In situ studies of barnacle cyprid cement using XRF and micro-Raman spectroscopy

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Introduction

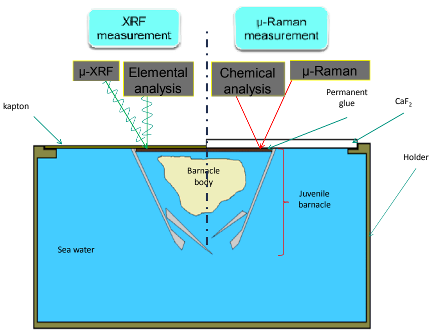
Marine biofouling is caused by the adhesion of marine organisms (barnacles, macroalgae, microbial slimes, etc.) on submerged man-made and/or natural surfaces. It is a worldwide problem in marine industry and causes increased maintenance costs. Barnacles are a good model for research on permanent underwater adhesion and the wider process of marine biofouling.[1]

Aim

Application of in-situ spectroscopy to understand the chemistry of marine adhesives of barnacles during the different settlement stages (cyprid to adult). Knowledge about the adhesive mechanism is supposed to inspire novel antifouling approaches in the future.

Methods

Barnacle cyprids (*Balanus amphitrite* and *Balanus improvisus*) cultured by Newcastle University were allowed to settle and investigated in-situ by spectromicroscopy. Kapton was used as substrate with good transmission for X-rays, whereas the calcium fluoride disks were used for Raman studies.



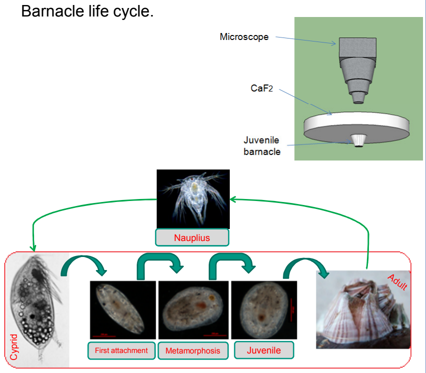
This diagram shows schematically the experimental setup for the two different in-situ spectroscopies: X-ray µ-fluorescence for elemental analysis and µ-Raman analysis for the determination of chemical composition of the adherent base plate. For both techniques a specific holder has been developed which allows to handle and investigate living barnacles in sea water.

Characterization

1) Micro-Raman spectroscopy for chemical composition.
2) X-ray fluorescence (XRF) for elemental distribution.

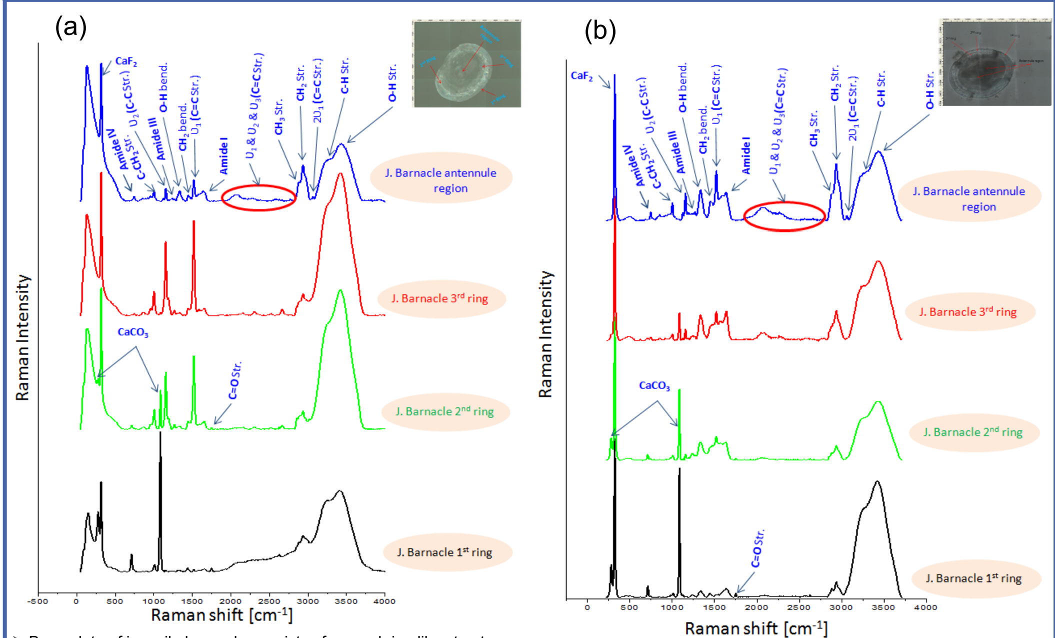
Results

Barnacle life cycle.



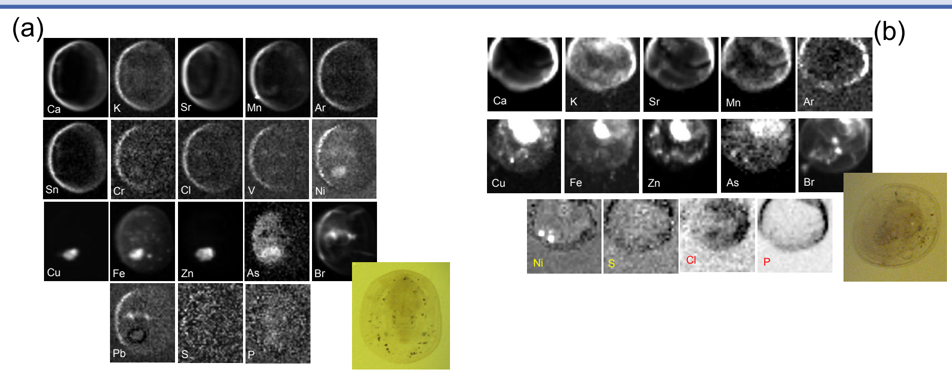
The barnacle life cycle is characterized by 3 major steps. The planktonic phase can be subdivided into six stages of nauplius, the plankto-benthic phase involves the cyprids and surface exploration, and the final benthic phase in which the barnacles are a sessile organism.[2]

Average spectrum for 4 individual Raman spectra recorded within an 1st, 2nd, 3rd rings and antennule region of the **4 days** settlement a) ***B. improvisus*** and b) ***B. amphitrite*** juvenile barnacles on CaF_2 disk.



- Base plate of juvenile barnacle consists of several ring like structure.
- The spectra show high calcium concentrations (277 and 1085 cm^{-1})[3] are only present at the rim and elevated at the 3rd ring of the barnacle in case of *Balanus amphitrite* compared to *Balanus improvisus* where it is only in the first and second ring.
- Amide rich components (e.g. proteins) are located on the inner ring close to where mineralization happens and is more in *Balanus amphitrite*.
- In the case *Balanus improvisus* there are strong peaks of C=C stretching in the range of 1520 cm^{-1}.[4]
- Although the two species are different but the spectra look similar so far as the chemical composition of cement glue is conserved.

X-ray fluorescence results for **4 days** juvenile barnacle a) ***B. improvisus*** and b) ***B. amphitrite*** settled on kapton sheet.



- Interestingly, calcium is mainly concentrated on the rims of the base plate and moves along with the growth of the young barnacle.
- In case of *Balanus improvisus* Cl , Sn,Cr and V are more concentrated at the rim but are absent in *Balanus amphitrite.*
- Element distribution correlates for iron, zinc, and copper near the center of the base plate.
- Bromium does not correlate well with the otherelements and frequently shows two bright spots on the surface.

Conclusion

- The base plate of juvenile barnacle of both spieces (B. amphitrite and B. improvisus) were measured by Micro-Raman spectroscopy without sample invasion or isolation.
- The presence of calcite at the rim of juvenile barnacle gives evidence that the cyprid started to build its shell by secretion cement glue after settlement.
- Presence of Amide components in the antennule region could be related to the cement glue and/or soft tissue of organism.
- The colocalization in the center points and especially the presence of iron points towards a contribution to the circulating vessel system.
- Hemolymphs of invertebrates are being considered to contain Cu but to our knowledge it was not shown that iron, bromine and zinc co-localize. Probably the data could be interesting in the context of curing of adhesives.

Future experiments

- Extending the work with confocal Raman spectroscopy to have a map of the distribution of their different chemical composition for settled barnacles at different life stages (cyprids, metamorphosis, Juvenile and adult)
- Extending work on adhesives to algae (Ulva linza, brown seaweed, Navicula and perminuta)

References

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4) M. Schmidt et al., 2009, J. of Adhesion, 85, 139–51.