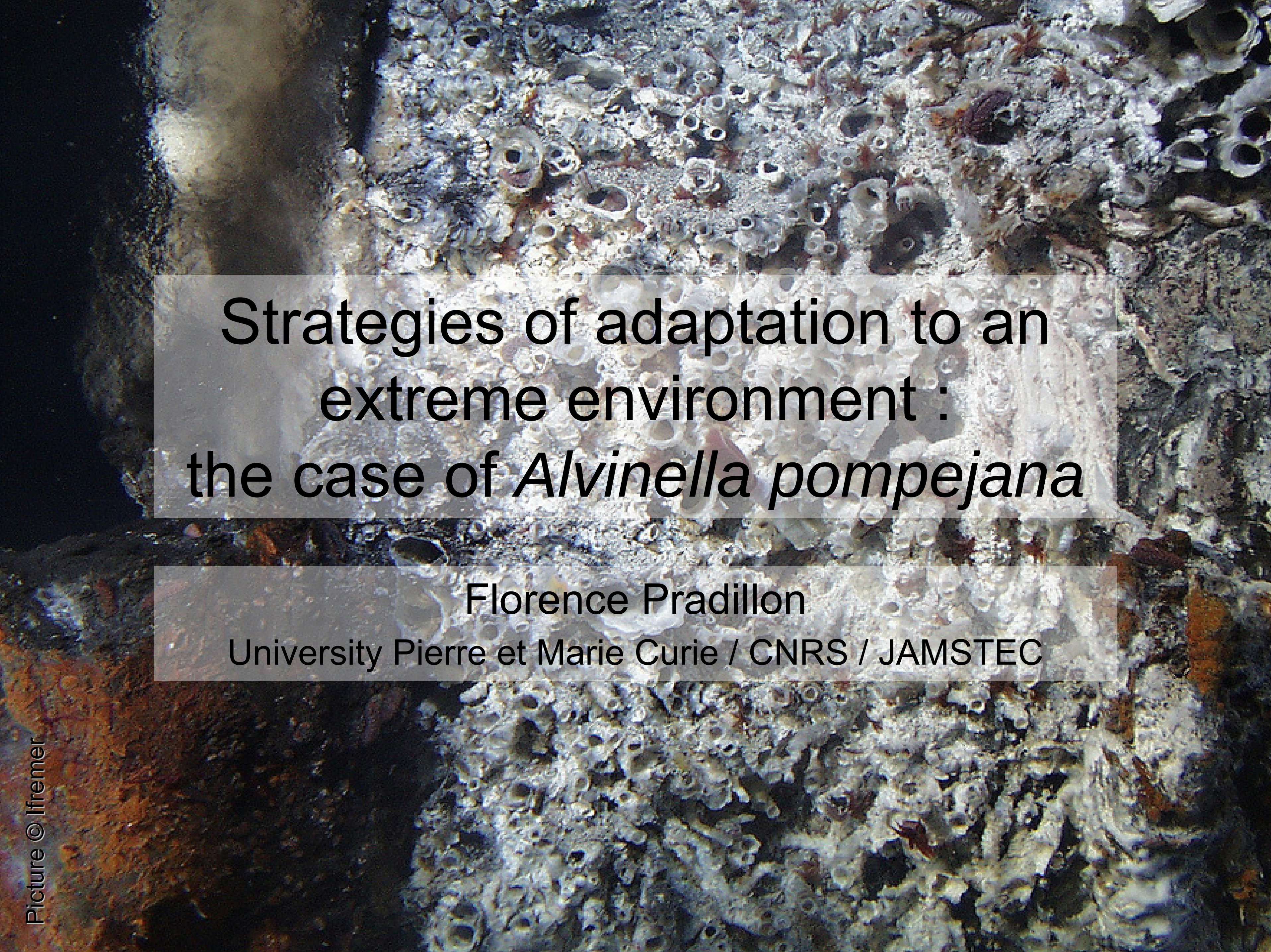


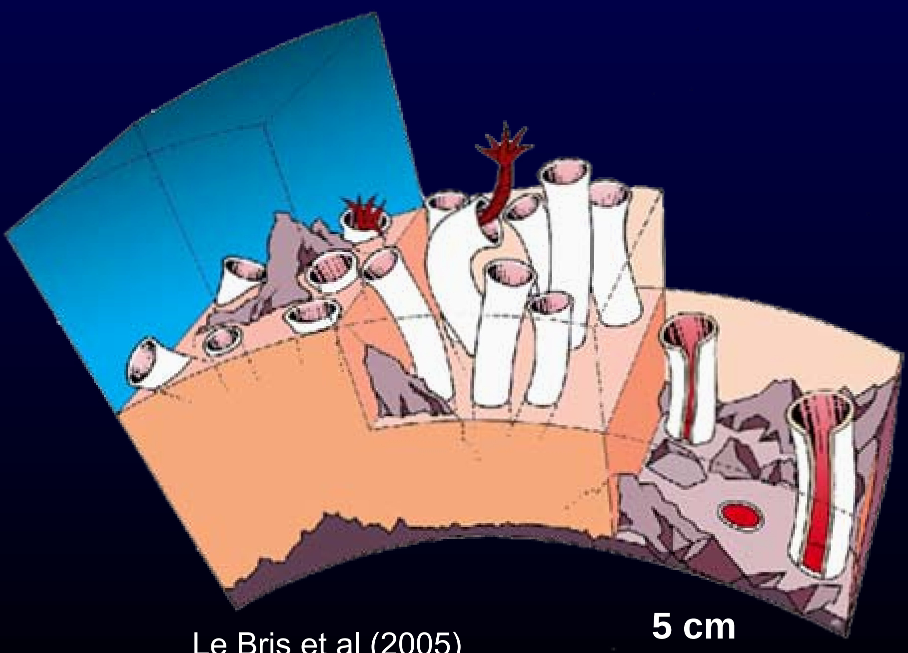
Strategies of adaptation to an extreme environment : the case of *Alvinella pompejana*

Florence Pradillon

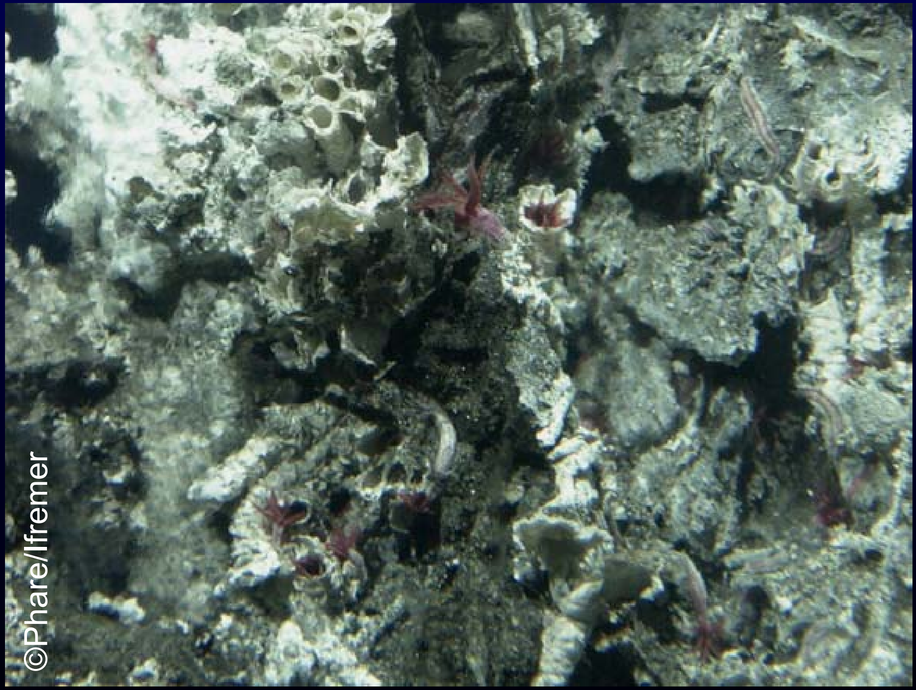
University Pierre et Marie Curie / CNRS / JAMSTEC

Environmental conditions in *A. pompejana* habitat

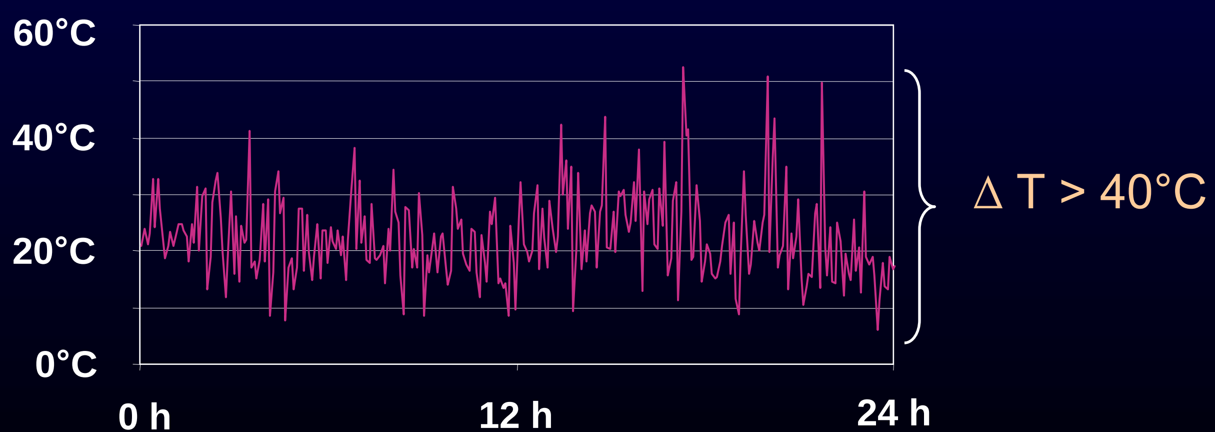
- Temperature up to 80°C
- Pressure 250 bars
- Sulfide up to 1.5 mM
- Low O_2
- High CO_2
- Heavy metals
- Oxygen radicals



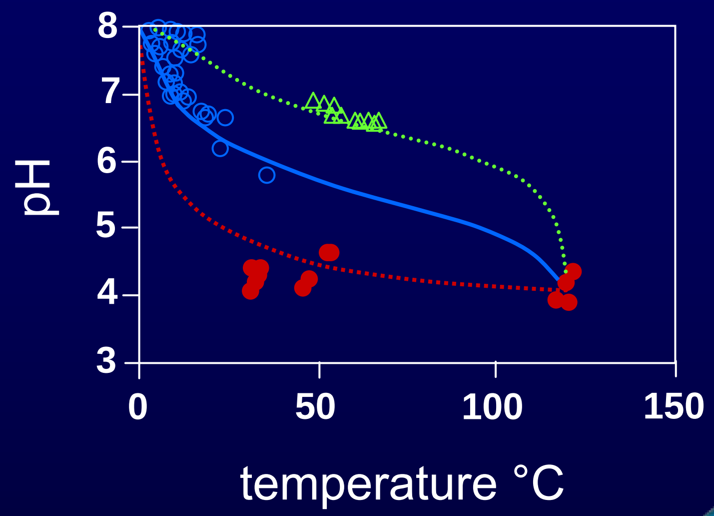
Le Bris et al (2005)



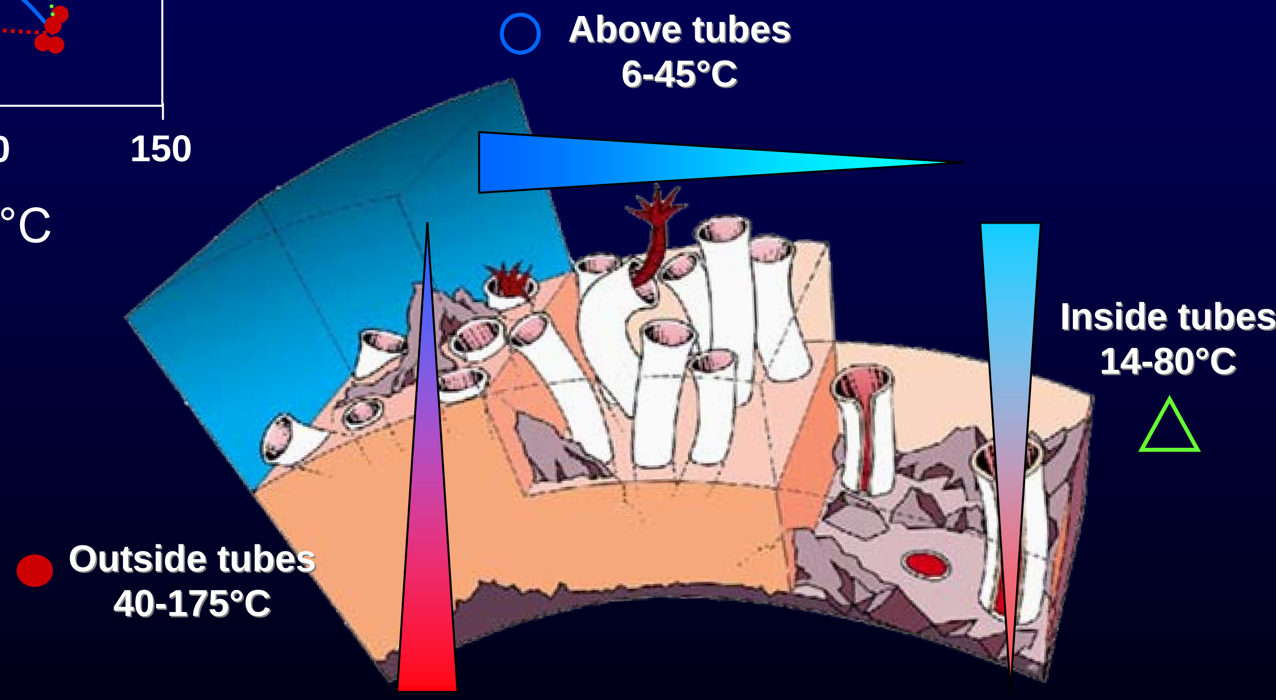
Steep spatial and temporal environmental variations



Fine scale studies revealed fluid circulations within the Alvinella colony

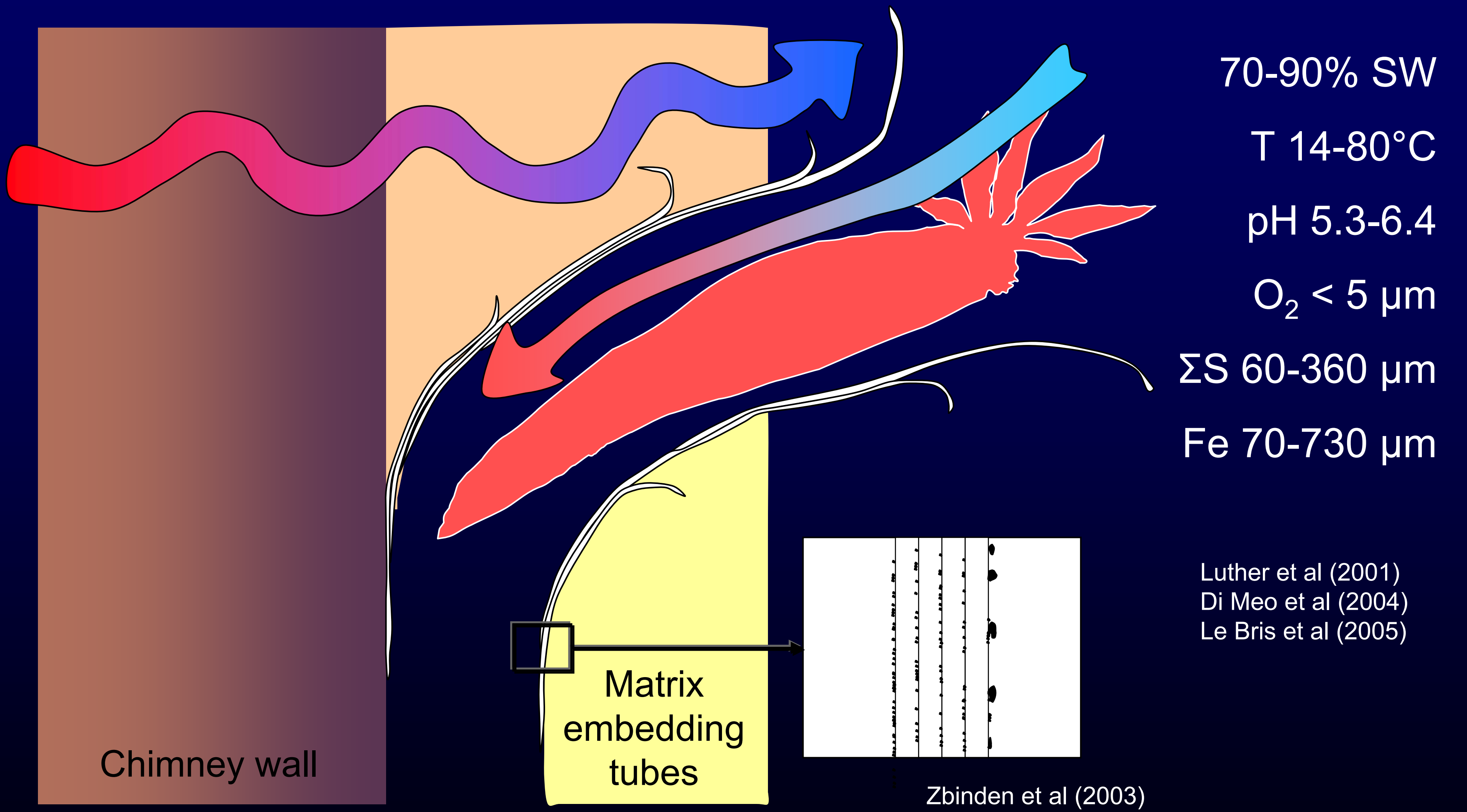


Le Bris et al. (2005)



Le Bris and Gaill (2007)

The Alvinella tube microenvironment



Which are the threats that challenge *A. pompejana* survival ?

Thermal stress

H2S poisoning

Hypoxia

Oxidative stress

Hypercapnia

Heavy metals

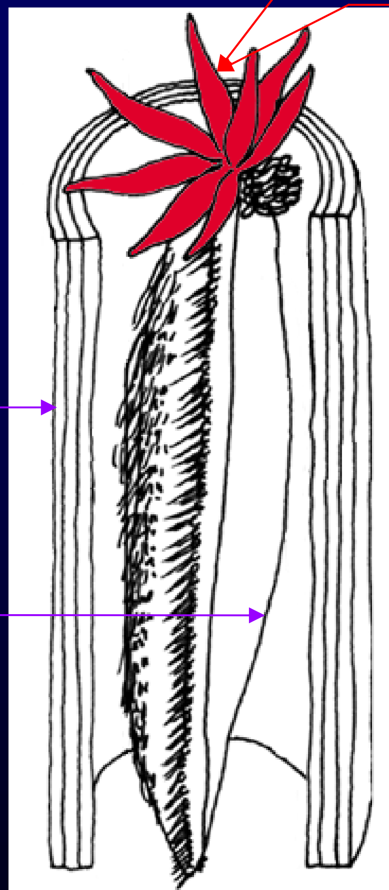
Thermotolerance biochemical data in *Alvinella pompejana*

Hemoglobin cooperativity : 30°C
Toulmond et al (1990)

Hemoglobin stability : 50°C
Terwilliger & Terwilliger (1984)

Tube : 100°C
Gail & Hunt (1986)

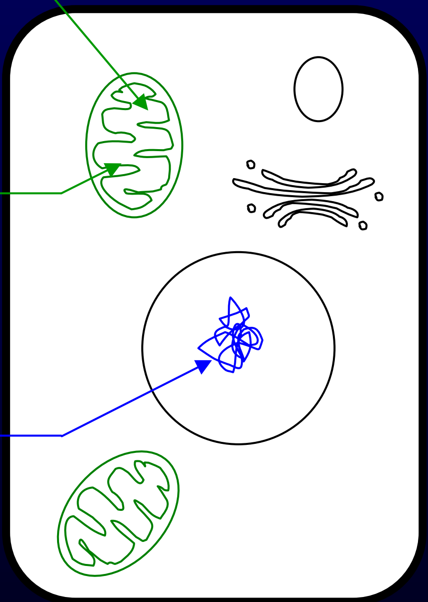
Collagen : 45°C
Gaill et al (1991)



Mitochondrial respiration : 49°C
Dahlhoff et al (1991)

Malate deshydrogenase activity : 31°C
Dahlhoff & Somero (1991)

DNA denaturation : 87°C
Dixon et al (1992)

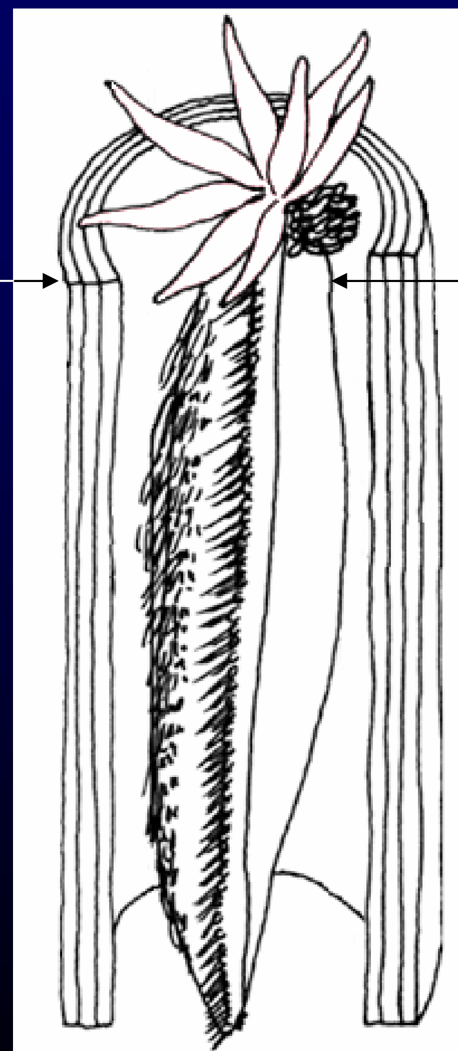


Optimal body temperature ?
Temperature ≤ 50°C

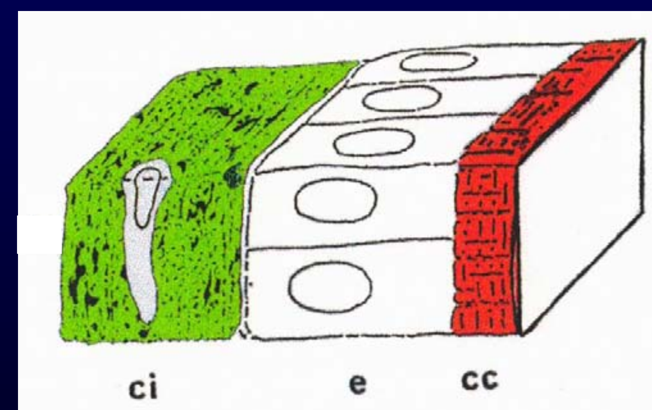
Extracellular matrices : barrier against external conditions ?

Tube

Main component : proteins
Stable up to 100°C
Chemically resistant

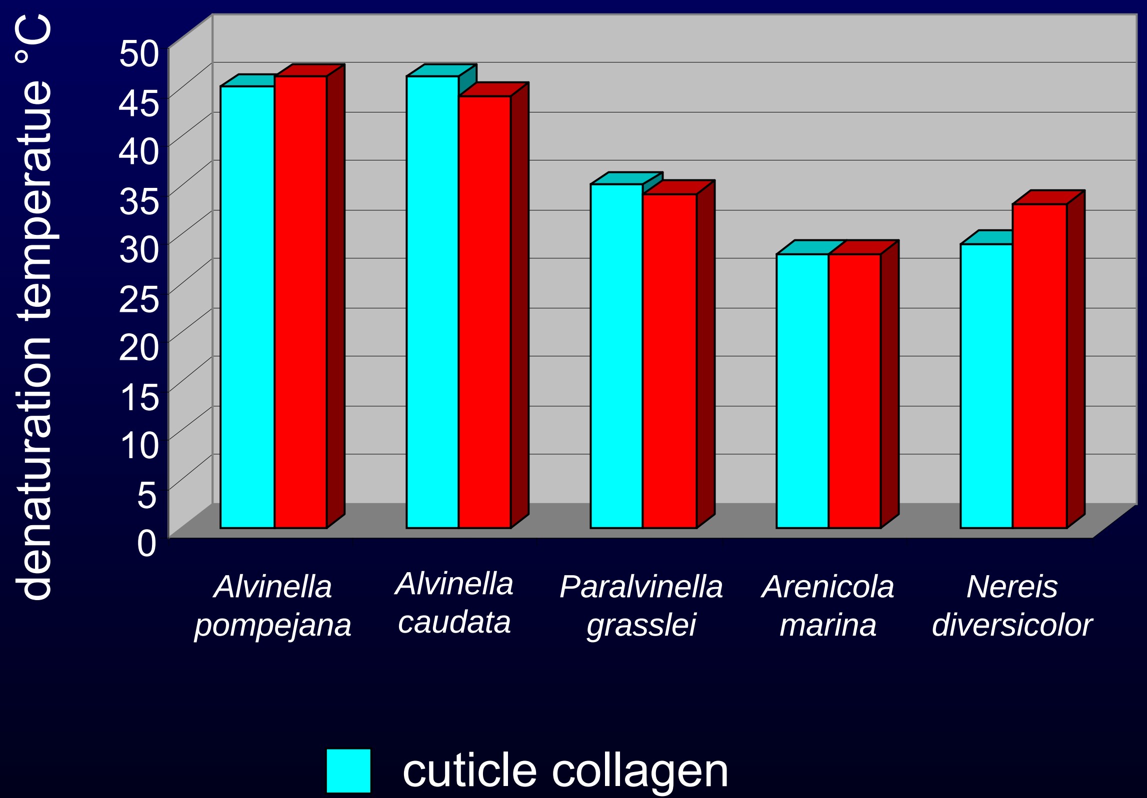


Cuticle



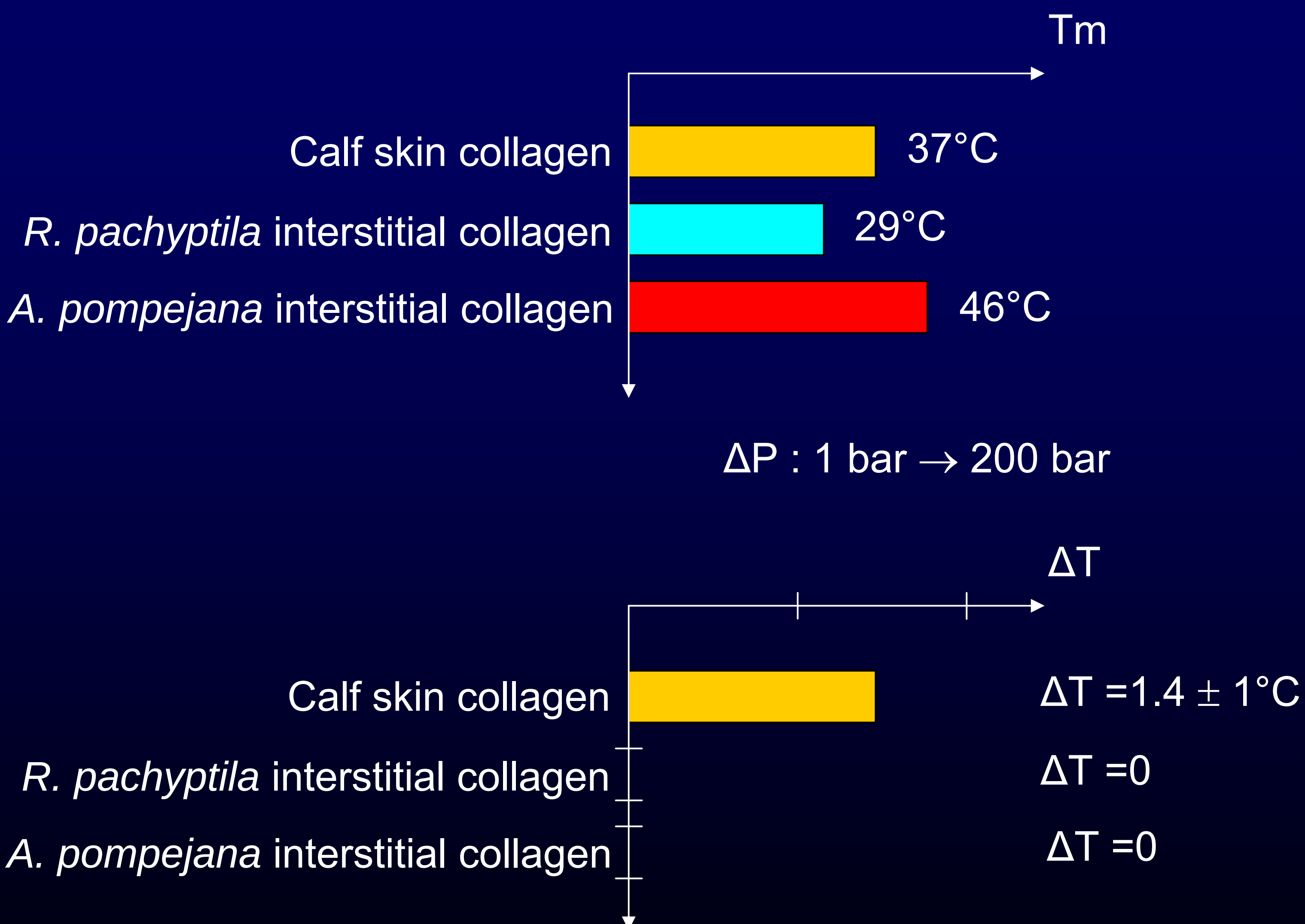
Main component : collagen
Stable up to 65°C

High thermal stability in *A. pompejana* collagen



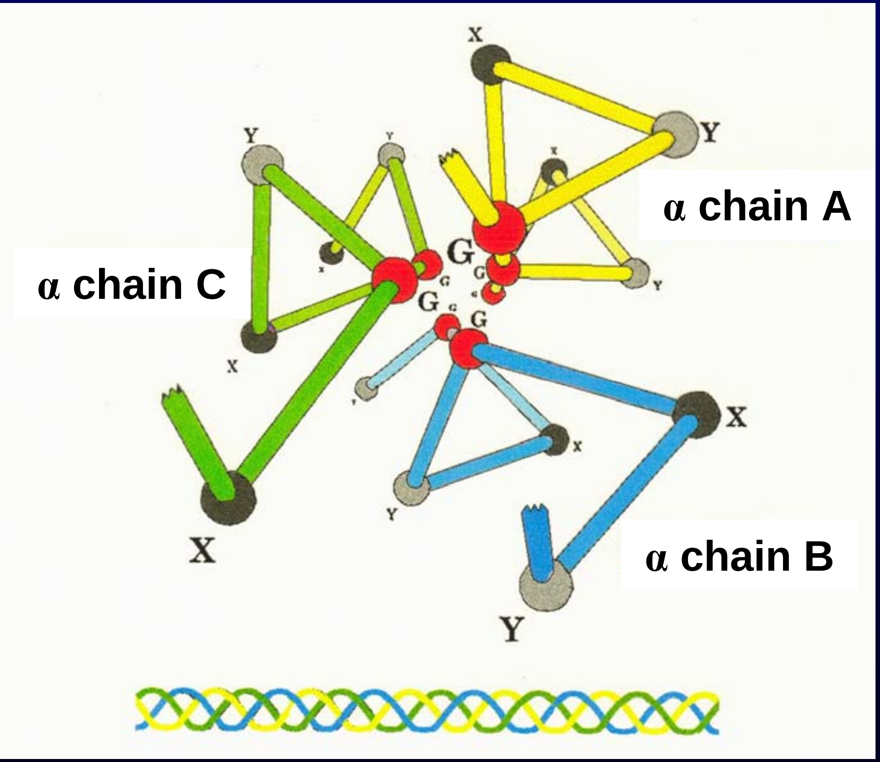
Gaill et al (1995)

Hydrostatic pressure does not affect thermal stability in *A. pompejana* collagen



Auerbach et al (1991)

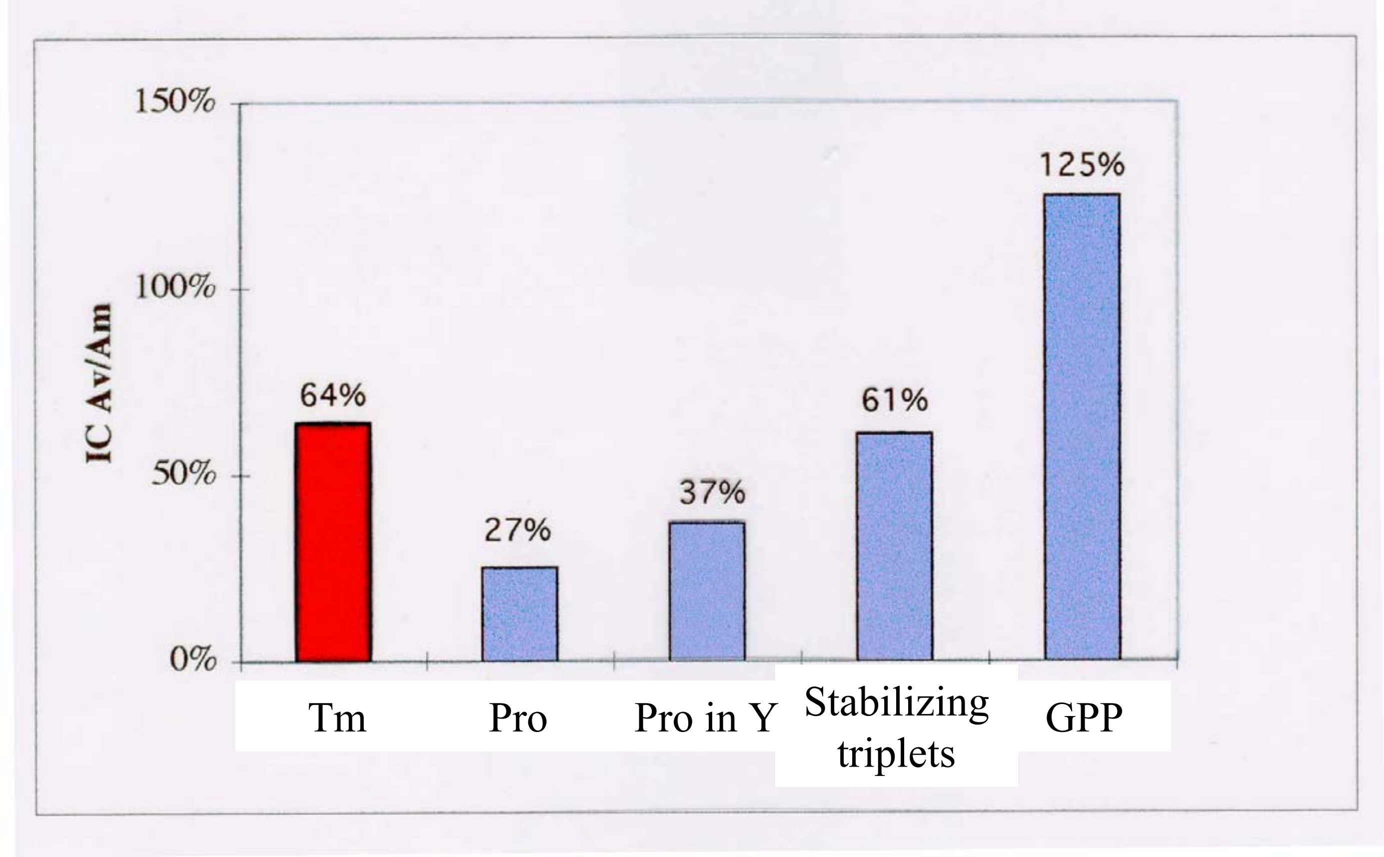
Amino acid sequence : Gly-X-Y-Gly-X-Y-Gly-X-Y-Gly-X-Y-



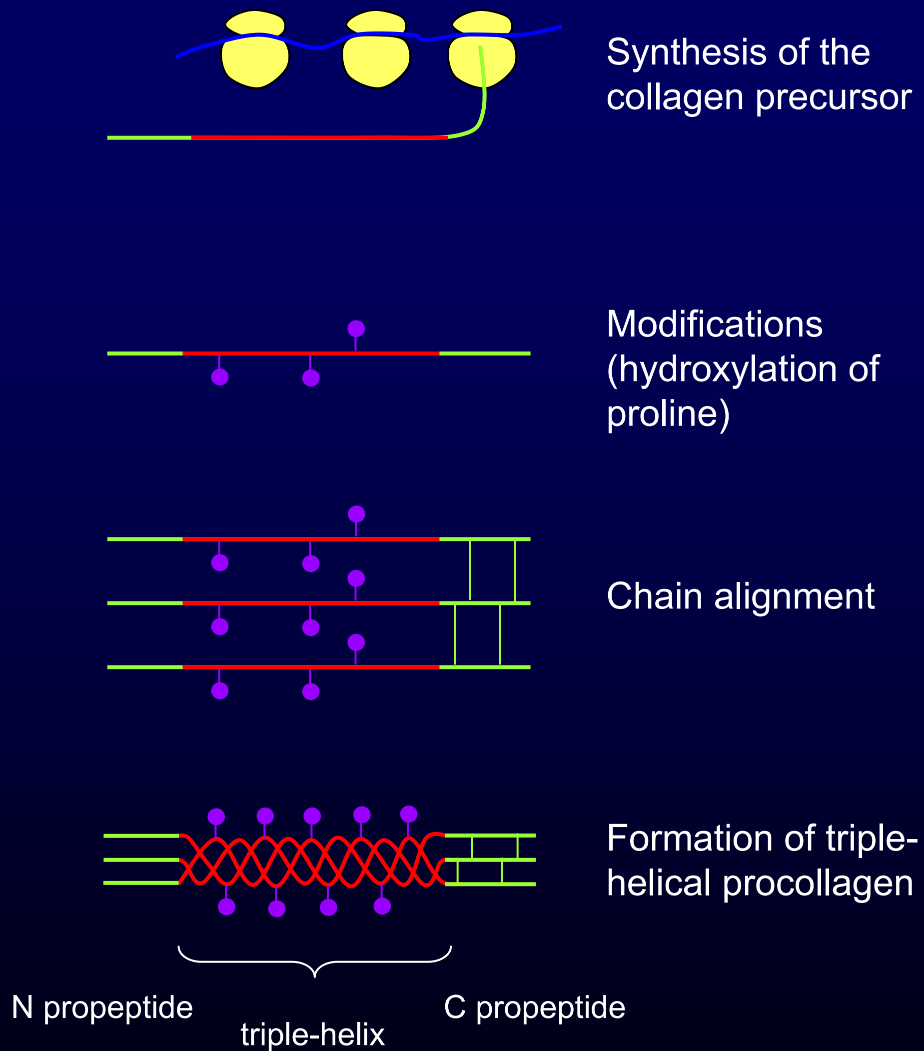
Van Der Rest & Garrone (1991)

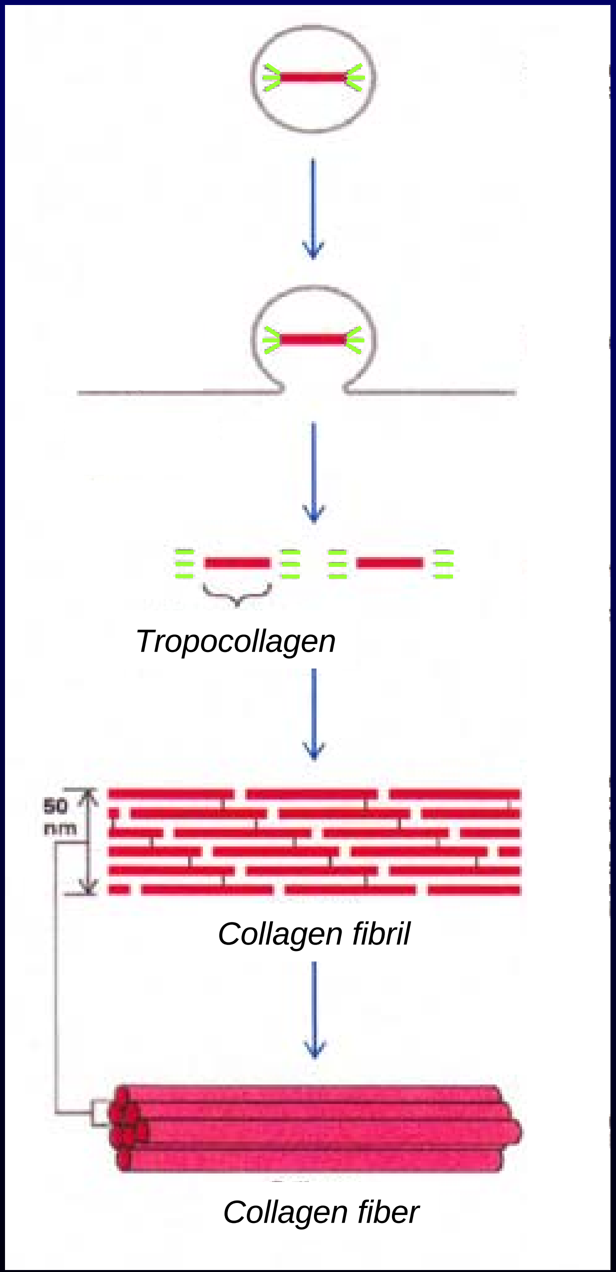
Molecular basis of thermal stability in *A. pompejana* collagen

Amino acid sequence : Gly-P-P-Gly-X-Y-Gly-X-Y-Gly-X-Y…

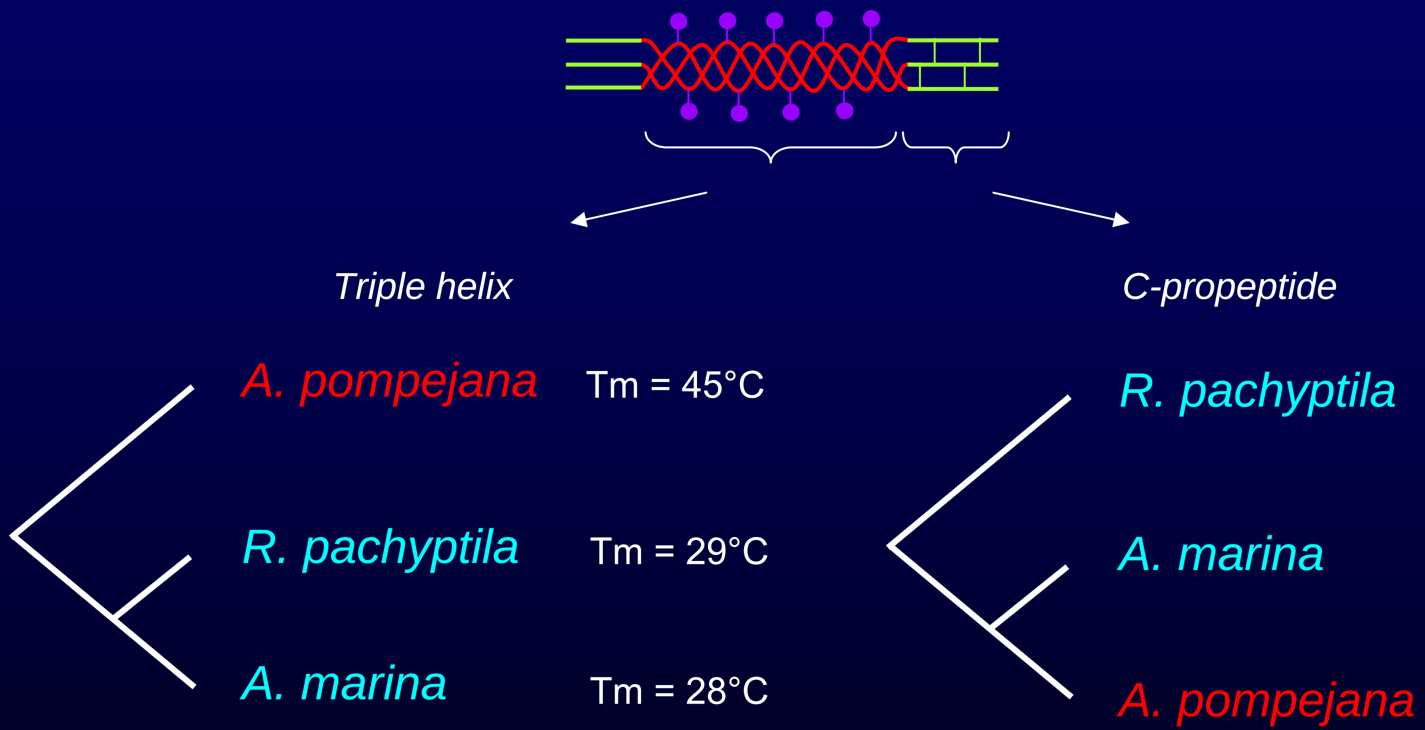


Hydroxylation of proline further increases collagen thermal stability.

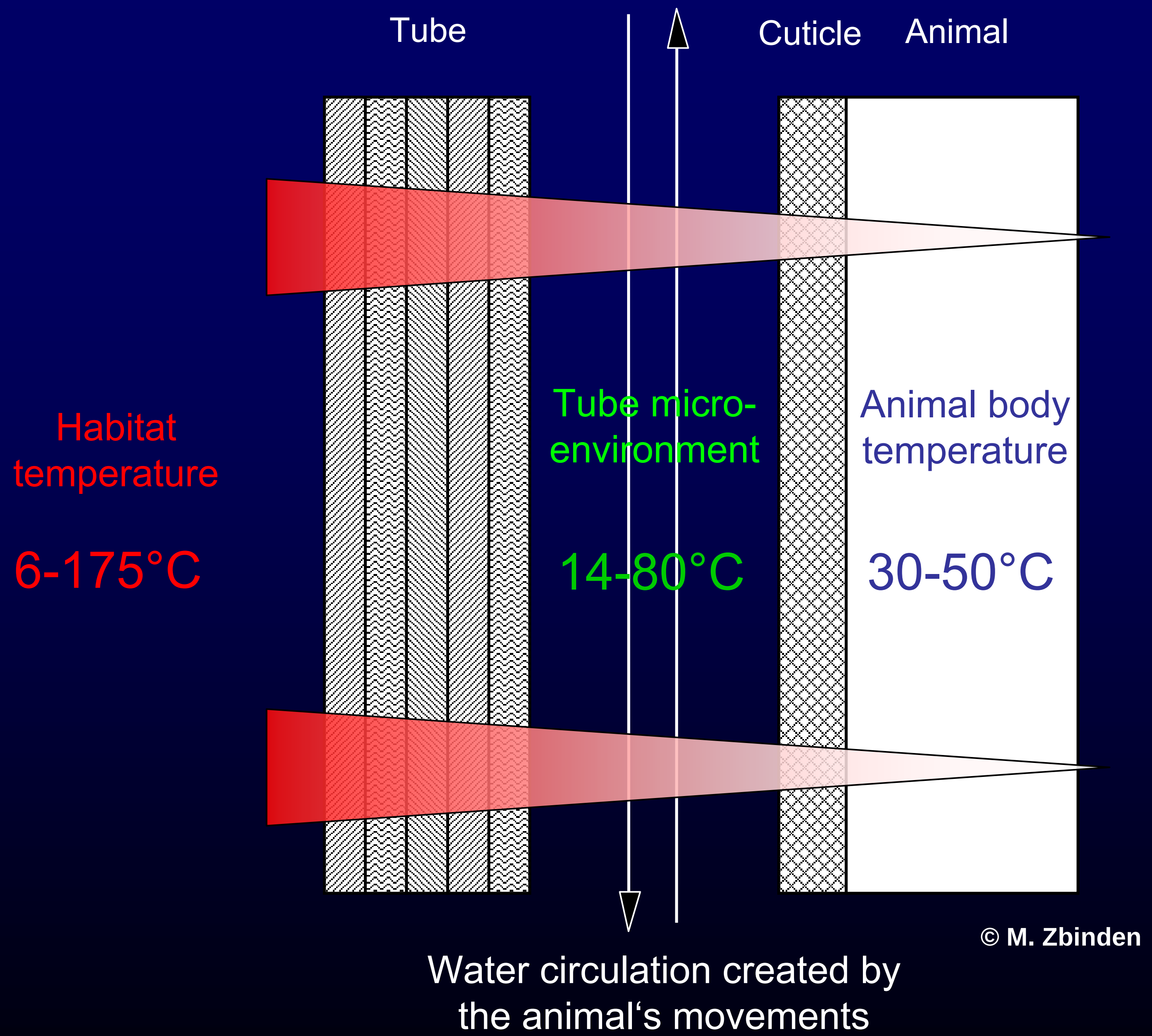

Synthesis of the collagen precursor
Modifications (hydroxylation of proline)
Chain alignment
Formation of triple-helical procollagen
N propeptide
triple-helix
C propeptide


Tropocollagen
50 nm
Collagen fibril
Collagen fiber

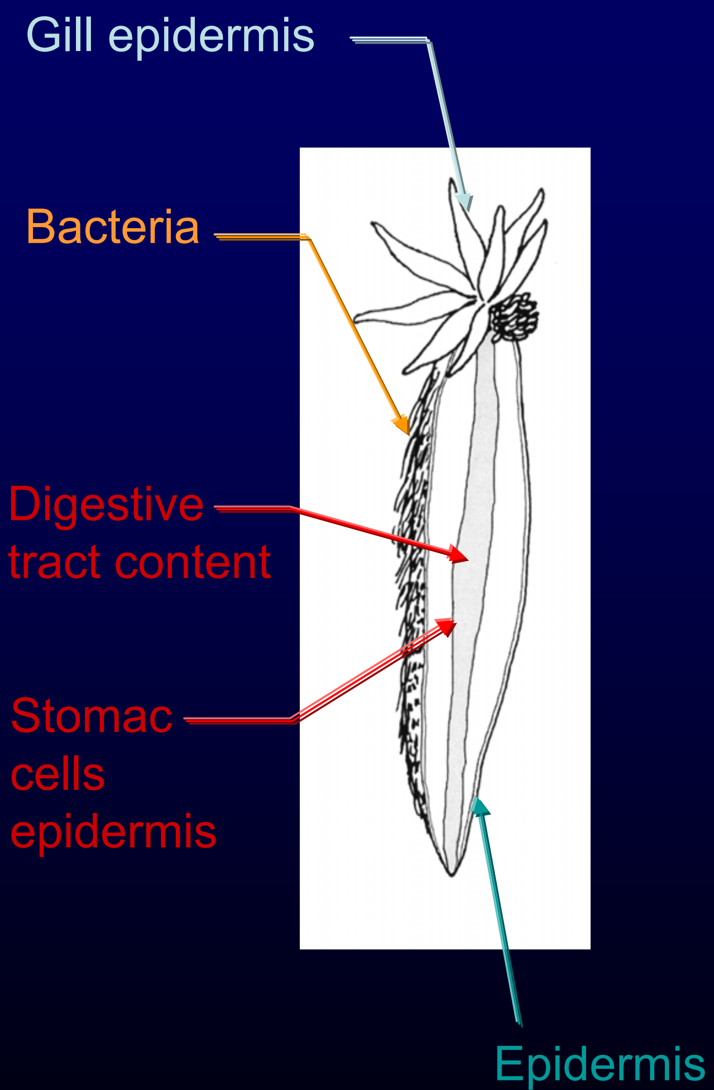
A. pompejana collagen : an example of molecular adaptation to high temperature



Sicot et al (2000)

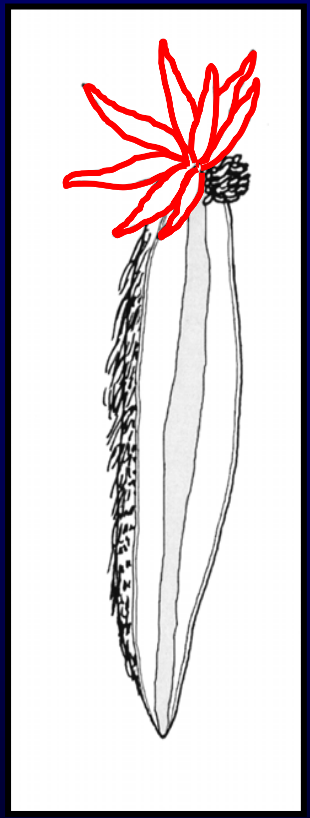

Tube
Cuticle
Animal
Habitat temperature
6-175°C
Tube micro-environment
14-80°C
Animal body temperature
30-50°C
© M. Zbinden
Water circulation created by the animal's movements

Sulfide detoxification



- Biological interfaces (tubes, cuticles)
- Epibiotic bacteria
- Peripheral tissues
- Detoxification sites
- Immoblisation in tissues

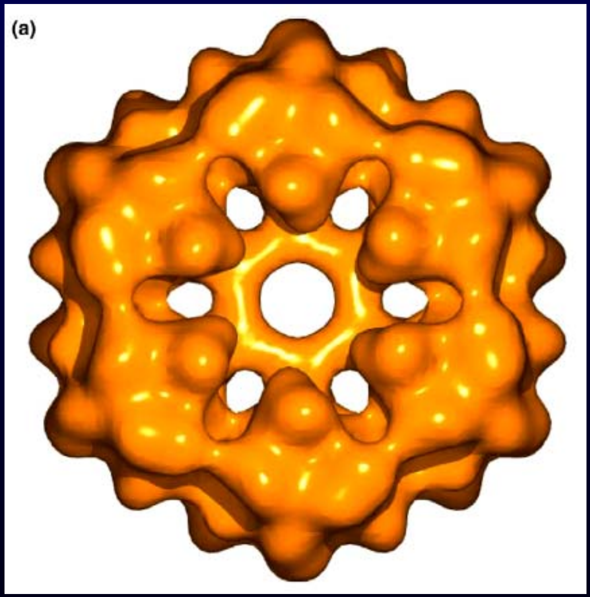
Adaptations to hypoxia



• Morphological adaptations :

Large gill surface, short diffusion distances Jouin & Gaill (1990)

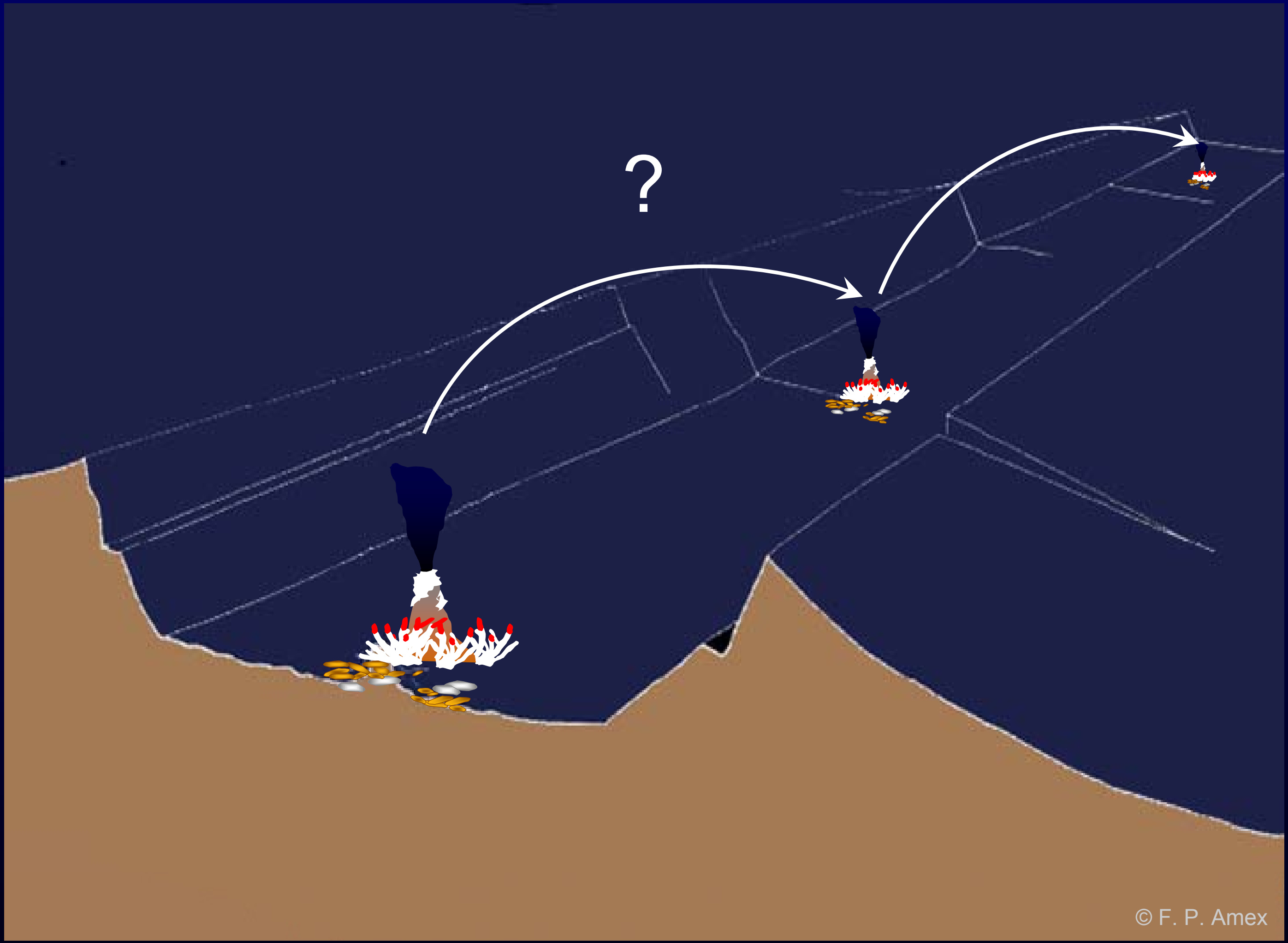
Gas transfer system Jouin-Toulmond et al (1996)



•2 Hemoglobins with high affinity for oxygen
Terwilliger & Terwilliger (1984), Toulmond et al (1990),
Zal et al (1997), Hourdez et al (2000)

Hourdez & Weber (2005)

Large scale variability : species persistance over generations?



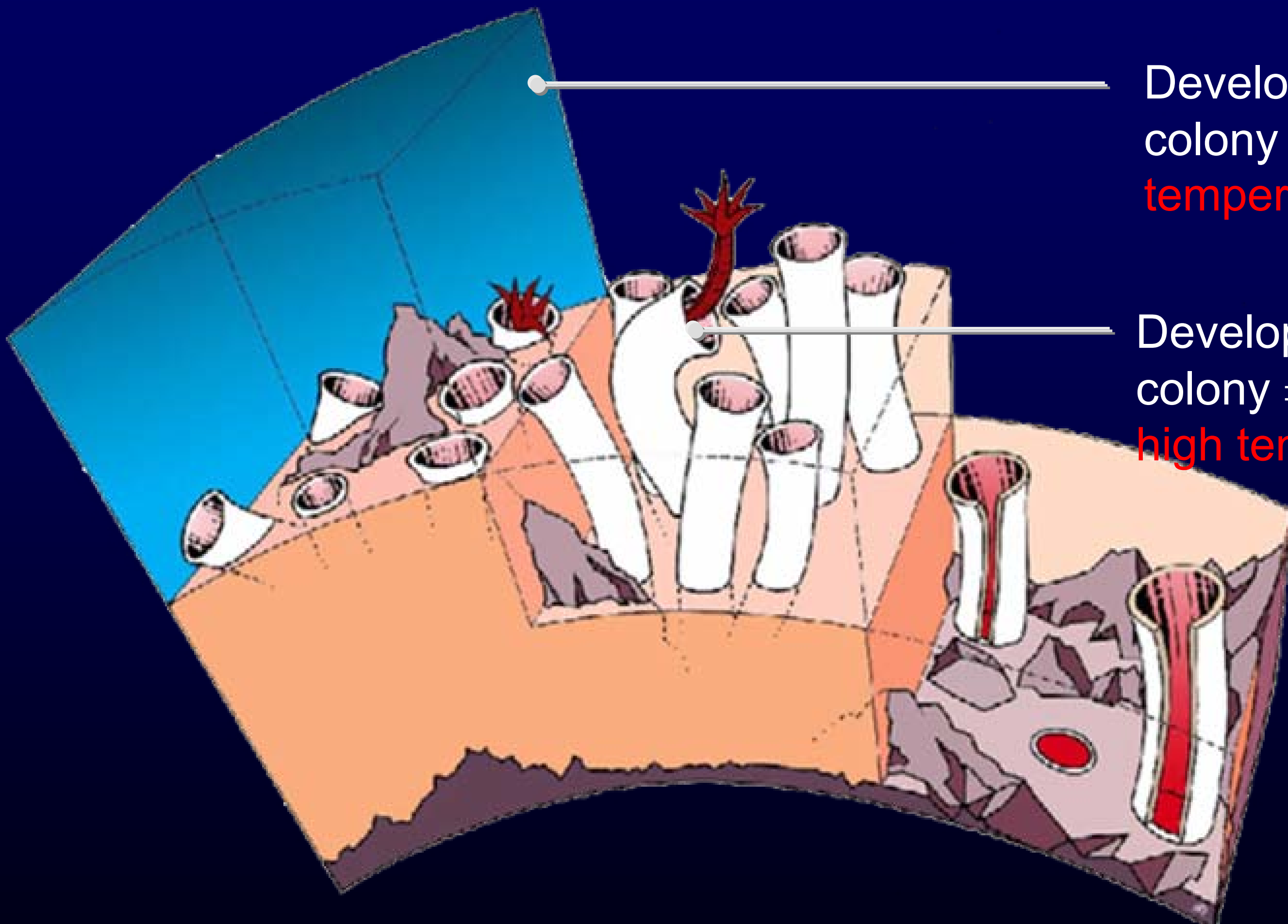
Reproduction and dispersal strategies ?

How do vent embryos develop ?

Which conditions are suitable for development ?

Is reproduction influenced by environmental conditions ?

Conditions suitable for early development in *A. pompejana* ?



Development outside of the colony ⇒ embryos tolerate low temperatures (< 20°C)

Development inside the colony ⇒ embryos tolerate high temperatures (≥ 20°C)

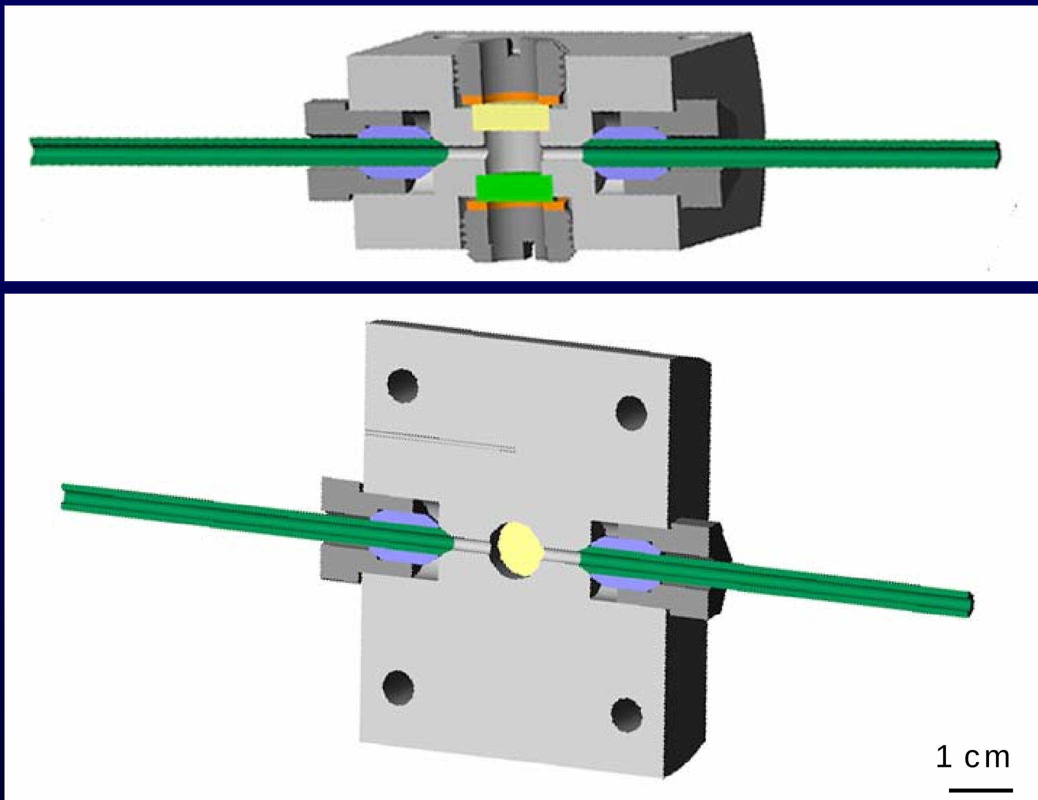
Picture from Le Bris *et al*. (2005)

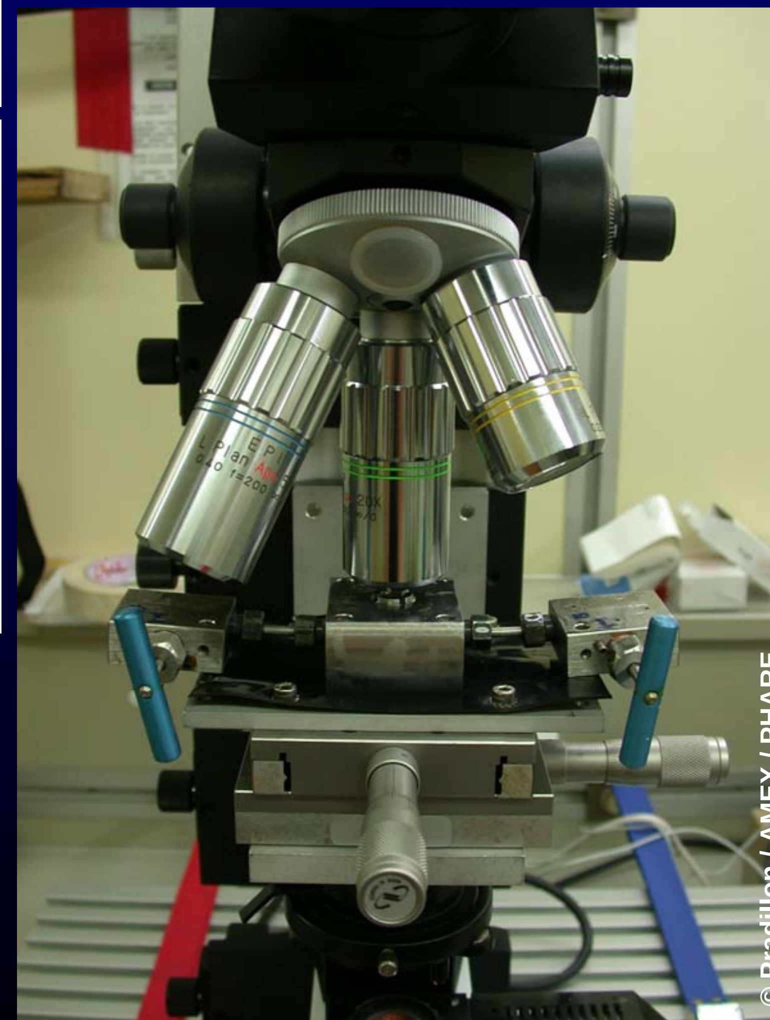
Pressure systems for the cellular level

PICCEL (*Pressurized Incubators for the Culture of Cells, Embryos and Larvae*)

Pradillon *et al.* (2004) High Pressure Res

Pressure systems for the cellular level

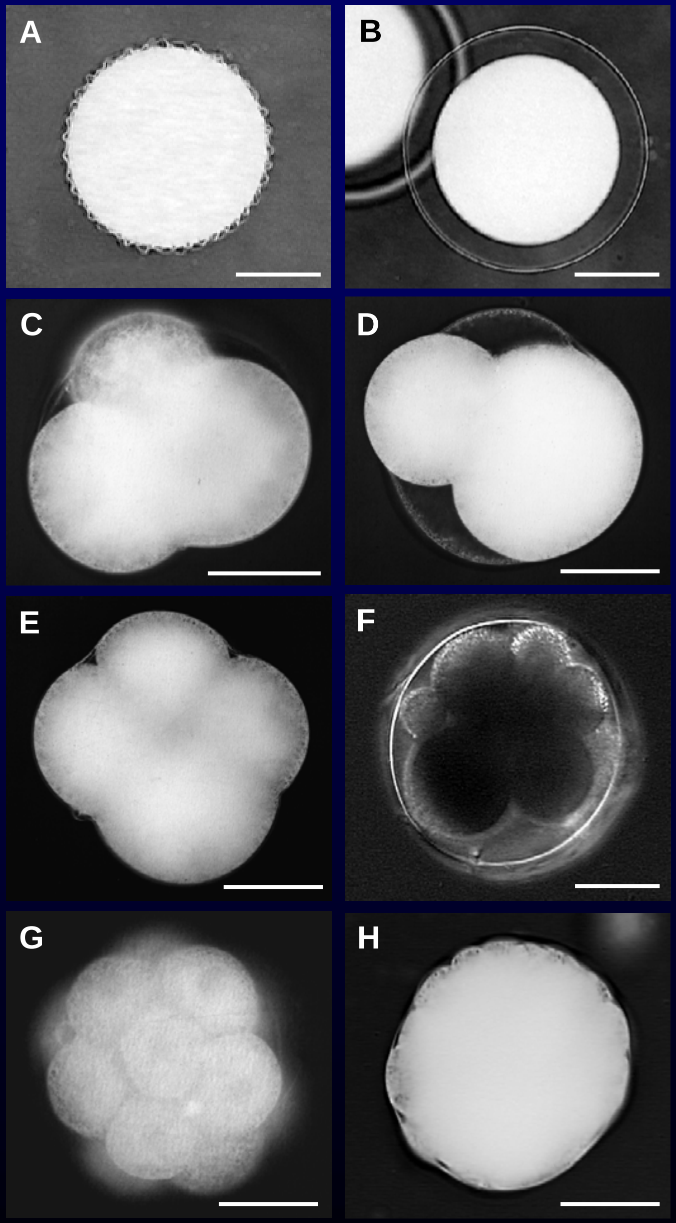




PIRISM (*PICCEL Related Imaging System for Microscopy*)

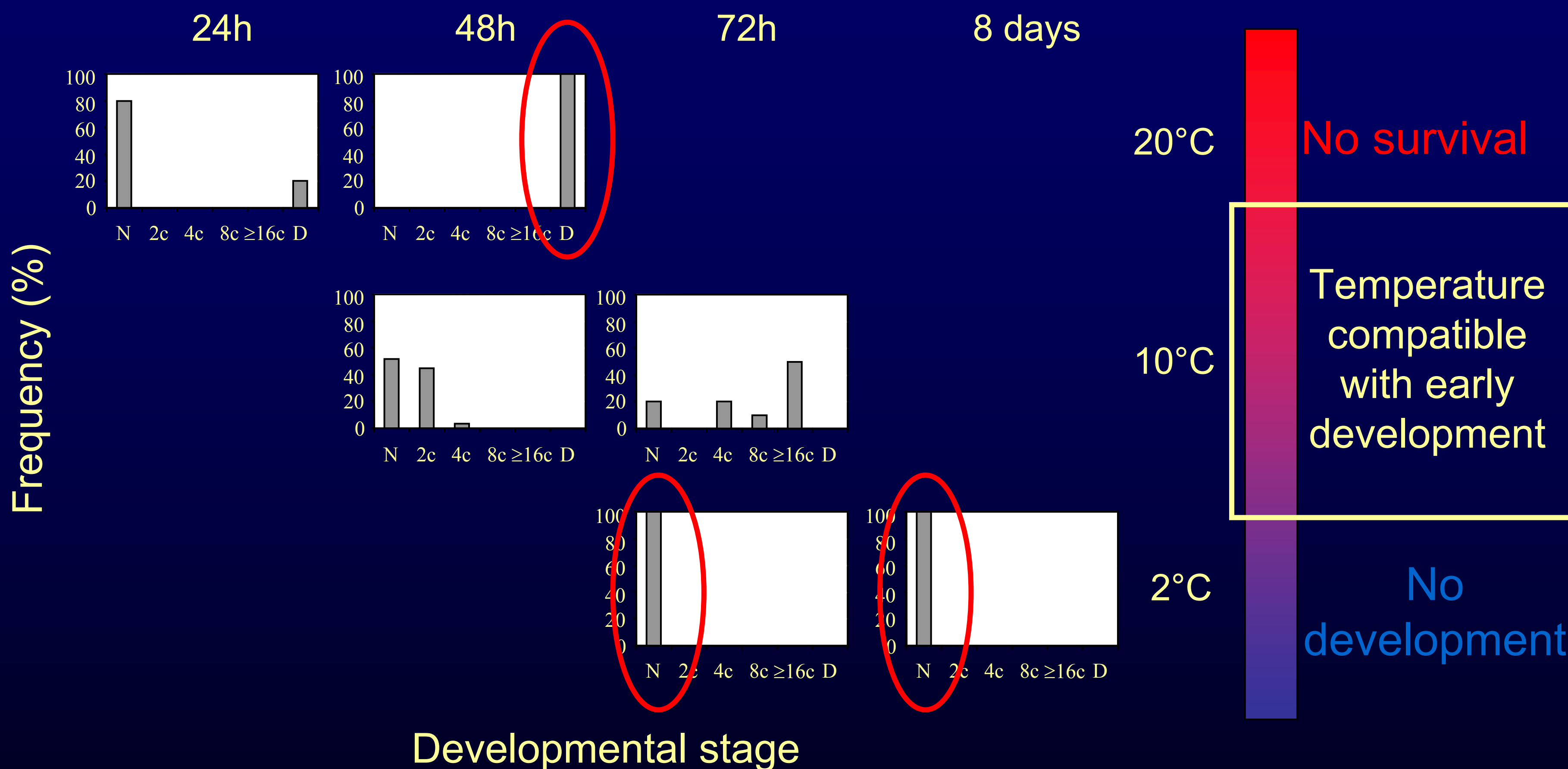
Pradillon *et al.* (2004) High Pressure Res

Morphology of *A. pompejana* early embryos



Pradillon *et al.* (2005) J Exp Biol

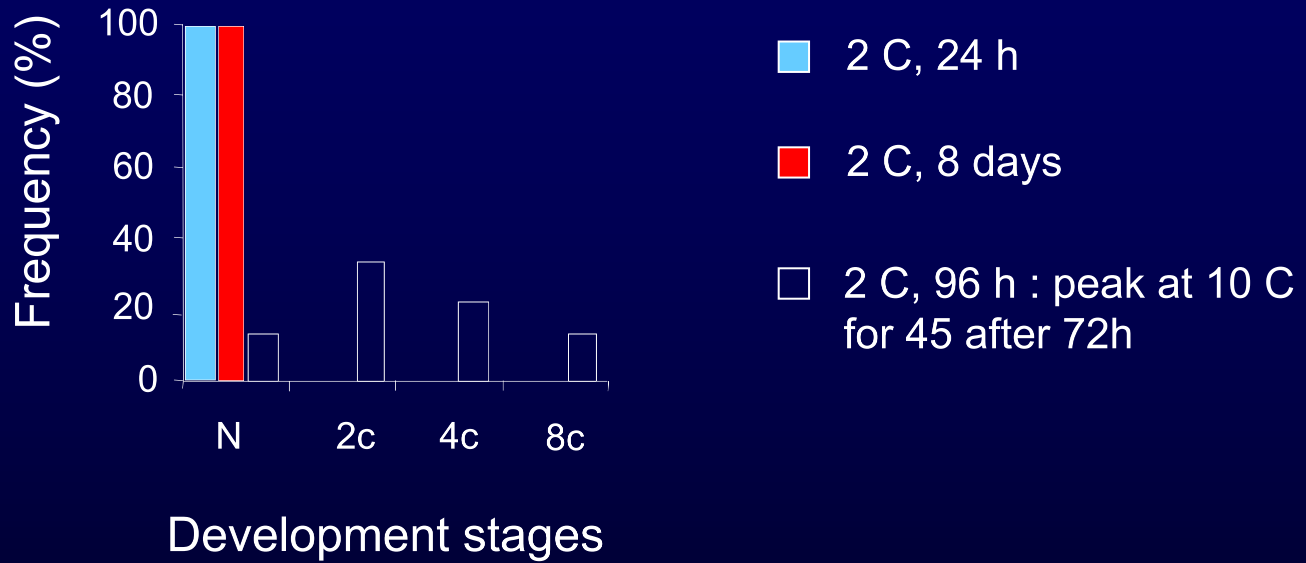
Effect of temperature on early development



N: non developping embryos
2c: 2-cell embryos
4c: 4-cell embryos
8c: 8-cell embryos
≥16c: 16 and more cell embryos
D: damaged embryos

Pradillon *et al.* (2001) Nature
Pradillon *et al.* (2005) J Exp Biol

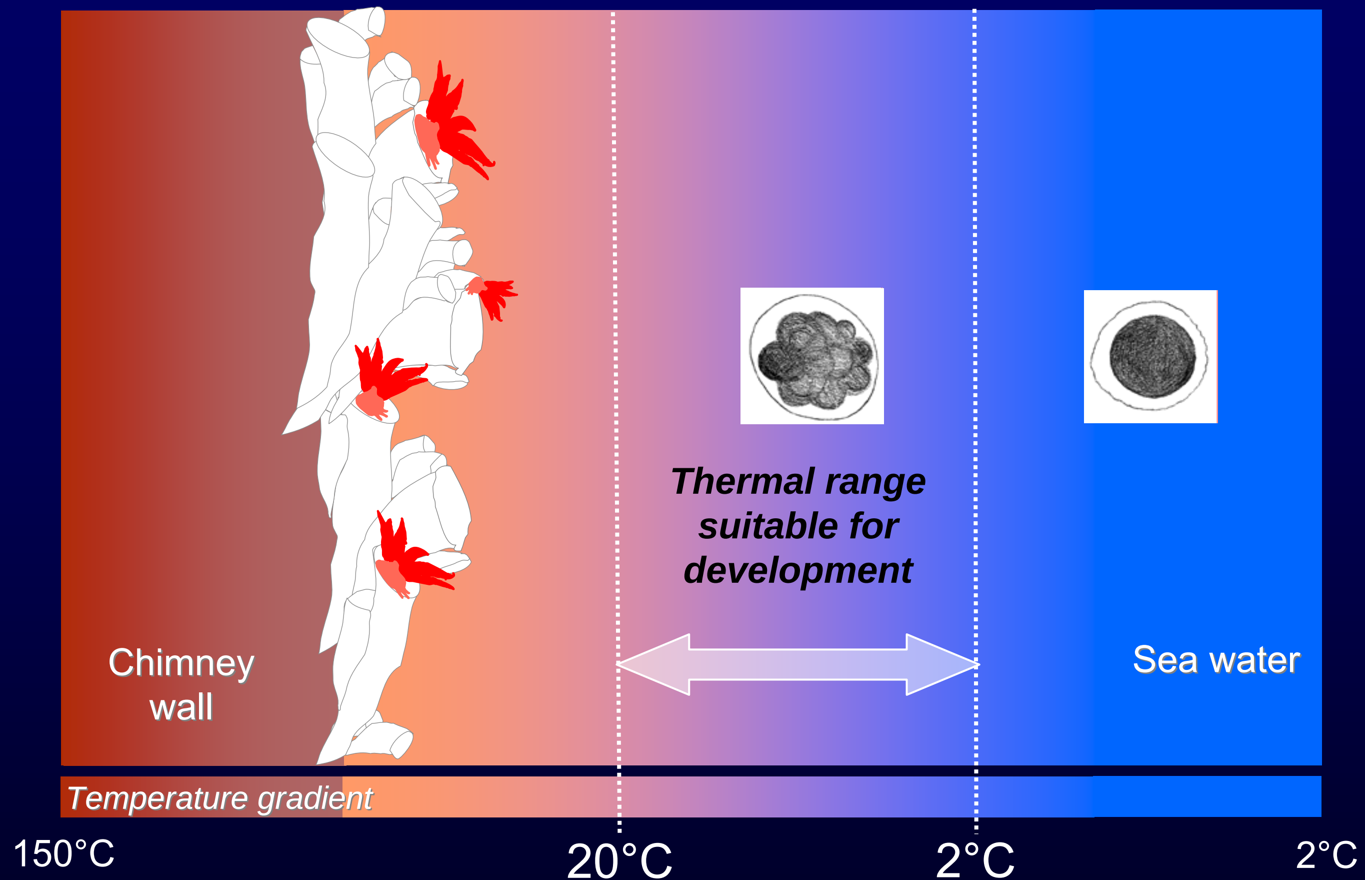
Effect of temperature on early development



N: non developping embryos
2c: 2-cell embryos
4c: 4-cell embryos
8c: 8-cell embryos

Pradillon *et al*. (2001) Nature

Effect of temperature on early development

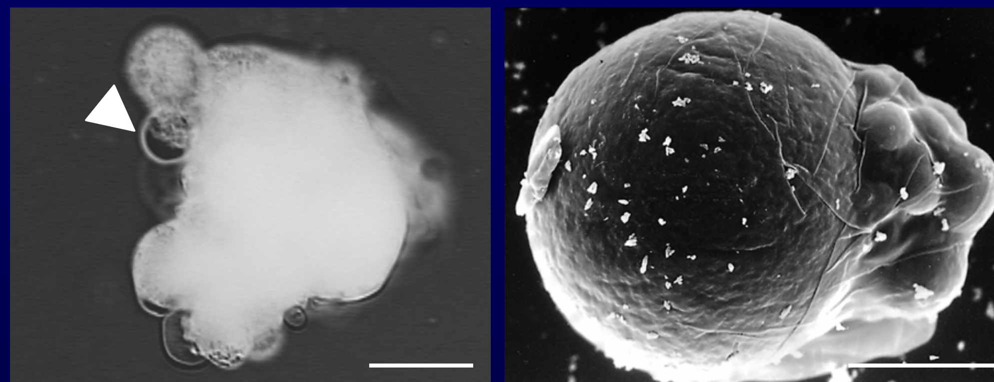


⇨ Temperature controls development

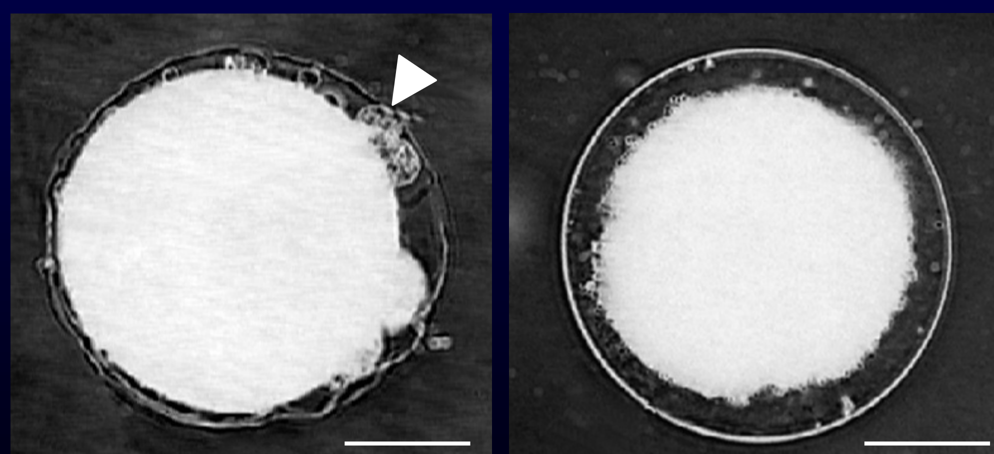
Pradillon *et al.* (2001) Nature
Pradillon *et al.* (2005) J Exp Biol

Hydrostatic pressure effects on *A. pompejana* embryos

1 atmosphere



- Abnormal cell size and arrangement

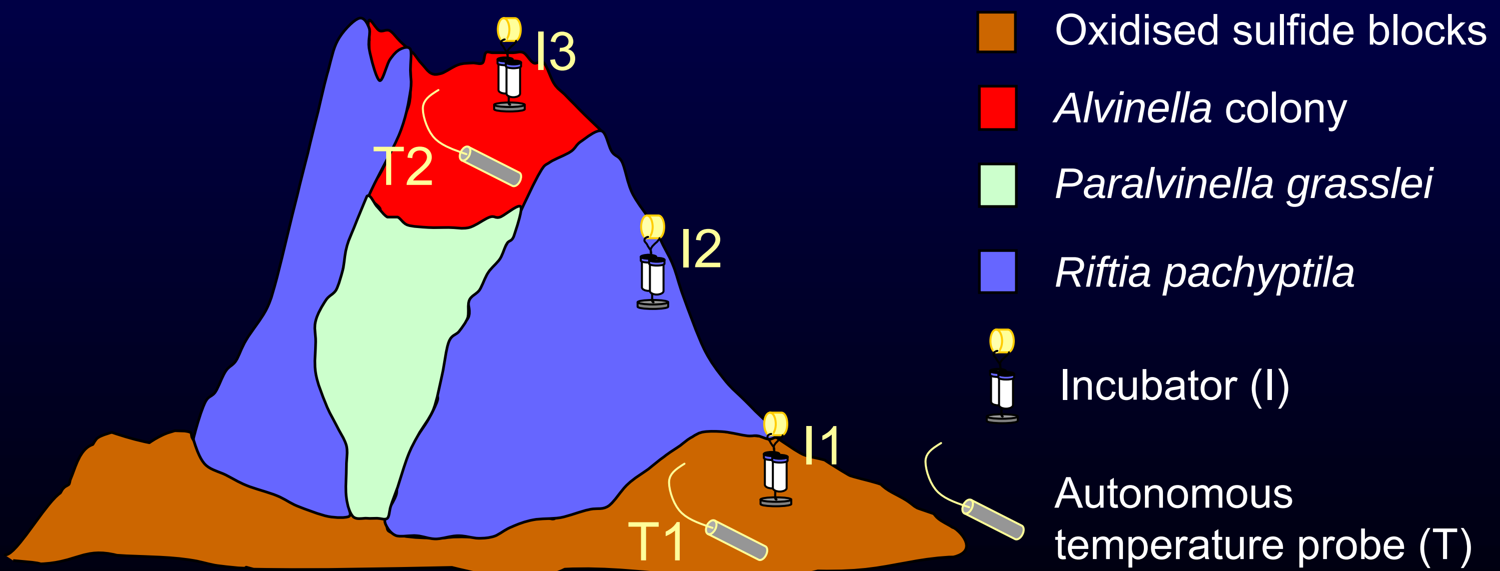


- Membrane integrity

Pradillon *et al.* (2005) J Exp Biol

In situ development *of A. pompejana*

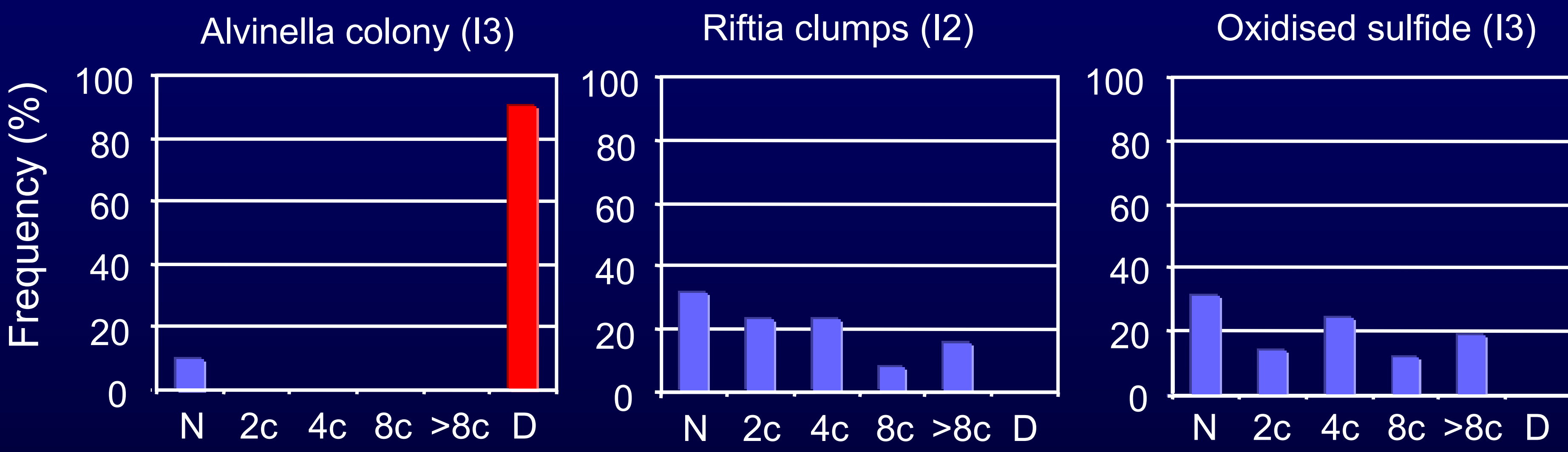
Elsa, HOT3, 13°N/EPR, PHARE 2002



Pradillon *et al.* (2005) J Exp Biol

In situ development *of A. pompejana*

Incubation time : 5 days



- Very low survival
- No development

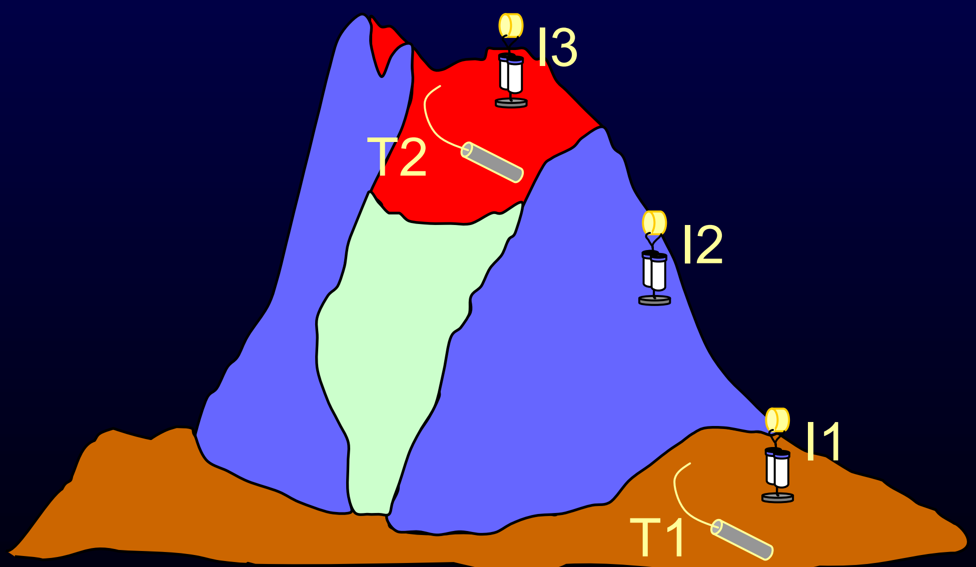
- 100% survival
- Development

N: non developping embryos
2c: 2-cell embryos
4c: 4-cell embryos
8c: 8-cell embryos
D: damaged embryos

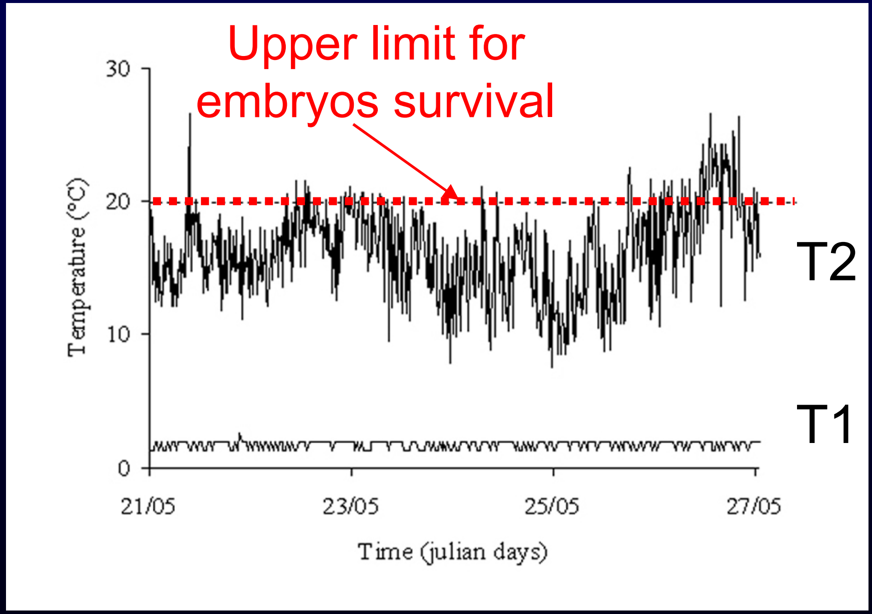
Pradillon *et al.* (2005) J Exp Biol

Incubator	Temperature (°C) Mean	Max	*N*	pH (estimated) Mean	Min	[Sulphide] (μmol l^{-1}; estimated) Mean	Max
I1	4±2	9	23	7.7	7.3	18	115
I2	6±2	11	32	7.5	7.3	59	145
I3	13±4	17	30	7.2	7.1	194	263
Background seawater	2			7.8		0	

→ Sulfide responible for embryos mortality...?



Pradillon *et al.* (2005) J Exp Biol



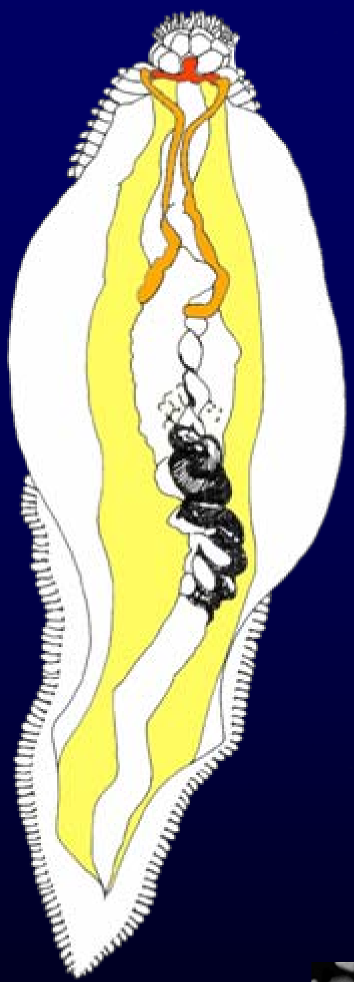
→ ...or / and short exposure to high temperature ?

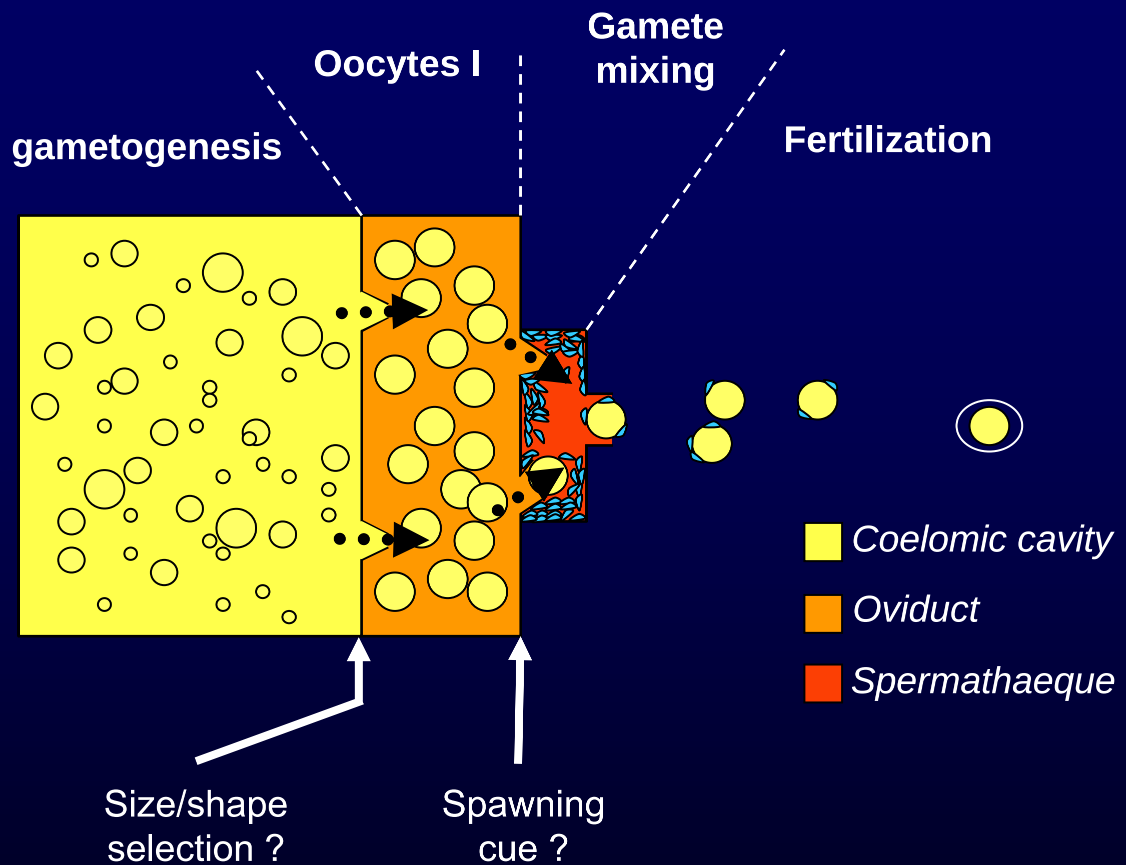
How do vent embryos develop ?

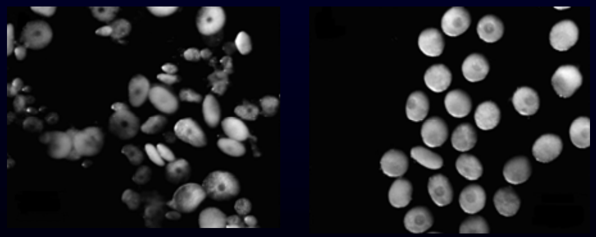
Which conditions are suitable for development ?

Is reproduction influenced by environmental conditions ?

Spawning process under environmental control?

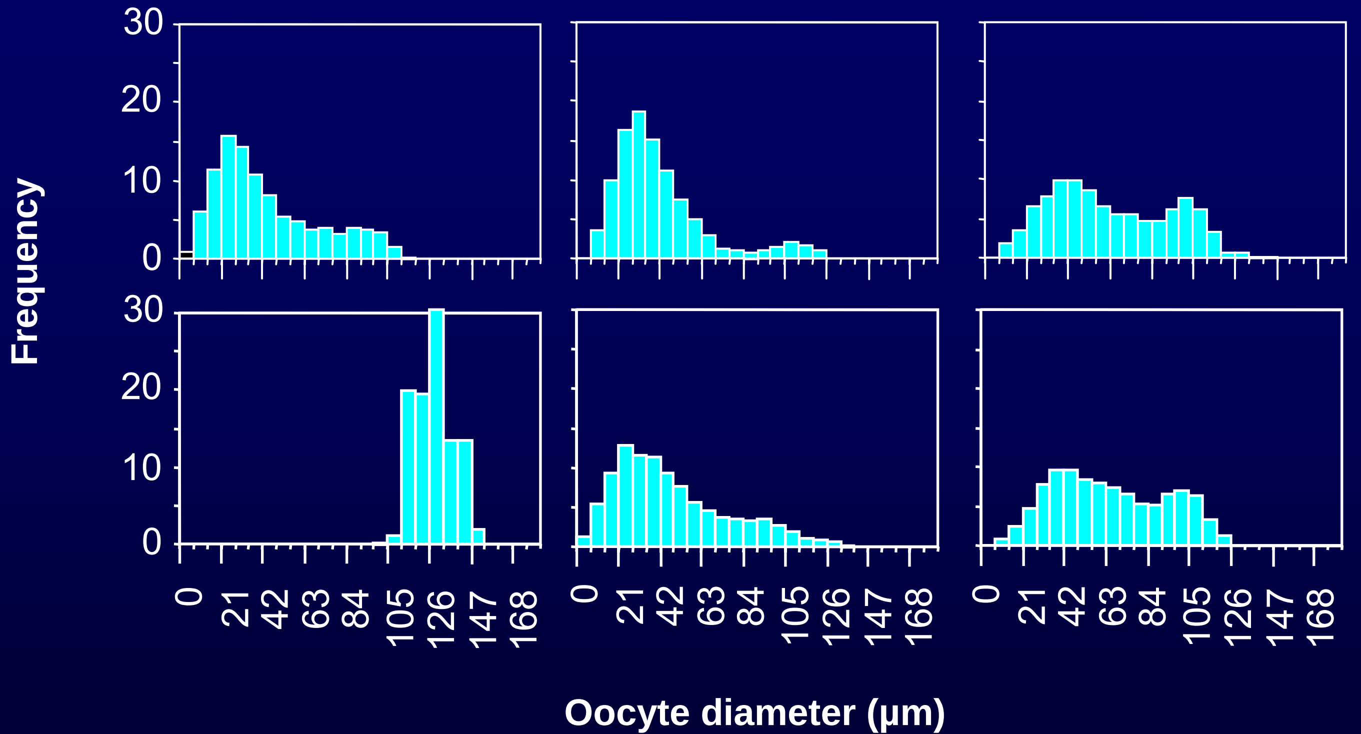






Pradillon et Gaill (2003) Invertebr Reprod Dev
Pradillon *et al.* (2005) Mar Ecol Prog Ser

Spawning process under environmental control?

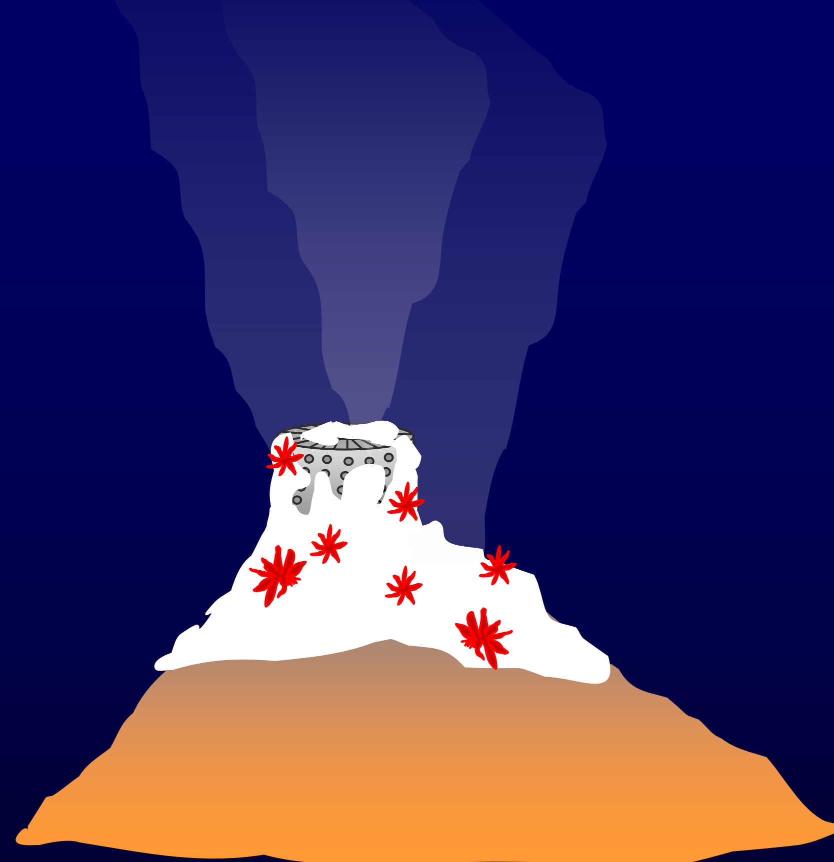


- Up to 1 000 000 oocytes / female
  - Continuous oocyte production
- Spawning by pulses in reproductive females

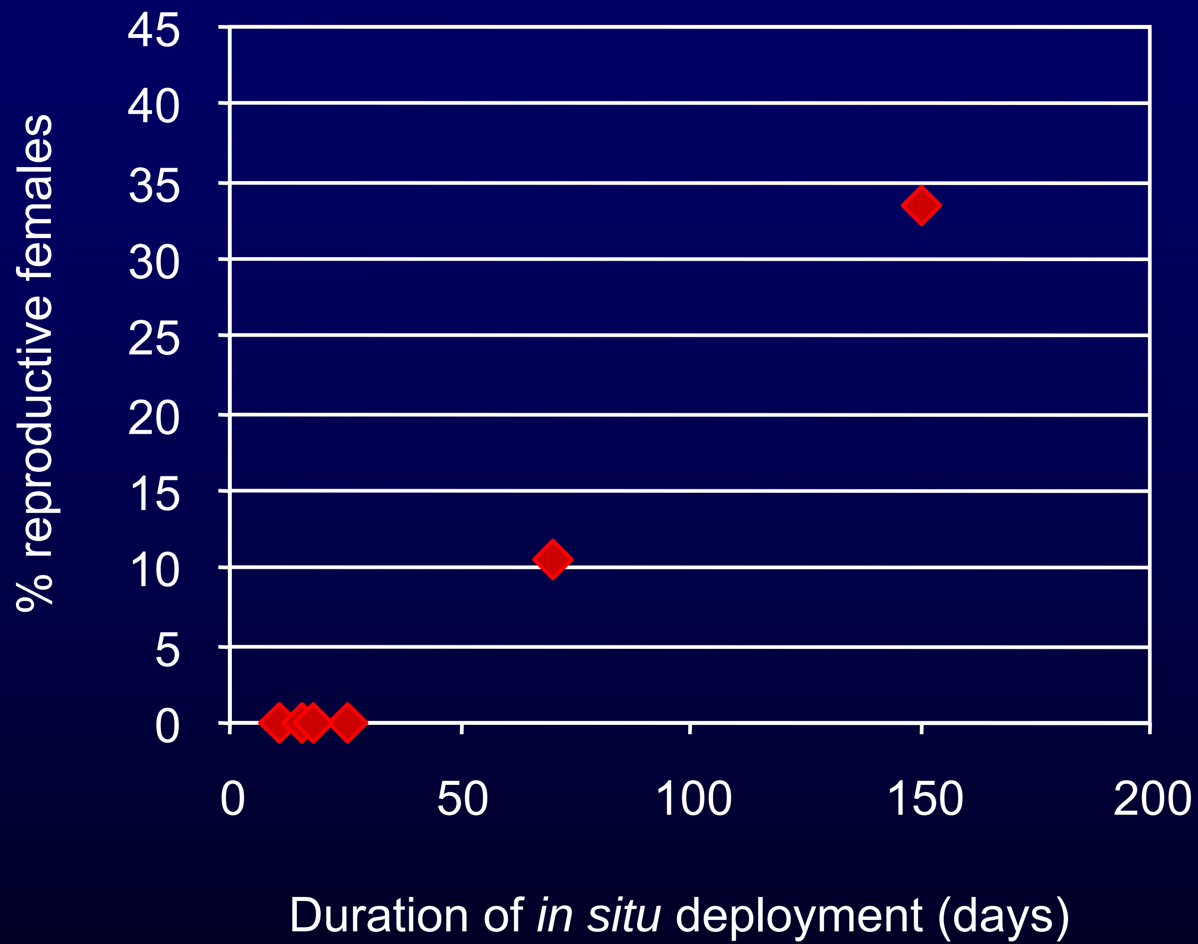
Pradillon *et al* (2005) Mar Ecol Prog Ser
Faure *et al* (in press) Mar Ecol Prog Ser

Experimental colonisation

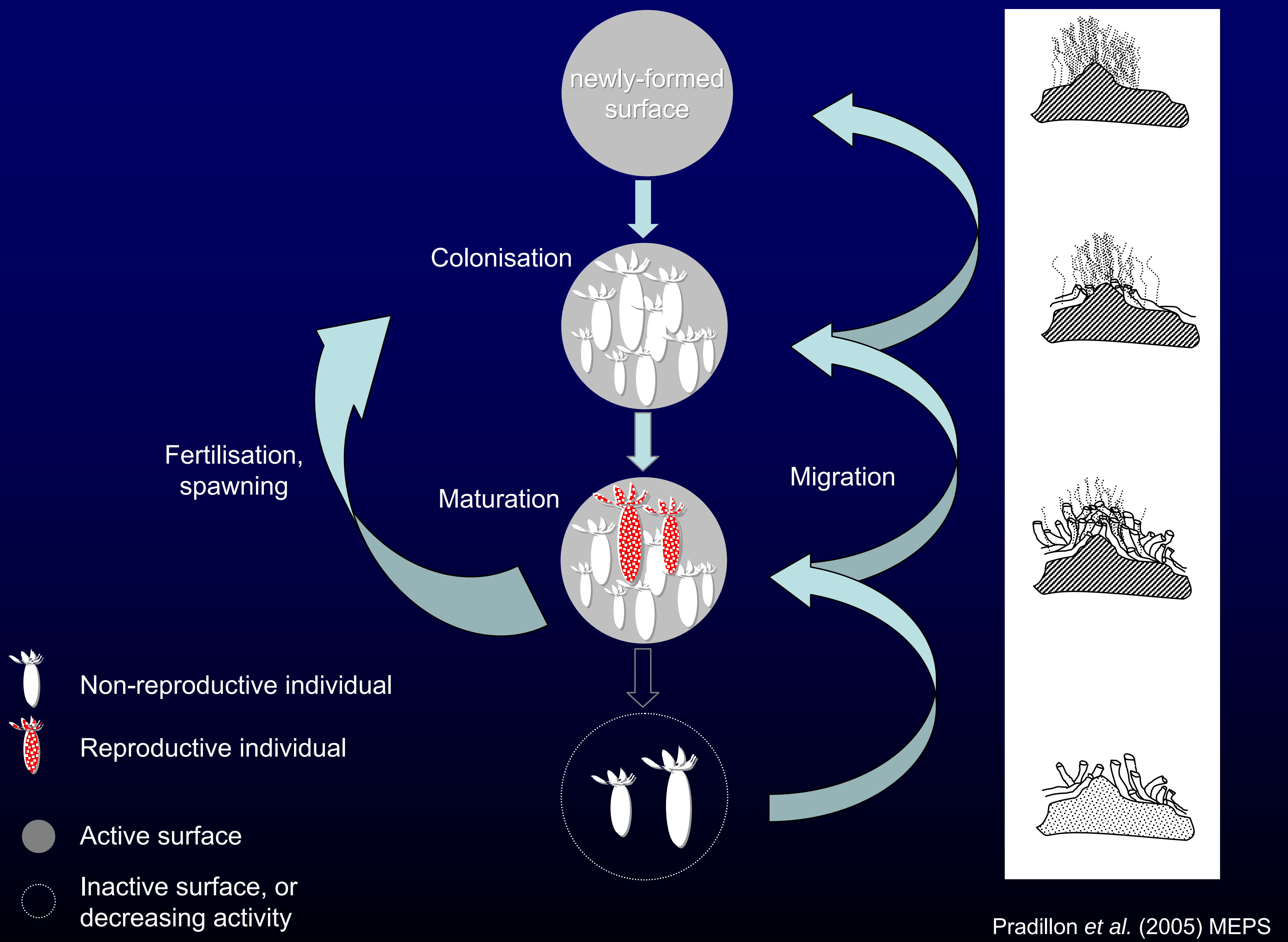
TRAC : Titanium Ring for Alvinellid Colonization

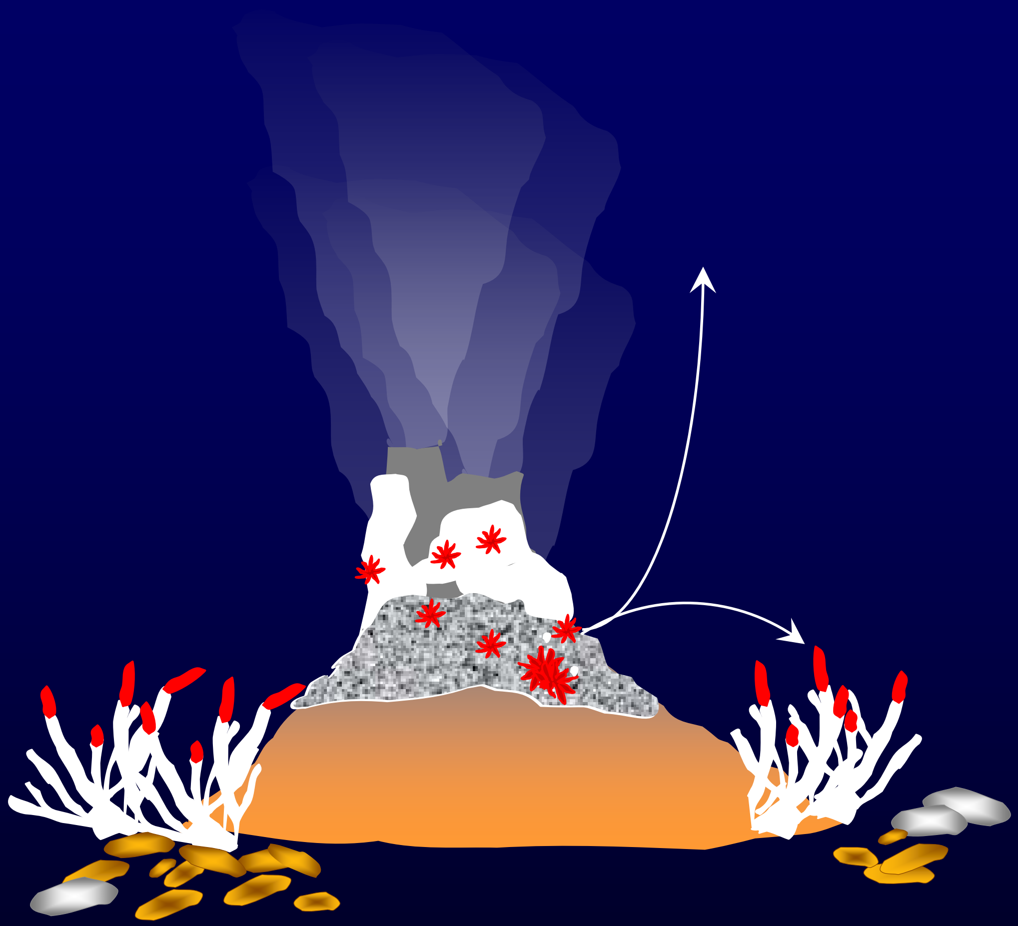


➢ Colonies of different ages (11 days to 6 months)



- Reproductive females are found only in patches older than 1 month


newly-formed surface
Colonisation
Maturation
Fertilisation, spawning
Migration
Non-reproductive individual
Reproductive individual
Active surface
Inactive surface, or decreasing activity
Pradillon *et al.* (2005) MEPS



- Distribution of larva *in situ* ?
- How sensitivity to hydrothermal environment evolve during development to allow colonisation of warm surfaces ?

Concluding thoughts

• Fine scale studies of *in situ* local conditions allow a better understanding of *A. pompejana* microenvironment.

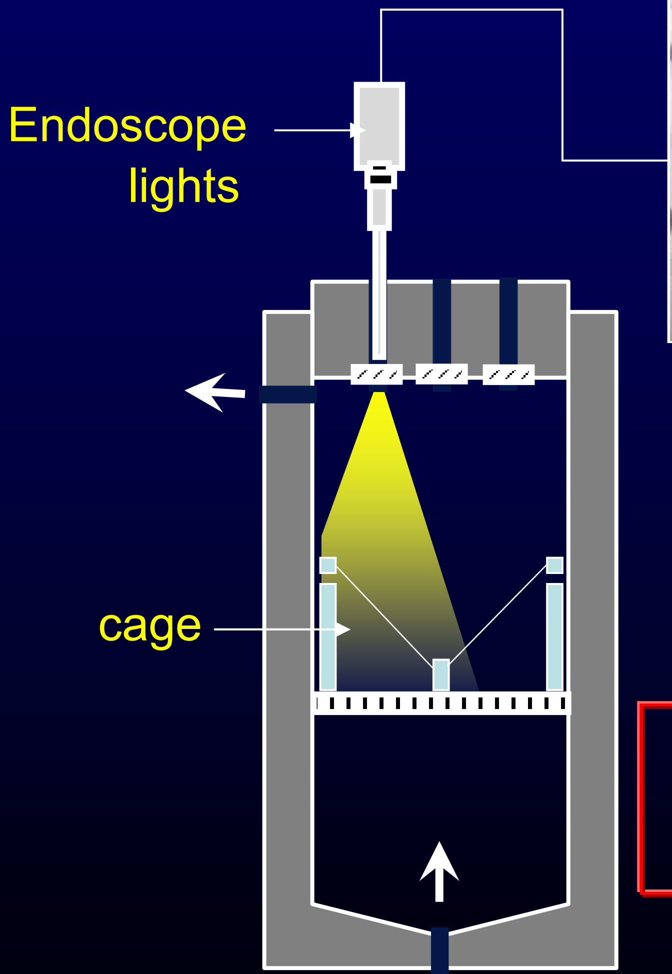
→ Need for tools allowing *in situ* monitoring of organisms.

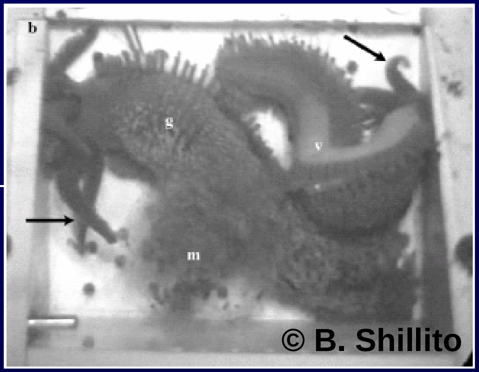
• Most biochemical data on thermotolerance indicate life temperature probably < 50°C.
However, most experiments were conducted *in vitro.*

→ Pressure aquarium allowing long-term maintenance of live specimen required for *in vivo* studies

Pressure systems for *in vivo* studies

IPOCAMP™ :
Incubateurs Pressurisés pour l'Observation et la Culture d'Animaux Marins Profonds



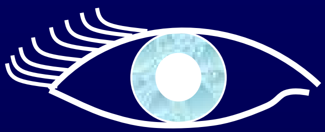


Monitor /
videorecorder

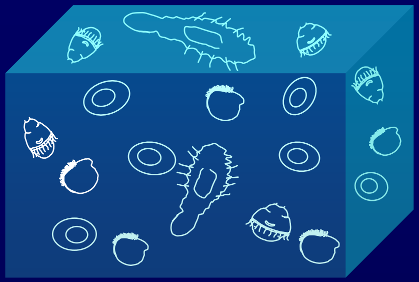
Live *A. Pompejana*
for 20h
Shillito et al (2003)

Molecular tools for the identification of larvae *in situ*

Phenotype



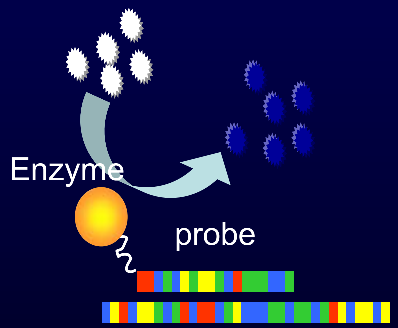
Taxa ?

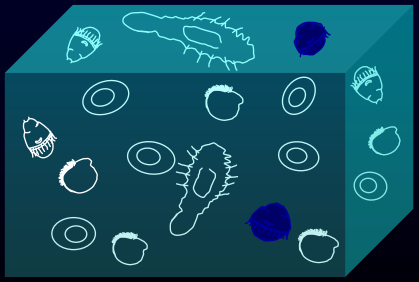


Whole larvae *in situ* Hybridization

Genotype

Morphology ?

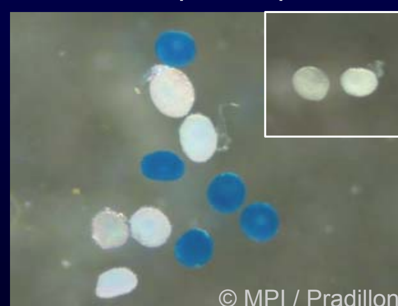




Molecular tools for the identification of larvae *in situ*

A. pompejana *A. caudata*

Specific probe *A. pompejana*



- identification of any developmental stage *in situ.*
- Correlation with *in situ* environmental conditions.

Pradillon *et al.* (2007) Mar Ecol Prog Ser

A. pompejana genome sequencing project

Alvinella consortium (F. Zal, O. Lecompte et al)

Development of microarray approaches to characterize the global stress response pathways to environmental injuries.

Other sources of stress such as pressure or high sulphide concentration at different stages of the life-cycle might also be addressed.

Acknowledgments

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Craig M. Young

Woods Hole Oceanographic Institution:

Lauren Mullineaux

Max-Planck Institute for Marine Microbiology Bremen:

Nicole Dubilier