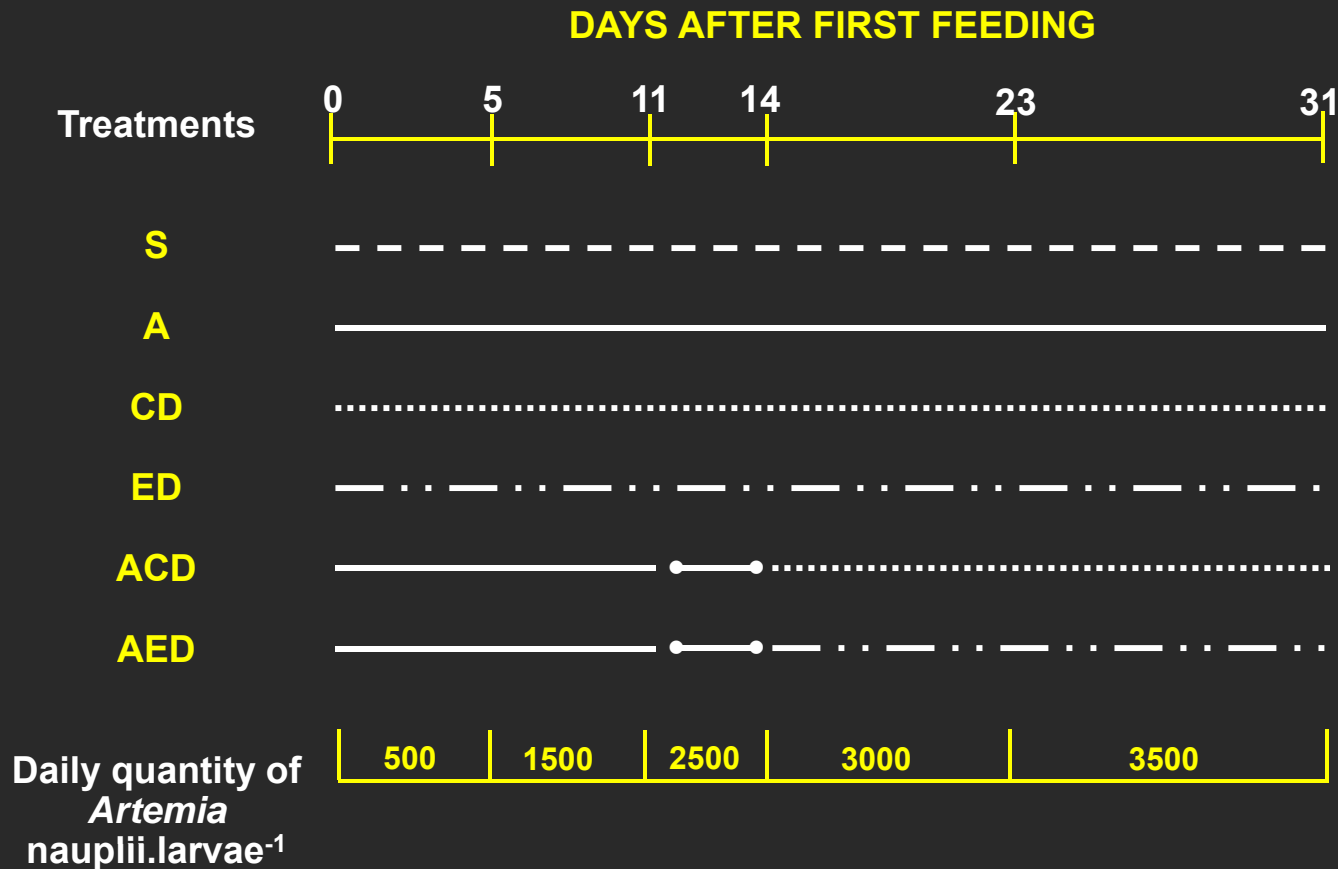
➤ **Beta-actina (mRNA) Sequence in *P. mesopotamicus* (5' – 3', 156 pb)**

(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 **hyperplastic** growth of muscle fibers and the expression of **MyoD** and **Myogenin**

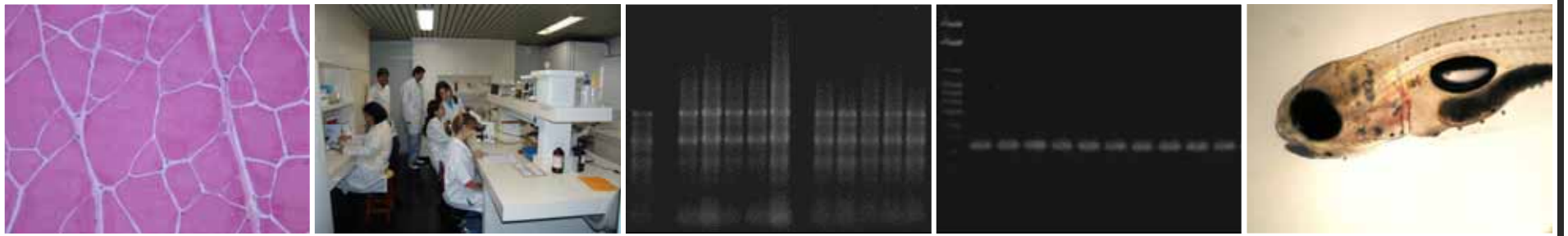
## ❖ Analyses

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

❖ **Morphology and Morphometry** (white muscle fibers were grouped into five diameter classes:  $\leq 10$ ,  $\leq 20$ ,  $\leq 30$ ,  $\leq 40$  and  $> 40 \mu\text{m}$ )

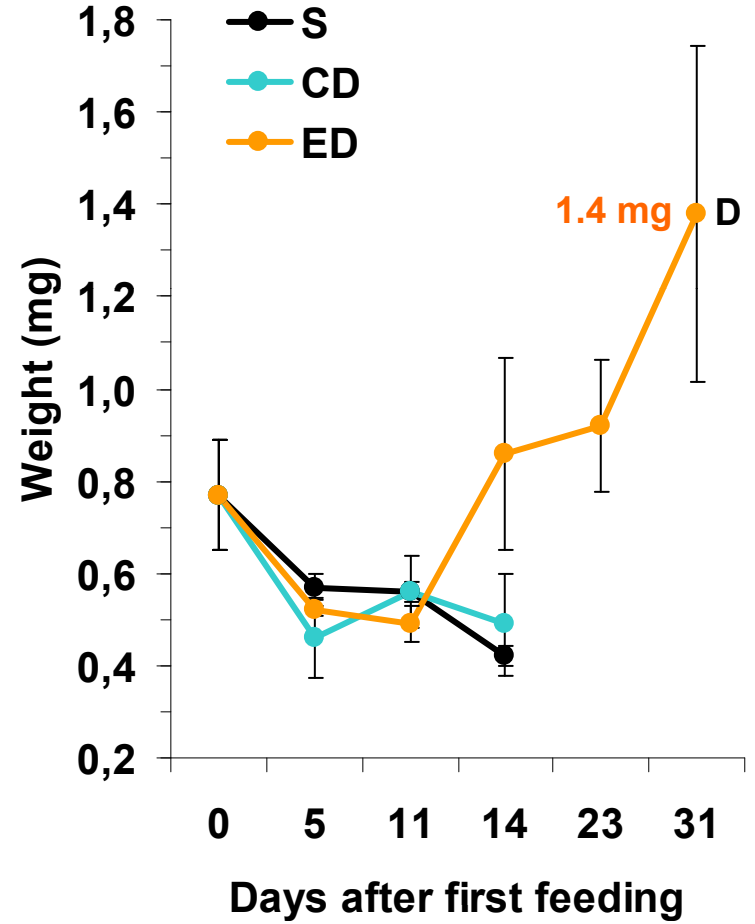
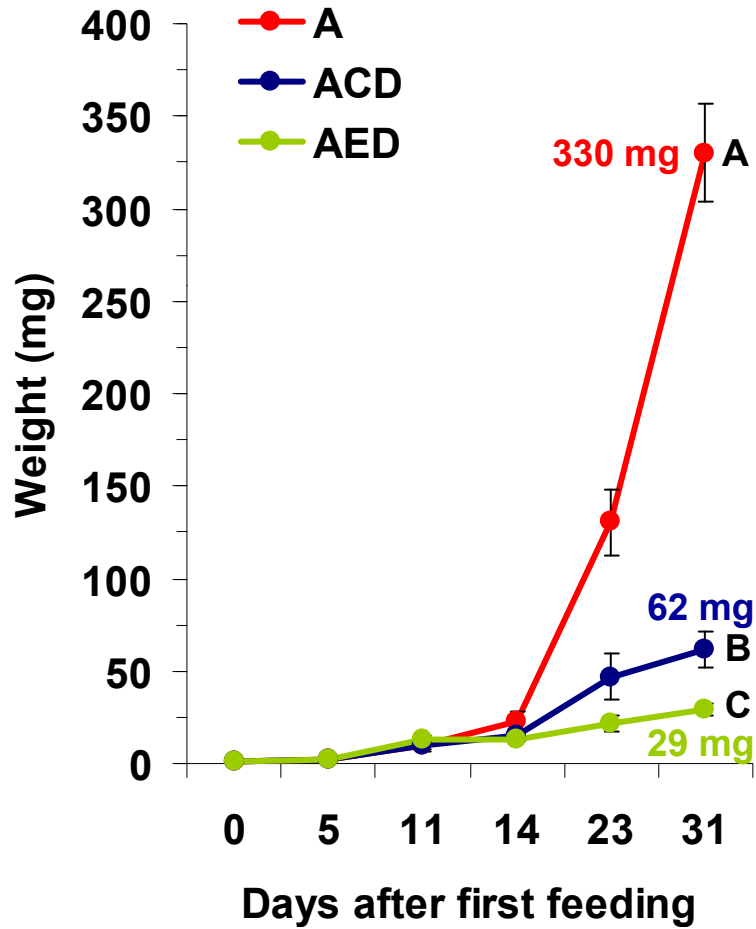
❖ **Expression of the MRFs MyoD and Myogenin by RT-PCR** (in the muscle of pacu larvae fed *Artemia* nauplii or formulated diets as a partial substitute for *Artemia* nauplii).

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

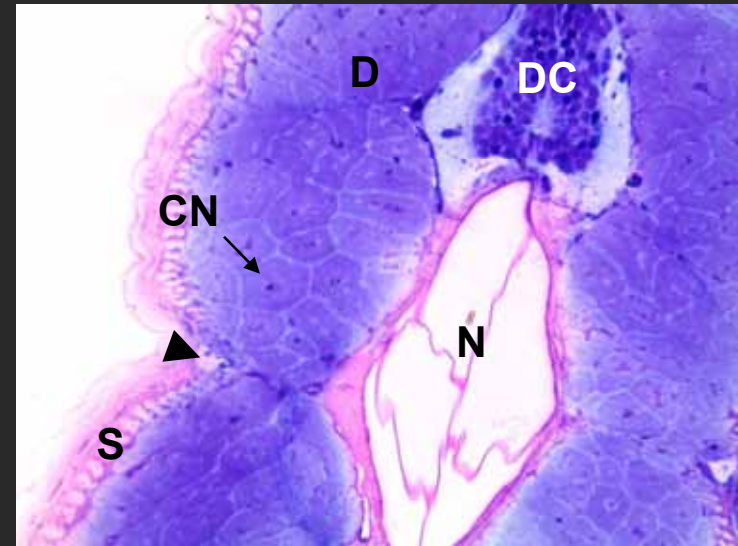
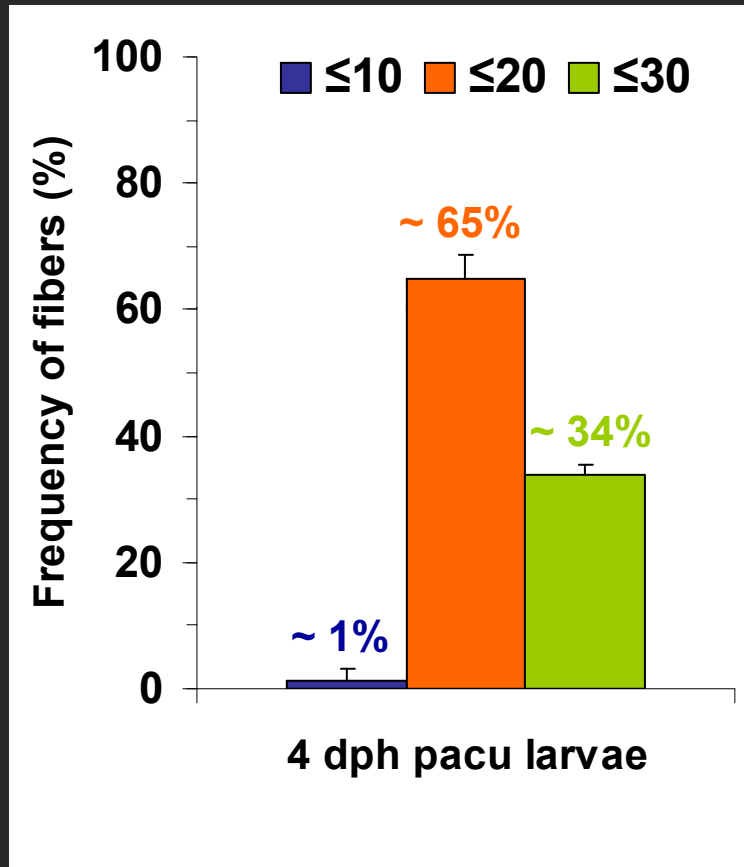




# Growth



# Morphology and morphometry

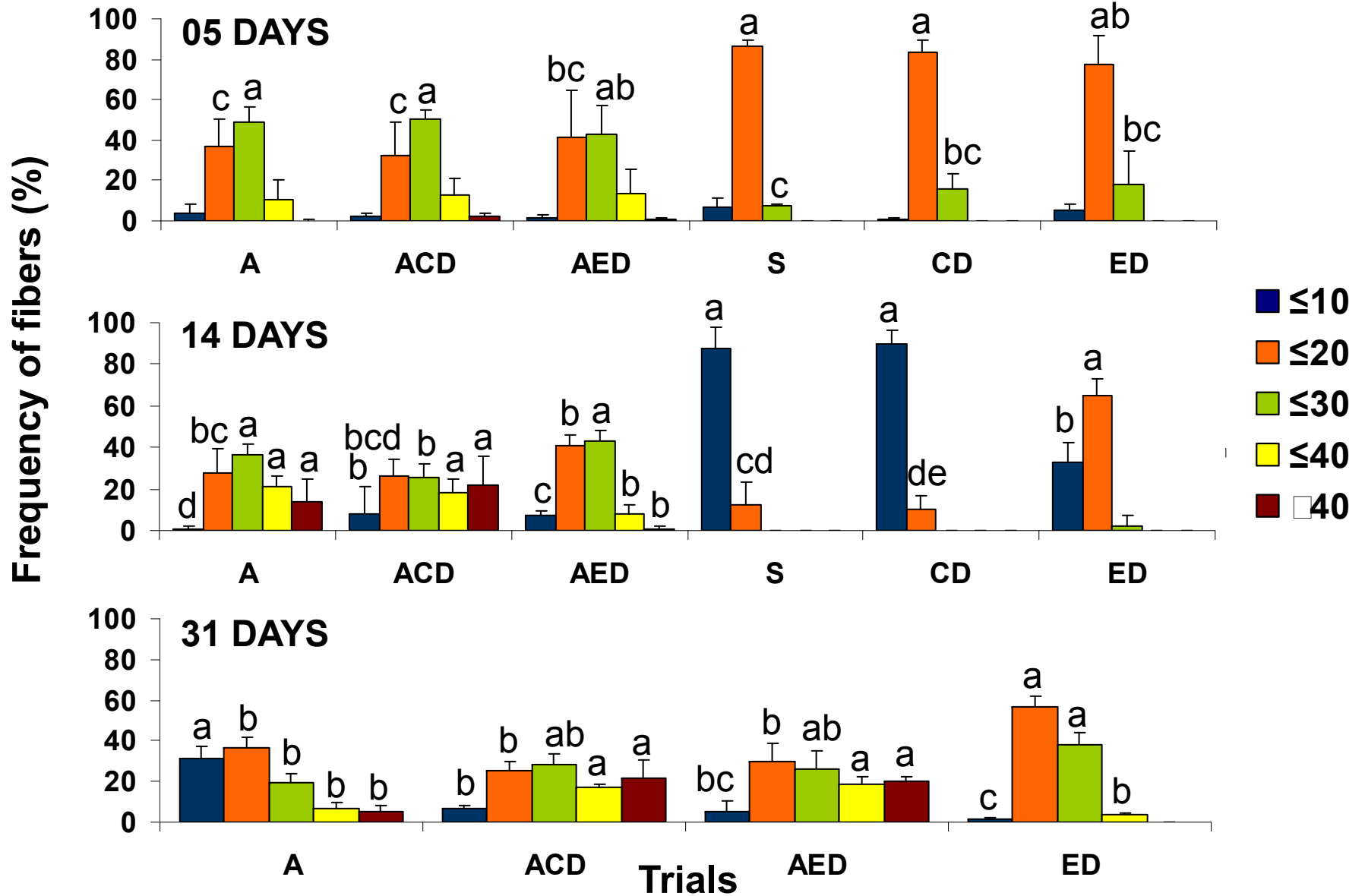


Transverse section of skeletal musculature in 4 dph pacu larvae. 400x.

Muscular fiber diameter distribution ( $\mu\text{m}$ ) in pacu larvae before first feeding

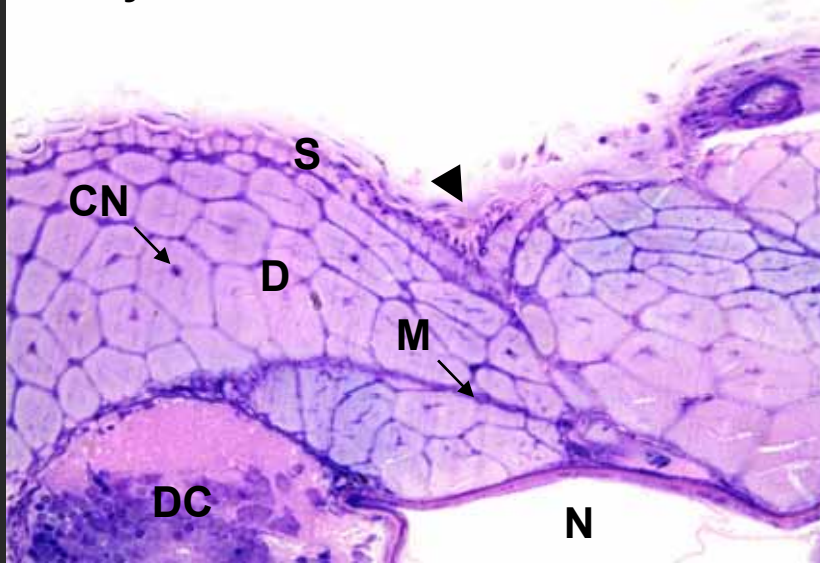
# Muscle fibers diameter distribution ( $\mu\text{m}$ ) in pacu larvae

05, 11, 14, 23 and 31 DAFF

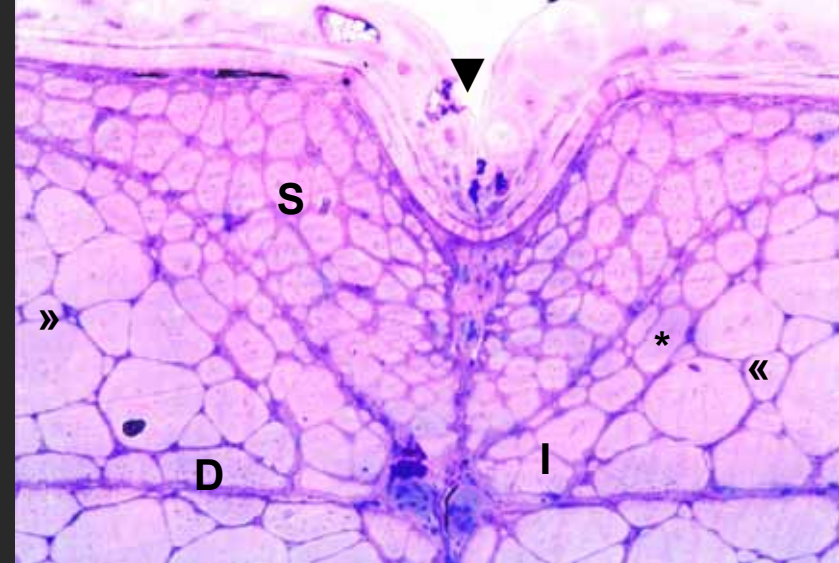


# Transverse section of skeletal muscle in pacu larvae fed *Artemia* nauplii. 400X

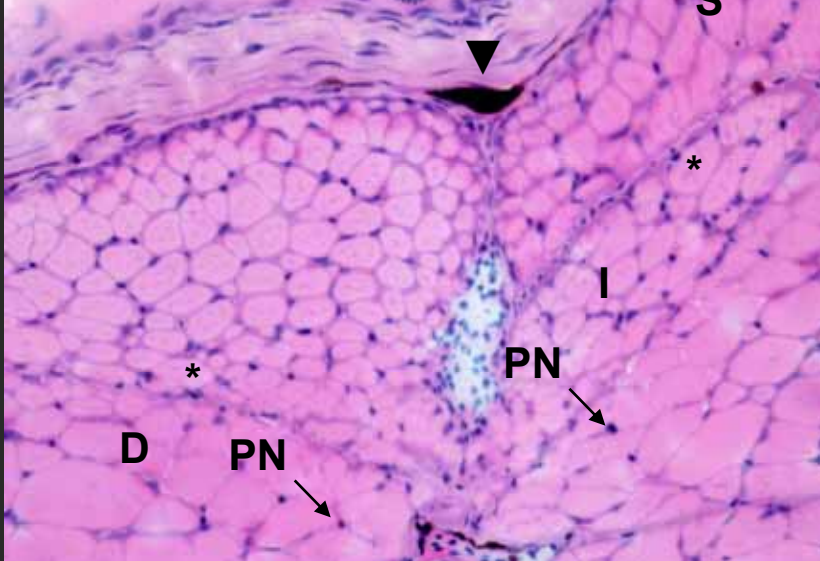
05 days



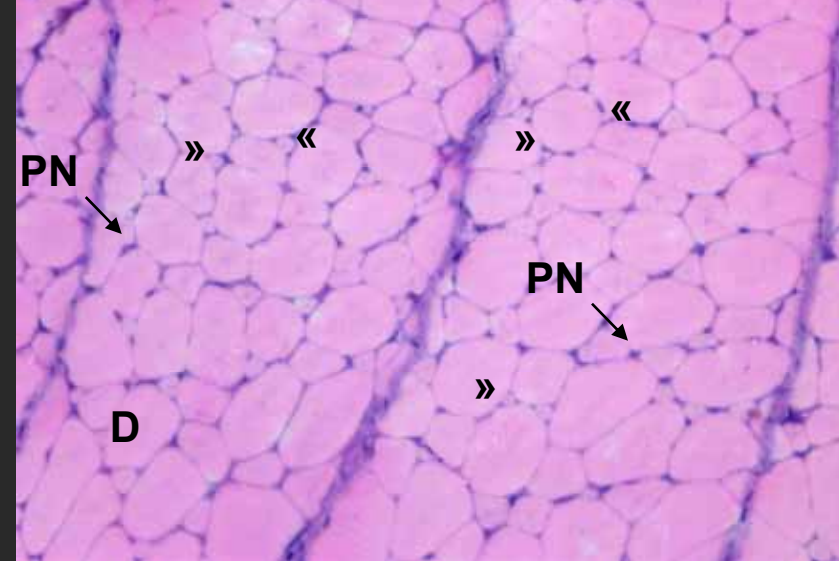
23 days



31 days



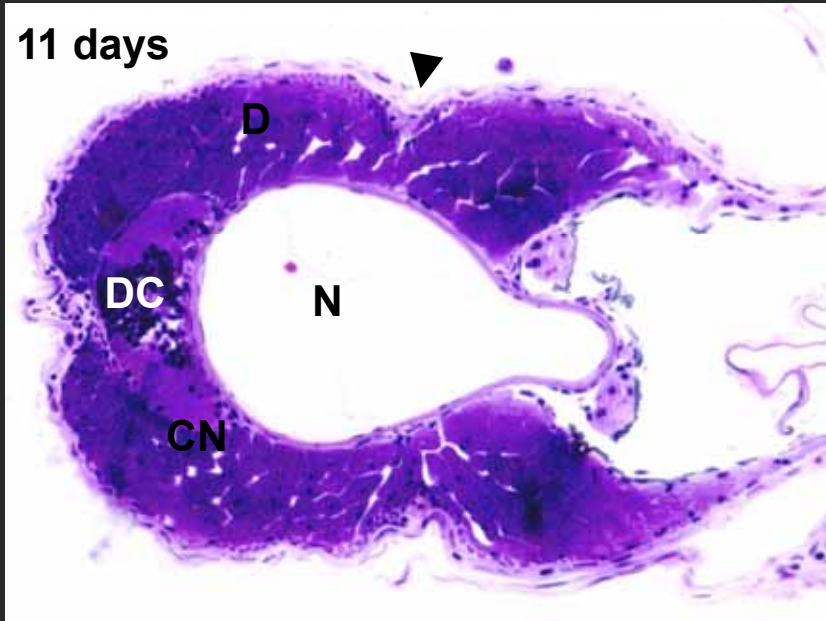
31 days



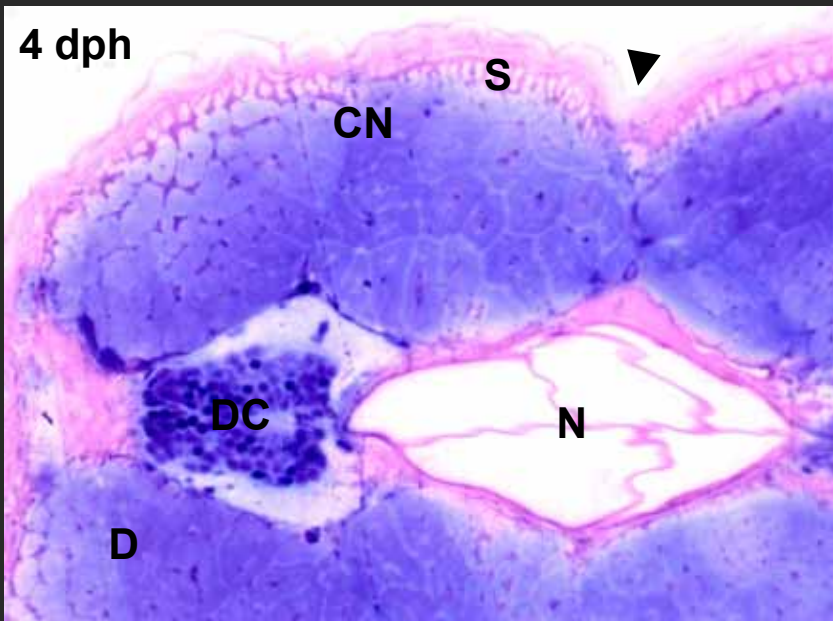


**Transverse section of skeletal muscle in pacu larvae on starvation S (left) and fed experimental diet AED (right) . 400X.**

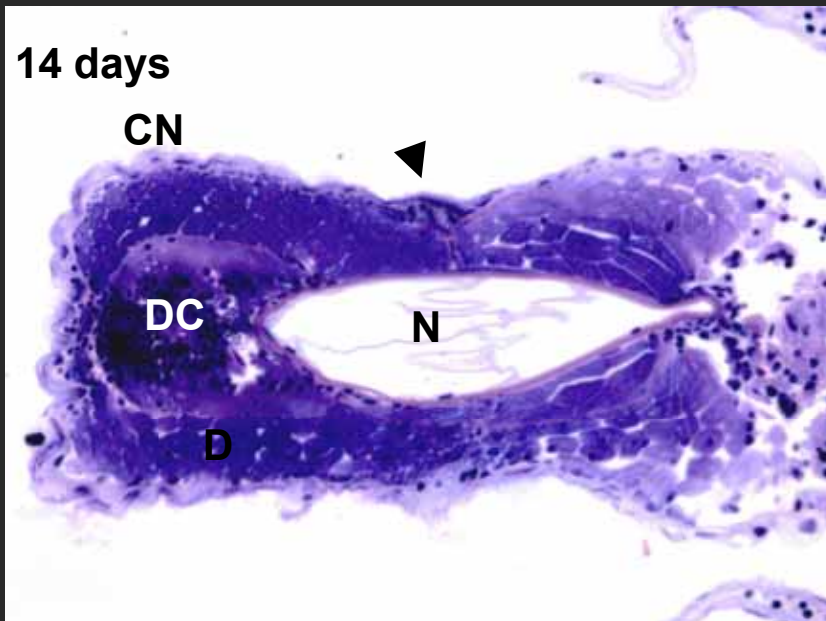
11 days



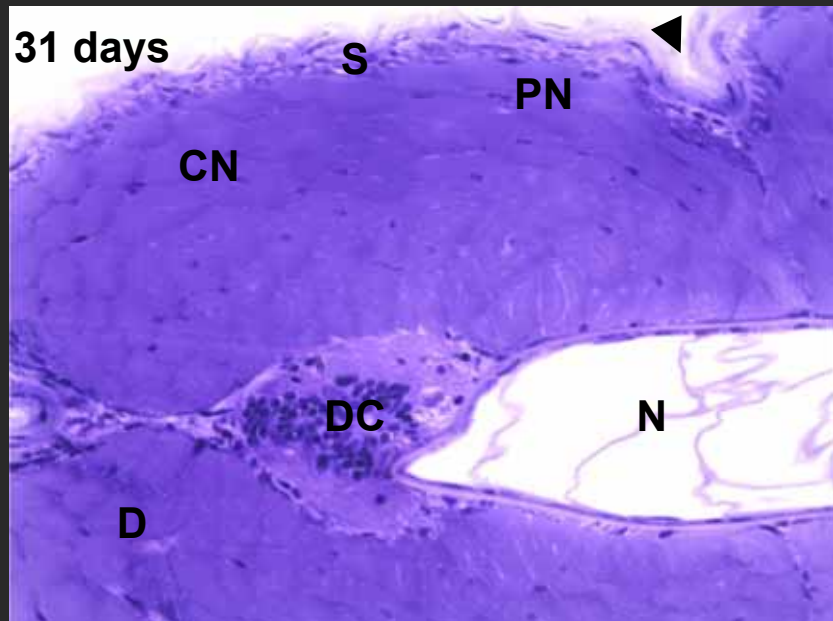
4 dph



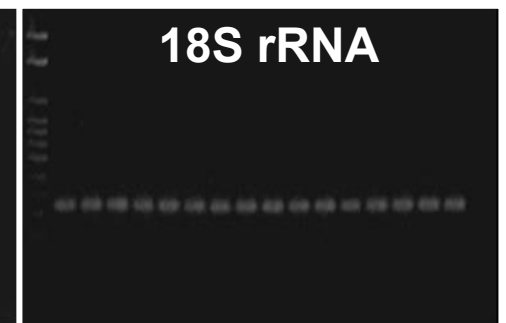
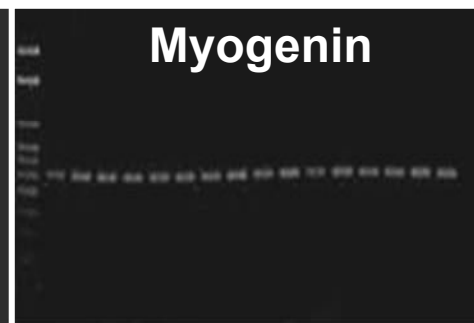
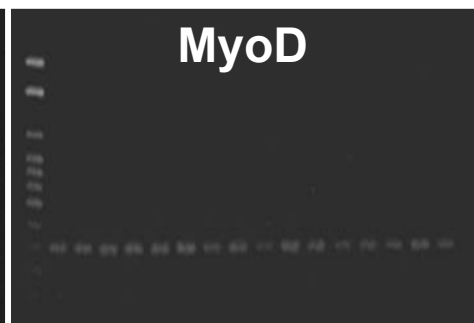
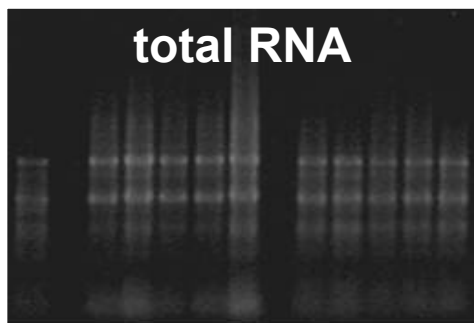
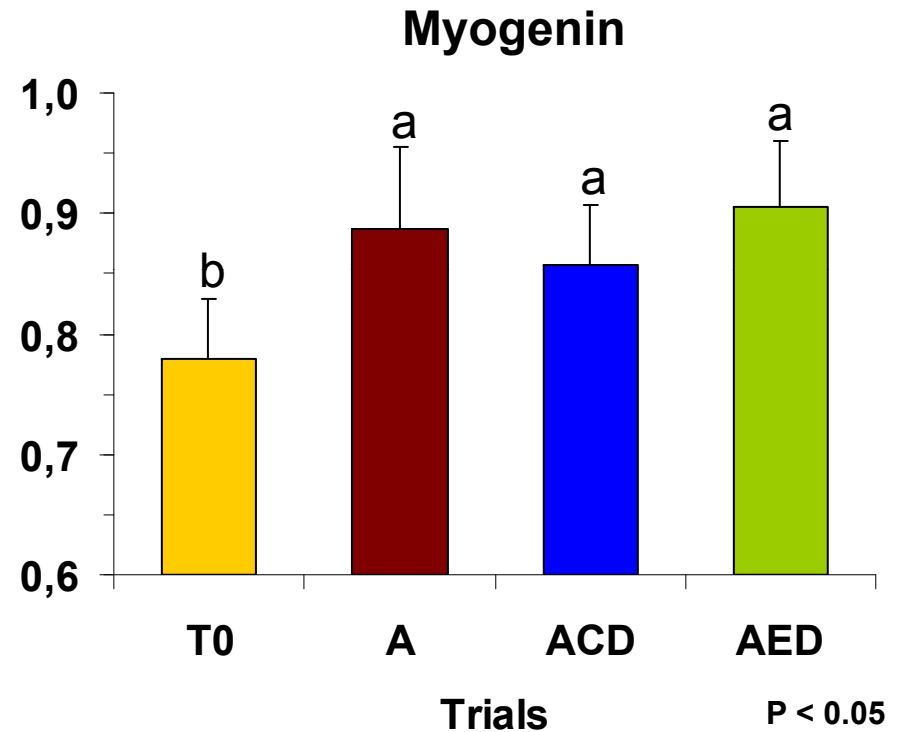
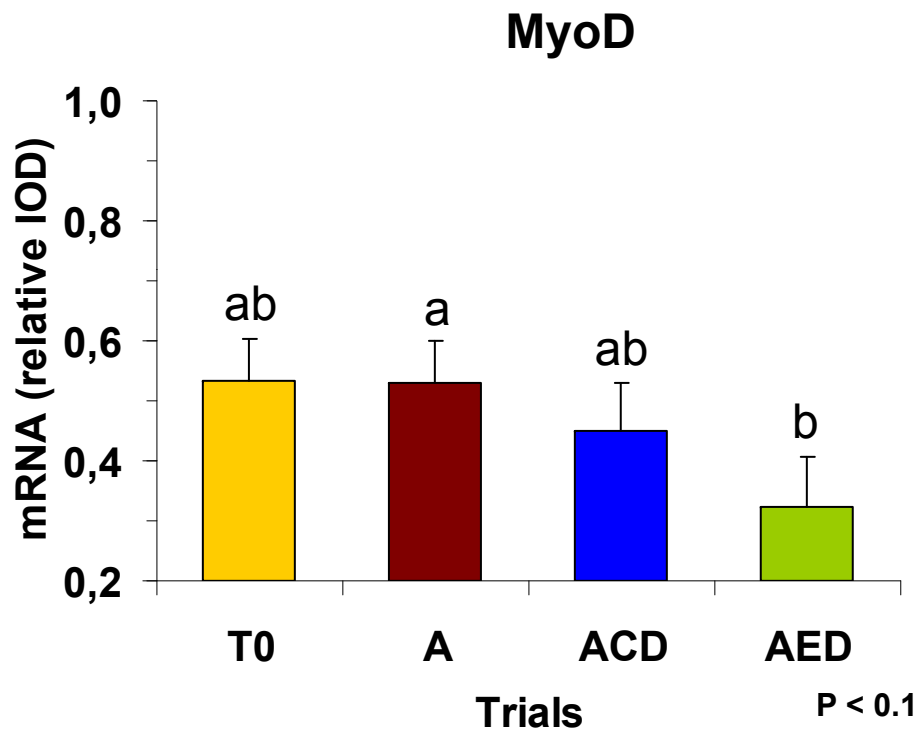
14 days



31 days



# 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  $> 40\mu\text{m}$ , 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

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State of the art

