Inter-annual variation in the reproductive pattern of Manila clam *Ruditapes philippinarum* and impacts of *Perkinsus olseni* infection on the reproduction observed from the west coast of Korea

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Ruditapes philippinarum, the Manila clam

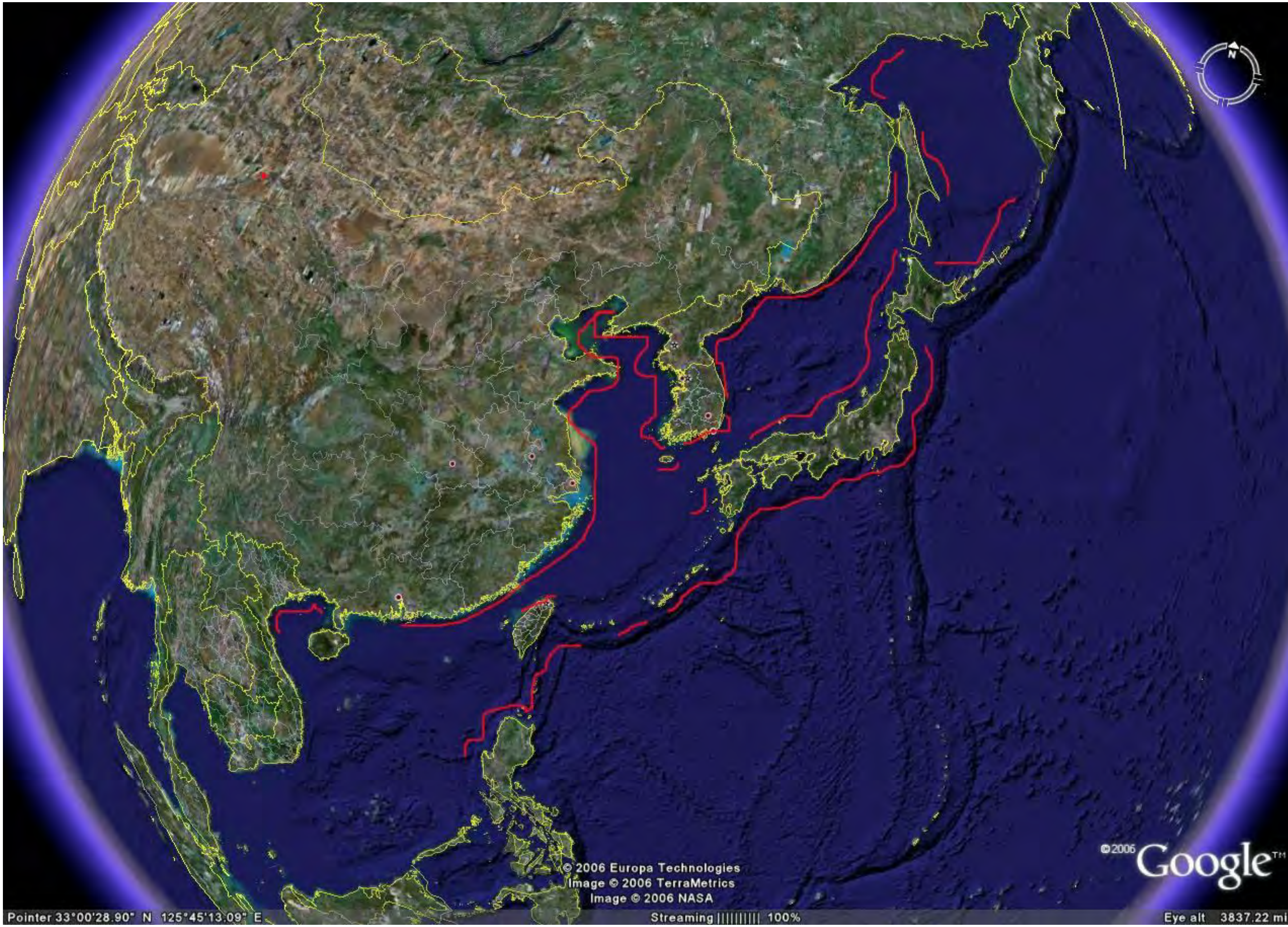
- Scientific name: *Tapes, Ruditapes, Venerupis philippinarum*

- Common name: Manila clam, short neck, little neck clam, Japanes little neck clam

- Introduced to the west coast of USA and to the European countries including Portugal, Spain, France and Italy

- Considered to be one of the most important species in world shellfish aquaculture industry

Ruditapes philippinarum in Pacific Asia Region



Ruditapes philippinarum, the Manila clam

- The Manila clam is endemic species to the costal Yellow sea and commonly cultured in Korea, China and Japan.
- The Manila clam *Ruditapes philippinarum* is one of the most important marine shellfish resources supporting Korean fisheries industry.
- *R. philippinarum* is intensively cultured on muddy or sandy tidal-flats along the coastal Yellow Sea and on the southern coast of Korea.

Clam (*Ruditapes philippinarum*) culture bed

- Clam seeds are naturally abundant in the beds
- Clam seeds are collected from spat-fall ground
- Seeds are also produced from hatchery
- 5-15mm seeds are sowed on commercial culture grounds
- After 2-3 years of grow-out period, they are harvested manually by local clam growers cooperative
- Whole sale price of 1.5-2.5 dollars and retail price of 3-4 dollars

Problems Identified in Korean Clam Industry

- **Recurring mass mortalities of the clams in the clam beds in early spring or late summer.**
- **Poor condition of the clams; poor growth and reproduction.**
- **Pathogenic organisms such as *Perkinsus olseni* and *Vibrio tapetis*-like bacteria have been identified from gaping clams as well as physiologically poor clams.**

Quantification of Reproductive Effort (RE)

- Understanding life histories and successful management
- Problems involved in assessment of RE in marine bivalves; gonads are integral part of the visceral mass in most bivalves

How to estimate RE of marine bivalves?

- Histological preparations
- Determining the difference in body weight
- Counting or weighing the eggs

→

- ➢ Spawning is often incomplete
- ➢ Occurs continuously
- ➢ Semi-quantitative

- Immunological methods
(Enzyme-linked immunosorbent assay, ELISA)

→

- ➢ Rapid
- ➢ Low cost
- ➢ High sensitivity

What is *Perkinsus*?

- **Perkinsosis is a protozoan parasitic shellfish disease occurring in some commercially important shellfish including oysters and clams**

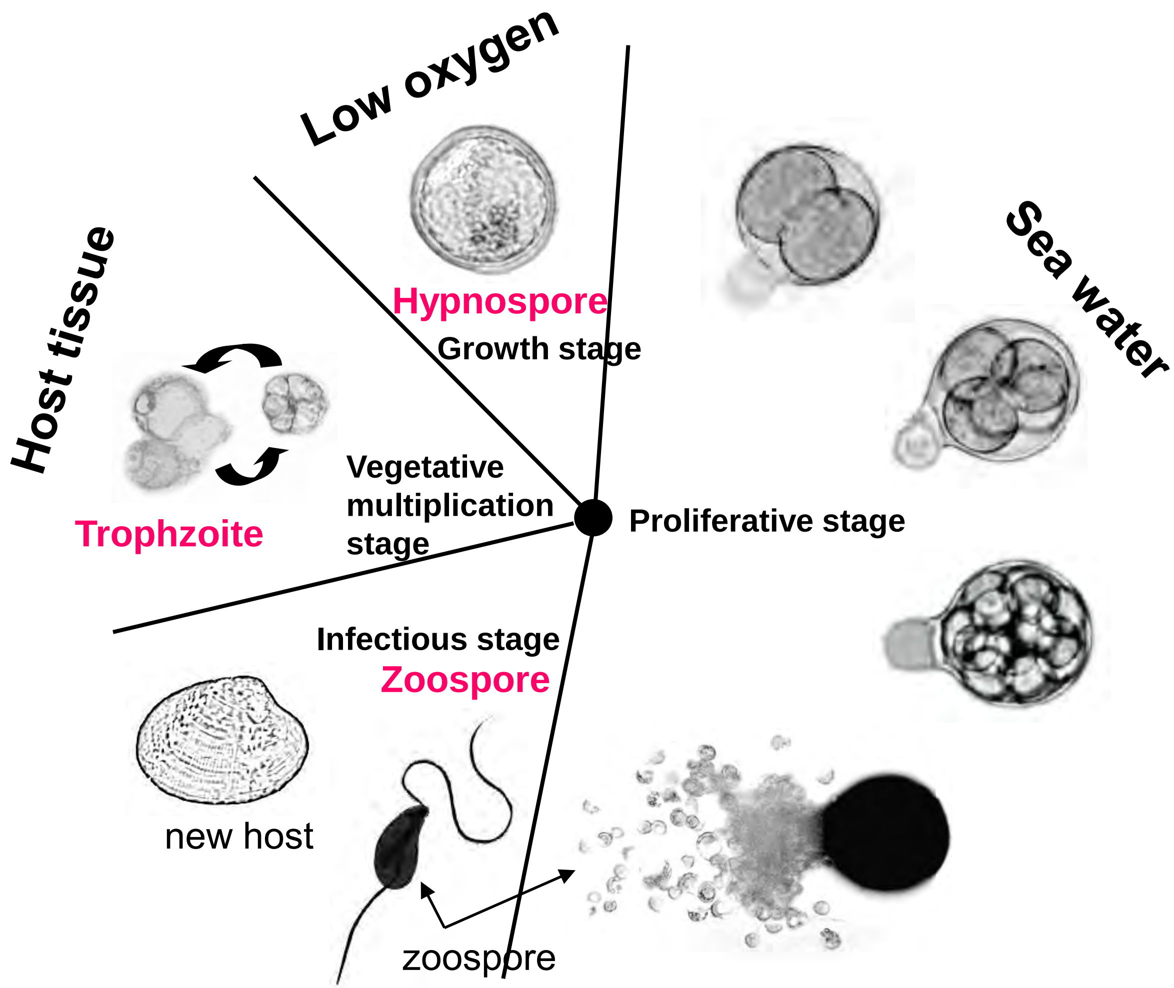
- **Responsible for the mass mortalities of the carpet shell clams *Ruditapes decussatus* in Europe and the eastern oyster *Crassostrea virginica*.**

- **Heavy infection with *Perkinsus* retards gonad maturation, spawning frequency and the reproductive effort**

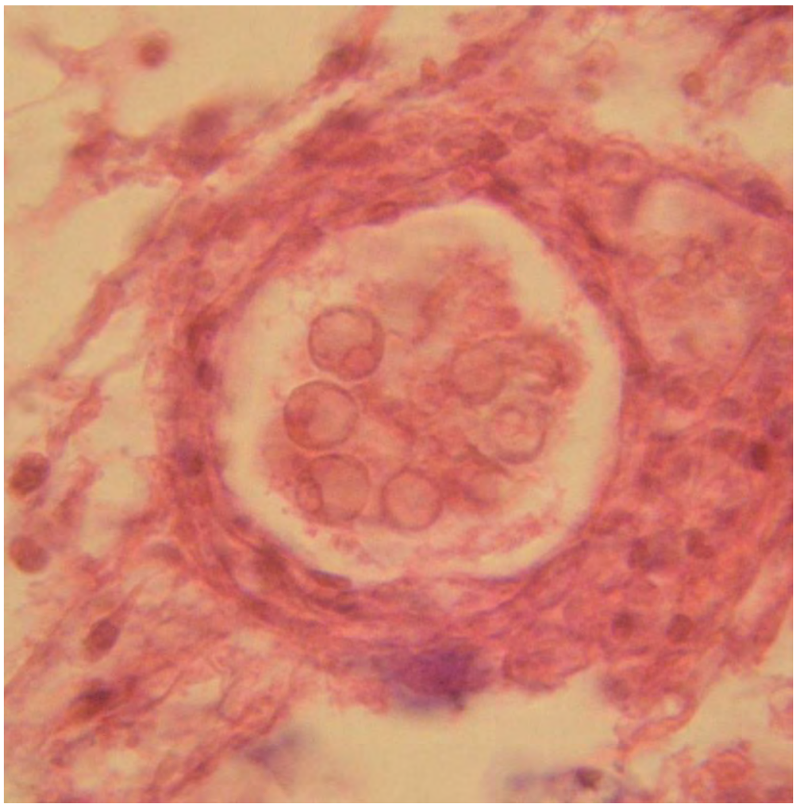
- **Difficulties involved in the study of impacts of *Perkinsus* infection on bivalve reproduction?**

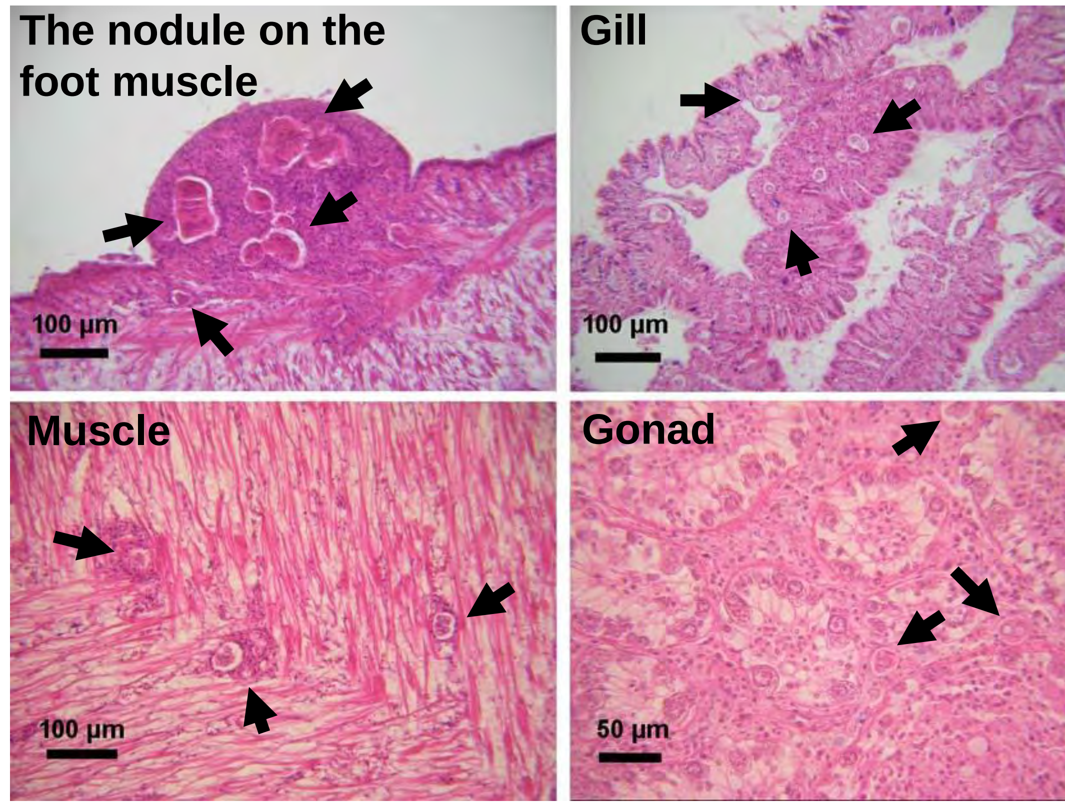
- **Classified by the OIE as a disease that warrants notification.**

Life Cycle of *Perkinsus olseni*



Microscopic appearance of *Perkinsus* in the tissue





Perkinsus olseni infection

- ***Perkinsus olseni*** **has been identified from clams on the coastal Yellow sea and the southern coasts of Korea.**

- **Heavily infected clams often observed harmful effects such as slow growth, poor condition and low fecundity.**

- **Perkinsosis is often associated with mass mortality and subsequent decline in cultured and wild shellfish populations.**

Objective

- **To monitor spatio-temporal variation in Manila clam conditions on the west coast of Korea**

  - **Annual reproductive cycle**
  - **Reproductive Effort**
  - ***Perkinsus olseni* body burden**

Materials and Methods

Sampling site

✓Hwangdo:

Commercial clam bed, silty-mud sediment

✓Padori

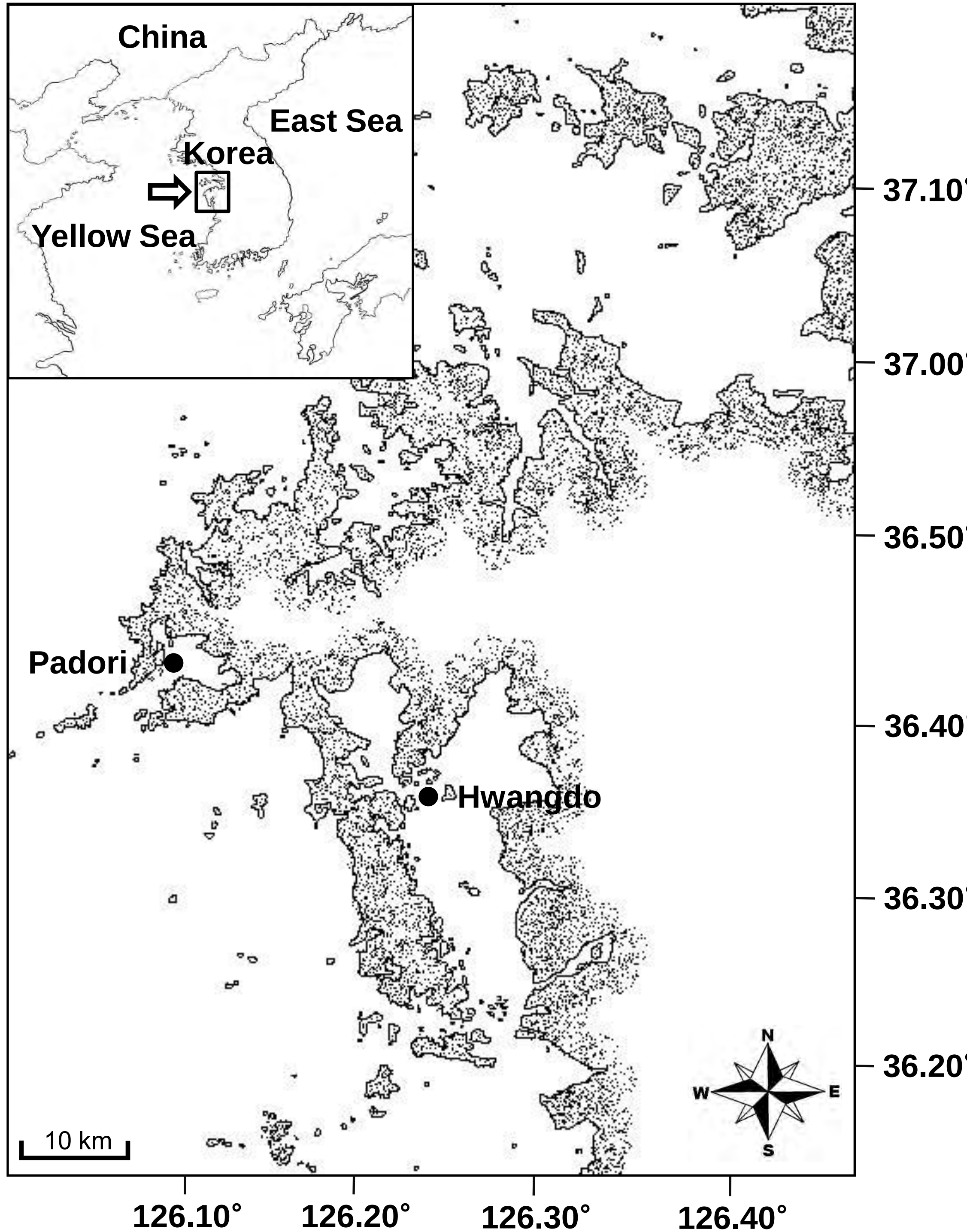
Commercial clam bed, subtidal, silty-mud sediment

✓Sampling period:

2007.1-2010.12 (48 months)

✓Distance:

Padori-Hwangdo (25km)



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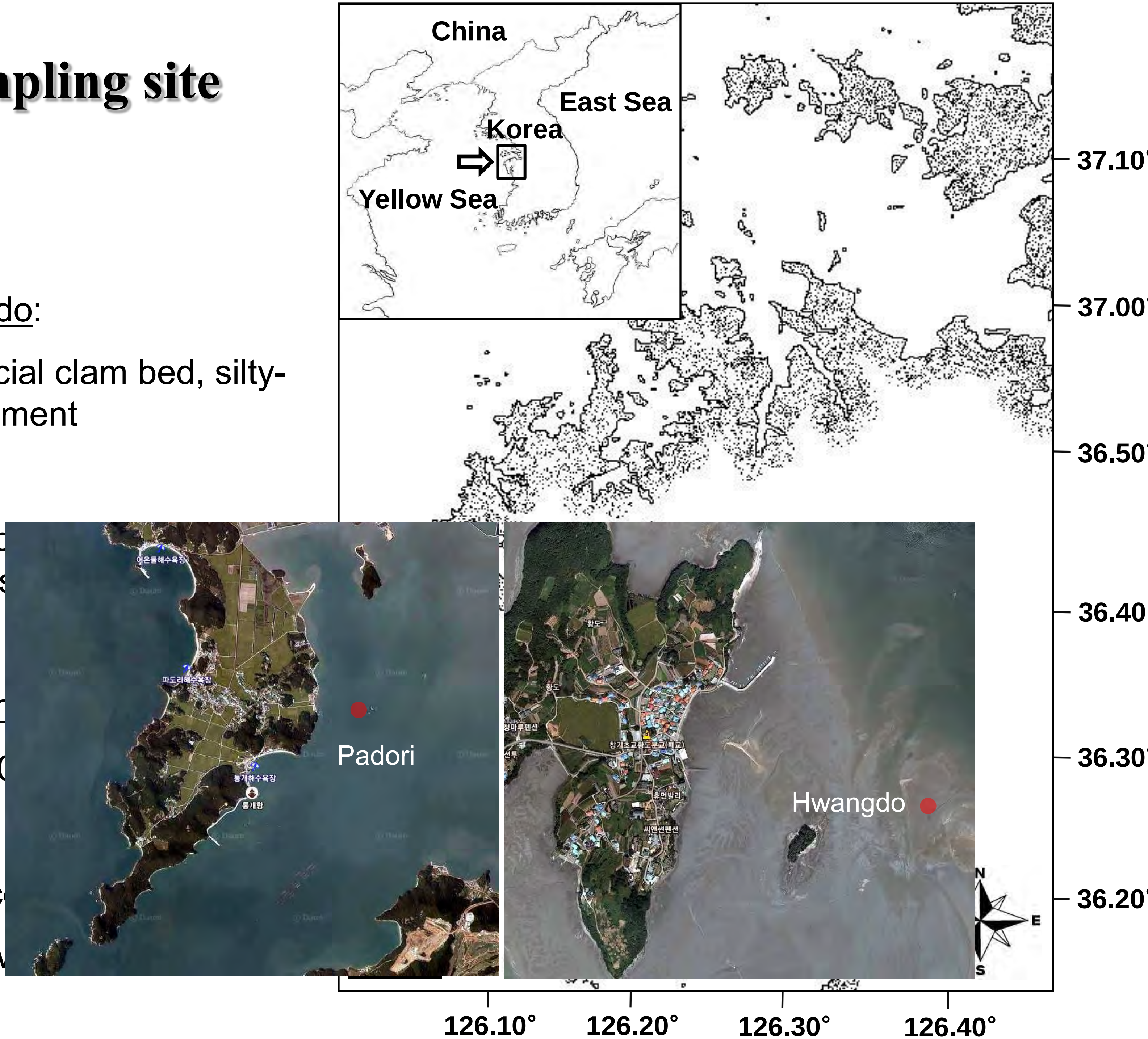
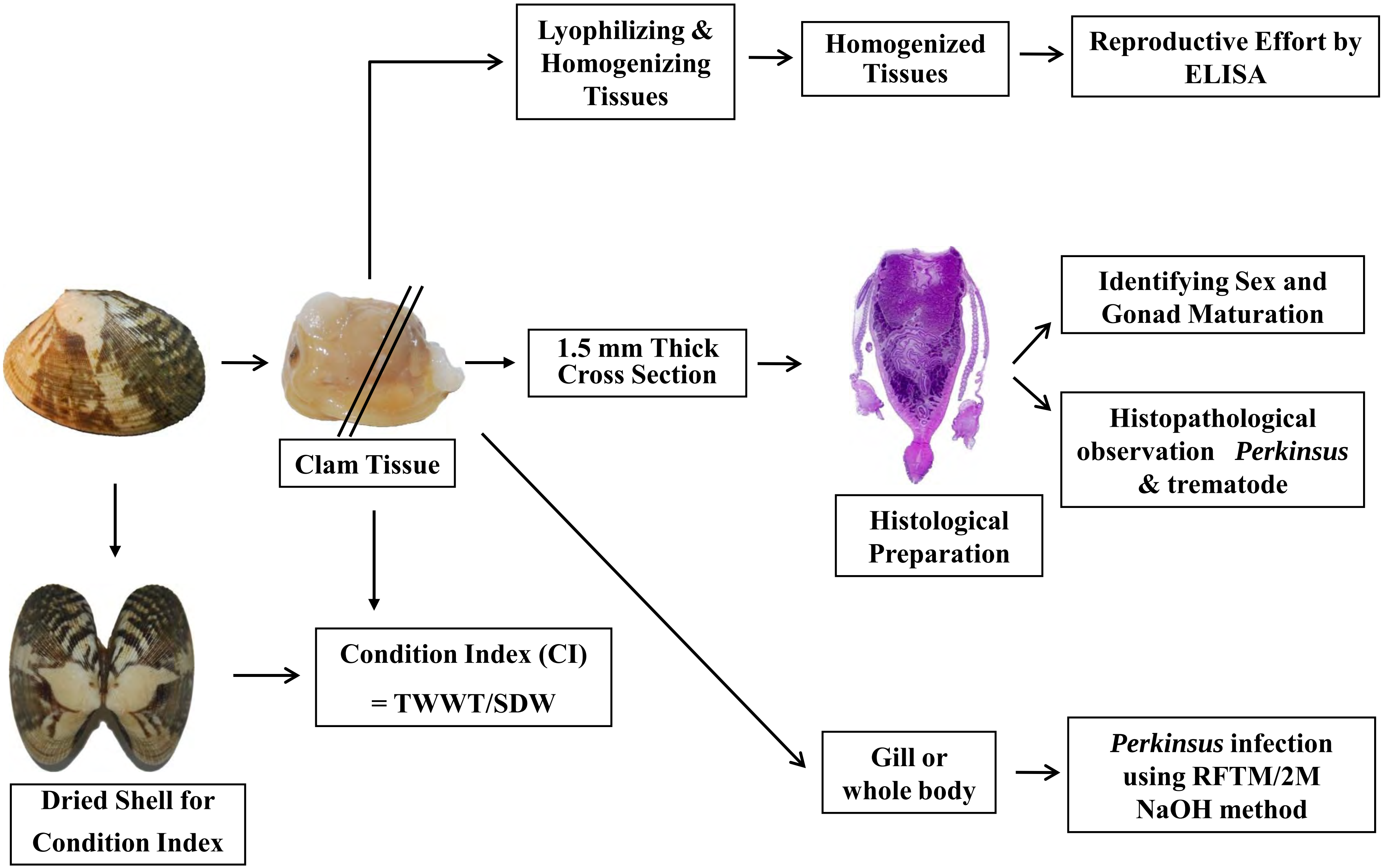
Padori-Hv



Steps involved in the analysis of clam in this study



Ray’s fluid thioglycollate medium assay (RFTM)

1. Gill or tissues were excised and incubate at room temperature in fluid thioglycollate medium (FTM) for 7 days at dark.

2. After FTM culture, the tissues digested in 2M NaOH.

3. All tissues were digested, add PBS in the conical tube till 5ml.

4. Number of *Perkinsus hypnospore* cells was counted under microscope using a hemocytometer.

5. *Perkinsus* infection intensity = hypnospores/g gill or tissue wet weight.

Histological observation

Preparation of the sample → 1.5 mm thick cross section of the body tissue

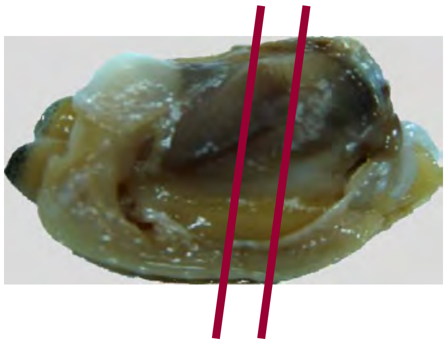
Fixation → Davidson's fixative; 70% alcohol

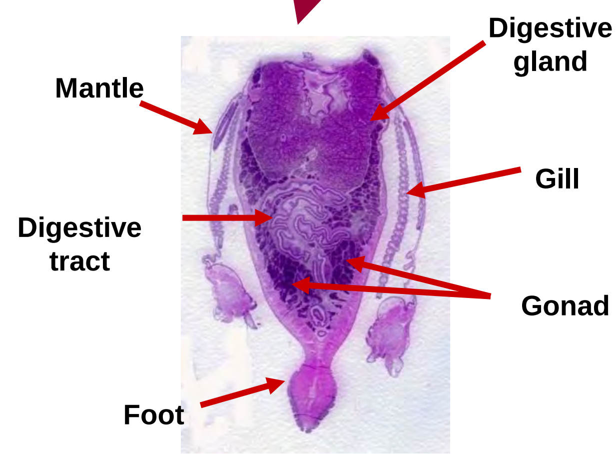
Embedding → Automatic Tissue processor; Paraffin bath & Cold plate

Sectioning → 6 µm from paraffin block

Staining → Hematoxylin & eosin

Mounting

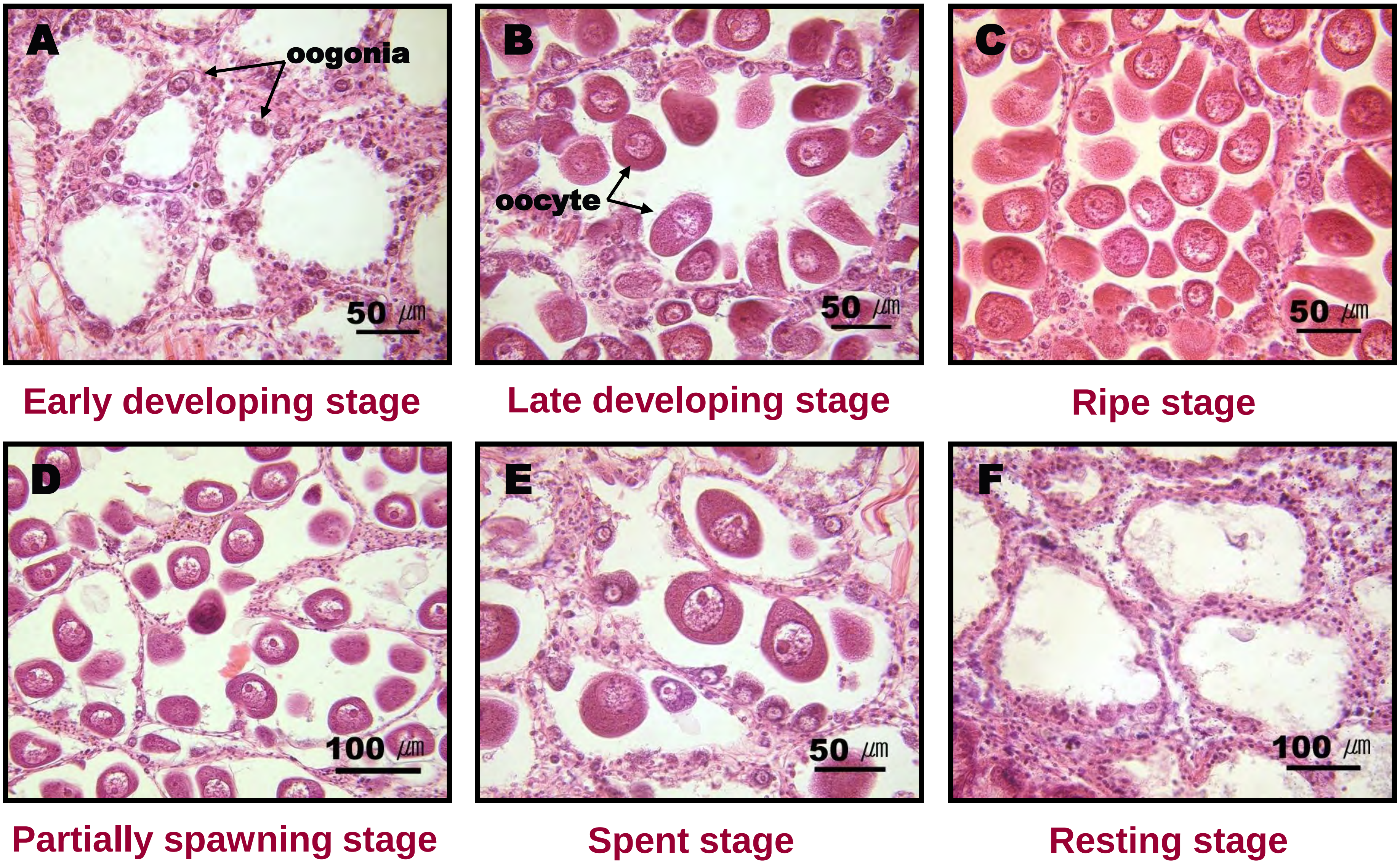




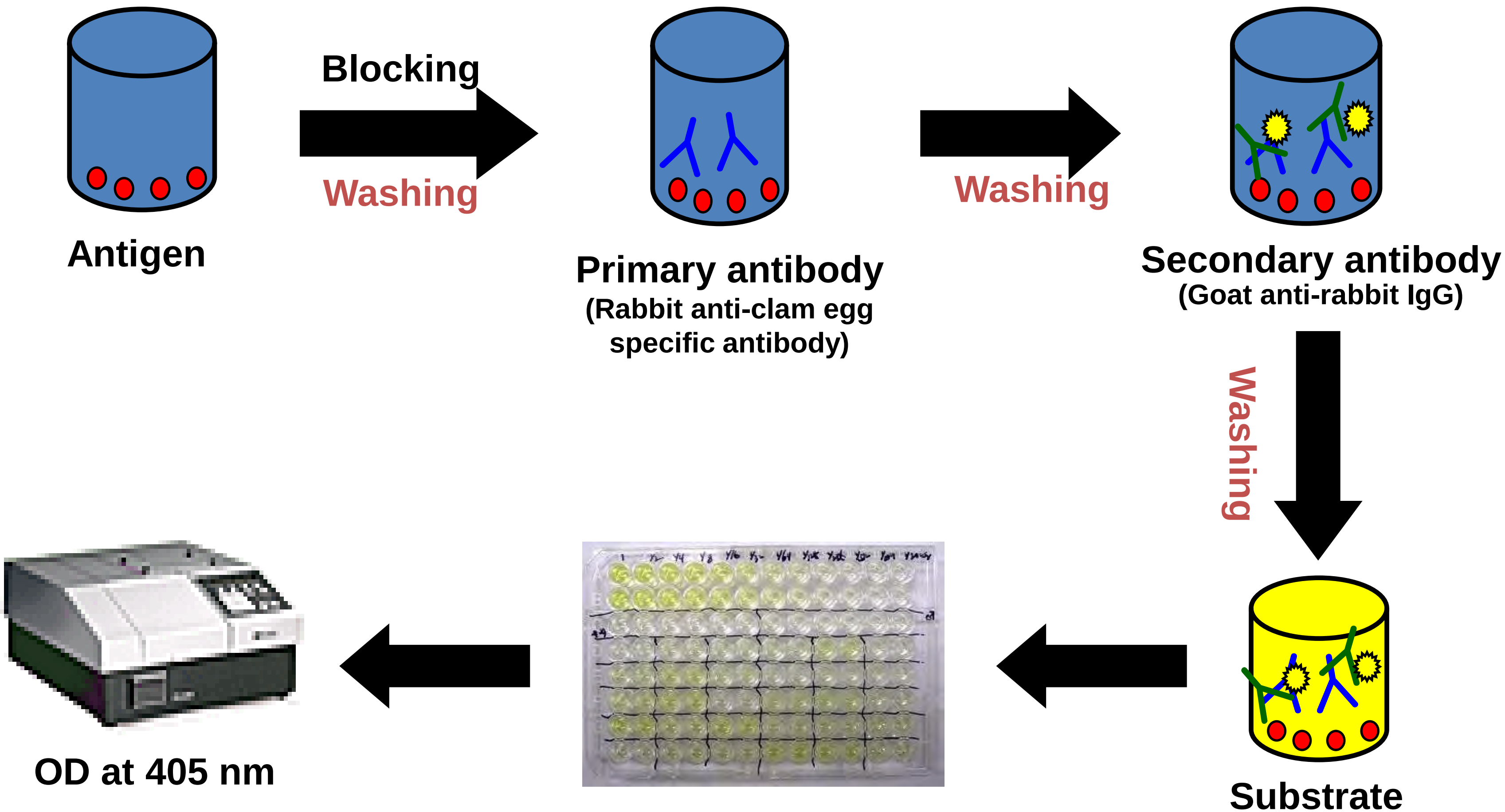
Classification of reproductive stage of the Manila clam, *Ruditapes philippinarum* (Drummond et al, 2006)

Reproductive stage	Scale	Description
Resting	0	Gonad follicle compose of connective tissue. The follicle is empty, oogonia cannot be observed.
Early developing	1	Gonad proliferation initiates; increasing number of oocytes at follicular wall, no free oocyte in the follicles.
Late developing	2	Free oocytes in the lumen but most oocytes attach on the follicular walls.
Ripe	3	Gonad filling large surface area, oogenesis follicle full with polygonal configuration oocytes.
Partially spawning	4	Numbers of free oocytes in lumen are decrease, empty space in follicle can be observed.
Spent	5	Follicles appear broken, scatter and relatively empty, only residual oocytes numerous numbers of phagocytes.

Gonad development of female *R. philippinarum*



Quntification of clam reproductive effort using Enzyme-Linked Immunosorbent Assay (ELISA)



- Quantity of RE in each clam=quantity of egg protein estimated from ELISA*2.44

- Egg protein=total egg weight x 0.4

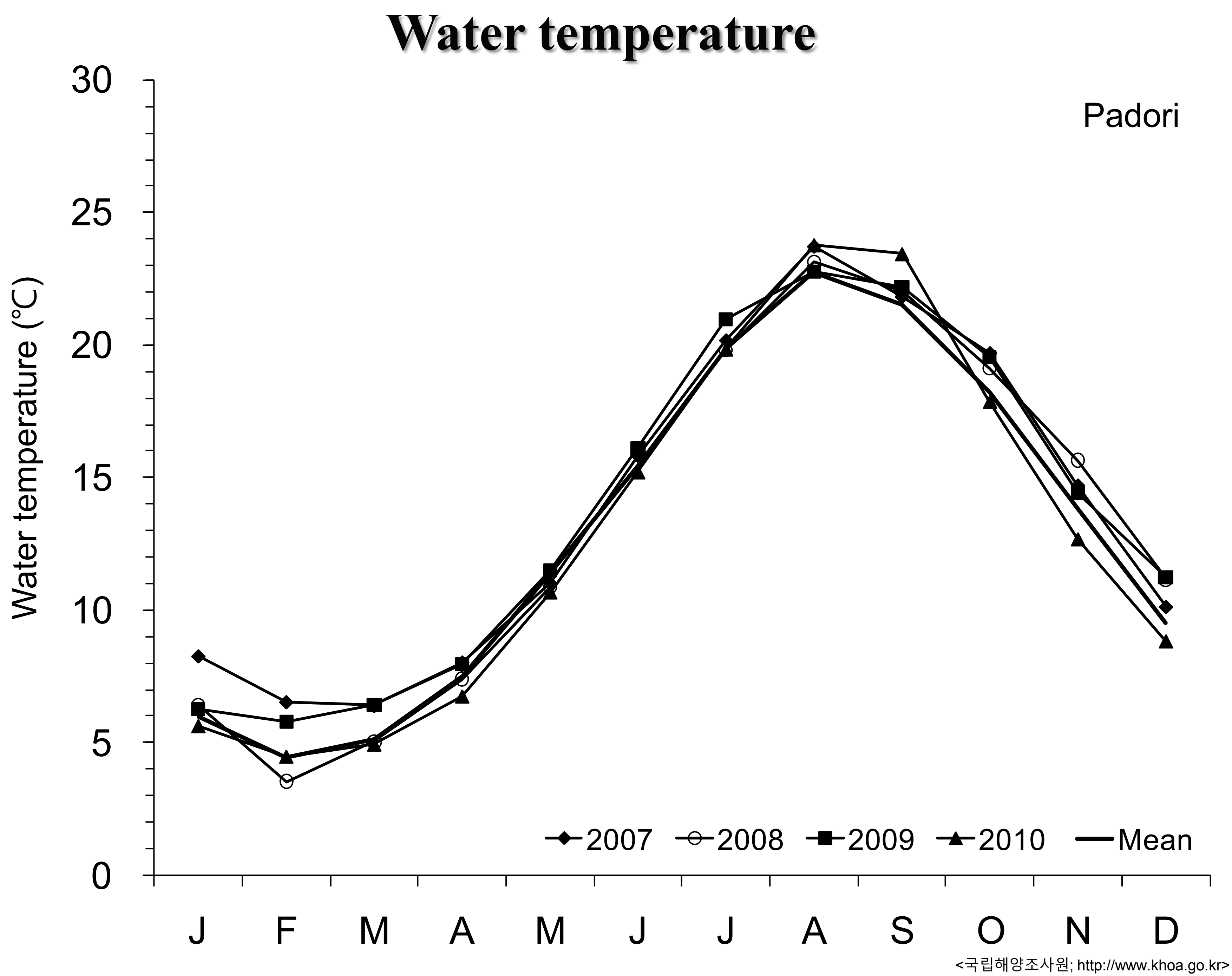
- Gonadosomatic index (GSI)

  GS I = Total egg dry weight/Total tissue dry weight

- Fecundity

  Fecundity = Total egg dry weight (g)/0.000022 g

Results


Water temperature
Padori
Water temperature (°C)
30
25
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15
10
5
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2007
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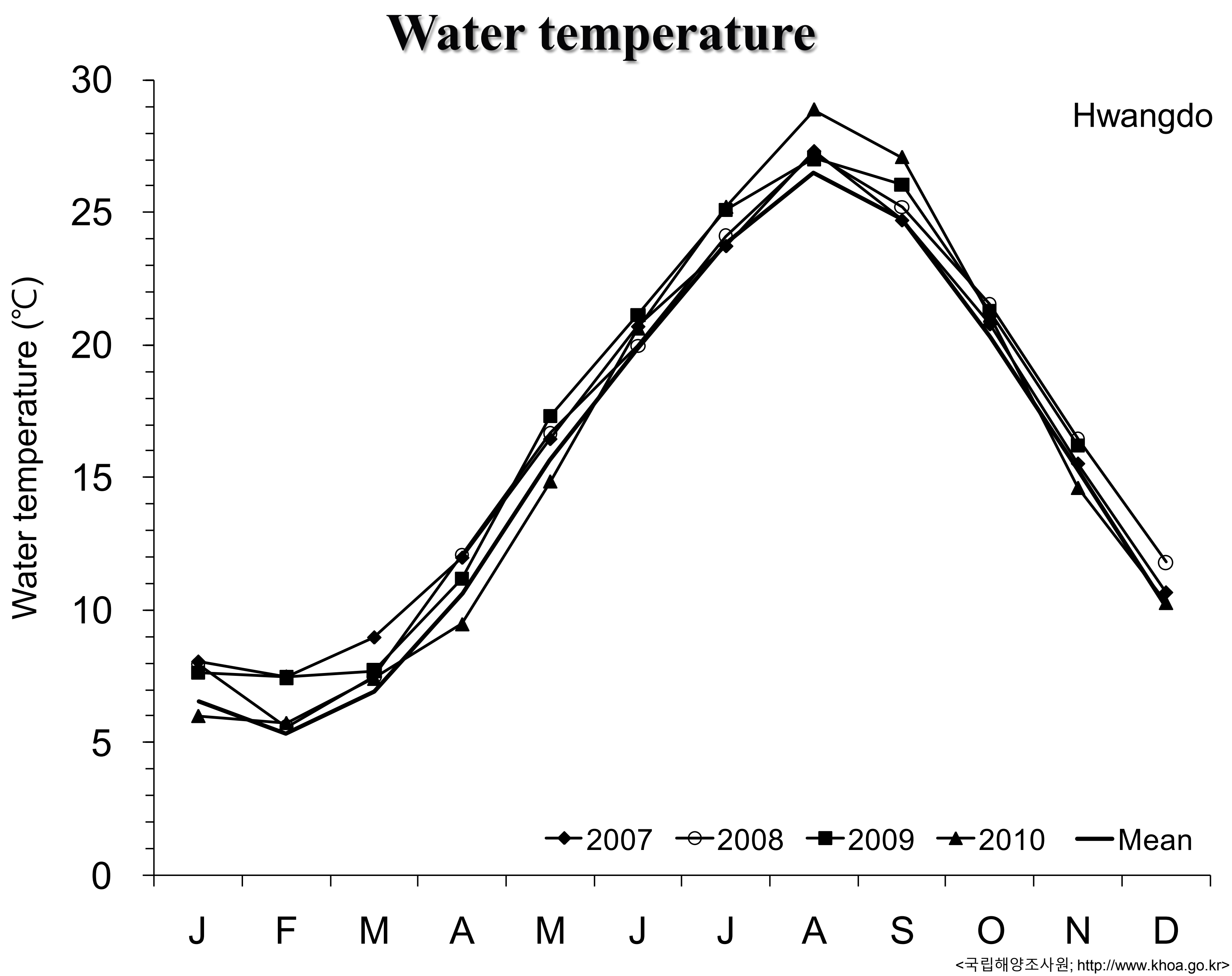
Mean
<국립해양조사원; http://www.khoa.go.kr>



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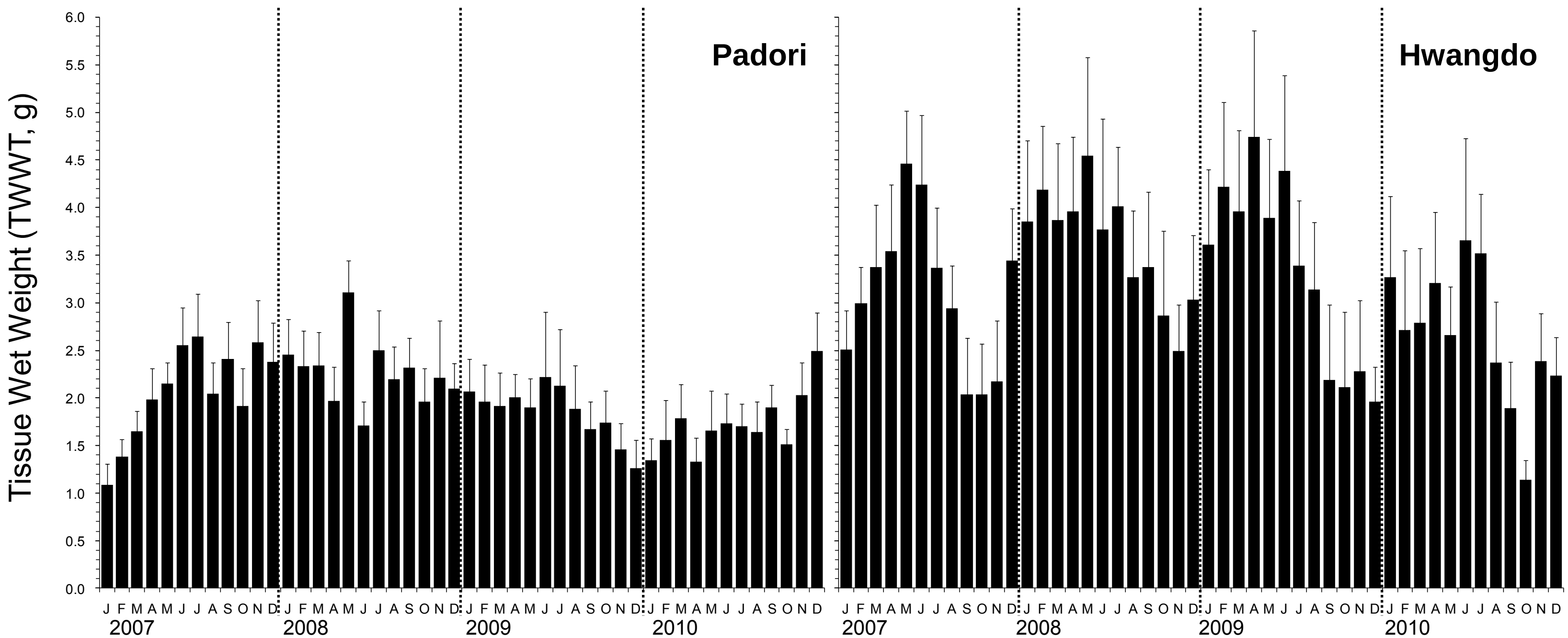
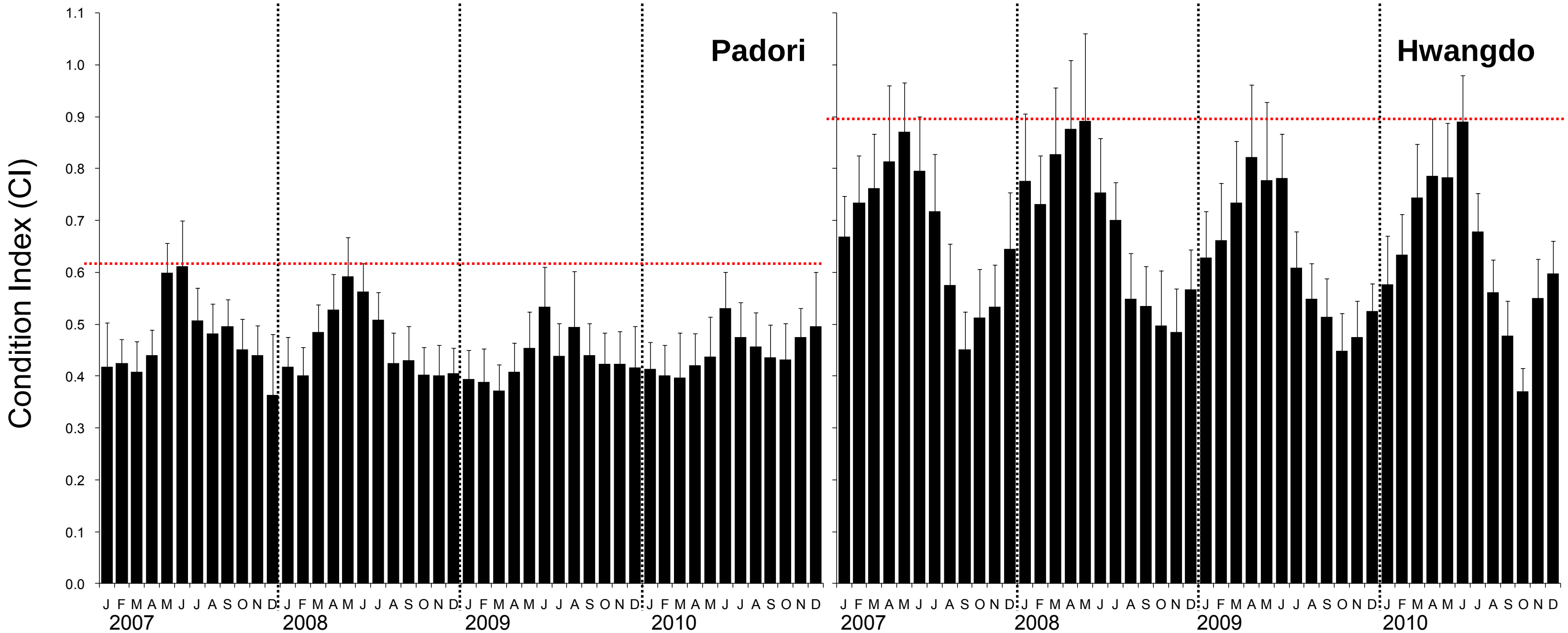
Field mortality

		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Padori	**2007**	0	0	0	0.6	0	0	0	0	0	0	0	0
	2008	0	0	4.0	0	0	0	0	0	0	0	0	0
	2009	0	0	0	0.1	0	0	0	0	0	0	0	0
	2010	0	0	0.7	0.1	0	0	0	0	0	0.2	0	0
Hwangdo	**2007**	0	0	0	0	0	0	6.4	11.6	48.9	19.3	0	0
	2008	0	0	0	0	0	0	0	0	0	0	0	0
	2009	0	0	0	0	0	0	0	17.6	11.8	0	0	0
	2010	0	0	0	0	0	0	0	10.0	12.1	60.4	0	0

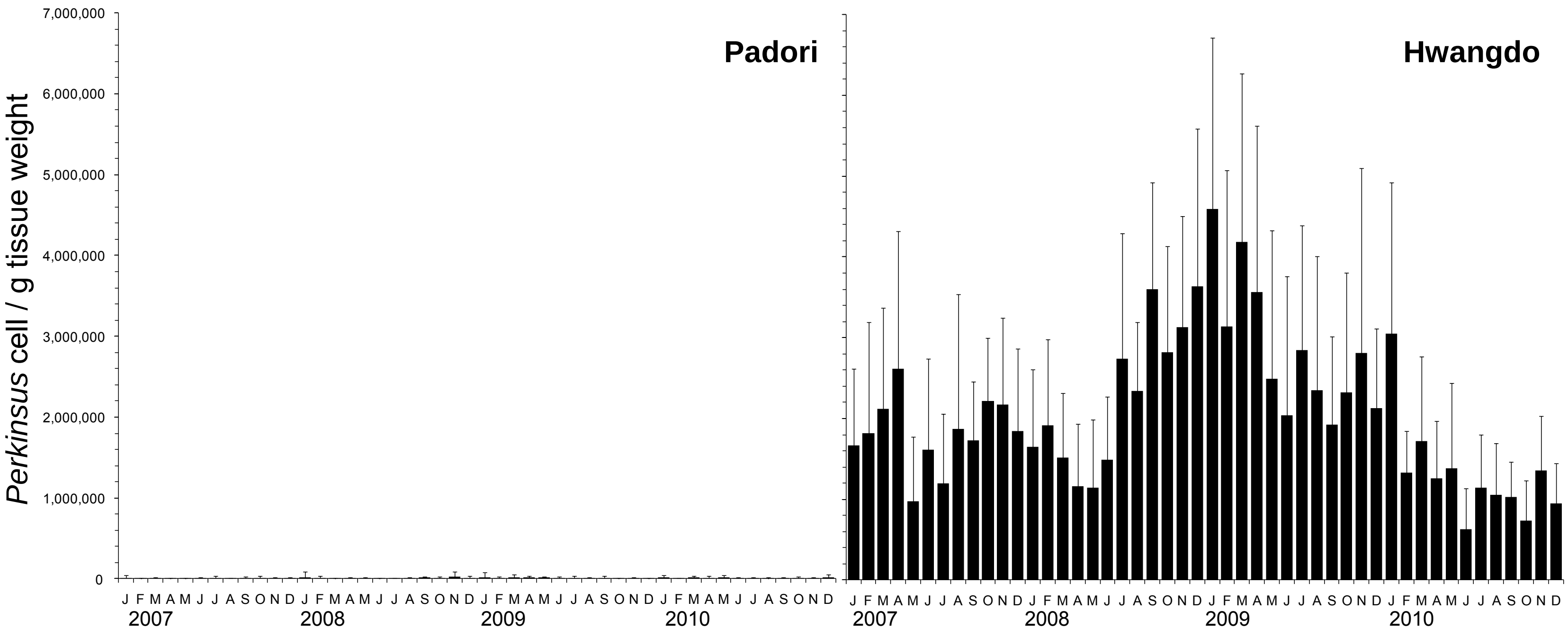
Annual variation of tissue wet weight



Annual variation of condition index



Perkinsus infection intensity

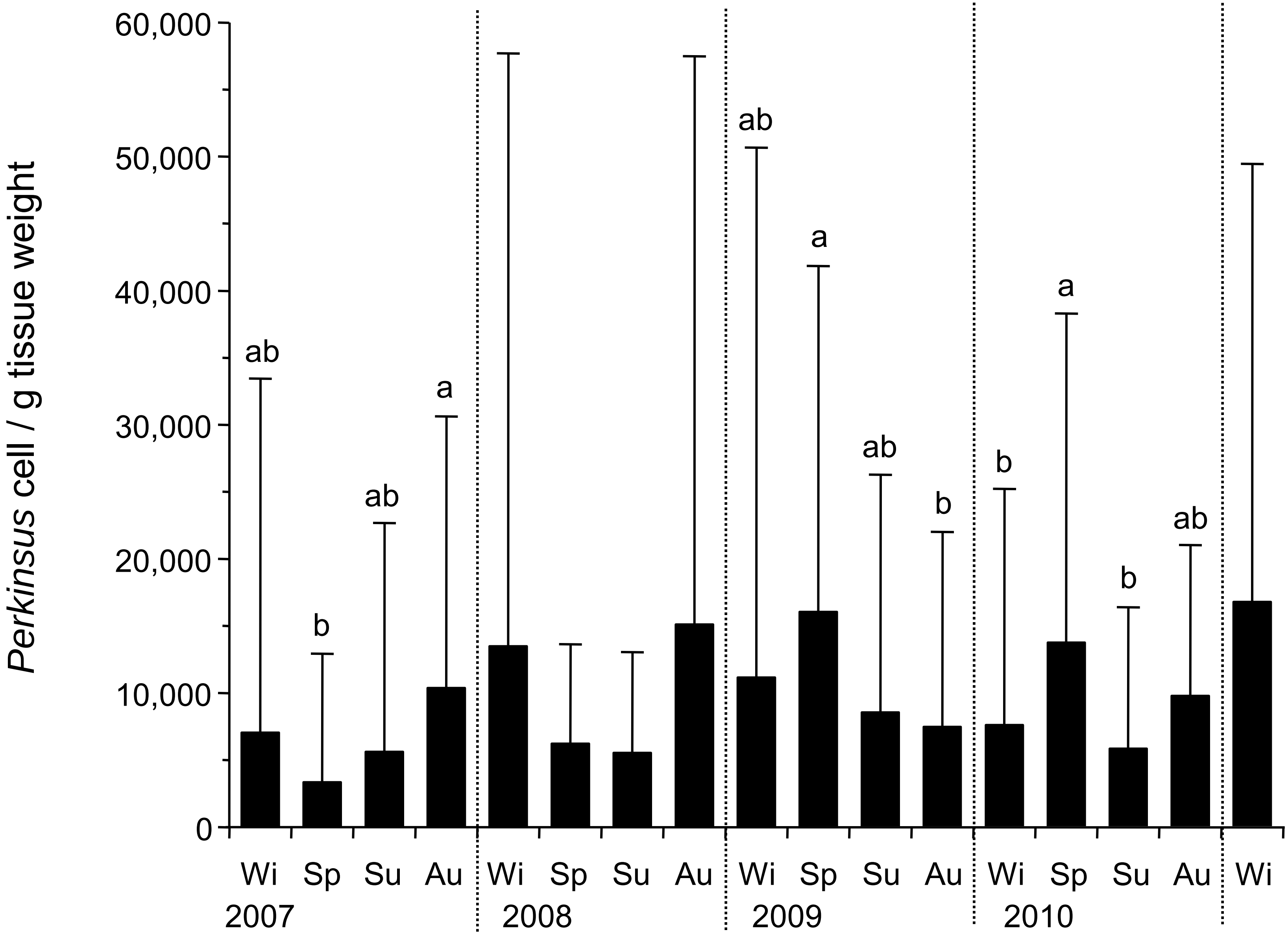


Perkinsus infection prevalence

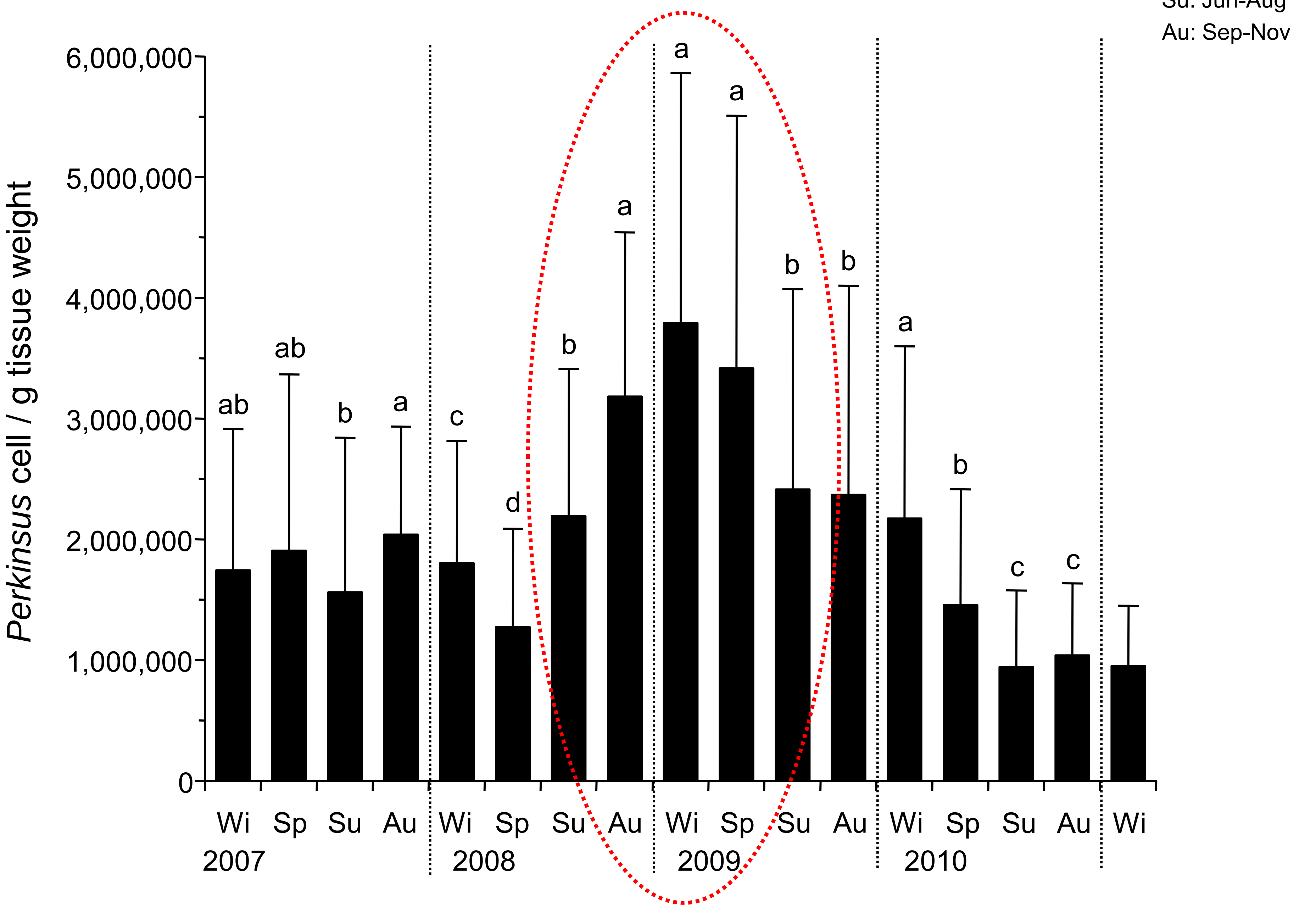
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Padori	**2007**	47	33	33	27	33	60	17	33	53	57	83	60
	2008	73	83	57	77	93	43	67	73	83	57	63	50
	2009	47	47	67	97	100	70	70	60	70	57	53	57
	2010	80	57	87	57	53	47	60	33	83	73	90	93
Hwangdo	**2007**	100	100	100	100	100	100	100	100	100	100	100	100
	2008	100	100	100	100	100	100	100	100	100	100	100	100
	2009	100	100	100	100	100	100	100	100	90	100	100	100
	2010	100	100	100	100	100	100	100	100	100	100	100	100

Perkinsus infection intensity (Padori)

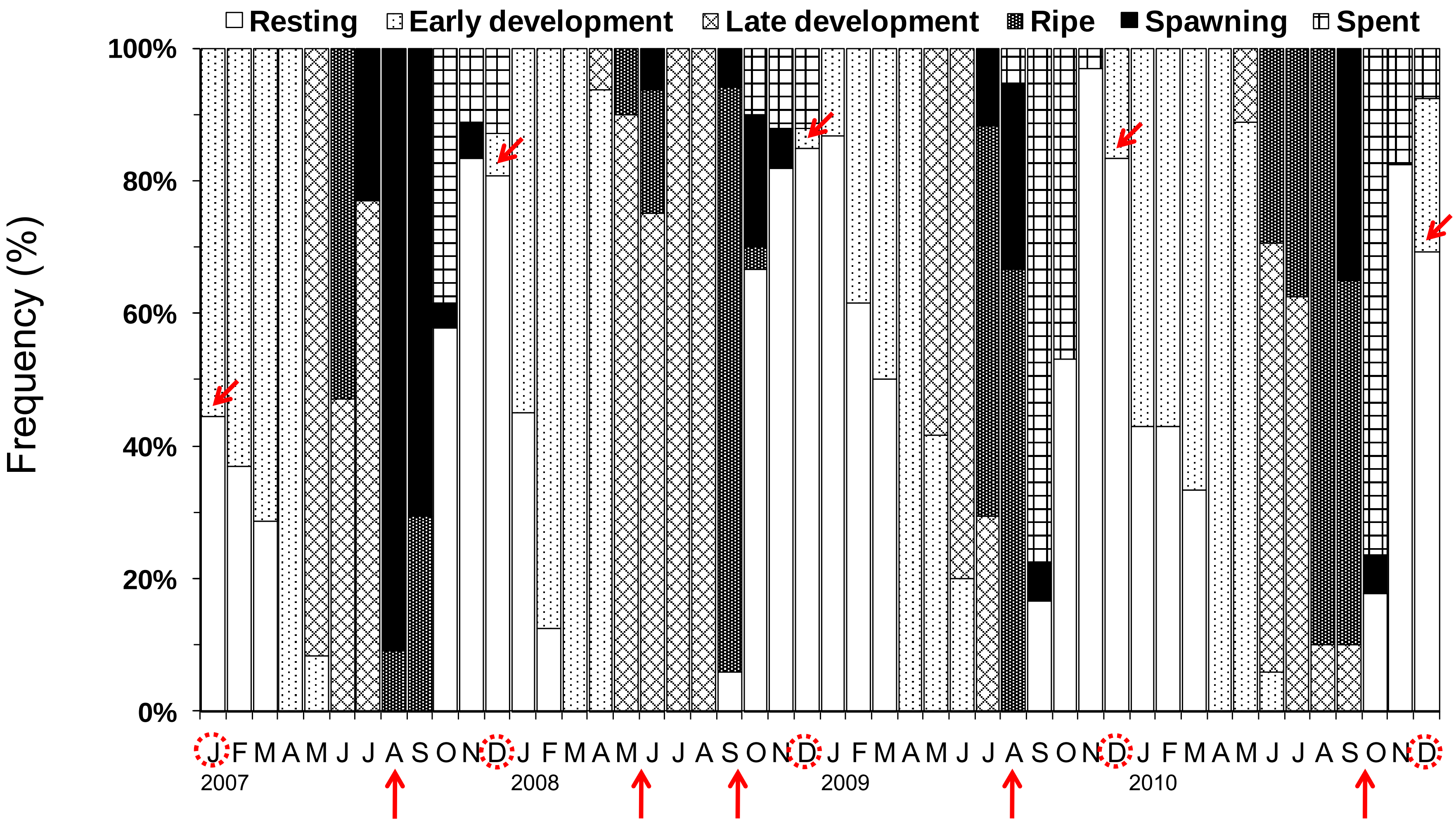
Wi: Dec-Feb
Sp: Mar-May
Su: Jun-Aug
Au: Sep-Nov



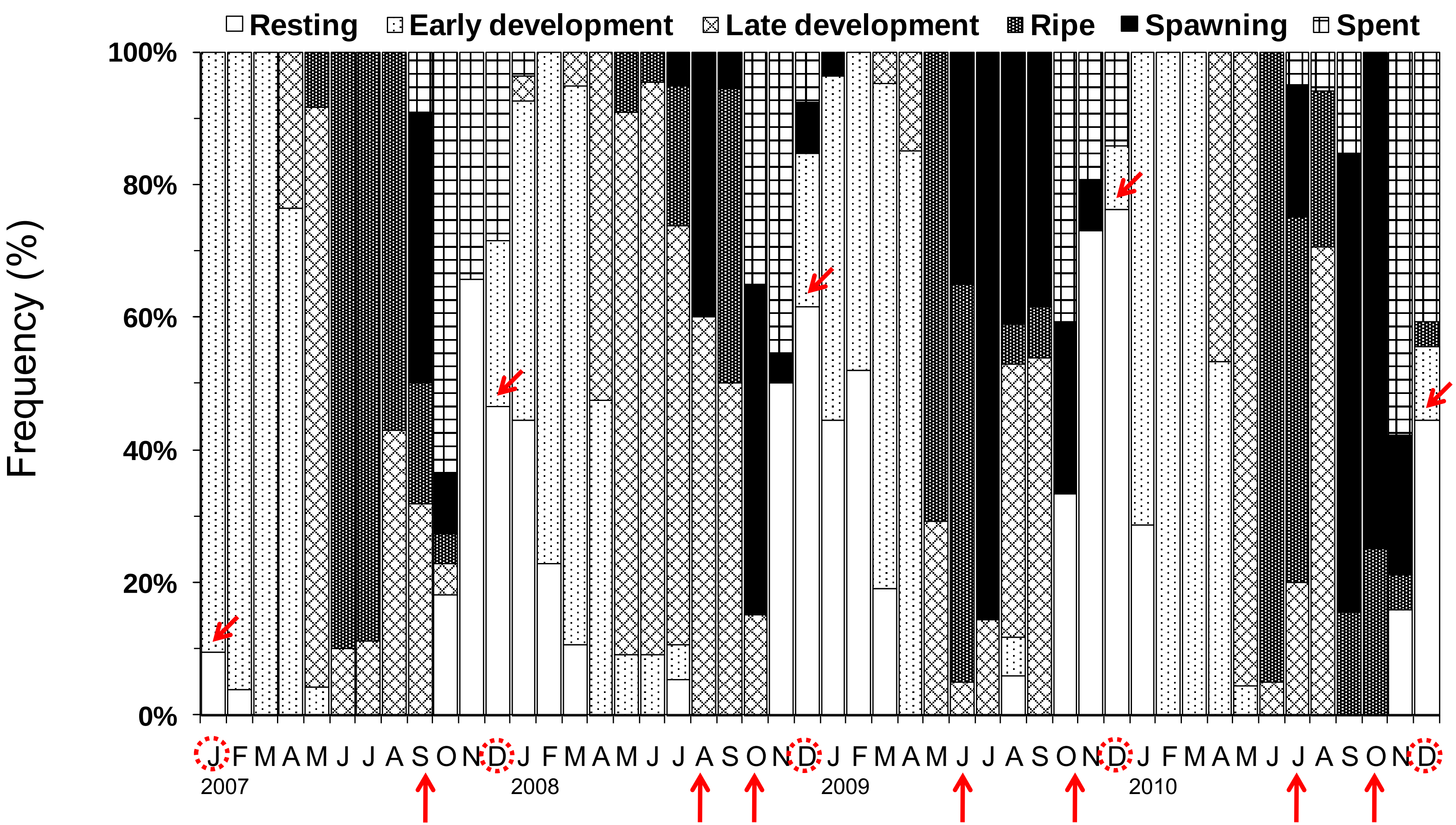
Perkinsus infection intensity (Hwangdo)



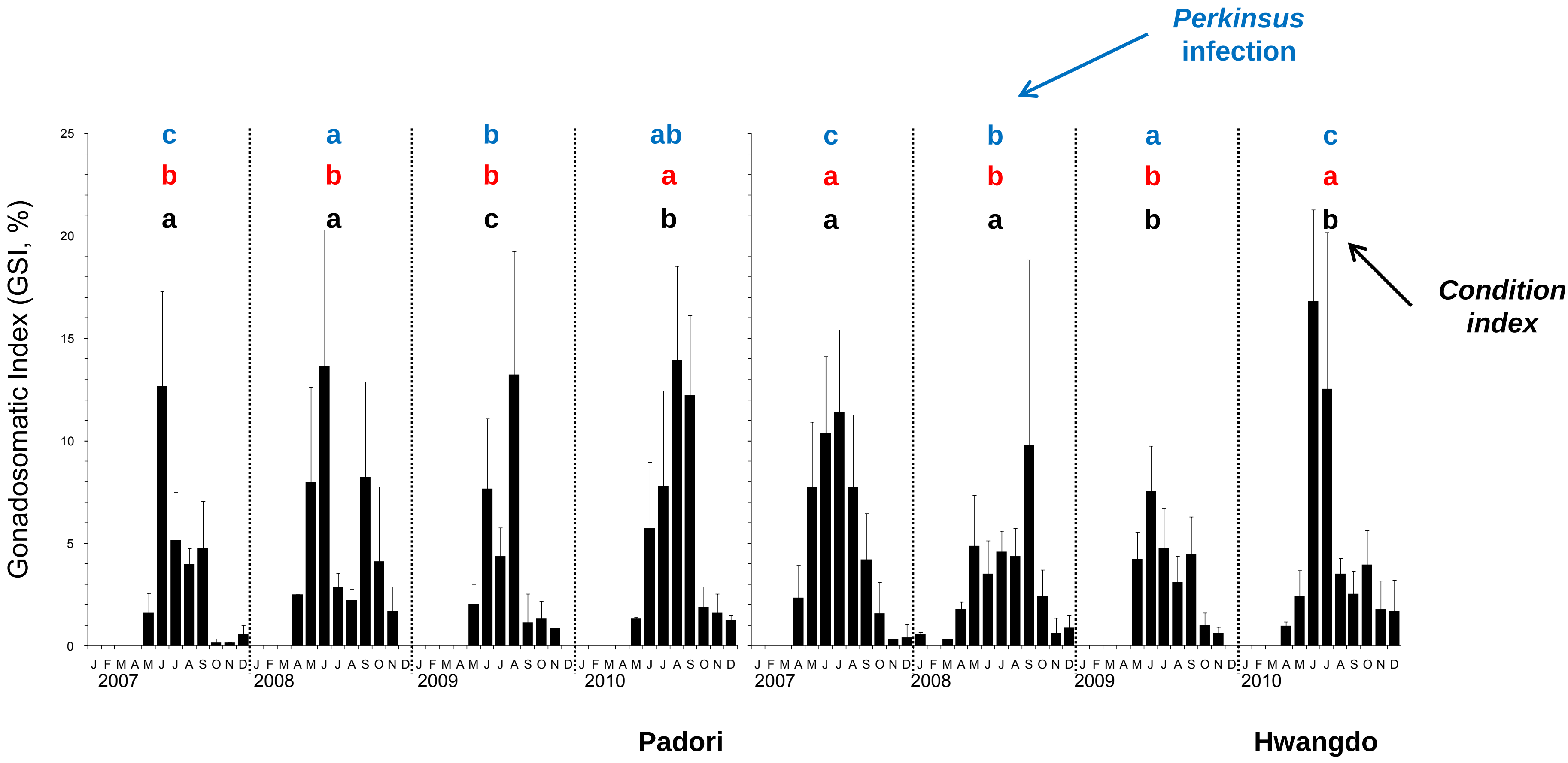
Annual reproductive cycle (Padori)



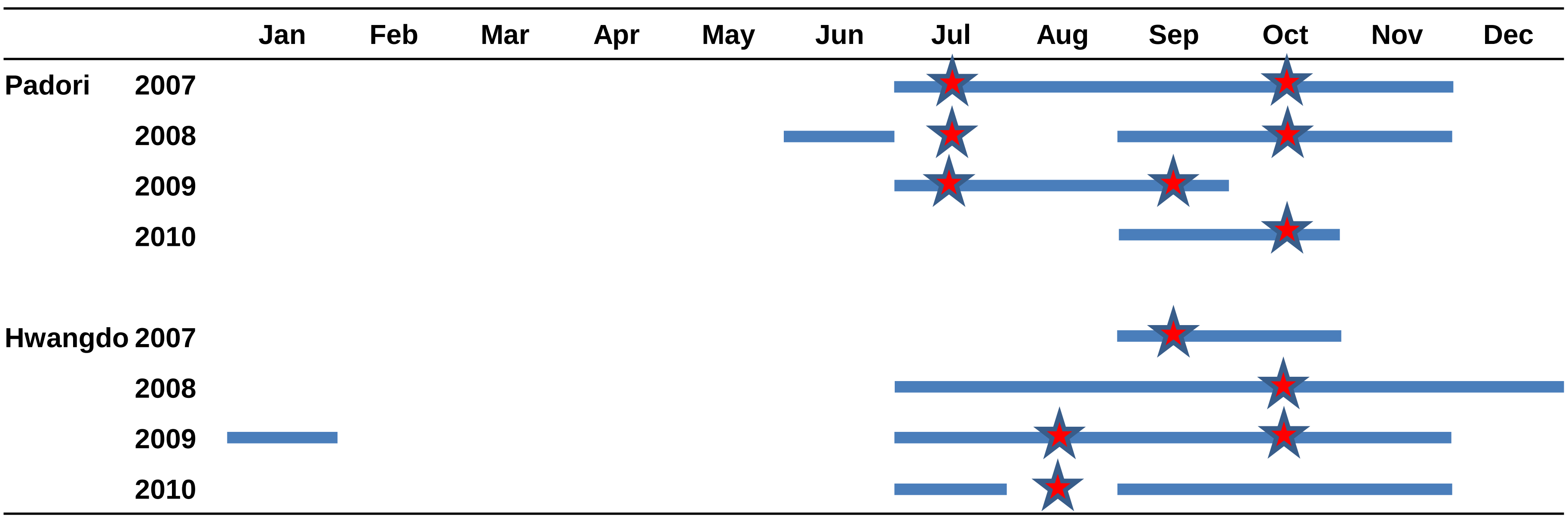
Annual reproductive cycle (Hwangdo)



Annual variation of Reproductive effort



Spawning peak



?

- Inter-annual variation in spawning season?
- Inter-annual variation in *Perkinsus* infection?
- Correlation between inter-annual variation in *Perkinsus olseni* infection intensity and the reproductive effort?
- Inter-annual variation in the water temperature and *P. olseni* infection intensity?

Conclusions

- Four years of the monitoring indicated that the spawning duration and the frequency varied year to year in the high infection area (Hwangdo).

- *P. olseni* infection intensity also varied yearly.

- GSI recorded in June 2010 (17%, prior to spawning) was significantly higher than the GSI measured in 2007, 2008 and 2009 (8-11%) in Hwangdo.

- On the other hands, clams from Padori showed stable pattern of the GSI, ranging from 13-14% (prior to spawning) and low level of *P. olseni* infection intensity and prevalence.


Thank you for your attention !
I would like to thank everybody that
participated in this project!