

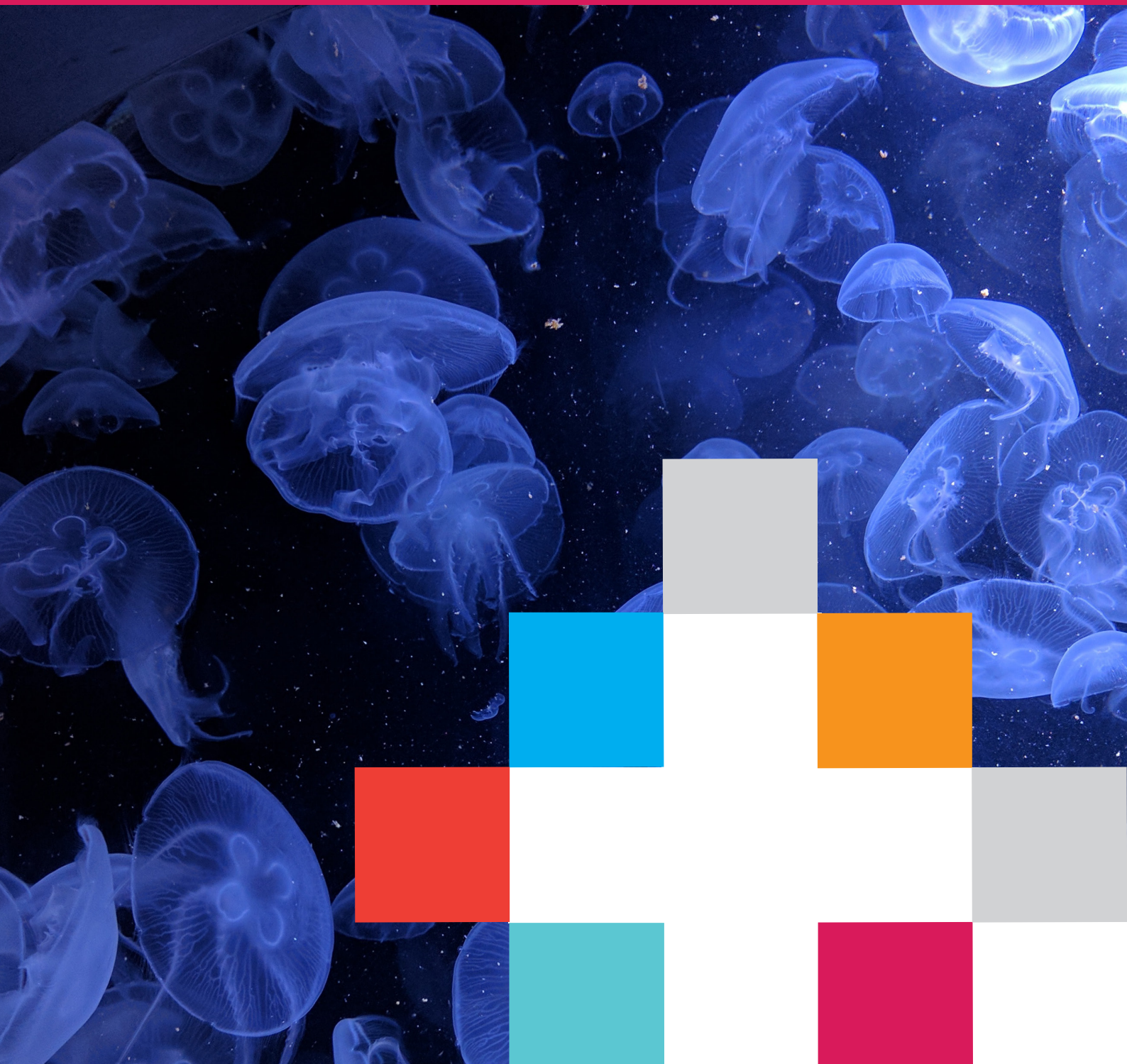
# ASSEMBLE

ASSOCIATION OF EUROPEAN MARINE BIOLOGICAL LABORATORIES EXPANDED



ISSUE 3 | **WINTER 2019**

**PROJECT NEWS**



[www.assembleplus.eu](http://www.assembleplus.eu)

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This newsletter has been developed to inform and update **ASSEMBLE Plus** partners of project activity. This is the third of five editions. External news items will be shared on the website, [www.assembleplus.eu](http://www.assembleplus.eu).

If you have an event report, news items or article that you would like to see appear on the website or in the next issue, please contact Georgia Bayliss-Brown ([georgia@aquatt.ie](mailto:georgia@aquatt.ie)).



## News: The Transnational Access programme

by *Davide di Cioccio*

The Transnational Access (TA) programme of ASSEMBLE Plus supports research projects in marine biology and ecology, providing access to facilities and research services for marine biology to researchers from academia and industry at more than 30 partner institutes. The objective of this programme is to enable researchers to carry out their own research projects, offering them free access to platforms and marine biological resources not available in their own institutes, also gaining the opportunity to establish new scientific collaborations.

Started on January 2018, the TA programme is currently at its sixth call for access and has received so far more than 250 project proposals from European and non-European

researchers. Until now, researchers who have been granted access to ASSEMBLE Plus marine stations have reported enthusiastic success stories (<http://www.assembleplus.eu/access/success-stories>). The last call for the TA programme will close in June 2020, and on-site and remote access to our facilities will be granted until February 2021. Next deadline for submission (6th call) is 9th February 2020. Visits can last for up to 30 days and can include up to two persons per project. ASSEMBLE Plus covers travel, accommodation and meals costs up to certain limits.

If you are interested in learning more about the TA programme and applying, please visit <http://www.assembleplus.eu/access/transnational-access>.



## News: An Outstanding Scientist, Ernest Everett Just

by *Ibon Cancio*

October this year was the 78th anniversary of the death of the man who has been considered the “Black Apollo of Science”, Ernest Everett Just (14th August 1883 to 17th October 1941). The first winner of the Spingarn Medal (1915), he was an outstanding marine biologist that greatly benefited from the “Transnational Access” possibilities into European marine biological stations.

Just, born in South Carolina, became one of the first Afro-Americans to obtain a PhD in Sciences (University of Chicago, 1916). His PhD supervisor was the great Frank R. Lillie, director of Marine Biological Laboratory (MBL) at Woods Hole. With a heavy teaching load as Professor in Howard University, federally chartered historically black university in Washington D.C., he visited MBL every summer for over 15 years; working mainly in different aspects of fertilisation with polychaetes, sea urchins, sea stars and other model marine invertebrates. These summer research retreats resulted in solid, technically magnificent, science on fertilization, describing for instance the fast and slow blocks of polyspermy.

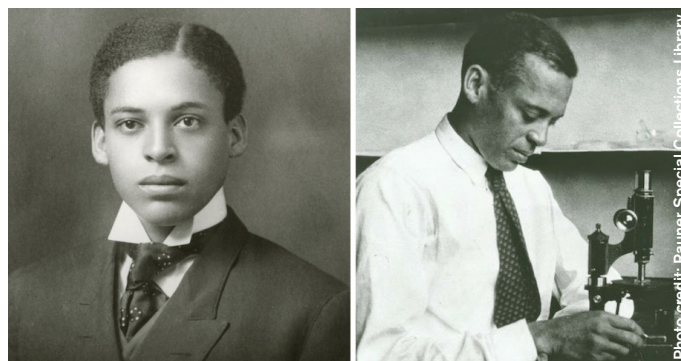
His achievements provided him with little prominence, always under funding constraints, in part because of the colour of his skin. After obtaining a Rosenwald foundation fellowship, he initiated his first trip to Europe in January 1929, for a research stay of six months in Stazione Zoologica Anton Dohrn, Naples (SZN), to work with *Nereis dumerilii*. He discovered an open collaborative scientific environment in the institution that has been considered the ‘mecca’ of marine science and developed a deep friendship with its Director from 1909 to 1954, Reinhard Dohrn.

The social environment, fraternity and freedom to conduct research he experienced convinced him of the need to conduct research in Europe, making every effort to travel yearly to Europe after his teaching duties were covered in Howard. He returned to Naples until 1938, even if 1930 was mainly dedicated to a sabbatical in Germany (Kaiser-

Wilhelm-Gesellschaft, Berlin), until finally he committed to leave USA definitively. In spring 1938, he moved to France, finding his place without salary in Sorbonne University. During 1939, and with the inspiration found in Europe, he published two books. One was on the use of eggs of marine animals while the second one was a more philosophical scientific treatise on the “Biology of the Cell Surface”, typical of a scientist in his plenitude with a strong experimental background on his shoulders. In these late two years, very short of funds, his research was centred at Station Biologique at Roscoff (Brittany), founded like SZN in 1872.

With the invasion of Germany in 1940, France had recommended foreigners to leave the country, but Just decided to stay to complete his work in Roscoff. He was made prisoner in a camp for a short period of time, and when he was rescued with the help of the family of his German wife (Maid Hedwig), in September 1940, there was no other option than returning to USA. He died of pancreatic cancer shortly after, at the age of 58. A great embryologist that loved European Marine Biological Stations.

A “coffee table book” is being written with ASSEMBLE plus on the history of marine biological stations, on the research and the researchers in them, since the first one was opened in Ostend (Belgium) in 1843.



## Workshop: FAIR data management for long-term biological and omics data 13 – 14th June, Oostende, Belgium by *Katrina Exter*

On 13th to 14th June 2019, the workshop on 'FAIR data management for long-term biological and omics data from marine stations' was held by Flanders Marine Institute (VLIZ). All marine stations and partners with long-term datasets held in the ASSEMBLE Plus datasets collection (<http://www.assembleplus.eu/results/datasets>) were invited. Twenty participants from twelve stations attended, plus a few remote participants. The objective of this workshop was to train the ASSEMBLE Plus consortium on the principles and practices of FAIR data management of their datasets, to improve data access and standardisation of genomic and long-term marine biodiversity observations (WP4/NA2.4).

The aims of the workshop were: (i) To introduce concepts and applications of the FAIR data principles; (ii) To improve skills to realise virtual access to data resources in general; (iii) To improve skills to realise virtual access to long-term biological data series; and (iv) To introduce data management approaches for creating data publications of data generated by genomic observatories. The workshop comprised a mix of presentations and tutorials. All the material can be found at <http://www.assembleplus.eu/FAIRworkshop>.

Subsequently, a guide to FAIR data management in ASSEMBLE Plus activities (and associated marine biological stations parts) was developed following discussions held at and lessons learned during this workshop. It was agreed that the FAIRification of the existing data records and data sets would be a joint VLIZ-marine station activity, with EurOBIS and EMODnet help in the standardisation process where desired. This is a process that will continue through the rest of the ASSEMBLE Plus timeline.

The guide to FAIR data management can be found at <http://www.assembleplus.eu/results/fairdatamanagement>.



## Workshop: Cryopreservation of Marine Genetic Resources 26th – 27th June 2019, Oban, UK by *Estefania Paredes*

On 26th – 27th June 2019, the ASSEMBLE Plus partners focussed on cryopreservation (WP8/JRA2) met at the Scottish Association for Marine Sciences (SAMS). The objective of the meeting was to exchange knowledge, best practices, critical aspects of the cryopreservation of marine organisms and specific processes related, between the members of the work package, the members of ASSEMBLE Plus and interested third parties.

Over twenty international participants from eight countries attended, all of whom had an interest in cryobiology applied to marine biological resources. The workshop provided a forum for discussion, understanding the precautions and constraints, as well as best practices, equipment, safety and regulations regarding cryopreservation of marine cells.

For more information, see the Joint Research Activity section on 'Cryobanking Marine Organisms'.



## Workshop: Zebrafish Tools for the Screening of Osteogenic compounds 15th – 17th July 2019, Faro, Portugal by *M. Leonor Cancela*

On 15th – 17th July 2019, a workshop on 'Zebrafish Tools for the Screening of Osteogenic compounds', supported by ASSEMBLE Plus. The workshop aimed at providing advanced training to both academia and company personnel on the use of specific zebrafish systems toward the screening and study of bone anabolic compounds.

It promoted knowledge exchange between attendees and contributed to the establishment of scientific collaborations in fields related to bioprospection, bone formation and skeletogenesis using zebrafish.

Specifically, this event transferred conceptual and practical know-how on the following topics: 1) Basis of zebrafish biology and husbandry; 2) Current knowledge on drug screenings; 3) Ways of exposing zebrafish to experimental compounds; 4) Imaging of bone structures and morphometric analysis; and, 5) Assessment of compound osteogenic and regenerative potential.

Social activities followed to promote new collaborations and broadcasting CCMAR research and ASSEMBLE Plus opportunities.

## Joint Research Activity: Genomic Observatories

by Georgios Kotoulas

### What is ASSEMBLE Plus working on?

The aim of this joint research activity is to produce a roadmap of long-term marine genomic observatories, by practicing and optimising coordinated genomics observations in planktonic and benthic communities, building on the long term investment, infrastructure, expertise and traditions of more than twenty ASSEMBLE Plus marine stations.

### What is ASSEMBLE Plus doing that is different?

ASSEMBLE Plus continue the coordinated practice of Ocean Sampling Day (OSD) events, which is very informative in terms of addressing genomics observatory challenges. The OSD takes place at every summer solstice (21st June each year). New stations have registered and are expected to contribute to subsequent OSD events. In each station, environmental and genomic data are produced following standardised sampling and experimental procedures for reproducibility and comparability. Samples are centralised for further processing (biobanking, high molecular weight DNA extraction, DNA metabarcoding of prokaryotic and eukaryotic microbial communities) to avoid biases between individual laboratories. OSD 2018 and 2019 have delivered high quality genomic DNA for 65 stations, which is ready to be jointly submitted to shotgun metagenomics sequencing. All data and metadata, genomics and environmental, will be made publicly available. OSD2018 and 2019 were a great exercise for acquainting the community with production and value of high quality FAIR data, where FAIR stands for 'Findable, Accessible, Interoperable and Reusable', which until now is more of a wish than of a practice.

Unlike the water column, the nature of benthic habitats presents a huge diversity and therefore it makes it difficult, if not impossible, to standardise sampling methods allowing to compare their respective biodiversity. Autonomous Reef Monitoring Structures (ARMS; <http://www.oceanarms.org/>) are passive collectors that can be deployed in any type of benthic habitats and be used to sample marine benthic fauna, which is understudied in standardised ways. ARMS deployment has been taking place in two phases. The first phase of training practitioners and exploring benchmarking methods has been completed and is being analysed. In the second phase which will be informed by benchmarking of the first phase, specific scientific questions will be asked; for example, the capacity of detecting non-indigenous species (NIS) around ports.

Several ARMS devices have been deployed in nineteen sampling areas at ASSEMBLE Plus sites in European coastal waters and Antarctica, in triplicates whenever possible. Each ARMS device is formed of eight stacked plates. Each plate is separately treated (with a high analysis photograph taken and DNA metabarcoding of eukaryotic communities are produced). ARMS can be used for a broad range of questions including long-term ecological research, monitoring and inventory (LTER). All files relating to the

ARMS sampling network are currently uploaded to <https://github.com/biomobst/ARMS/>. In addition, ASSEMBLE Plus aims at enriching local DNA barcoding reference databases, so that DNA metabarcoding captures taxonomically, well-annotated biodiversity data. Indeed, DNA metabarcoding is a high-throughput, effective and relatively cheap method for assessing biodiversity. Nevertheless, until now, with relatively poor reference database for DNA barcoding, a high percentage of biodiversity captured by DNA metabarcoding method, remains unknown. ASSEMBLE Plus' work is working towards improving this shortcoming.

### Potential impact

At present, at the international level, we are far from being able to produce long-term genomic data allowing deep understanding of biodiversity dynamics in terms of structure and function across geographical areas and ecosystem types. Such data simply are not produced. ASSEMBLE Plus has been building elements of an infrastructure that will allow producing such data and making it publicly available. OSD and ARMS are such examples involving broader international collaborations. ASSEMBLE Plus is in the process of moving from test level to operational level. Once completed, ASSEMBLE Plus will deliver a roadmap for long term observation of marine ecosystems by combining standardised methods for genomics and environmental data and for integrating new technologies and data types.

### What next?

- It is planned that monthly sampling of OSD data will be undertaken (2020) at some of ASSEMBLE Plus' stations.
- The ARMS data will be uploaded to EMBRC's Marine Data Archive and GeoME.
- Data and metadata will become available through ASSEMBLE Plus portal.



## Joint Research Activity: Cryobanking marine organisms

by Estefania Paredes

### What is ASSEMBLE Plus working on?

ASSEMBLE Plus' project partners are working on the design of cryopreservation protocols of various marine genetic and biological resources (e.g. marine invertebrates and algae) for bio-banking. They are also interested in exploring processes to extend the reproduction window of commercial species.

### What is ASSEMBLE Plus doing that is different?

Naturally, sea urchins only reproduce for 4 to 6 months of the year. It is possible to force their reproduction by tricking them that its spring - thus creating a stock of out-of-season reproducers - but this is expensive. Alternatively, by using cryopreservation and having the sperm, embryos or larvae in a biobank, it is possible to work with them all year around. Cryopreservation has proven an incredibly useful technique for the land animal breeding sector, and it can be equally useful for sea urchin seed production. As mussels only reproduce twice a year, in spring and autumn, it was believed that cryopreservation may offer an opportunity for them also. This year, the research team at the Universidade de Vigo obtained seed from cryopreserved mussel larvae (*M. galloprovincialis*) for the first time. These were then grown to juvenile stage and then to put to ropes in the marine environment to reach maturation. The proof of concept that cryopreservation of mussel larvae can be used to improve market seasonality was thus achieved. A protocol for mussel spawning was developed and the results are published. IFREMER had previously done this with oysters (*C. gigas*) therefore it is quite clear that cryopreservation has a great potential in aquaculture industry with molluscs.

In addition to the aquaculture industry, cryopreservation and biobanking has applications in preserving genetic biodiversity. One of the most interesting applications is the role in the preservation of genetic material of endangered species. CCMAR has worked on the cryopreservation of the Portuguese oyster (*C. angulata*) and for species of biomedical interest, such as different zebrafish strains. This biodiversity preservation is important and useful for culture collection and research facilities. Roscoff are investigating cryopreservation of other species of macroalgae, e.g. *Laminaria* sp. and *Prophyra* sp. SAMS have developed a method for cryopreserving gametophytes of the brown seaweed, *Saccharina latissima* and this has resulted in a paper. NIOZ has successfully developed a cryopreservation protocol for hypersaline microbial mats (a multi-layered sheet of different microorganisms of a few centimetres thick that grow in moist areas) that can now be preserved at  $-150^{\circ}\text{C}$  and can be grown again for 120 days. In total, ASSEMBLE Plus has developed cryopreservation approaches for over 200 algal species (micro and macroalgae), thanks to the combined efforts on standardising and training among several institutions. Cryopreserved samples can now be shipped using one-use dry shippers between institutions in a very simple and comfortable manner.

### Potential impact

The research on sea urchin embryo cryopreservation was patented by CCMAR in 2015, yet no one has shown interest in buying the patent. It would be possible that now that the interest towards land-based seed production is renewed,



cryobiology can be used as an out-of-season tool for seed storage. In general, molluscs and other marine invertebrate fisheries and aquaculture companies rely on the intake of seed from the ocean. This is the case for mussels in Spain where seed is extracted from the rocky shores of selected locations. For some species, cryopreservation can help avoid the risk of reductions of natural seed.

Many traditional fisheries, such as within clam culture, have been approaching harvesting in a very sustainable way; producing seed inland that is later transferred to the beach to increase natural production to maintain annual collection rates without overexploitation of the resources. They are always restocking. This approach means that they are better prepared for the future market and mitigating risks related to climate change. This approach can be also useful for sea urchins, with worldwide decline of stocks. There have been past attempts to grow sea urchins inland and on-shore but these have not been successful. It may, however, be possible to use these techniques to seed the ocean to restock natural populations and allow a healthy production-extraction balance.

### What next?

- The CCMAR team has been working on the development of improved cryopreservation protocols for *Chamelea gallina* and *Crassostrea angula* sperm and larvae.
- UPV are working on sperm cryopreservation for the thicklip grey mullet (*Chelo labrosus*) which are used as sentinels of environmental health in pollution monitoring campaigns on the Basque coast.
- Roscoff, SAMS, MBA and ECIMAT are working on the identification of recalcitrant microalgae species (species that are difficult to cryopreserve) Experiments for benthic-filter feeder, amphioxus, are scheduled for next spawning season to test a number of cryopreservation techniques on embryos, and then to evaluate using markers and/or sequencing techniques.



## Joint Research Activity: Functional Genomics

by Hector Escriva

### What is ASSEMBLE Plus working on?

ASSEMBLE Plus will adapt and implement protocols to generate genetically modified metazoans, macroalgae and microorganisms, all of which will represent strategically important resources for academia and industry (e.g. bioactives for food, feed, pharma and cosmetics).

### What is ASSEMBLE Plus doing that is different?

CRISPR is a simple yet powerful tool for editing genomes. It allows researchers to easily alter DNA sequences and modify gene function. Its many potential applications include correcting genetic defects, study gene function, treating and preventing the spread of diseases and improving crops through transgenesis. CRISPR can be used to 'knockout' a gene to then study the effect of its loss, and therefore its function.

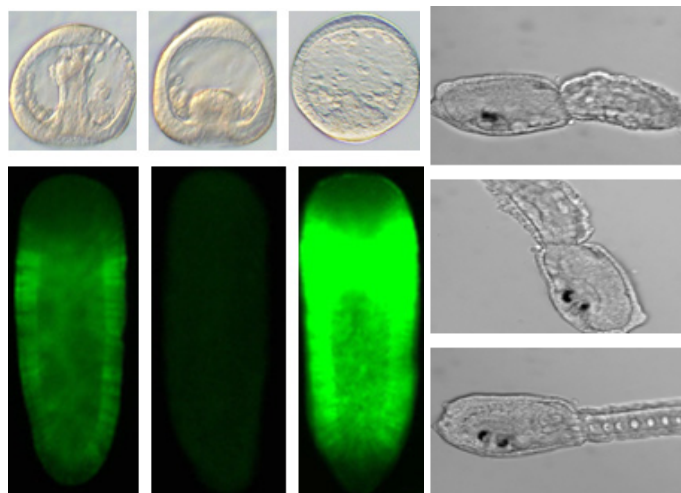
Protocols have been fully established for the sea urchin (both *Paracentrotus lividus* and *Strongylocentrotus purpuratus*) and three other marine metazoans (the cnidarian *Clytia hemisphaerica* and the ascidians (*Ciona intestinalis*, *Phallusia mammillata*). In amphioxus (the cephalochordate *Branchiostoma lanceolatum*), the results are promising but up to now the mutants are mosaic (not all cells are mutated) and more work is needed to obtain complete mutant lineages. Insertional transgenesis worked in all the four animal models, sea urchins, ascidians, *Clytia* and amphioxus. All the required material is ready to explore the brown algae *Ectocarpus*. Work is underway on the diatom *Phaeodactylum tricornutum*, with some gene knockout strains identified.

### Potential impact

Once ASSEMBLE Plus gains a better understanding of the functional genomics of the species covered, it will be possible to study many different gene functions and why not the bioactive properties of some of their gene products.

### What next?

- Work will continue on macroalgae including *Ectocarpus* and kelps, especially *Saccharina latissima*).
- Work will continue on microorganisms including (the diatoms *Seminavis*, *Cylindrotheca* and *Pseudonitzschia multistriata*, the picoeukaryotes *Ostreococcus* sp, *Bathycoccus* and *Micromonas*, the cyanobacteria *Synechococcus* and the bacteria *Kordia algicida*).



## Joint Research Activity: Developing Instrumentation

by Ian Probert

### What is ASSEMBLE Plus working on?

ASSEMBLE Plus' research team working within the 'Developing Instrumentation' activities are currently focused on developing a database featuring reviews on the aquarium systems and equipment being used by ASSEMBLE Plus research institutions. They are also interested in encouraging crossover and knowledge sharing between research institutions.

### What is ASSEMBLE Plus doing that is different?

#### *Database of Instrument Reviews*

As purchasing the aquarium systems and equipment is a rare occurrence within an organisation, the idea is that ASSEMBLE Plus will create a database that will provide partners with reliable advice if they wish to update technology or equipment within their laboratories. There is currently no other reference available and they want to fill this space. The database is currently at a prototype stage and the "Research Aquarium Infrastructure" on-line database was launched this summer. It features technical information about the categorised pieces of equipment, including cost, location and process.

#### *Knowledge Sharing in Technology Developments*

Innovations are often developed in isolation. ASSEMBLE Plus is encouraging individuals from research institutions to collaborate and share ideas as they develop new technologies, or adaptations of existing technologies. Seven "technical challenges" Working Groups have been developed to improve and harmonize design of selected categories of experimental systems. These Working Groups are entitled are tide simulation, CO<sub>2</sub> control, pH control, LEDs, multiplex systems, flow chambers and turbulence.

The achievements thus far are as follows:

- Station Biologique de Roscoff (France) and the Universidade de Vigo (Spain) have collaborated over the development of a tidal simulator. Together, they have developed three prototypes and have been managed to upscale to 500 litres, include a sensor for ultrasound levels and programmable interface, regulate pH, and, allow experimental replication with a multiple tank system. The Hebrew University of Jerusalem (Israel) improved pH regulation and the implementation of in-situ PAM (pulse amplitude modulated fluorometer) analysis in their Red Sea simulator.
- Sorbonne Université's Oceanographic Observatory of Banyuls-sur-Mer (France) adjusted their microplate experimental system to be easier to implement and get a better fit to natural spectra.
- The University of Gothenburg (Sweden) tested their systems for automating manipulation of carbonate chemistry.
- Flanders Marine Institute (Belgium) saw improvements made to their ocean acidification experimental facilities (larger tanks and smaller mesocosms) and sediment

micro-profiling system. CCMAR implemented ocean acidification facilities. The Marine Biological Association (UK) have improved their mesocosm facility.

- Stazione Zoologica Anton Dohrn (Italy) updated their seagrass culture (light, pH).

### Potential impact

Whilst there are no formal plans to commercialise the database (which will currently be accessible by ASSEMBLE Plus and EMBC members), there have been discussions about how the database could be exploited in a wider context. For example, it could be accessed externally via a "pay wall" or published freely and form of consultancy service could be provided based on this information. It is recognised that the intellectual property of such a platform could be complicated, unless information is provided on a voluntary and open basis.

### What next?

- In terms of the database, the focus is on adding and curating the submitted data, as if the database is not maintained, there will be no audience to satisfy. Data entry will continue until the end of 2019. After which, the data will be analysed to produce a technical design manual and best practice recommendations (for completion by October 2020).
- When developing new instrumentation or identifying new applications for existing instrumentation, it opens an opportunity to patent the ideas. As understanding of the patent process is limited within the scientific community – and there is a feeling that technology transfer offices are driven by numbers of patents submitted over the best interest of the knowledge owners – it would be beneficial to host a training course for ASSEMBLE Plus partners on intellectual property and patents.
- The University of Helsinki will be developing a mesocosm facility, which will be active from 2020.



## Joint Research Activity: Scientific Diving

by Martin Sayer

### What is ASSEMBLE Plus working on?

ASSEMBLE Plus' research team working within the 'Scientific Diving' activities are currently focused on developing their capabilities in stereophotogrammetry, otherwise known as "structure from motion" or "3D photogrammetry".

Stereophotogrammetry is a methodology that uses normal imagery (photos, videos, micrographs), to generate three-dimensional point clouds and stitches the images together to build a 3D image. The technique only needs images from a single camera and there is no need to control set distances or angles. Such images, if of high enough resolution, can be used to make retrospective, reliable measurements to the millimetre scale; as if the habitat is coming with you to the lab. Because the models are constructed mostly from close-up imagery, the water effectively disappears and in low visibility environments large-scale targets become totally visible, something that could never be observed with the human eye. More importantly, the amount of data that can be generated in one dive (or voyage using a drone or remotely operated underwater vehicle) is significantly better than doing it underwater. It is, therefore, time and resource efficient.

This process can have several applications such as being useful for habitat mapping, measuring the volume of coral, estimating carbonate production rates or calculating erosion or biofouling rates on underwater structures. It could also be used to capture a moment in time for a time-series or historical database; if, for example, subsequent years of volumetric changes of environments such as ice shelves were required. Those benefitting from this technique could be geologists, surveyors, archaeologists and intertidal biologists.

### What is ASSEMBLE Plus doing that is different?

With computer-based work, those doing this kind of work can sometimes place too much confidence on results that, while looking acceptable, may not be accurate. The ASSEMBLE Plus researchers are working in multiple teams, operating from the Arctic to the Mediterranean, to interrogate the results that their respective models are generating. One of the goals from the outset is to develop a standard methodology that could be applied by different teams in ways that produce the same results, and effectively be translatable between different projects.

### Potential impact

The oil and gas industry are using the same stereophotogrammetry software as used by the ASSEMBLE Plus researchers but have adapted it and are applying it in a different way, which is where their intellectual property lies. They have developed their own methodologies using unmanned underwater robots. Whilst the focus of ASSEMBLE Plus is on standardising the approach for scientific teams, they hope to find commercial opportunities for their skill sets particularly as the commercial licenses for the software and the advanced levels of computing power required are costly to resource.

### What next?

- It is hoped that the underwater observation network that has been created will further expand, and will demonstrate the value of diver-based, sub-tidal physico-chemical observation (cf. sea surface temperature).
- The next workshop will take place in December 2019 at the Hellenic Centre for Marine Research in Heraklion (Greece).



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ASSEMBLE Plus sponsors research teams for:

- Access to marine stations and institutes (see map overleaf) and their research infrastructures
- Travel and subsistence for two persons (one return trip per person, up to 30 days)

Deadline: 9th February 2020. Access window: April to September 2020.

Please check for updates on available services, call dates, application procedures and any further information on current and upcoming calls for access on: [www.assembleplus.eu](http://www.assembleplus.eu)

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