From *Crassostrea gigas* oyster larvae adhesion studies to potential biotechnological development of marine adhesives

Valentin Foulon, Pierre Boudry , Fabienne Guérard , Claire Hellio

UMR 6539 LEMAR CNRS/UBO/IRD/Ifremer, IUEM Technopôle Brest-Iroise - rue Dumont d'Urville, F- 29280 Plouzané France

contact : valentin.foulon@univ-brest.fr

- Pacific oyster, ***Crassostrea gigas*****:** species of major ecological and economical interest
- **Genome** of *C. gigas* available
- Larval adhesion **poorly documented**

- Study of flat oyster ***Ostrea edulis*** adhesion 45 years ago by H.J. Cranfield
  - ⟶ A specific organ, **the foot** produces adhesive
  - ⟶ Adhesive presents a **proteo-glucidic** composition

The pediveliger larvae (last larval development stage before benthic life) of ***C. gigas*** *and* ***O. edulis*** are **morphologically similar,**
C. gigas and ***O. edulis*** are relatively **closed phylogenetically:** is it the **same adhesion process?**
We used modern molecular approaches to characterize biochemically and structurally the adhesive of *C. gigas* larvae, in view of potential future valorization

How oyster larvae settle ?

Behavior observations:

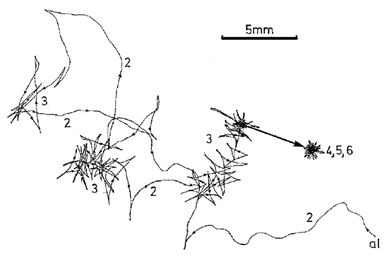

Fig 1. *O. edulis* crawling phase before adhesion. Cranfield, 1973.

Pediveliger larvae of *C. gigas* seem to adopt identical behavior to the observations of Cranfield (1973) on *O. edulis.* Larvae stop swimming, fall to the bottom and begin substrate exploration: the crawling. The foot has a locomotion and sensitive role during this crawling phase. Progressively, reducing explored area, the larvae remain in the same zone (Fig. 1, steps 4, 5, 6). At this time, the foot widens and spreads in front of the shell on the substrate for several minutes. Immediately after adhesive's released from the foot, larvae slide over the secreted adhesive and foot retracts. The attachment is definitive and metamorphosis starts.

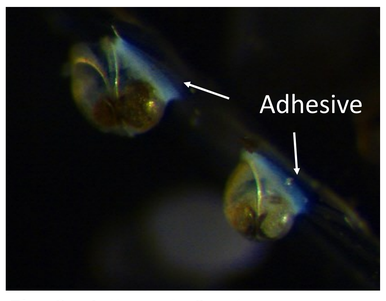

Fig 2. Adhered *C. gigas on glass slide - binocular*.

Structural characterization by electron microscopy

Morphological description of the foot

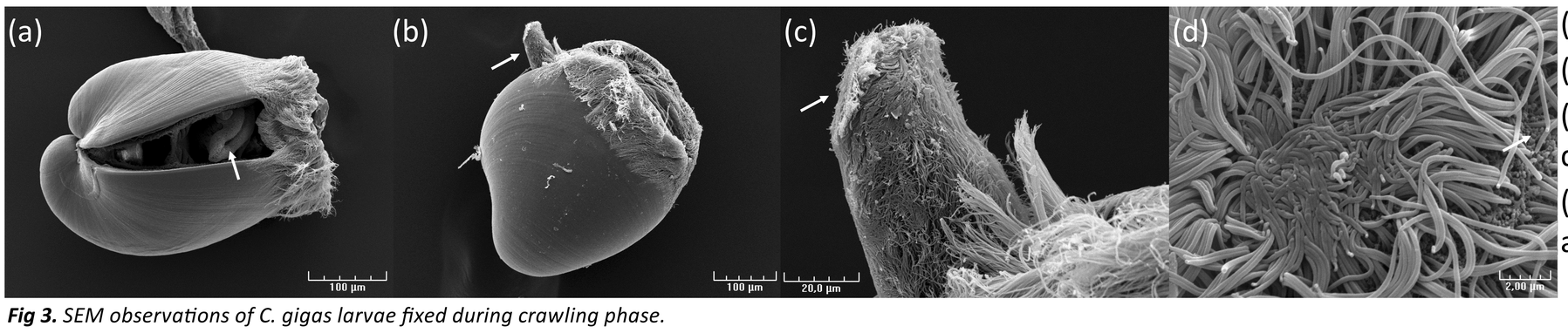

Fig 3. *SEM observations of C. gigas larvae fixed during crawling phase.*

(a) Byssal duct in *C. gigas* : similar to *O. edulis*

(b-d) Ciliate foot : motility / tactile function?

(c-d) Flattened zone extremity: pressure point during crawling

(d) Numerous vesicles at the base of cilia: adhesive excretion during crawling?

Adhesive ultrastructure observation

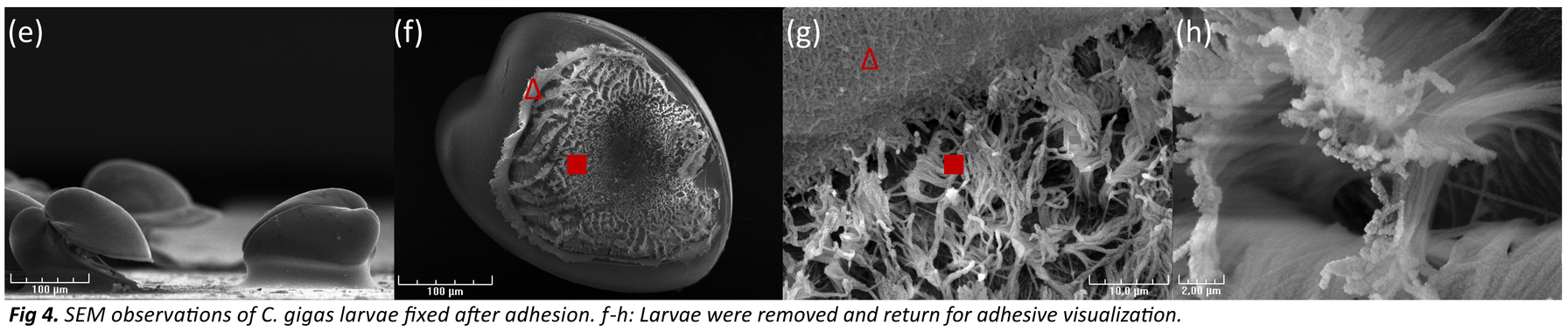

Fig 4. *SEM observations of C. gigas larvae fixed after adhesion. f-h: Larvae were removed and return for adhesive visualization.*

(e) Adhesive secretion englobe all the left shell

(f-g) Two main zones of different structure: Δ outer zone, ■ inner zone

(h) Inner zone is composed of fibers grid, less dense and tightened than the outer zone

C. gigas **foot structure is very similar to** ***O. edulis.*** **However, specific ultrastructure zones were observed in adhesive secretion.**

Composition of adhesive secretion

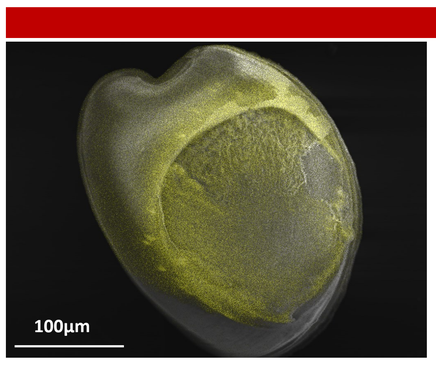

Fig 5. *Sulfur SEM –EDX map of C. gigas adhesive.*

Protein signal was detected by Fourier Transform Infra Red spectroscopy

Energy dispersive X-ray spectroscopy with SEM revealed presence of **Sulfur:**
Proteins or **sulphated polysaccharides ?**

Proteomic analysis from adhesive footprint

44 proteins

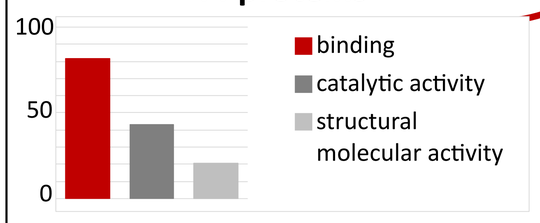

Fig 6. *Percentage of GO annotation "Molecular function".*

To Pursue

- Confirm the role of this proteins in adhesion by localization of gene expression and protein production
- Understand interactions between proteins and carbohydrate fraction of adhesive

Biotechnological development

- Proteins
- Sugars
- Lipids?

Production / Extraction / Purification → Formulation (Adjustments) → Strength - Weakness Opportunities

- Adhesion strength
- Aging resistance
- Biocompatibility

- Application fields
- Use constraints
- Eco production

→ Innovating adhesive

References

Cranfield H. J., 1973a, b, c - Marine Biology.

Cranfield H.J., 1974, 1975—J. mar. Biol. Ass. U.K.

Smith A. M., & Callow J. A. (Eds.). 2007. Biological adhesives. Springer Science & Business Media.

Von Byern, J. & Grunwald I. 2010. Biological adhesive systems. Springer Science & Business Media.