

# COCKLES

Co-Operation for Restoring Cockle Shellfisheries  
and its Ecosystem-Services in the Atlantic Area

**The cockle immune system, cell machinery to face  
pathogens and the fight against marteiliosis**

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**FINAL VIRTUAL CONFERENCE**

**March 2021**





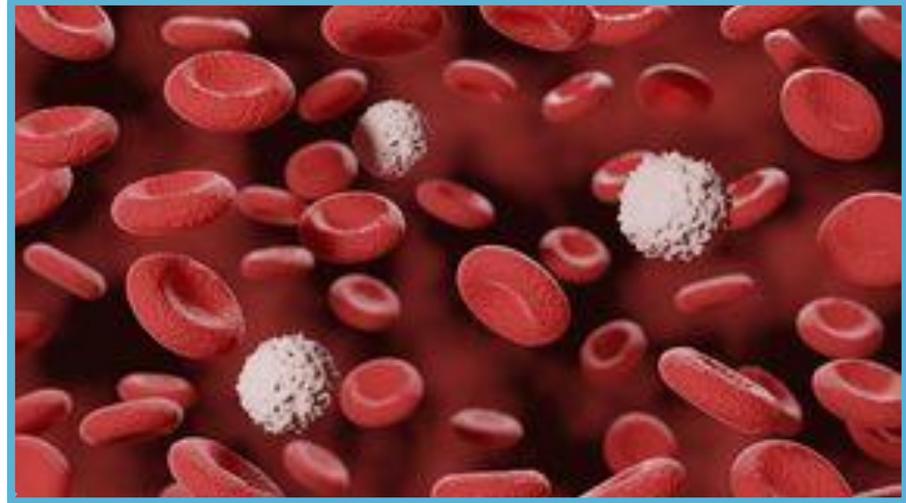
# IMMUNE SYSTEM



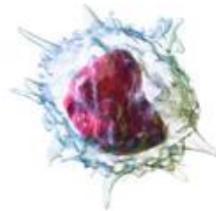
# IMMUNE SYSTEM



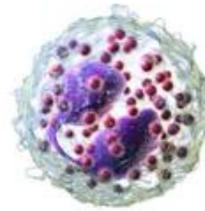
# COCKLE IMMUNE SYSTEM???



## White Blood Cells



monocyte



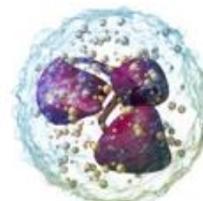
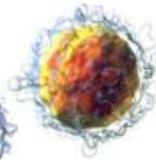
eosinophil



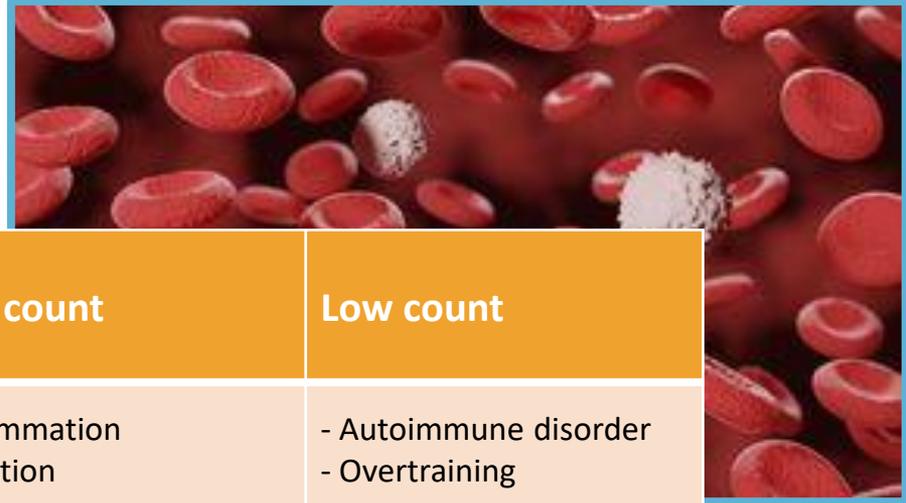
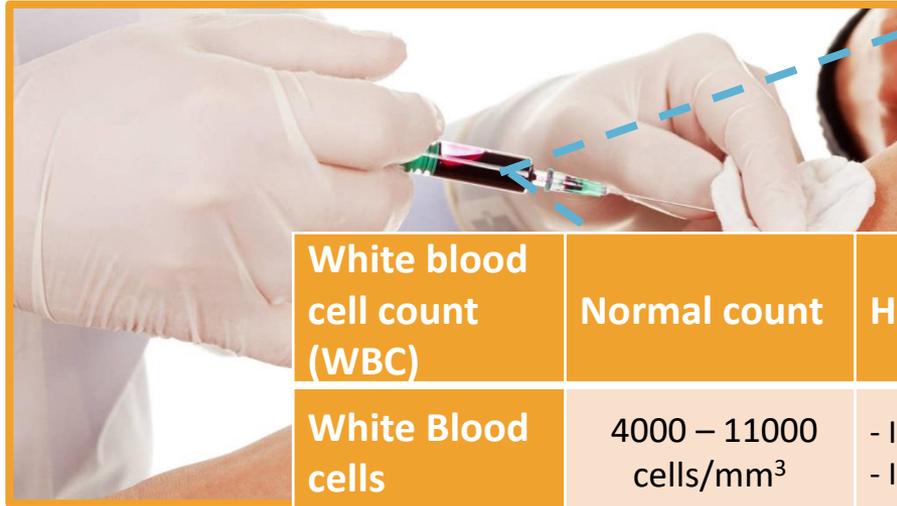
basophil



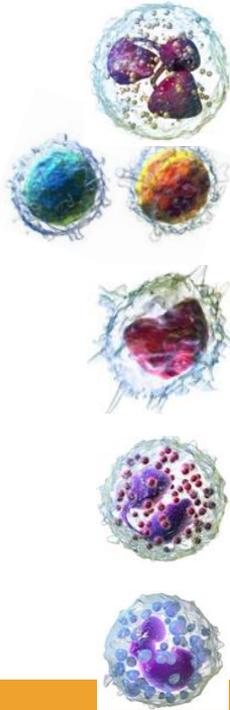
lymphocytes

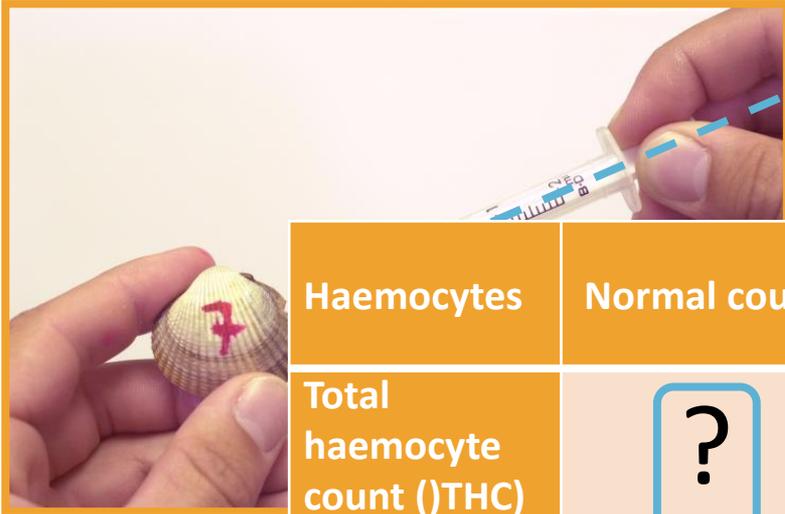


neutrophil



White blood cell count (WBC)	Normal count	High count	Low count
White Blood cells	4000 – 11000 cells/mm <sup>3</sup>	- Inflammation - Infection	- Autoimmune disorder - Overtraining
Neutrophils	50 – 70%	- Bacterial infection - Trauma and burns - Stress	- Leukaemia
Lymphocytes	20 – 40%	- Viral infection	- High level of steroids
Monocytes	2 – 10%	- Viral infection - Tuberculosis	- Rare
Eosinophils	1 – 4%	- Allergic reactions - Parasitic infection	- Stress
Basophils	0 – 1 %	- Some cancers	- Pregnancy - Ovulation - Stress





Haemocytes	Normal count	High count	Low count
Total haemocyte count ( )THC	?	?	?
	?	?	?
	?	?	?
	?	?	?
	?	?	?
	?	?	?

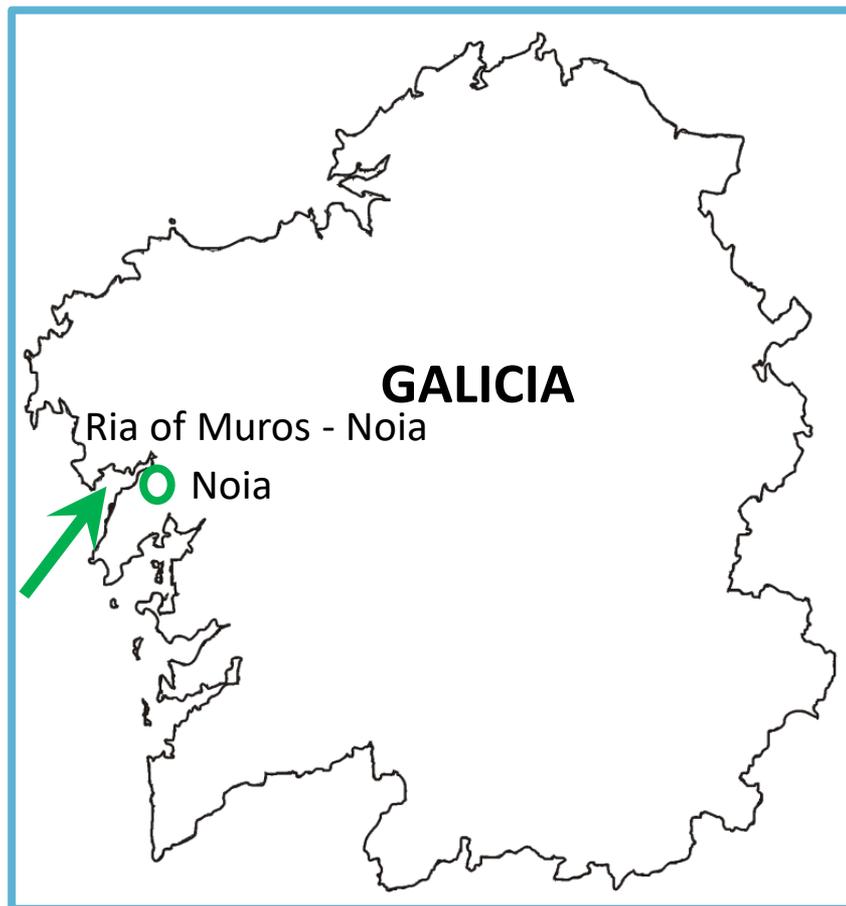
# Materials & methods

Monthly sampling from Apr 18 to Mar 20



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Monthly sampling from Apr 18 to Mar 20



Haemolymph collection

Total haemocyte count (THC)

Different haemocyte types

Differential haemocyte count (DHC)

# Materials & methods

Monthly sampling from Apr 18 to Mar 20



Disease diagnosis

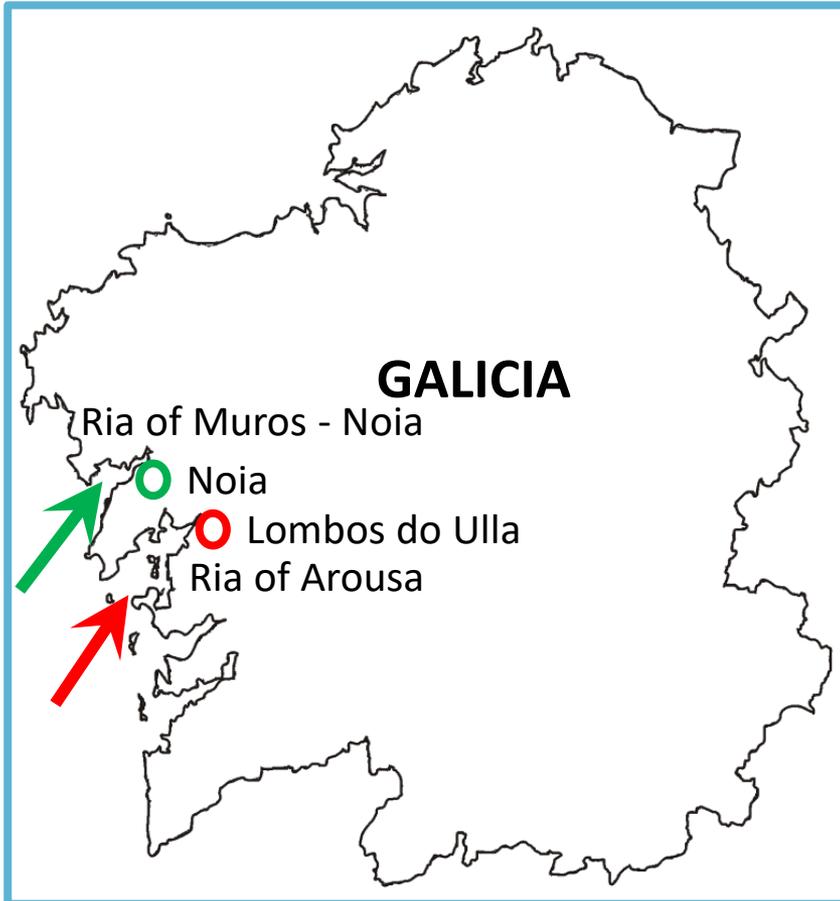
Association of pathological conditions with THC and DHC



Haemolymph collection

Total haemocyte count (THC)  
Different haemocyte types  
Differential haemocyte count (DHC)

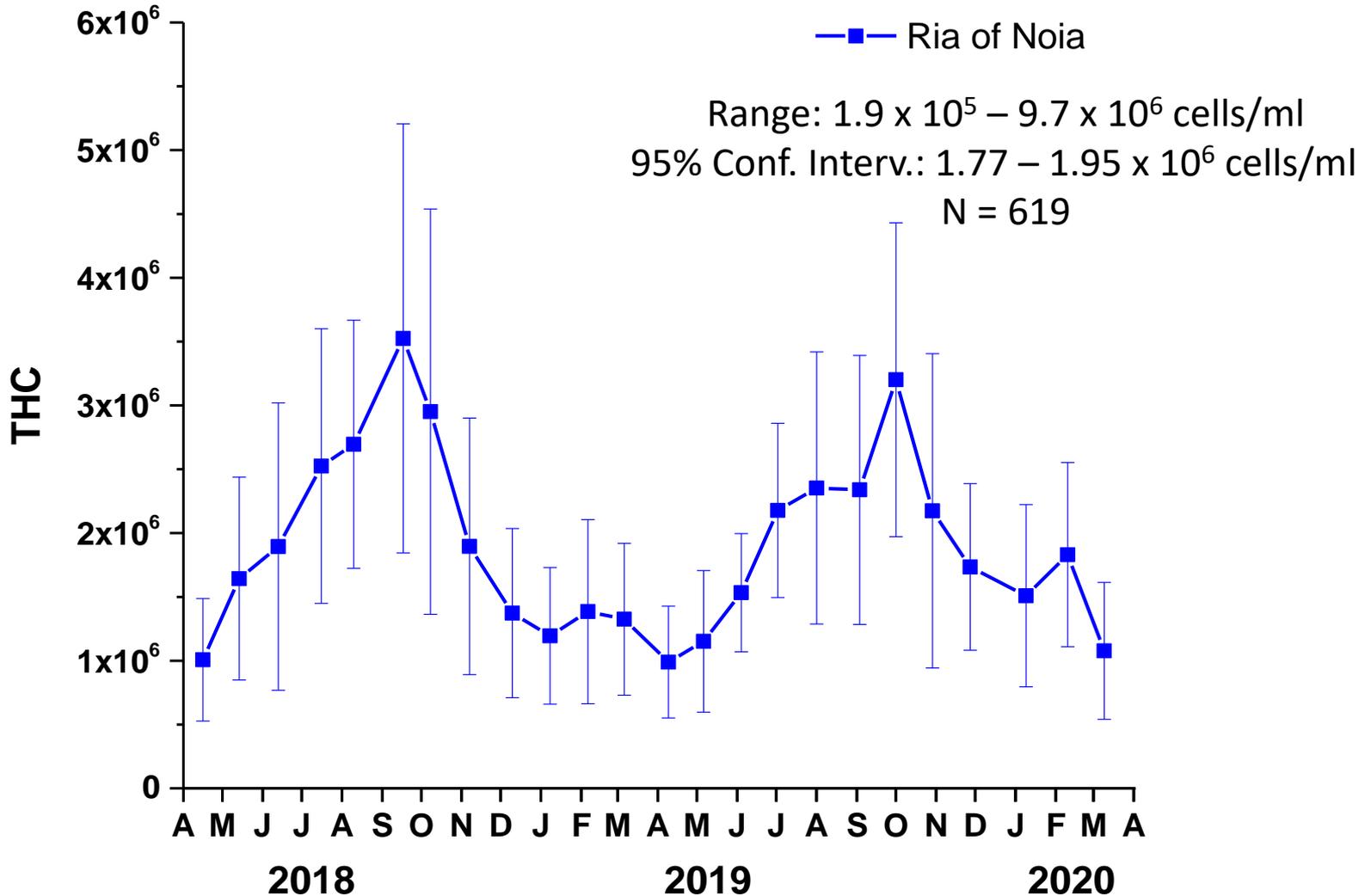
# Materials & methods



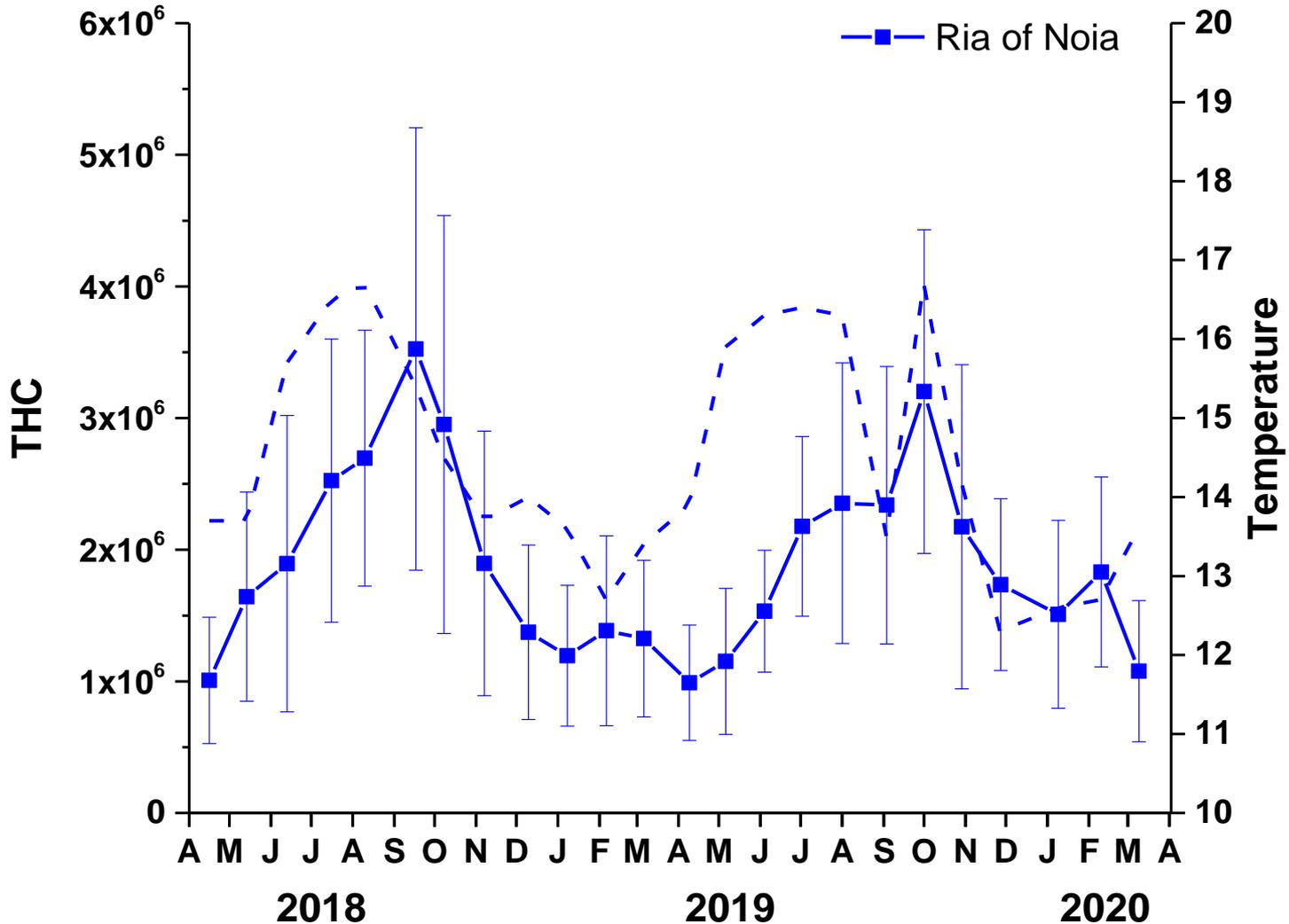
Cockles from Noia were transplanted to Lombos do Ulla in April 2018.

The transplanted cockle batch was sampled monthly. Cockles were processed for haemolymph analysis and disease diagnosis.

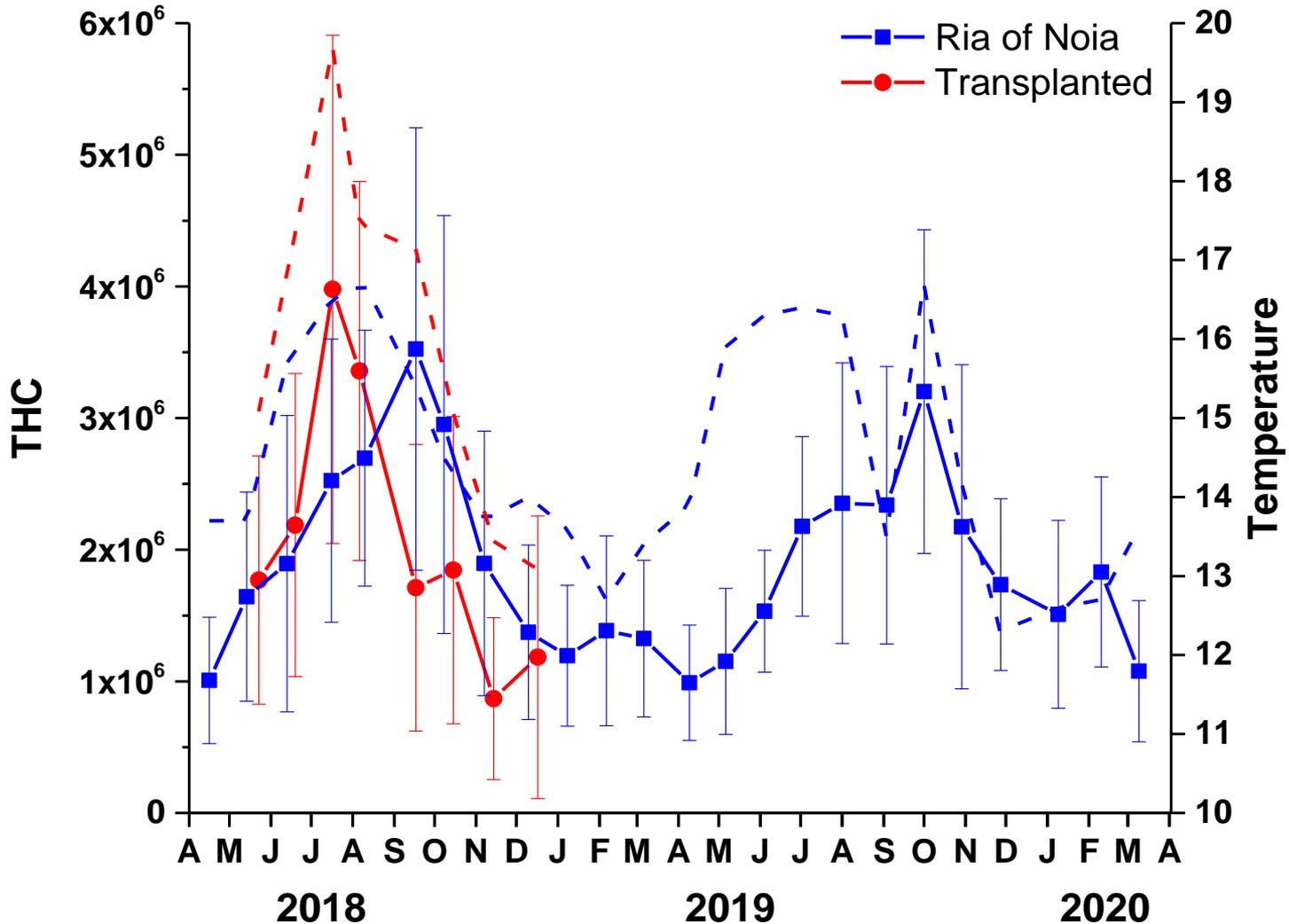
# Temporal variation of THC



# Temporal variation of THC

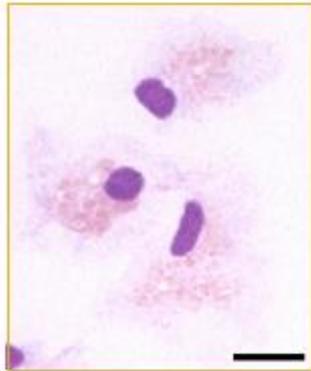


# Temporal variation of THC

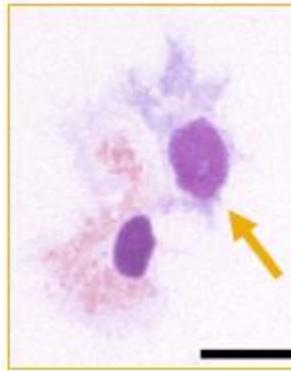


# Cockle haemocyte types

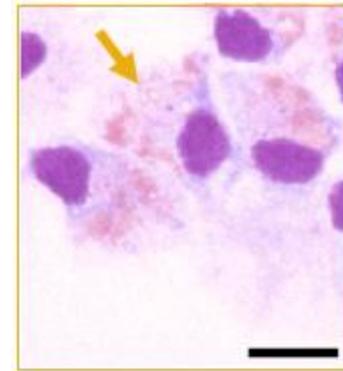
Acidophilic granulocyte



Basophilic granulocyte

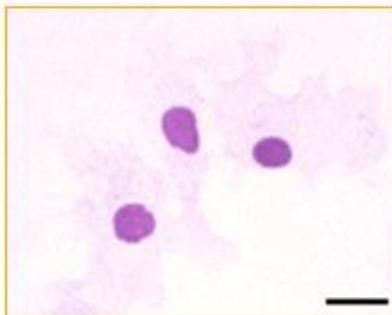


Mixed granulocyte

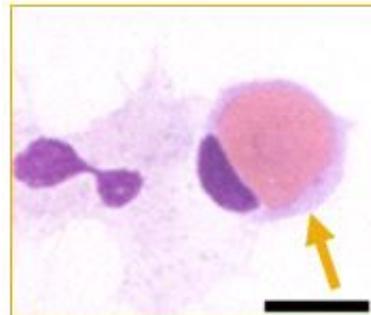


Bar: 10  $\mu$ m

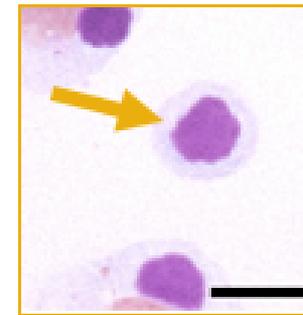
Large hyalinocyte



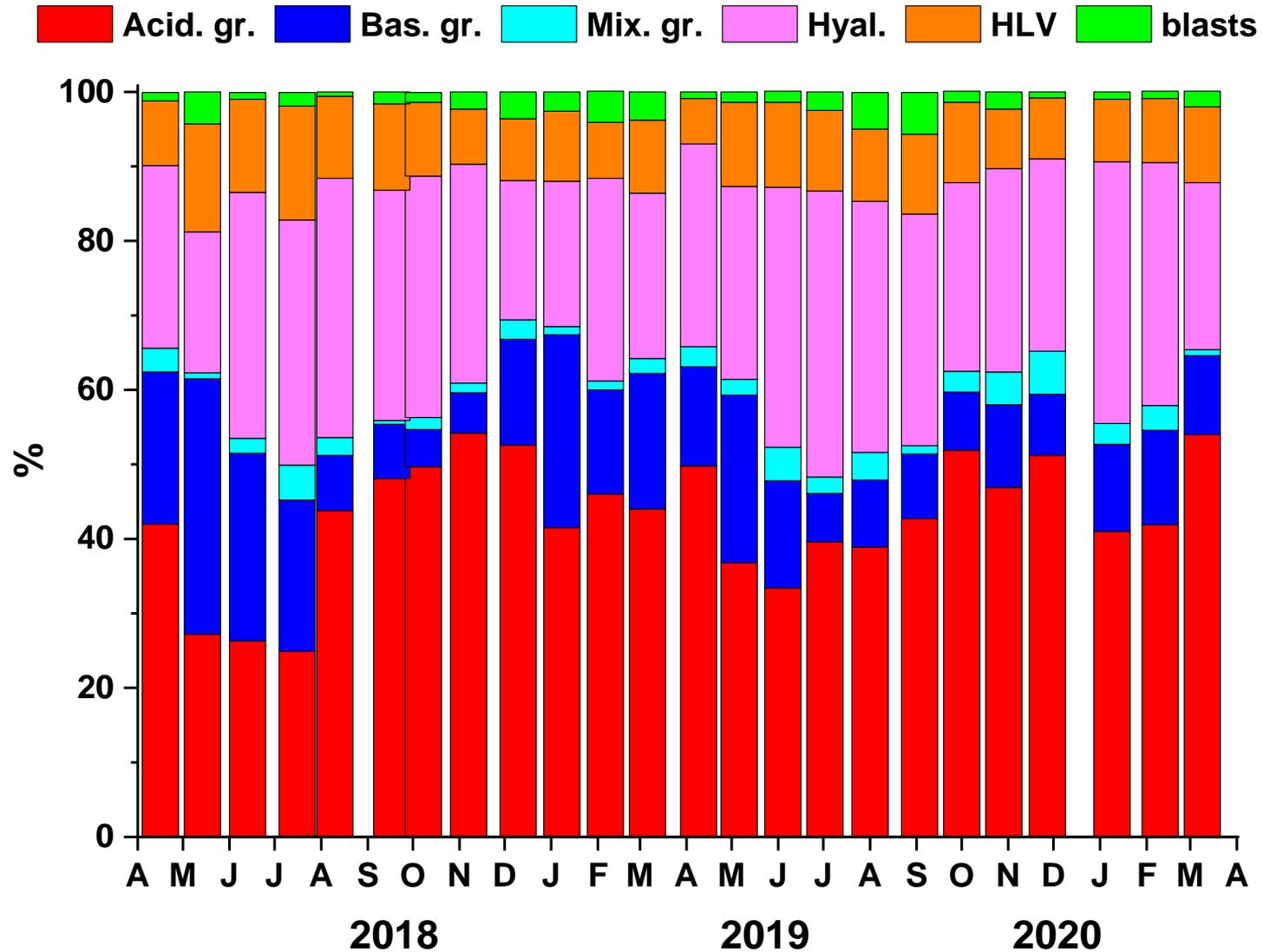
Haemocyte with a large vacuole (HLV)



Haemoblast



# Temporal variation of DHC



## Haemocyte counts (THC, DHC)

Haemocytes	95% interval confidence	High count	Low count
Total haemocyte count (THC)	1.77 – 1.95 x 10 <sup>6</sup>		
Acidophilic granulocytes	40.7 – 44.2 %		
Basophilic granulocytes	13.0 – 14.6 %		
Mixed granulocytes	2.0 – 3.0 %		
Large hyalinocytes	27.6 – 29.7 %		
HLV	9.5 – 10.5 %		
Haemoblasts	2.0 – 2.4 %		

## Haemocyte counts (THC, DHC)

Haemocytes	95% interval confidence	High count	Low count
Total haemocyte count (THC)	1.77 – 1.95 x 10 <sup>6</sup>		Advanced stage of marteiliosis
Acidophilic granulocytes	40.7 – 44.2 %		Early and moderate stages of marteiliosis
Basophilic granulocytes	13.0 – 14.6 %	Advanced stage of marteiliosis	
Mixed granulocytes	2.0 – 3.0 %		
Large hyalinocytes	27.6 – 29.7 %		Infection with trematode sporocysts
HLV	9.5 – 10.5 %		
Haemoblasts	2.0 – 2.4 %		

## Differences in immune capabilities among haemocyte types

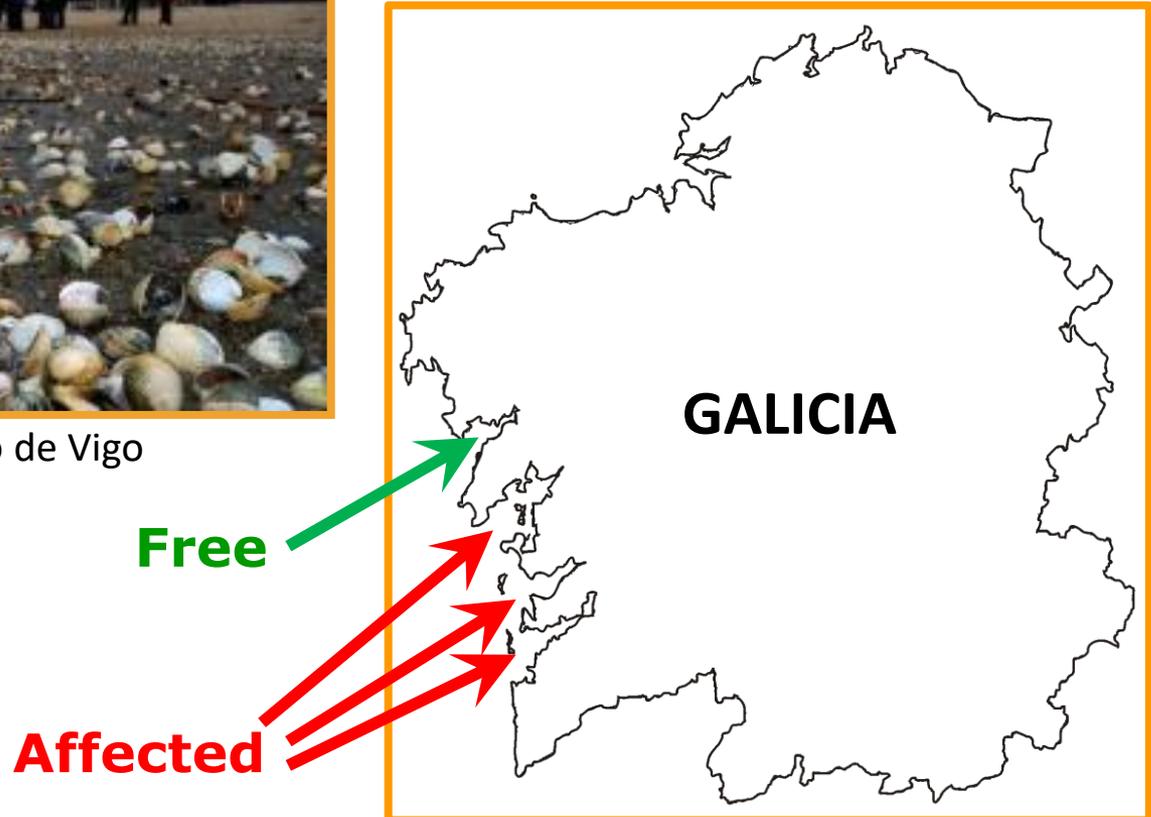
- The phagocytic ability was evaluated by *in vitro*-challenging them with fluorescent-labelled bacteria; granulocytes were the cells with the highest phagocytic ability, while the hyalinocytes and the haemoblasts showed very low phagocytic ability and the haemocytes with a large vacuole showed intermediate ability.
- Every haemocyte type was able to produce reactive oxygen species, without significant differences among types.
- Every haemocyte type showed both lysosomal and non-specific esterase contents, which were higher in large and more complex cells (likely granulocytes) and lower in small and less complex cells (likely haemoblasts).

# The fight against marteiliosis

Marteiliosis is the major threat for cockle production in Galicia because of the huge mortality it causes since 2012.



Photograph by Iñaki Abella, Faro de Vigo



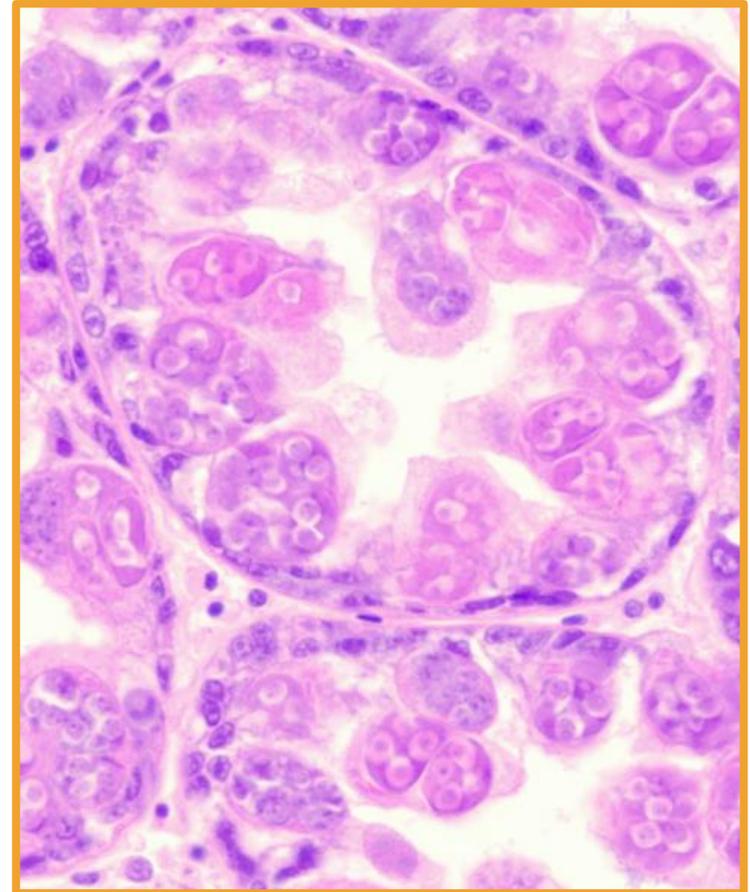
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The protozoan *Marteilia cochillia* proliferates through and destroy the digestive gland of the cockle.



## The fight against marteiliosis

Marteiliosis is the major threat for cockle production in Galicia because of the huge mortality it causes since 2012.



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### Fighting strategy

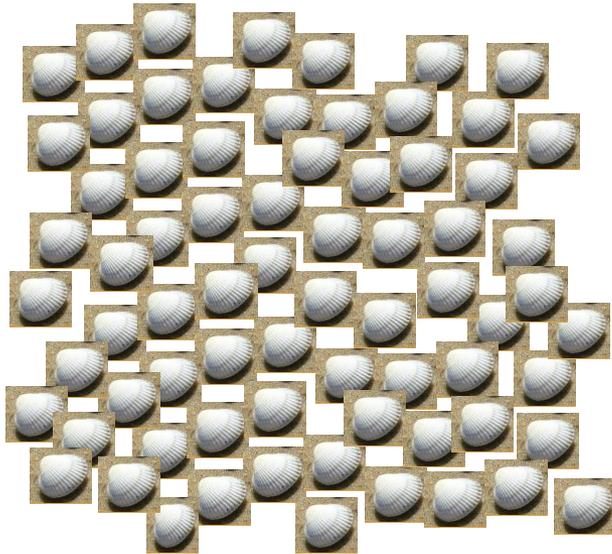
- Vaccination
- Chemotherapy

- Producing cockle strains resistant against marteiliosis through a selective breeding programme is a promising strategy.

Identifying molecular markers of resistance against marteiliosis to be used in selective breeding would increase the efficiency of the programme.

# Identification of molecular markers of resistance to marteiliosis through proteomic and genomic approaches

**BEFORE**



Marteiliosis



outbreak

**AFTER**



Experimental proteomic approach: Comparing the proteome before outbreak vs. the proteome of survivors

# Proteins identified as candidate markers of resistance against marteiliosis

- Matrilin-2-like isoform X2
- Glutamate receptor 2-like
- Mucin-2-like isoform X2
- Histone H2A-like
- Vacuolar protein sorting-associated protein 13A-like isoform X6
- Chloride intracellular channel protein 2-like
- Mitochondrial ornithine aminotransferase
- Histone H2B

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CONSELLERÍA DO MAR



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Bon appétit!

