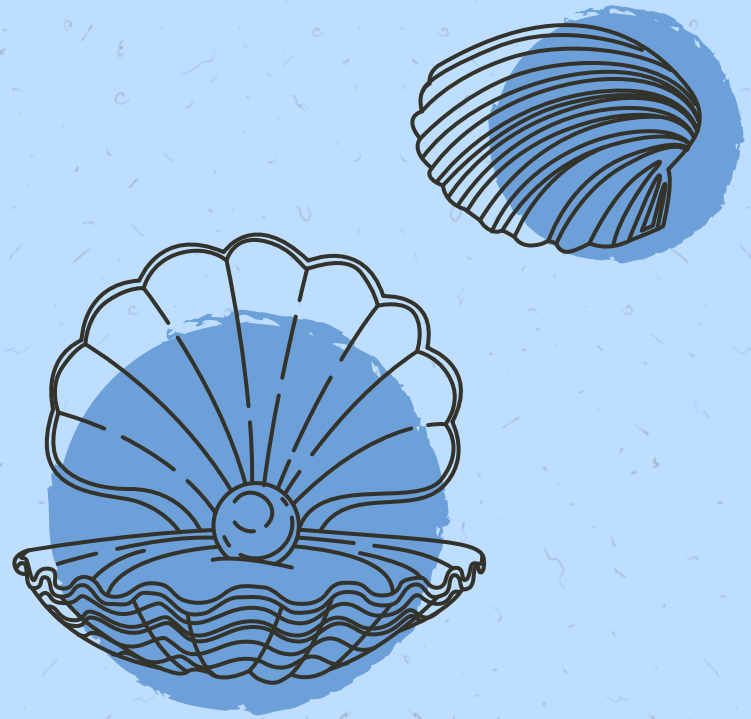




Ciências  
ULisboa

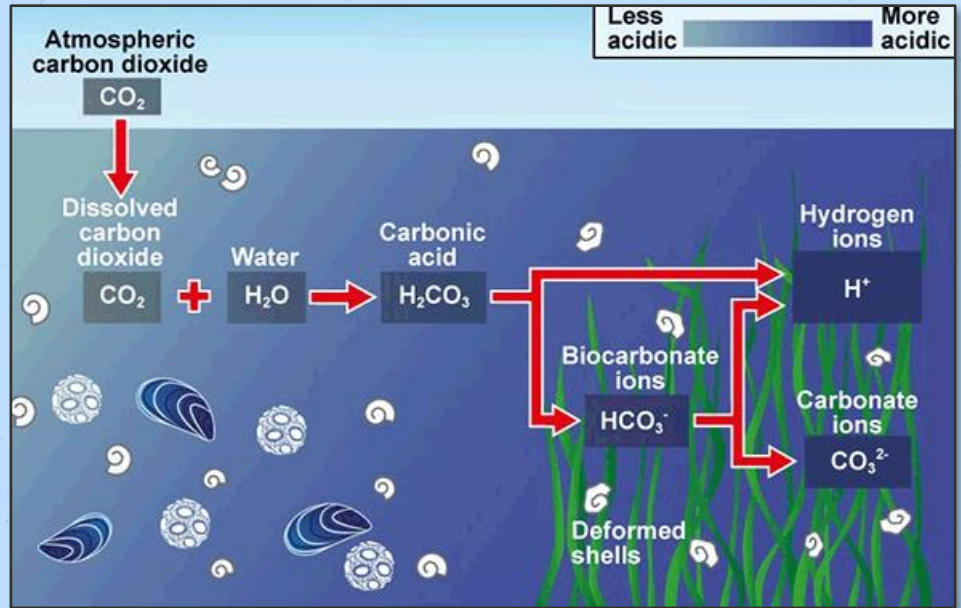
# Os efeitos da acidificação na *Crassostrea virginica*



Mariana Morais n°57921 | UC Genómica e Alterações Ambientais

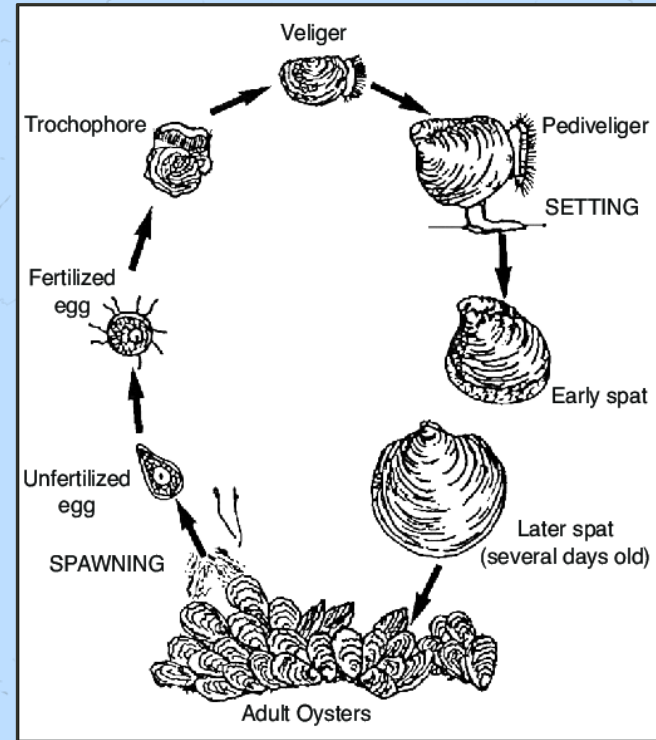
# Acidificação dos Oceanos

- Problema para calcificadores marinhos
- ↑ concentração de CO<sub>2</sub> na água leva a ↓ pH
- Último século – pH diminuiu 0,1
- Final do século – prevê-se que diminua de 8,2 para 7,8
- Afeta a deposição do material da concha

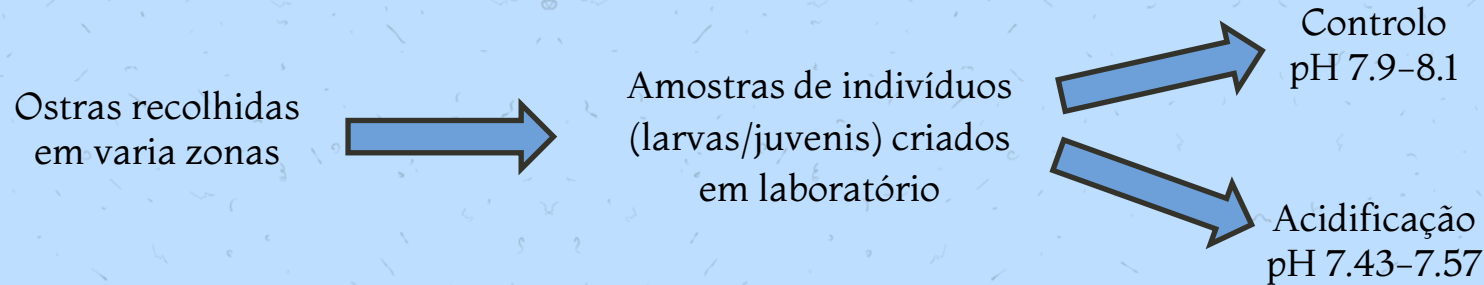


# Ostra oriental

- Potencialmente vulnerável à acidificação
- Larvas fazem a biomineralização da aragonite
- 2º bivalve mais importante no comércio dos Estados Unidos da América



# Desenho experimental / Workflow

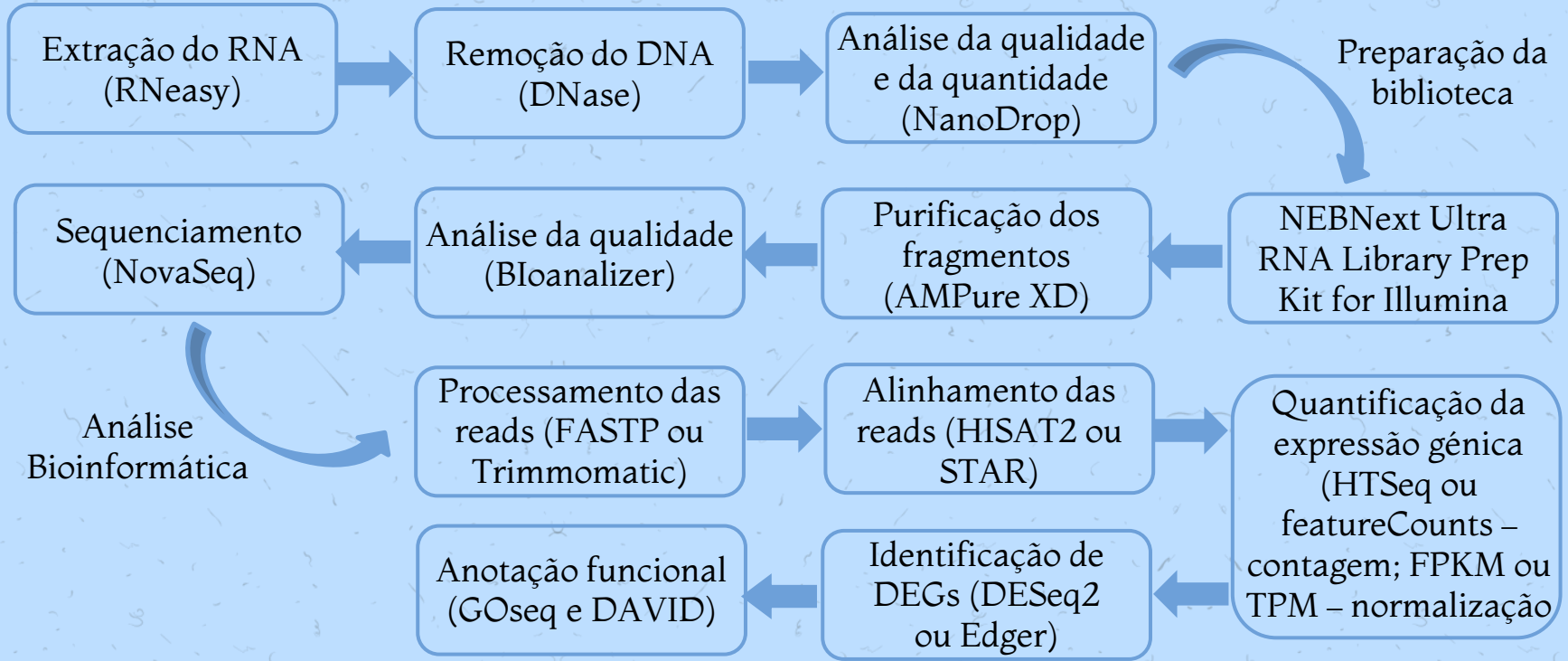


➔ Amostras recolhidas em diferentes alturas durante as experiências

➔ Foram feitas análises transcriptómicas, proteómicas e genómicas




# RNAseq



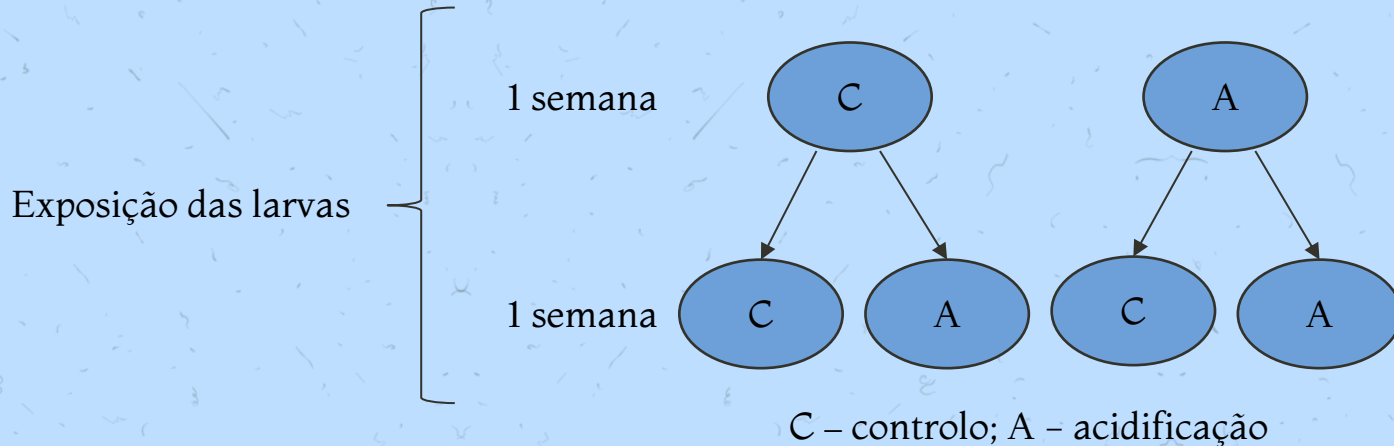
# Artigo 1

Article

## A Transcriptomic Analysis of Phenotypic Plasticity in *Crassostrea virginica* Larvae under Experimental Acidification

Michelle Barbosa †, Caroline Schwaner †, Emmanuelle Pales Espinosa and Bassem Allam \*

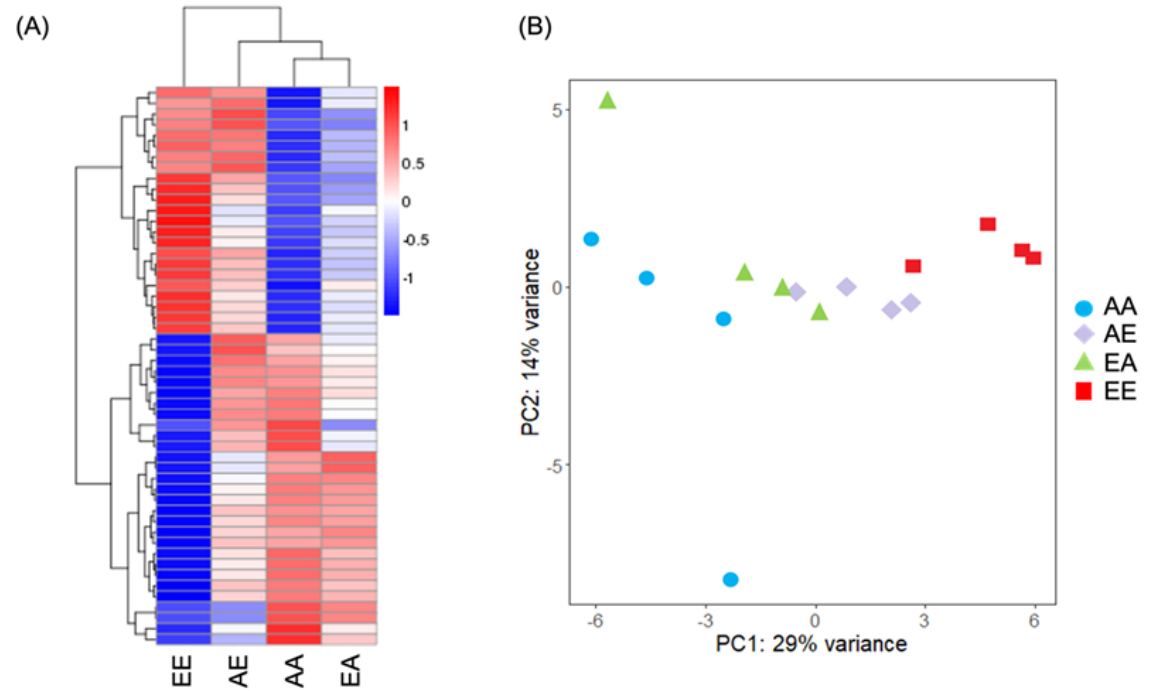
Objetivo: Ver se as larvas da ostra oriental apresentam recuperação à acidificação e quais os genes envolvidos nesta



# Artigo 1

Os clusters foram formados com base no tratamento final

Os DEGs identificados estão associados a vários fatores biológicos como a biomineralização, a homeostasia e o desenvolvimento



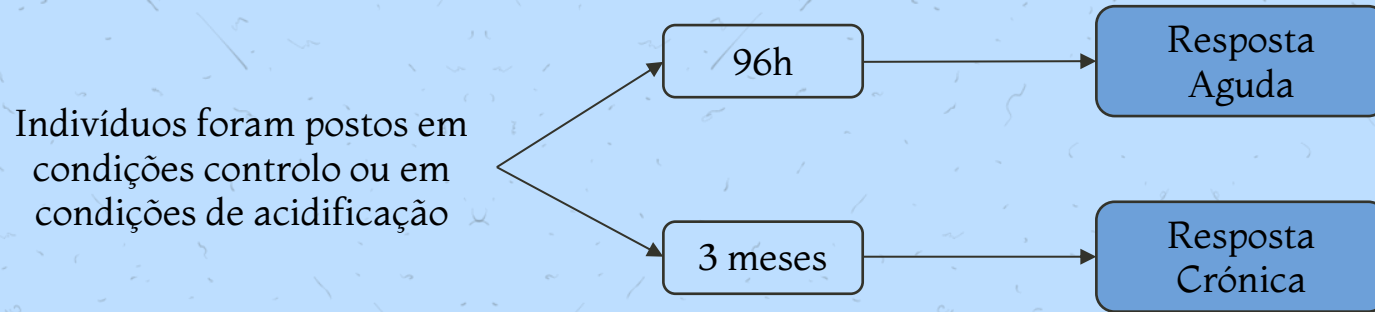
**Figure 4.** (A) Hierarchical cluster analysis of DEGs ( $n = 4$  replicates per treatment coalesced into 1 column). Over- and under-expressed genes are represented in red and blue, respectively. Data are normalized by average expression in all samples. (B) Principal component analysis of the normalized RNASeq data. Sample code names: first letter signifies original  $p\text{CO}_2$  treatment (A: ambient, E: elevated); second letter signifies final  $p\text{CO}_2$  treatment (first and second letters are different for transplanted treatments).

# Artigo 2

## Combination of RNAseq and RADseq to Identify Physiological and Adaptive Responses to Acidification in the Eastern Oyster (*Crassostrea virginica*)

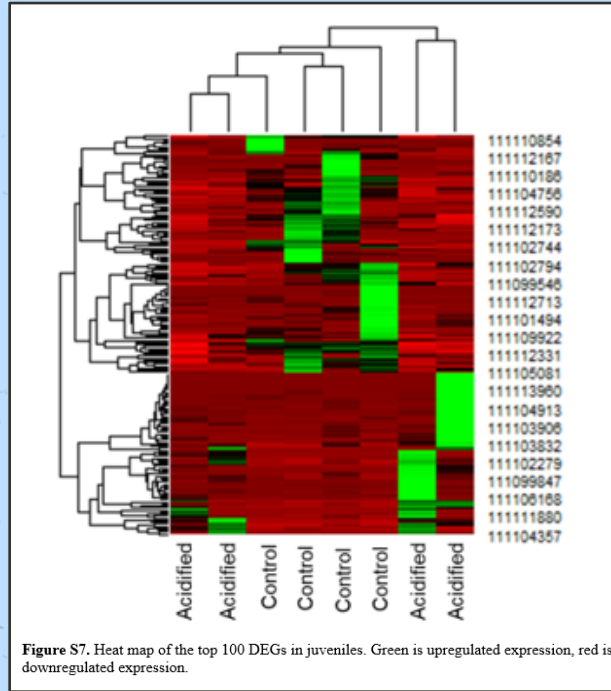
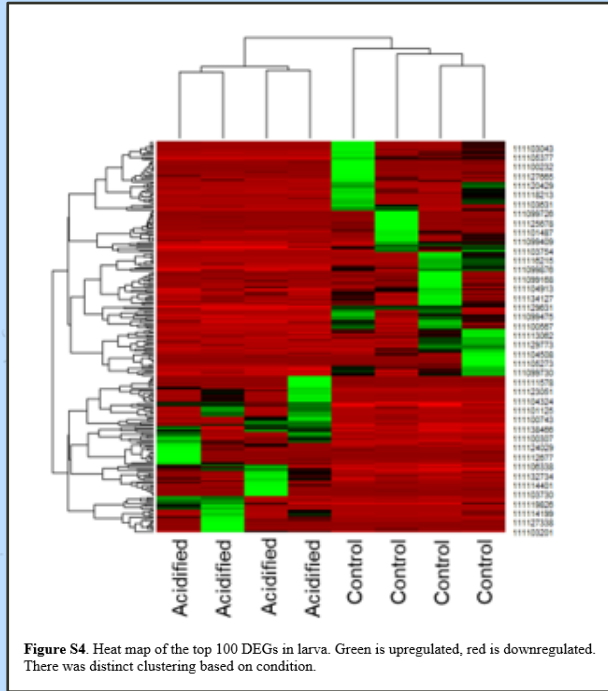
Caroline Schwaner<sup>1</sup> · Sarah Farhat<sup>1,2</sup> · Isabelle Boutet<sup>3</sup> · Arnaud Tanguy<sup>3</sup> · Michelle Barbosa<sup>1</sup> · Denis Grouzdev<sup>1</sup> · Emmanuelle Pales Espinosa<sup>1</sup> · Bassem Allam<sup>1</sup>

Objetivo: Identificar genes que estivessem regulados de forma diferente como resposta à acidificação tanto num curto como num longo espaço de tempo





# Artigo 2



Há um agrupamento da expressão diferencial em indivíduos que estavam no ambiente controlo e indivíduos que estavam no ambiente acidificado

# Artigo 2

Genes sub-expressos têm a ver com a biomineralização, resposta imunitárias, crescimento e citoesqueleto

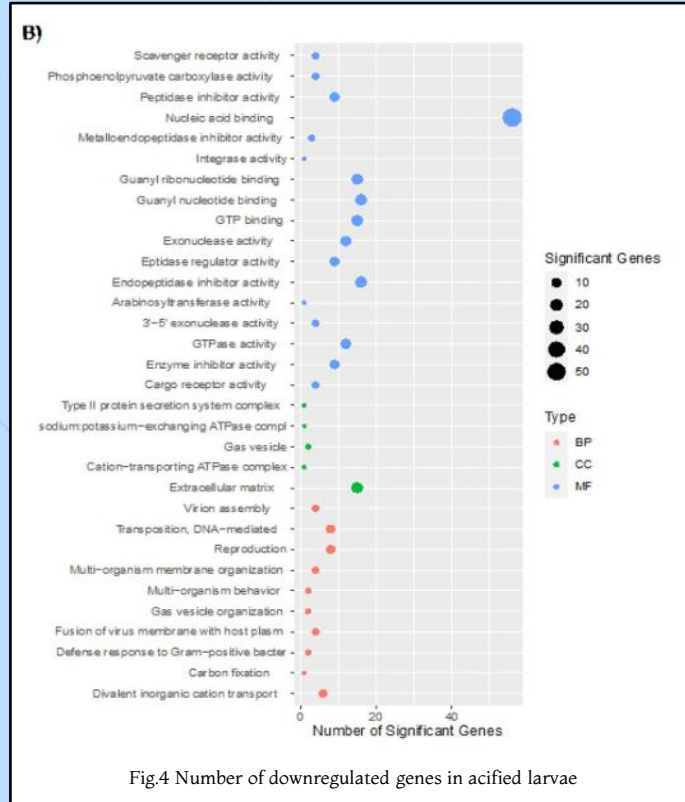


Fig.4 Number of downregulated genes in acidified larvae

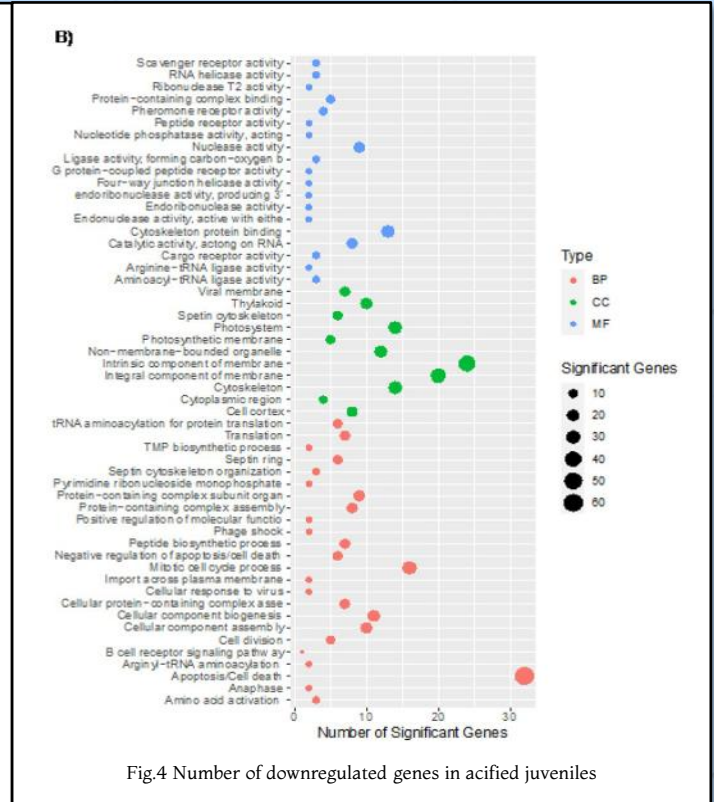


Fig.4 Number of downregulated genes in acidified juveniles

# Artigo 2

Genes sobreprensos têm a ver com o metabolismo

Com o aumento do tempo de exposição à acidificação há um aumento do número de genes com expressão diferencial

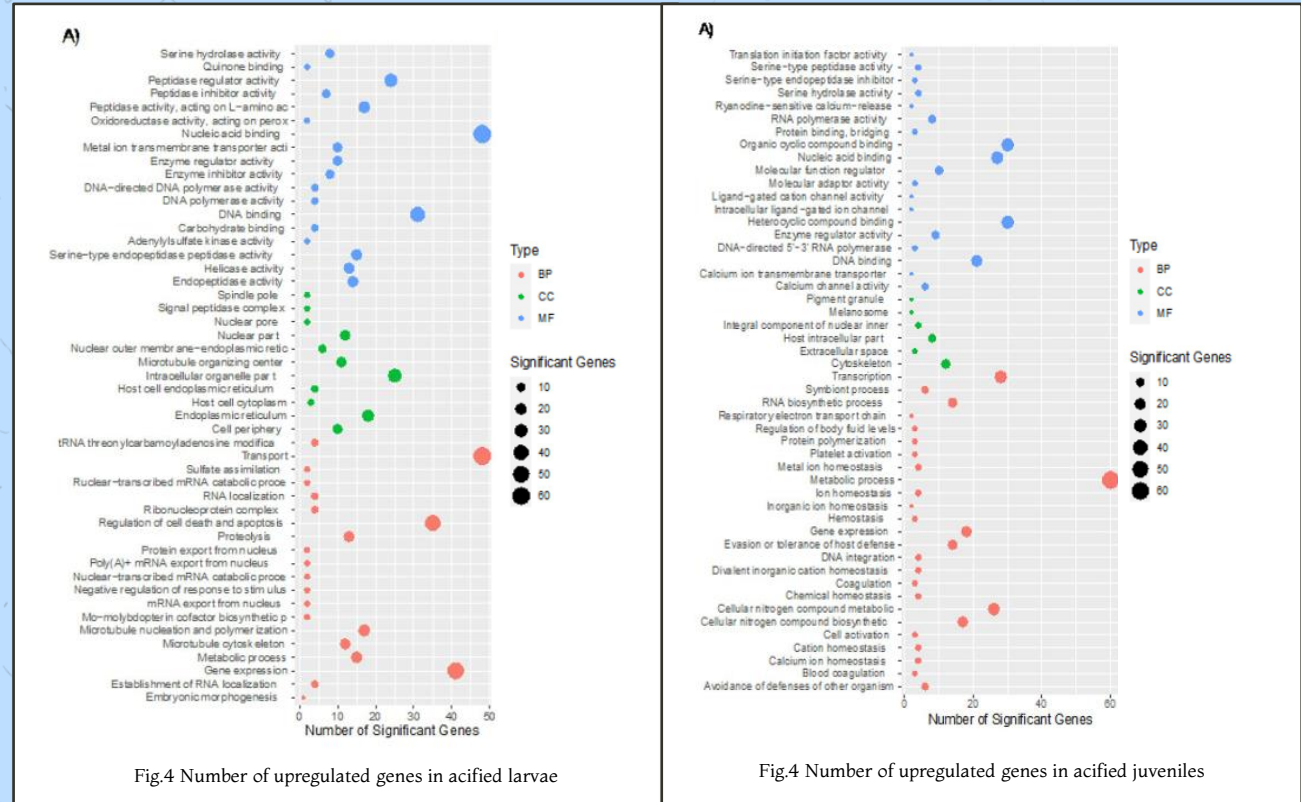


Fig.4 Number of upregulated genes in acidified larvae

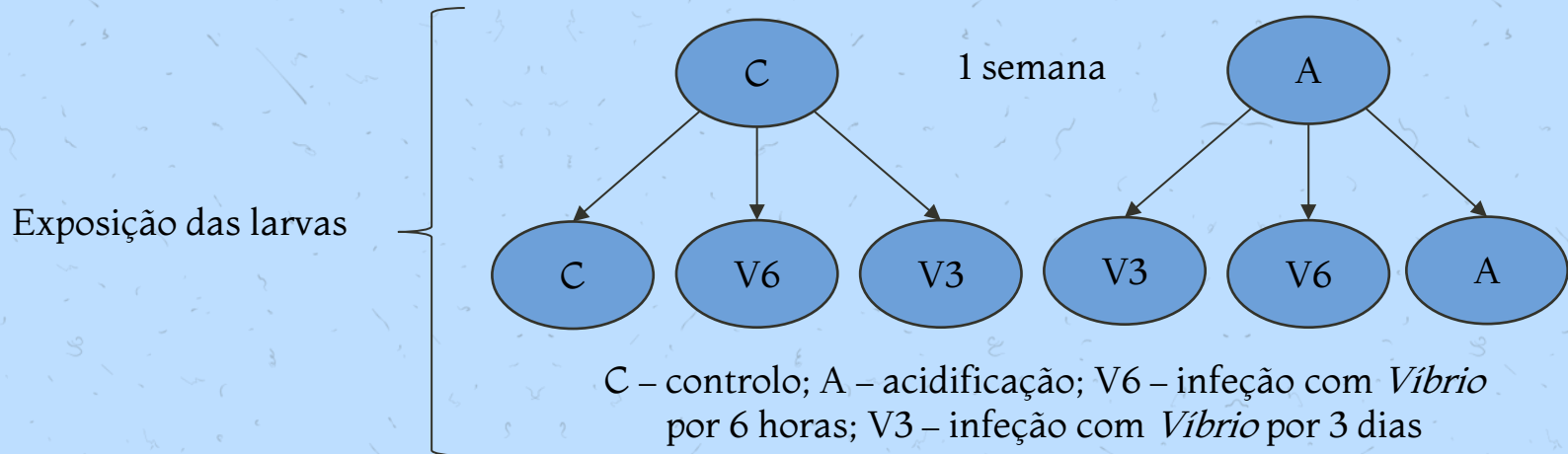
Fig.4 Number of upregulated genes in acidified juveniles

# Artigo 3

Transcriptomics, proteomics, and physiological assays reveal immunosuppression in the eastern oyster *Crassostrea virginica* exposed to acidification stress

Caroline Schwaner<sup>a</sup>, Michelle Barbosa<sup>a</sup>, John Haley<sup>b</sup>, Emmanuelle Pales Espinosa<sup>a</sup>, Bassem Allam<sup>a,\*</sup>

Objetivo: Identificar genes com expressão diferencial quando as larvas estão expostas à acidificação e a uma infecção com um patógeno



# Artigo 3

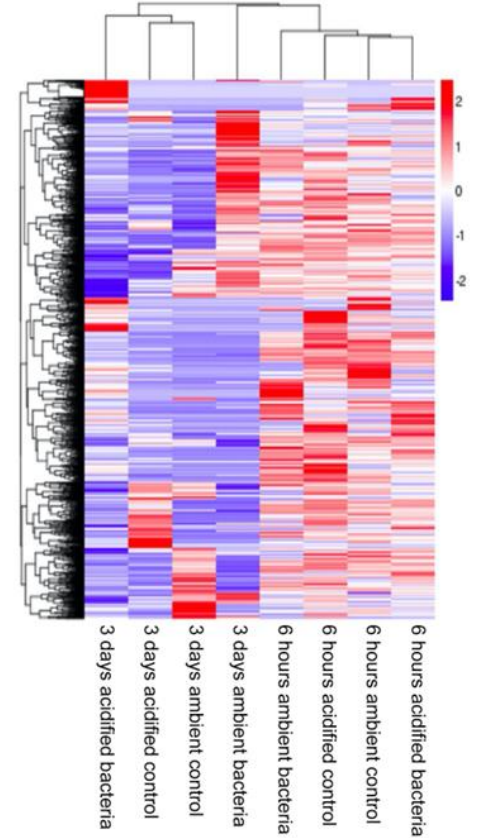
Clusters formados entre larvas que foram infectadas durante 3 dias ou durante 6 horas

**Table 3**

Sixteen characterized genes were among the top 25 (based on adjusted *p*-value) DEGs downregulated in larvae challenged with *Vibrio* spp. and maintained in acidified seawater for three days as compared to challenged larvae maintained in ambient seawater. See Supplementary Table S9 for the full list including those with unknown function.

Gene ID	Protein	Function
111100266	V-type proton ATPase subunit E-like	Immunity
111100865	Myophilin-like	Actin crosslink formation
111101814	8-Oxo-dGDP phosphatase NUDT18-like	Hydrolase activity
111101848	Protein XRP2-like	Cell morphogenesis
111103230	Homeobox protein Hox-B3a-like	Transcription
111103987	Glutathione S-transferase P 1-like	Oxidation-reduction process
111104800	Perlucin-like	Biomineralization
111107502	Ctenidin-3-like	Immunity
111107754	cystatin-A5-like	Immunity
111108501	Kelch-like protein 15	Immunity
111108710	Prostaglandin reductase 1-like	Metabolism
111109163	Protein limb expression 1 homolog	Unknown
111109360	Ankyrin repeat, PH and SEC7 domain containing protein secG-like	Unknown
111113270	ADP-ribosylation factor-like protein 5B	GTP binding
111119681	Dedicator of cytokinesis protein 7-like	Signal transduction
111119746	Glycine dehydrogenase (decarboxylating), mitochondrial-like	Electron transfer activity

Muitos dos DEGs identificados envolvidos na imunidade



**Fig. 2.** Hierarchical cluster analysis of differentially expressed genes. Over- and under-expressed genes are represented in red and blue, respectively. Data are normalized by average expression in all samples ( $n = 3$  replicates per treatment).

# Artigo 3

Via KEGG enriquecida relacionada com as vias metabólicas

Possível trade-off entre o metabolismo e a imunidade

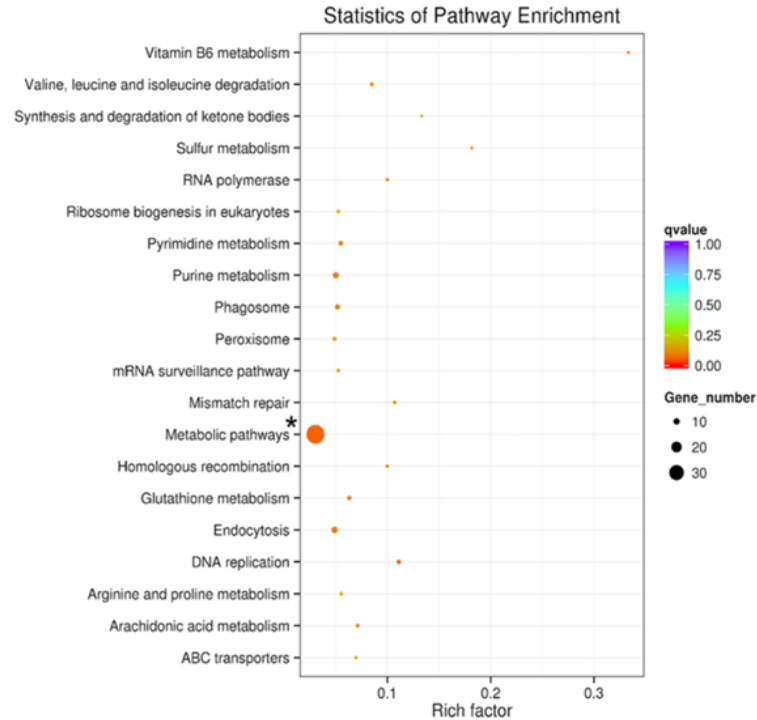
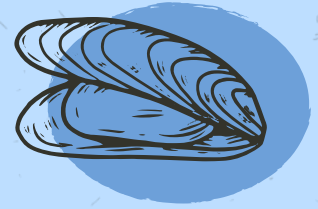


Fig. 3. KEGG pathways identified in larvae challenged with bacteria and maintained in acidified seawater for three days as compared to challenged larvae maintained in ambient seawater. Only one pathway ("metabolic pathways") was significantly overrepresented (\*).

# Conclusão



## Artigo 1

Plasticidade  
genética

Capacidade de  
recuperação

## Artigo 2

Genes sub-expressos  
têm funções como  
biomineralização e  
imunidade

Genes sobreexpressos  
têm a ver com o  
metabolismo

## Artigo 3

Maior suscetibilidade  
a infecção em  
condições de  
acidificação

Trade-off entre  
metabolismo e  
imunidade

# Bibliografia

- Barbosa, M., Schwaner, C., Pales Espinosa, E., & Allam, B. (2022). A Transcriptomic Analysis of Phenotypic Plasticity in *Crassostrea virginica* Larvae under Experimental Acidification. *Genes*, 13(9), 1529. <https://doi.org/10.3390/genes13091529>
- Schwaner, C., Farhat, S., Boutet, I. *et al.* (2023). Combination of RNAseq and RADseq to Identify Physiological and Adaptive Responses to Acidification in the Eastern Oyster (*Crassostrea virginica*). *Mar Biotechnol* 25, 997–1019. <https://doi.org/10.1007/s10126-023-10255-y>
- Schwaner, C., Barbosa, M., Haley, J., Espinosa, E. P., Allam, B. (2024). Transcriptomics, proteomics, and physiological assays reveal immunosuppression in the eastern oyster *Crassostrea virginica* exposed to acidification stress. *Fish & Shellfish Immunology*, Volume 146. ISSN 1050-4648. <https://doi.org/10.1016/j.fsi.2024.109366>.
- [What is Ocean Acidification?](#)
- [Physiological response and resilience of early life-stage Eastern oysters \(\*Crassostrea virginica\*\) to past, present and future ocean acidification - PMC](#)
- [False Discovery Rate - an overview | ScienceDirect Topics](#)
- [Scatter plot for KEGG enrichment results. The Rich factor is the ratio... | Download Scientific Diagram](#)
- [Eastern Oyster | NOAA Fisheries](#)

