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Two centuries of arts and science in Nice and Villefranche sur Mer: 2) Modern era: 1960 to 2024

Deux Siècles d'Arts et de Sciences à Nice et Villefranche-sur-Mer : 2) Les Modernes : 1960 à 2024

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ABSTRACT. In a first, companion article (Sardet 2025 / 1 Pioneers: 1800 to 1900) we tell the story of the exploration of biodiversity in the Nice region, and in particular of pelagic organisms. In this second article we examine how, more than a century later, research in cell and developmental biology and in physiology evolved at the marine station of Villefranche sur Mer. While research in the biology and ecology of plankton remained predominant at the site, and gradually led to the growth of a large multidisciplinary laboratory (Laboratoire d'Océanographie de Villefranche: LOV), physiology and cell biology were introduced in the 1960s as research teams on the physiology of fish and biology of protists joined the marine station. In the 1980s a new research group was created by the CNRS which has grown to the present Laboratoire de Biologie du Developpement (LBDV). We describe how imaging and molecular biology techniques were used to understand fertilization and development in sea urchins, tunicates, cnidarians, and many other marine organisms previously studied by the founders and visitors of the marine station in the 19th century. We also detail the development of new model organisms – the ascidian *Phallusia*, the appendicularian *Oikopleura* and the hydrozoan medusa *Clytia.* Finally we discuss the promotion of scientific discoveries via aesthetic photographs, drawings, exhibits and web sites.

RÉSUMÉ. Dans un article complémentaire (Sardet 2024/ 1 Les anciens : de 1800 à 1900), nous avons relaté l'histoire de l'exploration de la faune de la région niçoise, et en particulier des organismes pélagiques. Dans cet article, nous examinons comment, plus d'un siècle plus tard, la recherche scientifique en biologie et physiologie cellulaire et moléculaire du développement a évoluée à la station marine de Villefranche sur Mer. Alors que la biologie et l'écologie du plancton sont prédominants sur le site et ont progressivement conduit à la croissance d'un grand laboratoire d'Océanographie de Villefranche (LOV), à partir des années 1960 de nouvelles équipes de recherche sur la physiologie des poissons et des protistes ont été accueillies. Et dans les années 1980, une équipe de recherche créée par le CNRS a évoluée graduellement en l'actuel Laboratoire de Biologie du Développement (LBDV). Nous décrivons comment les techniques d'imagerie et de biologie cellulaire moléculaire ont permis d'analyser l'ovogénèse, la fécondation et le développement chez les oursins, tuniciers, cténophores, cnidaires et d'autres organismes marins dont certains étaient déjà étudiés par les fondateurs et les visiteurs de la station marine au 19ème siècle. Nous soulignons que de nouveaux modèles – l'ascidie P*hallusia*, l'appendiculaire *Oikopleura* et la méduse hydrozoaire *Clytia* – se sont développés sur le site. Nous détaillons aussi les efforts des chercheurs pour promouvoir leurs découvertes par le biais de photographies, de dessins, d'expositions et sites internet esthétiques.

KEYWORDS. Villefranche sur Mer, plankton, protists, sea urchins, ascidians, appendicularians, ctenophores, cnidarians, chaetognats, siphonophores, tintinnids, *Paracentrotus Phallusia, Oikopleura, Clytia.*

MOTS-CLÉS. Villefranche sur Mer, plancton, protistes, oursins, ascidies, appendiculaires, ctenophores, cnidaires, chaetognates, siphonophores, tintinnides, *Paracentrotus Phallusia, Oikopleura, Clytia.*

1. Introduction

From the 1960s-1970s, the Villefranche sur Mer marine station evolved into a multidisciplinary research and teaching center(Anon. 2010, 2024). The zoological research initiated 150 years ago by Fol, Barrois, Korotneff and their visitors (see companion article: Sardet 2025 / 1 Pioneers: 1800 to 1900) expanded under the aegis of the Université Pierre & Marie Curie (UPMC, now Sorbonne Université) and the Centre National de la Recherche Scientifique (CNRS). Other research teams joined the site in the 1960s, supported by the Commissariat à l'Énergie Atomique (CEA team led by Jean

Maetz on fish physiology) and the Université de Nice Sophia Antipolis (UNSA, protistology team led by Jean Cachon). At the same time, other research and teaching activities - involving departments of Paris University

(UPMC) - developed on the Villefranche site from the 1960s onwards. These focused on geological and oceanography studies and the physical chemistry of the oceans. Research, teaching and the hosting of visitors were carried out in the former galley slave building and now extend to other buildings along the Darse harbor of Villefranche – a former rope factory as well as 2 buildings dating from recent decades (see Anon. 2010 document on the 125 year anniversary celebrating the creation of the marine station).

In the 1960s, under the direction of Paul Bougis, the Station Zoologique de Villefranche sur Mer became officially independent of the marine station of Banyuls sur Mer (laboratoire Arago), consisting of 3 laboratories associated with the university of Paris (UPMC: Zoology & Ecology of Plankton/ Marine Physical-chemistry/ Geodynamics) and the CEA and UNSA teams mentioned above. The creation and installation of a new CNRS team (Marine Cell Biology headed by Roger Lallier & Christian Sardet) in the early 1980s added new research topics in cellular and molecular biology of development.

Over the last few decades, under the aegis of UPMC and CNRS, the Villefranche, Banyuls and Roscoff marine stations gradually acquired the status of “Observatoire Océanologique”. The Station Zoologique de Villefranche successively became the CEROV (Centre d'Études et de Recherche Océanographiques) in 1983, then the OOV (Observatoire Océanologique de Villefranche) in 1989, and in 2019, the IMEV (Institut de la Mer de Villefranche). During these administrative transformations driven by UPMC and CNRS, the physiological (CEA) and geological (UPMC) research teams left the Villefranche site to join larger laboratories (Unités Mixtes de Recherches: UMR) in Nice and Sophia Antipolis under the aegis of UNSA (Université de Nice Sophia Antipolis).

Our aim in this article is to highlight some biological research that is in remarkable continuity with the pioneering studies carried out more than a century earlier by naturalists from Nice and Villefranche and some of the visitors they welcomed (see companion article: Sardet 2025 / 1 Pioneers: 1800 to 1900). This continuity is due in part to the availability of benthic organisms (echinoderms, ascidians, etc.) and of planktonic organisms (protists, cnidarians, ctenophores, tunicates, etc.) which are easily and rapidly collected near the marine station with boats that go out to sea every day. The organisms are kept and/or raised in aquariums by experienced staffs that have perfected culture techniques.

We also emphasize the arts & sciences dimension of research linked to the intensive and creative use of various imaging techniques in biology. Many researchers are keen to draw attention to their discoveries through the aesthetics of cover photos, drawings, exhibitions, conference presentations, websites or posts on social networks.

Jean Maetz and his passion for fish and fish physiology

We begin this overview with a tribute to the naturalist school of Nice in the 19th century, and in particular Antoine Risso, Jean Baptiste Barla and Vincent Fossat, who were passionate students of local fish and invertebrates (Sardet 2025 / 1 Pioneers: 1800 to 1900). A century later, Jean Maetz inherited that passion for fish. For the staff and visitors to the Villefranche sur Mer marine station, Jean Maetz is the name of a research and teaching building constructed in 1983 by the Commissariat à l'Énergie Atomique (CEA) and Sorbonne Université (UPMC at the time). The building was named in memory of physiologist Jean Maetz, who died in 1977 at the age of 54 in a car accident in Scotland. In 1964, Maetz had moved his laboratory to Villefranche, a satellite of the CEA's Laboratoire de Biologie in Saclay, to study with René Motais euryhaline fish such as eels and trout, which are able to adapt from fresh water to sea water by excreting salt. As a young researcher, I was welcomed to Villefranche

in 1976 by Jean Maetz and his CEA team, to take part in research on the cells - chloride cells - responsible for ion exchange in the gills. These cells excrete salt by amplifying the pump proteins of their dense network of internal membranes, while modifying their junctions with neighboring cells (Fig.1). The work on these cells, renamed the MRC (Mitochondria Rich Cells) was published in cell biology and physiology journals. It still underpins current theories of osmoregulation in euryhaline fish (Sardet et al. 1979, Evans et al. 2005).

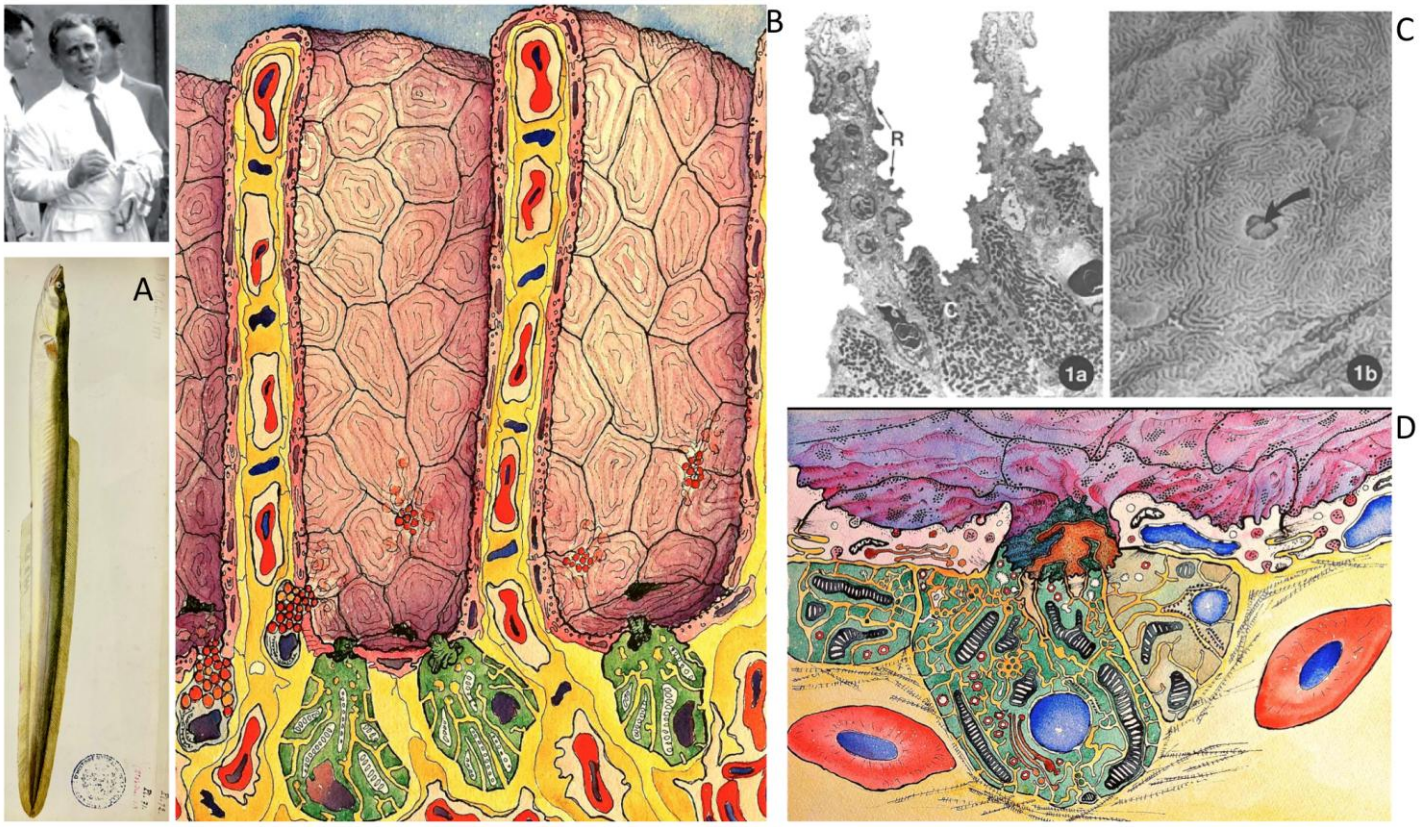

Fig. 1. *Jean Maetz and the physiology of osmoregulation in euryhaline fishes*
Top left, Jean Maetz in his white lab coat
A – Eel: watercolor painting by Vincent Fossat (Nice Natural History Museum collection)
B – Watercolor painting of gill filaments with vessels filled with red cells (red) and chloride cells (green) at the base (C Sardet)
C – (1a) Thin section electron microscopy of gill filaments (R) showing chloride cells (C) (1b) Scanning electron microscopy of gill surface with crypt of chloride cells (arrow) from (Sardet et al. 1979
D – Watercolor painting of chloride cells (green) at the base of gill filaments. A complex of several adjacent chloride cells forms in response to sea water adaptation (C Sardet)

The sudden death of Jean Maetz led to the relocation of the physiology laboratory from CEA at the marine station in Villefranche to the University of Nice Sophia Antipolis under the direction of René Motais. It changed my own destiny, giving me the opportunity to research fertilization at the marine station by creating, with colleagues from Villefranche, Paris and Nice, a new research team among those encouraged by the CNRS life sciences department in the early 1980s, when François Mitterrand had become the new French president.

Hermann Fol's legacy - from fertilization to protists

Hermann Fol, the founder with Barrois of a first laboratory in Villefranche sur Mer in 1881, was an extraordinary personality who disappeared at sea in 1892. His genius and uncompromising, difficult

character shine through in his publications and abundant correspondence with his 2 main mentors, Henri de Lacaze-Duthiers and Carl Vogt (Jessus & Laudet 2022, Dolan 2024, Sardet 2025).

Fol is without doubt the biologist who left in Villefranche the most interesting legacy from a scientific point of view, if we consider the scope of his discoveries and interests. Over a period of twenty years (1869-1889), Fol published pioneering research on protists (rhizaria, tintinnaria), appendicularians, echinoderms, chaetognaths, planktonic molluscs and human embryos (Bedot 1894, Dolan 2024). When he transferred his personal laboratory by boat from Messina to Villefranche in 1878, Fol had just published his discovery of fertilization in echinoderms and chaetognaths (Fol 1878), and his research on the development of planktonic molluscs (Fol 1875). Previously, he had described and remarkably illustrated his work on the anatomy and development of ctenophores (his thesis) and appendicularians (Fol 1972, Dolan 2024). In fact, all the organisms mentioned have subsequently been the subject of in-depth research at the Villefranche marine station. I myself worked on the fertilization and development of ctenophores, echinoderms and chaetognaths, and realized that these subjects had been pioneered a century earlier by Hermann Fol! What was striking about Fol was his ability to discover, describe and illustrate new phenomena accurately and aesthetically. Almost always publishing alone in various magazines, he was also very particular about the engravers he chose himself (Dolan 2024).

Sticholonche zanclea - a rowing protist

When examining the contents of a plankton net towed at depth in the Bay of Villefranche, the eye is inevitably drawn to *Sticholonche zanclea*, an atypical rhizarian measuring 0.2 mm that moves with the aid of oar-like extensions called axopods. Fol, and then Alexis Korotneff, published their research on this protist in the 1880-90s (Fol 1883, Korotneff 1891). A century later, Jean and Monique Cachon turned their attention to *Sticholonche*, spurred on by Lewis Tilney, professor at the University of Pennsylvania, a visitor to Villefranche on sabbatical. Tilney was famous for his contributions to the understanding of the cytoskeleton, the support network and dynamic musculature of cell motility. Jean and Monique Cachon had introduced microcinematography and electron microscopy techniques to Villefranche in the 1960s. They were renowned for their work on the structure of heliozoans, acantharia and other protists, carried out with Jean and Colette Febvre, members of the Nice university protistology laboratory hosted in Villefranche. In the case of *Sticholonche*, the Cachon couple and Tilney wondered what mechanisms enabled the rows of oars - axopods made up of microtubule bundles - to move. They showed that these oars/axopods were anchored at the base by ball-and-socket joints that pivoted in depressions in the nuclear membrane, reminiscent of a hip joint (Fig.2). They observed contractile filaments that appeared to be involved in calcium-controlled movement. This work was published in the Journal of Cell Biology, the best journal in the field at the time (Cachon et al. 1977). As a young researcher newly arrived in Villefranche, I have fond memories of discovering protists thanks to Jean and Monique Cachon and Jean and Colette Febvre (Febvre-Chevallier & Febvre 1994). Jean Cachon sorted and prepared the specimens, Jean and Monique examined them together under the microscope, and Monique meticulously drew the pictures. And I learned a lot from Lewis Tilney, a master at asking the right question to the appropriate organism.

Taking advantage of the European network of marine stations hosting facilities (see EMBRC: Anon. 2024c), a new generation of cell and molecular biology researchers work at the marine station of Villefranche to collect and study radiolarians and rhizaria, and particularly their genes and symbiotic relationships with microalgae (Decelle et al. 2012).

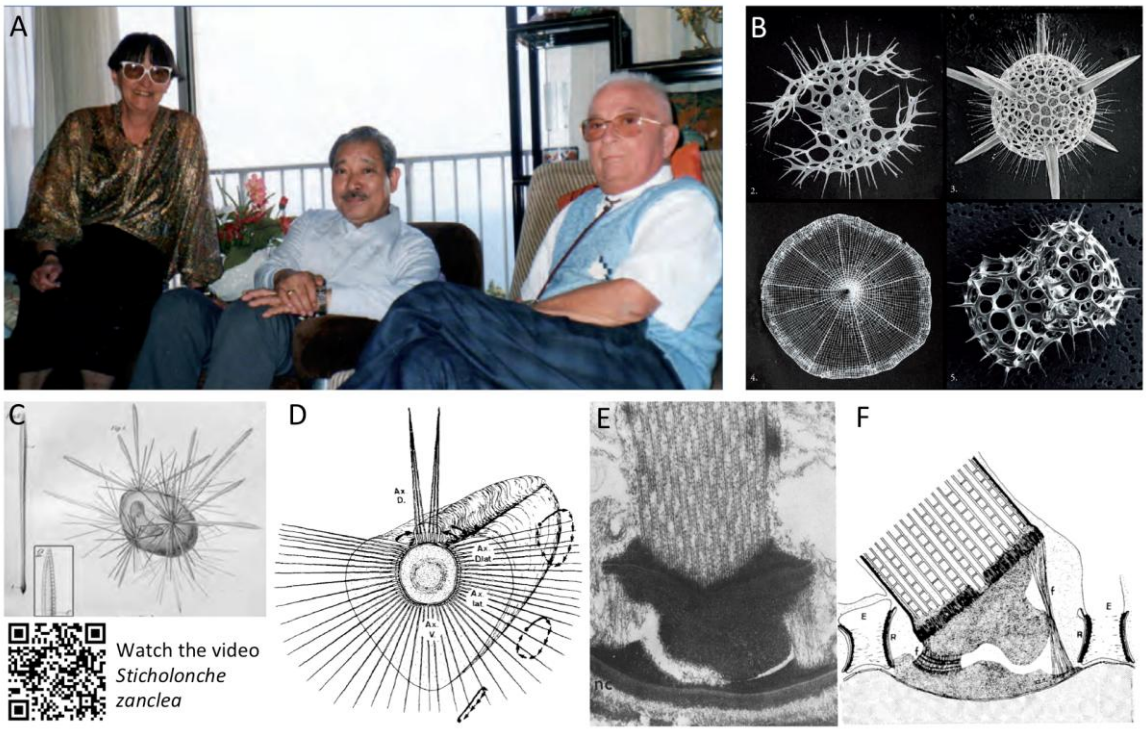

Fig. 2. *Monique & Jean Cachon and the heliozoan Sticholonche zanclea*
A - Monique & Jean with their Japanese microscopist friend Hidemi Sato
B - Scanning electron microscope photos of siliceous skeletons of polycystine radiolarians (M & J Cachon: page 84 of Plankton-Wonders of the Drifting World, Sardet 201, Ulmer
C - Sticholonche zanclea: drawing by Hermann Fol (Fol 1883)
D - Drawing by Monique Cachon showing a cross-section through Sticholonche.
E & F - Electron microscope thin section of the base of an oar/axopod anchored in a depression in the nuclear membrane of Sticholonche (E) and corresponding drawing by Monique Cachon (F), see (Cachon et al. 1977).
QR Code in the left-hand corner. By photographing it with your phone, you can watch Sticholonche rowing (film made by a Japanese colleague).

Tintinnids - decorated ciliates

Tintinnid ciliates are barely visible to the naked eye, but they are among the most interesting micro-organisms in plankton, as they are constantly on the move and have a shell decorated with other protists - called a lorica - in which the ciliated cell contracts or stretches. Protruding out of the lorica, the ciliate captures and feeds on the smallest algae in the plankton. Tintinnids, of which over 500 species have been described, are thus part of a functional group called microzooplankton (Dolan et al. 2012, Dolan 2013). Hermann Fol studied tintinnid ciliates of Villefranche in 1879 and 1880 after Haeckel had made earlier less precise descriptions (Fig. 3, Fol 1881, Dolan 2024).

Tintinnids are frequently and easily collected with a fine mesh net in the bay of Villefranche, which probably explains why Fol studied these ciliates. He was also the first to attempt to determine the chemical nature of tintinnid loricas. A century later several researchers worked on tintinnids in Villefranche, starting in the late 1950s with the Argentinian Ernesto Balech, who after a stay of several months in Villefranche published a landmark taxonomic monograph (Dolan 2017). In the 1960s and 1970s in Villefranche, Michelle Laval-Peuto and Fereidoun Rassoulzadegan became interested in the cytology and ecology of these ciliates (Rassoulzadegan et al. 1988). More recently, John Dolan published articles on the diversity of tintinnid species assemblages in the bay of Villefranche and in deep waters offshore (Dolan 2012, 2013). Nonetheless, many aspects of tintinnid biology and ecology

remain to be elucidated and Villefranche is a perfect location for researchers interested in the taxonomy, ecology, parasitism and symbioses of tintinnids (Vincent et al. 2018).

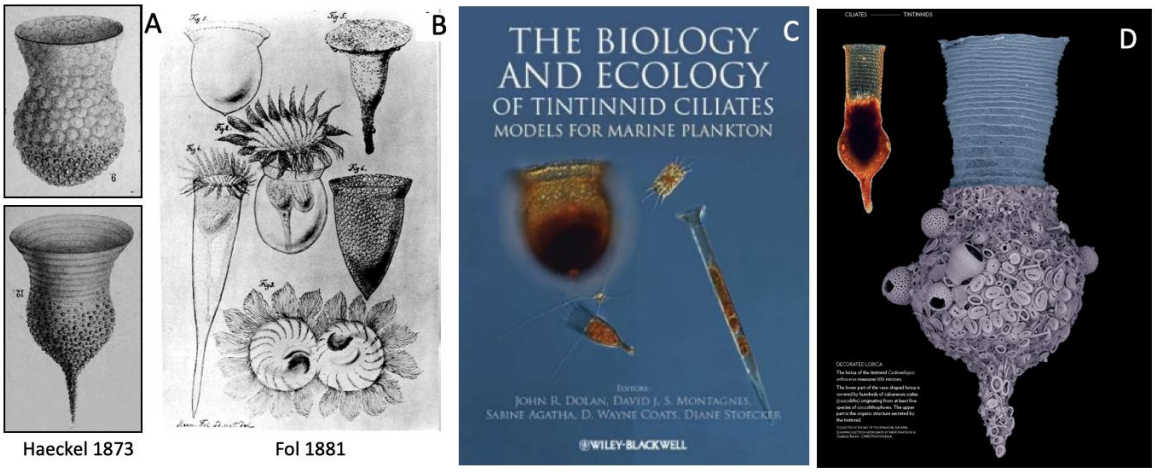

Fig. 3. *Tintinnid ciliates in the bay of Villefranche sur mer*
A - Drawings by Ernst Haeckel (see Dolan 2024)
B - Drawings by Hermann Fol (Fol 1881, see Dolan 2024)
C - Cover of the book on tintinnid ciliates edited by John Dolan (Dolan et al. 2012)
D - The tintinnid Codonellopsis (photo J. Dolan) whose lorica is decorated with calcareous scales of coccolithophores. Scanning microscope by I Machour & C Bachy, CNRS photo library (Plankton – Wonders of the Drifting World, Sardet 2015, Univ. of Chicago Press).

The larvacean *Oikopleura dioica* - a research model developed in Villefranche

Fol had just published his observations of larvaceans (also called appendicularians) done in Messina when he first stayed in Nice for a few months (Fol 1872). In a letter to his future wife Emma Bourrit, he describes these tadpole-like urochordates: "*I went out yesterday morning. The sea was calm, the weather splendid and a small boat was docked near the beach. I couldn't resist the temptation. I grabbed a jar and headed out to sea. After a quarter of an hour, my jar was full and I returned to examine it at my leisure. It contained about twenty larvaceans, microscopic long-tailed creatures that swam in all directions, as transparent as crystal and as agile as little fish. But here's one that stops. It's in the process of forming an envelope serving as a net, and soon it's on the move again in its crystalline envelope 20 times its size. It's well protected now. Let's capture it in a glass tube and put it under the microscope. Just as it feels it's been caught, a flick of the tail and the larvacean is off, leaving its empty envelope as its only booty*".

Fol's pioneering work on appendicularians was taken up again at Villefranche in the 1950s by Robert Fenaux and his successors, including Gabriel Gorsky who, with Fabien Lombard, succeeded in mastering the cultivation of the species *Oikopleura dioica* to analyze their reproduction and physiology (Fig.4, Fenaux 1963, Gorsky et al. 1987, Lombard et al. 2009). In the late 1990s, the technical expertise developed at Villefranche was transferred to the Michael Sars Center in Bergen, enabling Daniel Chourrout, Eric Thompson and their collaborators to establish *Oikopleura dioica* as the reference experimental model for appendicularians (Seo et al. 2001, Marti-Solans et al. 2015)). Since then, half a dozen laboratories in Europe, Japan and the United States have adopted *O. dioica* and helped elucidate and manipulate its genome - the smallest known genome in the chordates (Nishida 2008). The appendicularian model is very attractive. The adult animal consists of fewer than 5,000 cells, with perfectly established lineage and differentiation into a half dozen tissue types. The larvacean *O. dioica* can reproduce in just a few days, and using its “house”/net and a sucking technique it feeds on nearby bacteria, micro-algae and particles. The appendicularian secretes and deploys several

"houses"/nets (called logettes) per day thanks to highly polyploid epithelial cells that secrete the net's fibers in a precise pattern (see fig 4 and Thompson et al. 2001).

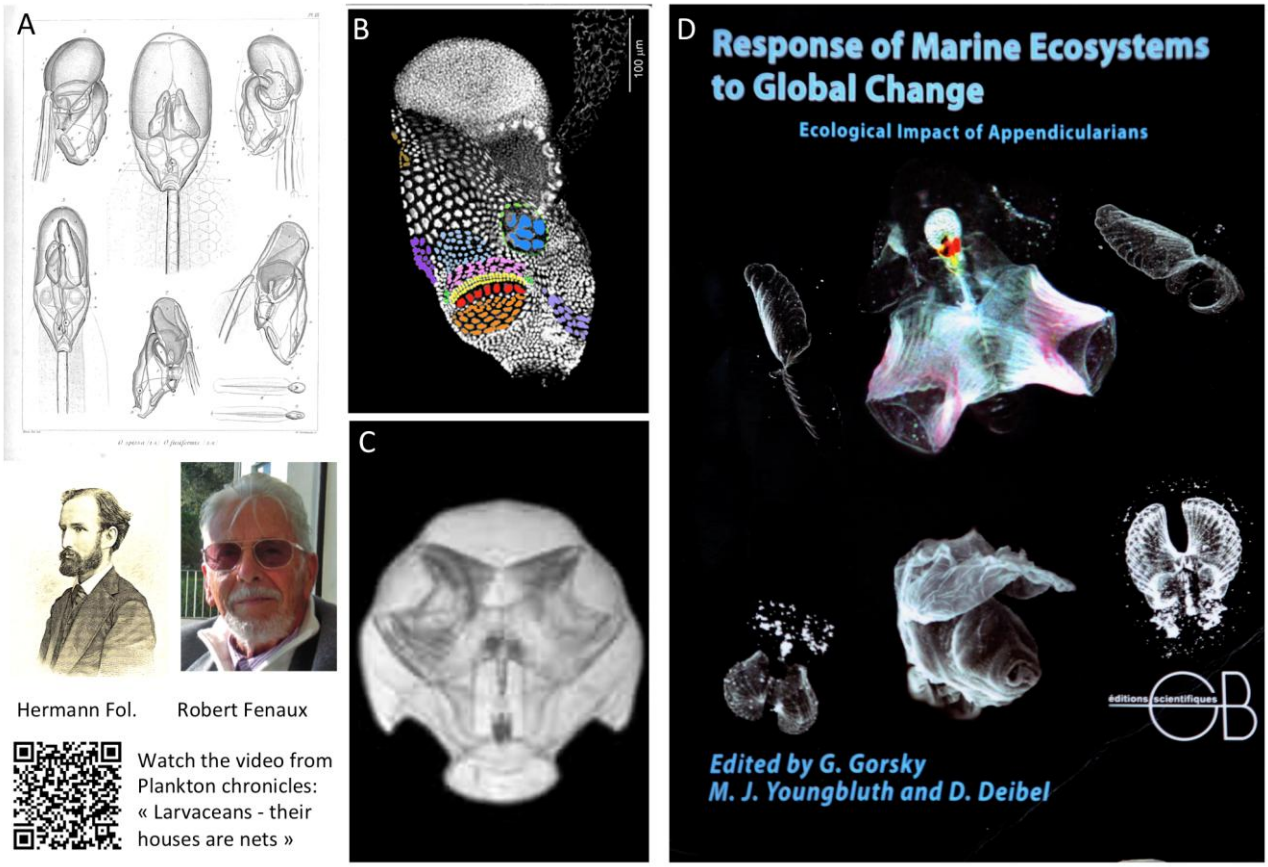

Fig. 4. *The larvaceans (also called appendicularians)*
A – Drawings of the anterior part of the appendicularian Oikopleura dioica (Fol 1872)
B – Domains of polyploid cells secreting the "house"/net (Thompson et al. 2001)
C – Appendicularian "house"/net (Fenaux 1986)
D – Book on the ecological impact of appendicularians (Gorsky et al. 2005)
QR Code: use your mobile phone to watch a film about larvaceans

Due to their abundance and proliferation in all oceans, and their ability to filter particles and microorganisms, appendicularians and their "houses"/nets play an important role in the constitution of marine snow particles, contributing to carbon sequestration as they sink to the abyss. This is the direction that recent research has been taking in Villefranche (Fig. 4, Gorsky et al. 1984, Guidi et al. 2009).

Fertilization and development - a new team

In the 1980s, a CNRS research team (ER250 Biologie Cellulaire Marine), of which I was one of the instigators, was established at the marine station, reviving early research on the development of marine organisms in Villefranche by Barrois, Fol, Metchnikoff, Kowalski and others a century earlier (see companion article: Sardet 2025 / 1 Pioneers: 1800 to 1900). In fact, this research never ceased in Villefranche, as local zoologists continued to explore the characteristics and development of marine organisms - salps, appendicularians, pteropods, cnidarians, fish, etc. They feature prominently in the reference book on Mediterranean plankton (Manuel de Planctonologie Méditerranéenne from Trégouboff & Rose 1957).

The ER 250 research team was first headed by CNRS embryologist Roger Lallier, who had been at the Villefranche site since the 1960s doing research on the development of the sea urchin

Paracentrotus lividus (Lallier 1975). The oocytes and embryos of this species are characterized by a sub equatorial pigment band, making it possible to study the expression of vegetative/animal polarity during development. Lallier modified the development of sea urchin embryos by using a variety of chemical compounds that either animalized the embryos (enriching them with ectodermal tissue) or vegetalized them (enriching them with endodermal and mesodermal tissue). In addition to resident investigators (Roger Lallier, Danièle Carré, Christian Sardet), the new group, soon attracted CNRS researchers from Paris (Jacky and Marie Paule Cosson), Nice (Christian Gache) and the first PhD students (Richard Christen, Thierry Lepage). Moving from the CEA to the CNRS, I succeeded Roger Lallier as head of the CNRS research unit in 1985, and we welcomed other CNRS and INSERM researchers, as well as post-docs and students working on fertilization, development and motility. We also incorporated Jean Cachon's protistology team into a new Unité Mixte de Recherche (UMR 671 Biologie Cellulaire Marine) with around thirty members. In 2000, the laboratory became UMR 7009 Biologie du Développement under the direction of Christian Gache (2000-2008). It was later headed by Evelyn Houliston (2009 - 2018) and Alex Mc Dougall (2019 - present), two CNRS researchers who had first joined our laboratory as post-docs.

Fertilization and cell activation – the importance of ionic signals

Exchanges with Roger Lallier on sea urchin fertilization and development, and the discovery at that time of calcium signals triggered by the fertilizing spermatozoa in fish and sea urchins (see Sardet 2023) prompted us to study ion fluxes in embryos with professors and researchers at the university of Nice, who had been my colleagues at the Villefranche CEA laboratory (Christen et al. 1979, Girard et al. 1982, Sardet et al. 1984). These research subjects, new to me, corresponded to the birth of my first son Noé and the meeting of visiting biologists interested in developmental biology with whom I worked in Villefranche (Marko Zalokar and Lewis Tilney) and in the USA (David Epel, Dan Mazia as well as Lionel Jaffe, Mark Terasaki and Shinya Inoue, pioneers of calcium signals and the microscopic imaging revolution).

In the 1990s, our Villefranche laboratory became one of the best equipped in France for light and electron microscopy sample preparations and observations largely thanks to the efforts of two inventive CNRS engineers/researchers - Christian Rouvière and Patrick Chang (Sardet & Chang 1985, Rouvière et al. 1994, Sardet et al. 1998). Our research group developed a new experimental model inherited from Marco Zalokar – the ascidian *Pallusia mammillata* – a solitary ascidian species whose oocytes and embryos are more abundant and far more transparent than those of the reference species – *Ciona intestinalis* (Zalokar & Sardet 1984). This allowed us to analyze in detail calcium signals at fertilization and the reorganization of the cortex and cytoplasm in fertilized oocytes and the consequences for embryonic development (Fig. 5). It was already known that in mouse oocytes, fertilization triggered oscillations in intracellular calcium concentration lasting for hours. Thanks to experiments on several species of ascidians initiated with our colleagues at Woods Hole, and pursued with Alex McDougall and Rémi Dumollard at Villefranche, we were able to show that the periodic bursts of calcium were in fact propagating calcium waves emitted by a localized "pacemaker". Alex McDougall demonstrated for the first time that these periodic calcium waves were strictly necessary to complete the meiotic cell cycle (Speksnijder et al. 1990, McDougall & Sardet 1995, Dumollard et al. 2002).

Research was pursued on *Phallusia* with Janet Chenevert, Philippe Dru, Fabrice Roegiers, François Prodon, Alexandre Paix and Nang Le Nguyen. We focused on cortical structures and determinant macromolecules during early development, and in particular on cortical determinant RNAs, which play a key role in embryonic polarity (Prodon et al. 2005, Paix et al. 2011). During this latter period, we benefited from intense exchanges with our Japanese colleagues Hiroki Nishida, Lixy Yamada and Kazuo Inaba (Prodon et al. 2010). This research continues in Villefranche and other laboratories in Europe have now adopted this experimental model.

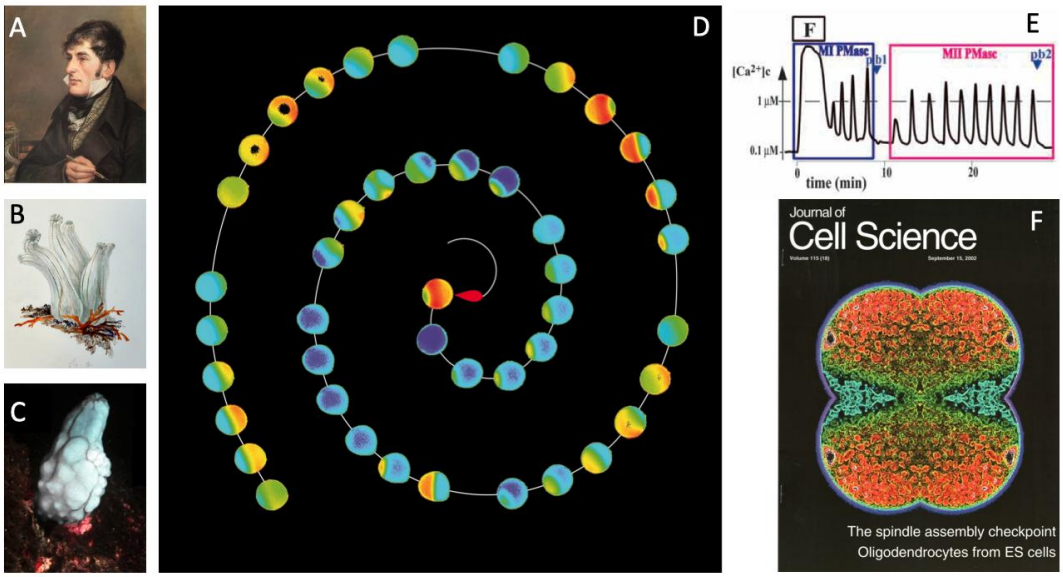

Fig. 5. *Calcium signals in ascidians oocytes following fertilization*
A & B – Alexandre Lesueur and Ciona intestinalis he drew in Nice in 1804
C – The ascidian Phallusia mammillata photographed by Christian Rouvière
D – Calcium waves (yellow to red) repeatedly crossing ae fertilized oocyte. In the center, a schematized spermatozoon (graphic by Mohamed Khamla)
E – Left: calcium signals triggered by fertilization (F) and during the first meiotic division (MI Phase). Right: calcium oscillations during the second meiotic division (MII Phase).
F – Cover inspired by one of our publications on calcium signals (Dumollard et al. 2002)

Asking the right question to the right organism - chaetognaths and ctenophores

Hermann Fol's ghost must have suggested that we take an interest in ctenophores – Fol had written his thesis on their development – and also in chaetognaths, which Fol used to extend his observations on fertilization (Fig. 6, Fol 1979, see Sardet 2025 / 1 Pioneers: 1800 to 1900). Inspired by early work and the expertise of our zoologist colleague Danielle Carré, we began asking these planktonic animals two pertinent questions in the 1980s.

Chaetognats

With regard to the hermaphroditic chaetognaths(arrowworms) *Sagitta* and *Spadella*, whose reproductive cycles and tissue morphology had been figured out by Danielle Carré, we asked how an exceptionally large germinal granule was formed and how it enabled the cells that inherited it to become the germ cells at the origin of male and female gonads. It was in fact in chaetognaths that these germline-determining granules were discovered in the 1900s (Wilson 1925). We were able to observe how this very large germinal granule (it measures twenty microns), forms at the moment of mitosis at one pole of the fertilized egg. This granule and its descendants remain visible throughout the embryo's divisions (Fig. 6, Carré et al. 2002). This makes it possible to analyze and manipulate germ cell development.

We now know from genetic and biochemical analyses in the fly *Drosophila* and the nematode worm *Caenorhabditis* that these granules contain aggregates of RNA molecules and associated proteins in the form of biomolecular condensates (Sardet 2023). These germ granules condensates direct the differentiation of the cells that inherit them into germ cells. An algerian colleague, Chakib Djediat , successfully focused on this project (Carré et al. 2002), which deserve to be continued in the light of recent advances about the differentiation of germ cells and the discovery of biomolecular condensates.

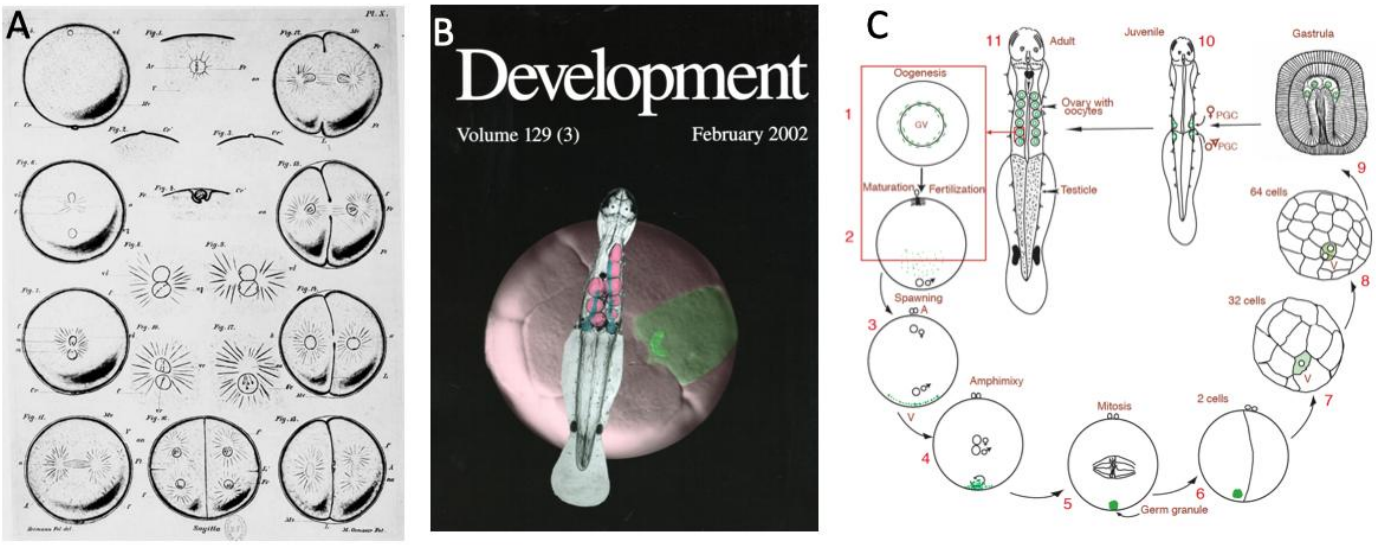

Fig. 6. *Chaetognaths*

A - Drawing by Hermann Fol of the meeting of the male and female nuclei in the fertilized egg of the Sagitta chaetognath (Fol 1978)

B - Illustration taken from our publication on chaetognaths (Carré et al. 2002).

C - Development cycle of chaetognaths and their germ cells (in green) from Carré et al. 2002.

Ctenophores

We also explored basic embryological questions using the oocytes and embryos of the ctenophore (comb jelly) *Beroe ovata*. In ctenophores, fertilization plays a major role in the acquisition of the unique oral-aboral axis in embryos and adults. As its name suggests, *Beroe ovata* is characterized by oocytes with a large diameter (1.5 mm) much larger than those of other ctenophores. These oocytes and embryos are particularly well suited for microscopic imaging due to their extraordinary transparency and the fact that all events - from fertilization to the successive divisions of the embryo - take place within a thin cortical layer some ten microns thick beneath the surface.

Every spring during the 1990s, we - Danielle Carré, Evelyn Houliston, visiting collaborators, fishermen and myself - would watch for the arrival of these iridescent animals in the bay. Early in the morning, we collected animals aboard boats or a pneumatic zodiac in the Bay of Villefranche, a sort of treasure hunt. Sometimes we'd bring dozens of animals back to the laboratory, sometimes we'd return empty-handed, but happy with these mornings spent scanning the surface of the meandering currents. In any case, thanks to imaging techniques coupled with frame-by-frame video recording (at first, we were using bank surveillance equipment!), we observed that the female nucleus in the oocyte would often travel to explore several male nuclei introduced by different spermatozoa that had entered the oocyte as fertilization in *Beroe* is polyspermic (Carré & Sardet 1984, Rouvière et al. 1984). The female nucleus would then fuse with the chosen male nucleus to participate to the first mitotic division defining the site of the first unipolar division, thus determining *Beroe*'s unique oral-aboral embryonic axis (Fig. 7). When we first published them, these observations of the behavior of a cell nucleus stimulated the imagination of many biologists calling for further experimentations (Carré et al. 1991). We also demonstrated the potential of this experimental model for understanding the role of cell cycle factors in the establishment of the embryonic axis (Houliston et al. 1993). Unfortunately, to our knowledge, work on the biology and development of this species seems to have ceased for the moment.

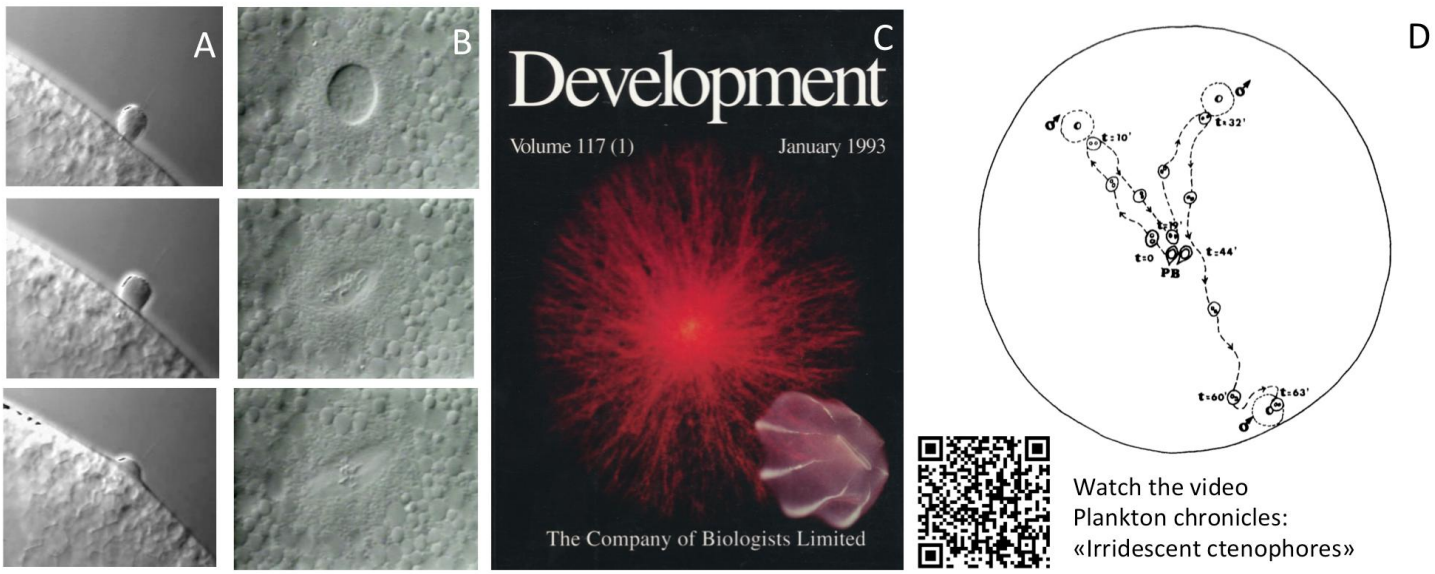

Fig. 7. *The ctenophore Beroe ovata*
A - Three images from a film of the fusion of the spermatozoon with the oocyte
B - Three images from a film showing the exceptional clarity of first mitosis
C - Cover illustration inspired by one of our publication (Houliston et al. 1993)
D -Drawing of a fertilized oocyte in which the trajectories of the female nucleus to successively explore several male nuclei were filmed for 1 hour (Carré & Sardet 1984)
QR Code: use your phone to watch a film about ctenophores

Exploring cnidarians - a century after Vogt and Metchnikoff

In 1853, Carl Vogt – known as the revolutionary scientist – (Fig.8) and in 1886, Elie Metchnikoff,the co-discoverer of immunity, collected and made pioneering observations on cnidarians (siphonophores, jellyfish, corals and anemones) in Villefranche. Cnidarians are ancestral animals sharing the common characteristic of possessing and using stinging cells - called cnidocytes - to paralyze prey. Carl Vogt described and beautifully illustrated the siphonophores collected in the bay in his monograph "Recherches sur les animaux inférieurs de la Méditerranée" (Vogt 1854, Sardet 2025). Siphonophores live as colonies of organs – floating, propulsive, reproductive, and digestive - arranged along a filament called a stolon. Some of the 175 known species extend their colonies over tens of meters fishing small organism including fish with stinging filaments. For this reason siphonophores are the longest animals in the world.

In the 1980s, our zoologist colleague Danielle Carré showed us that the oocytes of siphonophores released by reproductive colonies - the gonophores – remarkably attract sperm in a species-specific way. Each oocyte is capped by a hemispherical cupule attracting spermatozoa, at one pole of the oocyte corresponding to the fertilization site (Fig. 8). By dissecting and solubilizing cupules, we were able to show that the attraction was due to a molecule which, in an electrophoretic micro-gel, attracted sperm of the right species in the form of a band (Carré & Sardet 1981 and drawing F in Fig. 8).

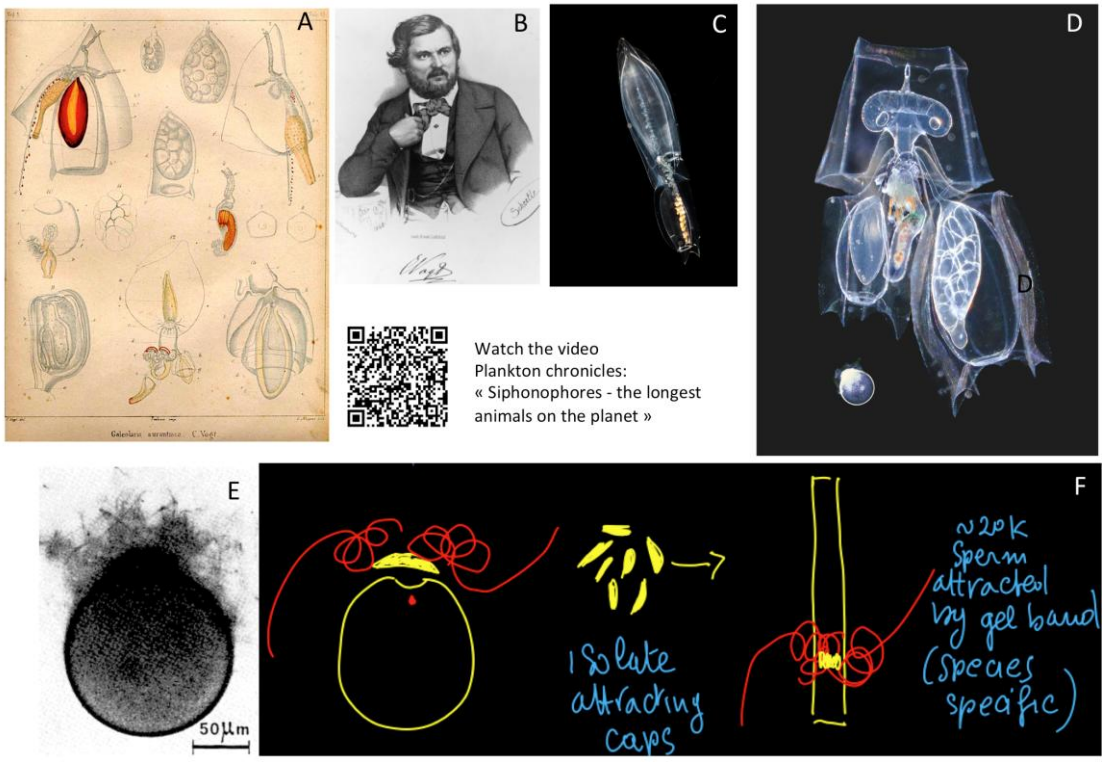

Fig. 8. *Siphonophores*

A – Plate 19 in "Recherches sur les animaux inférieurs de la Méditerranée" (Vogt 1854)

B - Carl Vogt at the age (40) when he made his observations in Villefranche sur Mer

C - A calycophore siphonophore of the genus Chelophiès (page 116, Sardet 2013)

D- Eudoxia (reproductive medusa) of a siphonophore carrying male and female gametes (page 121, Sardet 2013)

E - A siphonophore oocyte attracting spermatozoa of the same species to one pole.

F - Drawings illustrating the attraction of spermatozoa (red) to the cupule (yellow).

QR Code: use your phone to watch a film about siphonophores

However, the analytical methods used at the time did not make it possible to identify the molecular nature of the attractant, which would probably be possible now.

Our colleagues Marie-Paule and Jacky Cosson went on to show that attraction was in fact linked to a change in sperm behavior (Cosson et al. 1983). In the vicinity of the cupule emitting the attractant molecules, the spermatozoa switched from a rectilinear swimming trajectory to swimming in small circles, keeping them close to the attracting cupule capping the oocyte fertilization site. This swimming change is mediated by the concentration of calcium ions (Cosson et al. 1984).

Further research into the development of siphonophores is currently underway at Villefranche (Mańko et al. 2023). Incidentally, our original publications on sperm attraction (Carré & Sardet 1981, Cosson et al. 1983) are unfortunately not listed in the PubMed bibliographic databases.

A new experimental model - the micro medusa *Clytia hemisphaerica*

We have already described experimental models - the ascidian *Phallusia*, the appendicular *Oikopleura* - developed at the Villefranche marine station and how these models have been adopted by other laboratories(see previous chapters). The most recent and remarkable example of the development of an experimental model concerns a small medusa - *Clytia hemisphaerica*. This hydrozoan jellyfish is the subject of a large-scale project initiated in the mid-2000s by Evelyn Houliston with Tsuyoshi Momose (Momose & Houliston 2007).

Since then, some twenty research colleagues, teachers, post-docs, students and visitors have been involved in elucidating the cellular and molecular mechanisms at work in oogenesis and in spawning, in the establishment of embryonic axes, in tissue differentiation, regeneration and the ecology of this jellyfish. This research is described in some thirty publications (see reviews by Houliston et al. 2010, and Houliston et al. 2022).

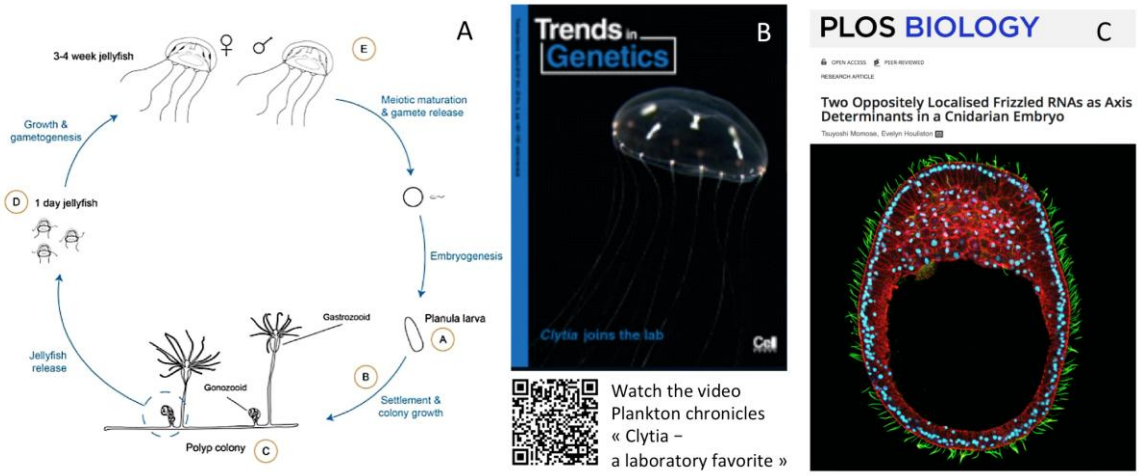

Fig. 9. *Clytia hemisphaerica an attractive jellyfish experimental model*
A - Drawing of the polyp and jellyfish life cycle (Houliston 2022)
B -Cover photo of Clytia in Trends in Genetics (Houliston & Momose 2010)
C - Cover photo showing a section of a Clytia embryo section using fluorescence microscopy: the cell nuclei are blue, the cilia are green (Momose & Houliston 2007)
QR Code: use your phone to watch the film Clytia, a laboratory favorite

The judicious choice of *Clytia* as a model was originally based on observations made in Villefranche by Danielle and Claude Carré (Carré & Carré 2000). They showed that it was possible to cultivate this jellyfish measuring a few millimeters in the form of colonies of "immortal" polyps that propagate vegetatively, providing offspring by constant reproduction via budding. Since then, cultures and techniques, including those for imaging, visualization, gene manipulation and transgenesis, have been optimized and published (Lechable et al 2020, Weissbourd et al. 2021, Houliston 2022). As a result, *Clytia* has become the reference experimental model for hydrozoans adopted by other laboratories around the world. Although the genomes of some model cnidarians - the anthozoan *Nematostella* and the hydrozoan *Hydra* - are known, the sequencing of the genome of the hydrozoan *Clytia*, spearheaded by Lucas Leclère and Richard Copley (Leclère et al. 2019), allows investigations into the mechanisms at work during the complete life cycle of cnidarians in all their complexity. As a typical hydrozoan, *Clytia* lives and reproduces as fixed budding polyps and at the same time as swimming male and female jellyfish. Yet, all these very different life forms share the same genome.

Remarkably, the *Clytia* model lends itself equally well to work in neuroscience and ecology (Vogt 2022, Houliston et al. 2022), and to model gastrulation (Kraus et al. 2020). This brings us back to the origins, when in 1886, the Russian zoologist Elie Metchnikoff came as a visitor to the Villefranche marine station and described the formation of the gastrula in *Phialidium*, since renamed *Clytia* (Metschnikoff 1886).

Genes in action – the development of sea urchins and ascidians

Since the 1970s, the development of animals and plants has been analyzed in terms of the expression in time and space of key gene networks, some of which are universally shared. Specific genes are transcribed and expressed in the form of proteins, in different regions the oocyte (so-called maternal genes) and/or embryo (so-called zygotic genes) at crucial moments after fertilization, the first

cell divisions, gastrulation or metamorphosis. Choosing to work on the *Paracentrotus lividus* sea urchin model already worked on at Villefranche by Roger Lallier, our colleague Christian Gache left the Biochemistry Department at the University of Nice to bring his knowledge of molecular biology and gene expression to our Villefranche laboratory from the outset.

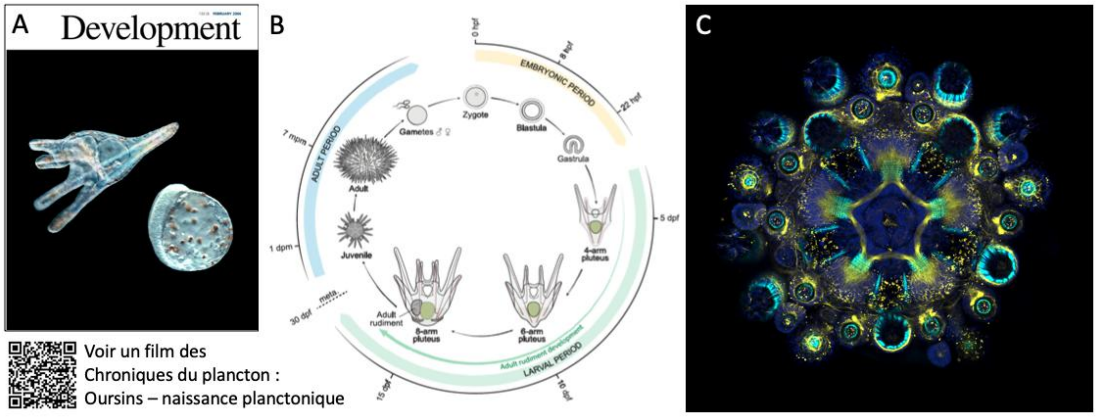

Fig. 10. *Development of the sea urchin Paracentrotus lividus*
A - Cover photo showing a normal sea urchin embryo at the pluteus larva stage (left) and an animalized embryo (right) (Croce et al. 2006)
B - Life cycle of the sea urchin Paracentrotus lividus (Formery et al. 2021)
C - Confocal microscopy of a young adult(juvenile)Paracentrotus lividus. Muscles are in blue, the nervous system in yellow, and the nuclei in white (Formery et al. 2021).
QR Code: use your phone to watch a film about sea urchins

The research initiated by Christian Gache has been continued by Thierry Lepage, Jenifer Croce, Christian Ghiglione, Guy Lhomond, and many other researchers, technical staff, students and visiting researchers and professors such as David McClay (Duke Univ. USA), right up to the present day. Gache and Lepage first isolated the enzyme that enables sea urchin larvae to hatch (break their envelope) and showed that its gene is expressed in a polarized fashion (Lepage & Gache 1989, Lepage et al. 1992). Then Gache, his team and their successors - the teams of Thierry Lepage and then Jenifer Croce and their collaborators - explored the roles of key genes in the differentiation of embryonic tissues (ectoderm, mesoderm, endoderm). They also investigated how the pluteus larva acquires its skeleton made of calcium carbonate semi-crystals (Croce et al. 2006, Robert et al. 2014). Collectively, these research teams provided the research community with remarkable genomic (Lepage et al. 2004, Marletaz et al. 2023) and morphological (Formery et al. 2021) observations as well as essential tools.

Solitary and colonial ascidians, tunicates known as sea squirts (*Ciona intestinalis, Phallusia mammillata, Botryllus schlosseri*) have also been used extensively in Villefranche sur Mer to understand gene expression. Having joined the laboratory some twenty years ago, Clare Hudson and Hitoyoshi Yasuo and their research team defined some of the rules of tissue differentiation in embryos of *Ciona intestinalis*, the most widely used experimental model for ascidians. In a single day, ascidian embryos develop into a motile tadpole consisting of only 6 tissues made up of less than 3,000 cells, with all cell lineages perfectly known. The experimental strategy employed by Hudson and Yasuo combines micromanipulation and ablation of some specific cells in embryo at early stages, followed by analysis and modeling of the expression of essential genes and proteins using imaging. These approaches enabled them to understand how a small number of cells involved early on (at the16-64 cell stages) differentiate in just a few hours to form the muscle and nerve tissues of the tadpole. They compared gene expression in this simpler ascidian model with the mechanisms at work in the more complex vertebrate embryos (Yasuo et al. 2007, Hudson et al. 2011, 2021).

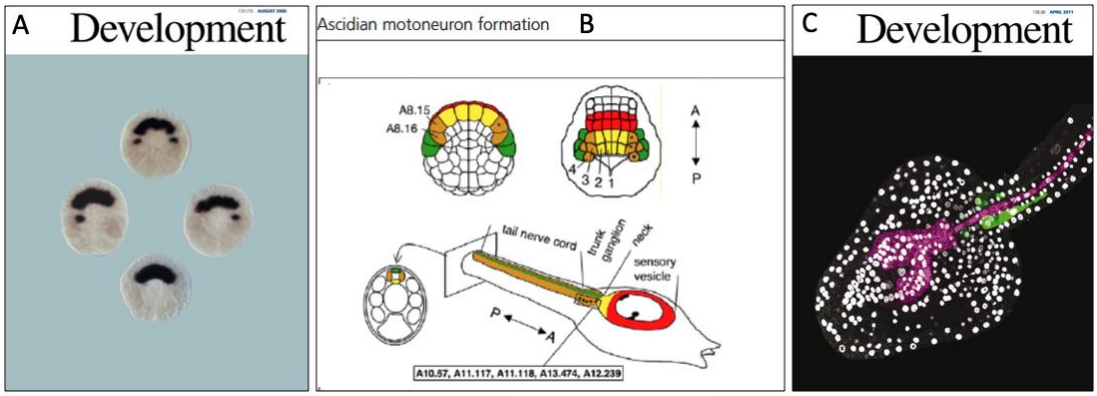

Fig. 11. *The development of the ascidian Ciona intestinalis*

A - Cover photo showing that in a normal embryo (top), 10 neural chord precursor cells express the Brachyury gene. Its expression is altered in embryos (middle and bottom) following micromanipulations of cells (Hudson & Yasuo 2006)

B - Drawing showing which cells in the early embryo specify different parts of the ascidian tadpole's nervous system (Hudson et al. 2011)

C - Cover photo showing different motor neurons expressing fluorescent genes (in green), motor neurons injected with a lipophilic molecule (in magenta) and cells in which nuclei are revealed using a DNA fluorescent dye (in white). From Hudson et al. 2011.

The other experimental model developed at Villefranche - *Phallusia mammillata* - is being used by Alex McDougall, Rémi Dumollard, Janet Chenevert and their collaborators to analyze how the orientation of cell divisions and the de-synchronizations of cell cycles determine the positioning and differentiation of cells (stages 16 to 128 cells) whose fates are rapidly fixed (Dumollard et al. 2013, McDougall et al 2019, Chenevert et al. 2020).

The Villefranche sur Mer development biology laboratory, considered to be one of the world's leading laboratories in the field of comparative development biology has shared widely its expertise and methodologies and organized several meetings of the cell and developmental biology research community in Villefranche (Sardet et al. 2008, 2011, Yasuo & McDougall 2018, Dumollard et al. 2017).

Finally, to close this chapter, I'm sorry I do not have the space to do justice to our colleagues from Villefranche sur Mer who are successfully pursuing comparative molecular and cellular approaches on a variety of other marine organisms (*Amphioxus*, *Botryllus*, *Clytia*, *Mytilus*, *Salpa*, etc.).

Tara oceans – a human and scientific adventure

Villefranche has been world-famous for its plankton ever since pioneering naturalists from Nice, Germany and Switzerland revealed the biodiversity of pelagic organisms that drift and dwell in the bay ever changing with the seasons and the weather conditions.

Following in the footsteps of the pioneers, Grégoire Trégouboff and the Villefranche zoologists and their successors at the LOV laboratory popularized the history of plankton biodiversity and ecology (Trégouboff & Rose 1957, Trégouboff 1983, Anon. 2024a).

In 2008, with Eric Karsenti and a few colleagues I came up with the idea of a global expedition to study plankton in all oceans on board the schooner Tara, the Villefranche marine station emerged as an essential partner in the project (Karsenti & Di Meo 2012). On a table corner in the Villefranchge harbor – le port de la Darse - Eric Karsenti, Gaby Gorsky and I began to sketch out the broad outlines of the *Tara oceans expedition* a global exploration of plankton in all oceans. We convinced colleagues

at marine stations - Villefranche, Roscoff, Banyuls, Naples - and at a dozen other research laboratories in Europe and USA to work together and share their indispensable oceanographic and biological expertise. The schooner Tara came in the bay of Villefranche for an initial planning conference, and after a year of planning, the expedition left Lorient for the Mediterranean in September 2009 (see our films of the expedition's departure: Anon. 2019). Gaby Gorsky, Marc Picheral, Lars Stemann and colleagues from Villefranche and colleagues from the marine stations of Roscoff, Banyuls and Naples began equipping and adapting the schooner and guiding its course for a global exploration of plankton. Eric Karsenti (EMBL, Heidelberg) and Etienne Bourgois (agnèsb) led the first expedition with some twenty scientific coordinators as part of a consortium of half a dozen major institutions (CNRS, CEA, EMBL, Sorbonne University, etc.).

Aware that the main strategy of the expedition - the analysis of the planktonic ecosystem using genomics - would not be an effective way to popularize plankton, we decided to tell the stories of organisms in plankton via multimedia documents of the plankton chronicles project (Sardet 2017). I'm very grateful to fellow zoologists from Villefranche, and in particular Claude Carré, who taught me and told me many stories about planktonic creatures. Véronique Kleiner (CNRS Images), Noé Sardet and Sharif Mirshak (Parafilms, Montreal) made it possible to photograph and to produce short films and the well used Plankton Chronicles multilingual website on the beauty and diversity of planktonic creatures. A book on plankton for a wider audience was published in French (Sardet 2013), and translated in English (Plankton - Wonders of the Drifting World (Univ. Chicago Press 2015), Japanese (2014), Germany(2018) and Chinese (2019).

Fig. 12. *Plankton – an arts and science perspective*

A - Cover of "Manuel de Planctonologie Méditerranéenne " (Trégouboff & Rose 1957)

B – A page from Volume 1 of the Manuel with illustrations of Sticholonche and Zoothamnium (Chapter XV, plate 55)

C - Cover of the May 22, 2015 issue of Science magazine announcing the 5 articles detailing the first scientific results of the Tara oceans expedition (photos of planktonic organisms from the book "Plankton - aux origines du vivant" C. Sardet, Ulmer 2013).

D - The video episodes on the "Chroniques du plancton" website in French, English and Spanish have been visited by 200 to 1,000 people a day since 2013.

QR Codes: use your phone to view Chroniques du plancton (films, news, etc.) and the different expeditions the Tara oceans expedition

The first *Tara oceans* expedition ran from 2009 to 2012 (Anon. 2019), then the expedition set off again under the direction of Chris Bowler (ENS, Paris) around the Arctic in 2014 and has continued in various forms ever since (Anon. 2024b). Over 150 publications, most of them in major international journals, attest to the impact and success, of the expedition and its scientific discoveries which we will not detail here (Anon. 2024b).

The project was atypical in the sense that an ad-hoc group of biologists, geneticists, bioinformaticians and oceanographers from various institutions (CNRS, EMBL, CEA Genoscope, etc.) joined forces with a private organization (Fondation Tara Océans / agnèsb, owner and manager of the schooner Tara) with a simple idea in mind: to collect and analyze the entire planktonic ecosystem together with many physical-chemical parameters in hundreds of well-chosen sites worldwide. This ambitious project relied on massive gene analysis by the Genoscope (metagenomics) and the use of interpretation tools (AI, correlation analyses etc.). Automated image acquisition and recognition was also key to the expedition success, a field in which the Villefranche marine station played a leading role thanks to the involvement of Gaby Gorsky, Fabien Lombard, Lionel Guidi, Marc Picheral and their colleagues (Gorsky et al. 2019, Lombard et al. 2019, Picheral 2022). We set off on our expedition in September 2009 with a great deal of enthusiasm and sympathy, but also with a lot of criticism and skepticism. As a consequence we did not get much of grant money that might have made things easier. It was a gamble!

From the outset, I was keen to assemble on board Tara a library of planktonic works, and above all a photocopy and digitized version of the "Trégouboff", the plankton bible: "Manuel de Planctonologie Méditerranéenne" published in the 1950s by Grégoire Trégouboff, then director of the Villefranche sur Mer zoological station (Trégouboff & Rose 1957). The book is in two volumes, one volume containing plates of organisms compiled from many zoologists' drawings, the other volume a dense text with digressions and references. Although somewhat difficult and confusing to use, the "Trégouboff", is a must-have reference for plankton and a source of pride for Villefranche.

In addition to its scientific aspects, the *Tara oceans* expedition aimed to raise awareness about the planktonic ecosystem among the general public and young people in schools. Like the scientific component of the expedition, the communication impact went beyond our expectations, thanks to the generosity of the participating scientists as well as the communication and education team set up by the Tara Oceans Foundation (Anon. 2024b). Acting as liaison between the Foundation's communications team and the scientific team, I began photographing and filming the organisms from the outset to tell their stories. Many other educational and artistic projects (exhibitions, applications, publications, games) have been created and continue to be created around the *Tara oceans* expeditions such as the “La grande expédition“ the art and science exhibit organized recently by the Tara Océans Foundation at the CentQuatre in Paris (Anon. 2024b, d). This desire to share information is in the air of the times, and scientific communication and mediation efforts in Villefranche have multiplied in recent years, in particular through the "Ocean Culture" outreach project.

Conclusion

This overview of research over the last 60 years represents my personal view of events, and I apologize in advance to anyone I may have overlooked or forgotten in recounting this saga. This story is also a way of paying tribute to the work of some of the Villefranche biologist colleagues whom I frequented and mentioned in this article and who are unfortunately no longer with us: Jean Maetz, Lucienne Fenaux, Jean Cachon, Marie Paule Cosson, Jean Febvre, Monique Cachon, Maurice Fenaux, Roger Lallier, René Motais, Richard Christen.

To find out more about current research into the biology and development of organisms, plankton and oceanography, visit Institut de la Mer de Villefranche (IMEV) website, the *Tara oceans* website (Anon. 2024b) and Traversing EuropeanCoastlines (TREC) the last expedition of the Tara schooner.

Thanks

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Anon. 2019. Our films on the beginnings of the *Tara oceans* expedition
-1) PART 1 Preparing the expedition and leaving from Lorient in September 2009
https://www.youtube.com/watch?v=tcx8-GpLUfM&list=PL0cA6oCJaXodaj3225MArm6Uc5mXZa436&index=3
- 2) PART 2 Tara oceans beginnings / From Lorient to Barcelona in 2009
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