**Offre de thèse - 36 mois**

**Study the effect of environmental factors on asexual development in salps**

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**Etudier le lien entre l'environnement et la reproduction asexuée chez les salpes.**

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Salps (Chordata, Tunicata) are a group of pelagic gelatinous zooplankton diffused worldwide[1]. They form vast seasonal swarms, also called **blooms** that can cover areas up to several thousand $Km^2$ often dominating the zooplankton communities (Fig. A). Their high and non-selective filter-feeding activity impacts both primary and secondary producers, for instance by grazing on phytoplankton blooms or competing with krill larvae[2,3]. The massive production of rapidly sinking fecal pellets, which are rich in carbon, nitrogen, calcium and aluminum, also strongly influences the ecology of the deep-sea benthos[4]. Therefore, salps play a central role in both oceanic **trophic webs** and **biogeochemical cycles**. While common in healthy pelagic ecosystems, dense blooms of salps can also directly impact human activities, for instance by clogging fishing nets or damaging pumping water systems.

A B C

(A) A chain of blastozooids during a salp bloom ; (B) *Thalia democratica* bearing a circular, budding stolon ; (C) Cell proliferation (white cells), in a young budding stolon (confocal microscopy)

The triggering of salps explosive blooms is tightly linked to their rapid life-cycle, which alternates sexually and asexually generations[1]. During asexual reproduction the solitary **oozooid** produces chains of hundreds **blastozooids** along a particular structure called a stolon (Fig. B,C), by a process called **stolonial budding**[5]. During a bloom, the rate of stolonial budding increases enormously and leads to a density up to thousands of individuals/$dam^3$, making **asexual propagation the major driver of the blooms**. The magnitude of the blooms is influenced by two main environmental factors, *i.e.* sea surface temperature and food availability, which notably impact the speed of blastozooid production[6-8]. Thus environmental factors impact the rate of bud production and release, *i.e.* the very developmental core of the salp asexual reproduction. Up to now, the cellular and genetic developmental mechanisms of asexual reproduction are very poorly known, and the few studies that quantified the link between environmental parameters and budding rate led to contradictory results[7,9].

Salps are very abundant during spring and autumn blooms in the Bay of Villefranche-sur-mer and can be easily collected in front of the hosting laboratory, which makes this place a pristine location to study salp biology and ecology. We are currently working to describe the stolonial budding in *Thalia democratica* and *Salpa fusiformis,* the two most abundant species in the bay. We found that bud production involves a very high rate of cell proliferation and discrete stem cells in the budding stolon (Fig. C). We recently obtained transcriptomic datasets that now allow us to study the expression of genes involved in budding regulation. We also established protocols to routinely collect salps on the field and maintain them in the laboratory, which allow us to study the budding mechanisms during and between the blooms. In the present PhD project, which combines environmental ecology, developmental and cellular biology, the candidate will conduct field observations and laboratory experiments with various methods such as microscopy, quantitative imaging and genomics, in order to characterize the cellular and molecular mechanisms of asexual reproduction in *Thalia democratica* and *Salpa fusiformis,* and study the impact of temperature and food availability on these mechanisms. This project can be divided in two complementary axes:

Axe 1. The aim of this axe is to establish the link between environmental conditions (*i.e.* water T° and phytoplankton abundance), budding rate and salp abundance. To this end, the student will first use the zooplankton collection of the IMEV, in which plankton samples collected every week are preserved (https://lov.imev-mer.fr/web/facilities/ccpv/). Thanks to this data bank, s/he will measure the number of buds in the stolon of salps collected in the last twenty years (including intra- and inter-bloom periods) and correlate these variables with the salp abundance and the environmental parameters that are also daily recorded in the bay of Villefranche sur Mer (http://obs-vlfr.fr/data/view/). In parallel, s/he will conduct on-board experiments every week to measure the rate of cell proliferation in freshly collected oozoids using EdU staining, a thymidine analog routinely used to visualize and quantify cell proliferation (Fig. C). This will allow to establish whether cell proliferation in the budding stolon is correlated to the environmental conditions and to the salp abundance. Finally, s/he will raise salps in laboratory-controlled conditions using our established flow-through system, to quantify more finely how T° and phytoplankton availability tune the cell proliferation and the budding rates.

Axe 2. Once identified environmental conditions having a clear effect on the stolonial budding rate, the student will conduct a transcriptomic comparison on salps harvested in these conditions. To identify genes whose expression level affects the budding rate, s/he will dissect the budding stolons in order to extract and quantify the transcripts expressed in this particular tissue. Sequencing reads will be mapped on the reference genomes obtained by our team, and functional annotation of differentially expressed genes (DEG) will establish the functional relationships between the tested conditions and the transcriptomic response. The student will then use *in situ* hybridization technique to better understand what are the tissues and the processes affected by T° and food availability. For instance, we expect to find variations in the expression of cell cycle regulators (*e.g.* cyclins, pcna), stress response genes, as well as developmental genes involved in blastozooid morphogenesis (*e.g.* transcription factors, morphogens) and genes involved in the regulation of bud release. These results and the results from the first axe will bring together knowledge at the population, individual, cell and molecular levels to improve our general understanding of the mechanisms underlying salps seasonal blooms.

The research activity will be conducted at the Institut de la mer de Villefranche (IMEV). This ecology and developmental biology project is part of an ongoing ANR funded project (DEVOBLOOM). The student will be co-supervised by S. Tiozzo and A. Alié, specialists in developmental biology and genomics of tunicates, and by F. Lombard who is a recognized expert in plankton zoology and ecology. The student will beneficiate from the expertise of a post-doctoral researcher of the Tiozzo team, specialist in bio-informatics. S/he will also be supported by the technician and engineer staff of the animal facility, the imaging and the bioinformatics platforms to generate and analyze the data. Salp collection and monitoring of environmental parameter is ensured by the Observational Service.

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