STATION MARINE
CONCARNEAU

SEA CUCUMBER AQUACULTURE: NEW CHALLENGES

October 3 & 4, 2022 at the Concarneau Marine Station

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SOUTHEAST ASIA-EUROPE JOINT FUNDING SCHEME FOR RESEARCH AND INNOVATION

Monday, October 3rd 2022

Session Fisheries and Aquaculture #1

Moderator: Igor EECKHAUT (Mons University, Belgium)

9:00-9:10	**Igor EECKHAUT, Nadia AMEZIANE & Solène AVIGNON** *Openning*
9:10-9:40	**Chantal CONAND** (MNHN/Univ. La Réunion, France) *Global sea cucumbers fisheries: an update of the past decade*
9:40-10:10	**Jean-François HAMEL** (SEVE, Canada) *Community-based emerging fisheries in Nunavut (Canadian Arctic)*
10:10-10:40	**Mercedes GONZALEZ-WANGUEMERT** (WANGUMAR SLU, Spain) *Sea cucumber aquaculture, why not?*
10:40-11:10	***Coffee time***
11:10-11:40	**Matthew James SLATER** (Alfred Wegener Institute, Germany) *The uses of European sea cucumbers in Integrated Multitrophic Aquaculture*

11:40-12:10 **Frank DAVID** (MNHN, France)

A screening of methods to differentiate wild vs. farmed European sea cucumbers

12:10-14:00 Lunch time

Moderator: Chantal CONAND (MNHN / Univ. of La Réunion, France)

14:00-14:30 **Arnold RAKAJ** *(University of Rome Tor Vergata, Italy)*

Aquaculture and IMTA applications with mediterranean sea cucumbers: progress and problems

14:30-15:00 **Marc-André LAFILLE** (French Polynesia Marine Ressources Department)

Strategic approach and sustainable development of sea cucumber farming adapted to French Polynesia context

Session Physiology and Ecotoxicology

15:00-15:30 **Igor EECKHAUT** (Mons University, Belgium)

Ovarian and oocyte maturations of Holothuria scabra *in the context of aquaculture production*

15:30-16:00 **Philippe DUBOIS** (ULB, Belgium)

Sea cucumber aquaculture in a changing world

16:00-16:30 Coffee time

16:30-17:00 **Patrick FROUIN** (University of La Réunion, France)

*Wild sea cucumber stress: what do enzymatic activities and oxidation protein products tell us about three tropical species (*Holothuria atra, Holothuria leucospilota *and* Stichopus chloronotus*)*

17:00-17:30 **Alessandra WHAITE** (Mons University, Belgium)

Tube feet and cuvierian tubules: two different adhesive systems from sea cucumbers

17:30-18:00 **Mohammad MAGDY** *(University of Rome Tor Vergata, Italy)*

Towards sea cucumbers as a new model in embryo-larval bioassays in ecotoxicological studies

Evening at Le Chantier restaurant with panoramic sea view

(20 Quai Carnot, Concarneau)

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Tuesday, October 4th 2022

Session Fisheries and Aquaculture #2

Moderator: Guillaume CAULIER (Mons University, Belgium)

9:00-9:30 **Laurent BURGY** (Tahiti Marine Products, French Polynesia)

*Development of sustainable sea cucumber farming (*Holothuria fuscogilva*) in French Polynesia*

9:30-10:00 **Bastien SADOUL *in videoconference*** (Institut Agro Rennes-Angers, France)

Latest advances in the breeding and rearing of Holothuria forskali

10:00-10:30 **Gyda CHRISTOPHERSEN** (Møreforsking AS, Norway)

Initiating reproduction in captivity – emerging species Parastichopus tremulus

10:30-11:00 ***Coffee time***

11:00-11:30 **Annie MERCIER** (Memorial University, Canada)

Latest advances on Cucumaria frondosa*: biology, fisheries and aquaculture*

11:30-12:00 **Gaëtan TSIRESY** (ISTRCE, Madagascar)

*Technical feasibility of sea cucumber farming (*Holothuria scabra*) at the pioneer site in North-Eastern of Madagascar*

12:00-14:00 Lunch time

Moderator: Philippe DUBOIS (ULB, Belgium)

Session Diseases and Parasites

14:00-14:30 **Jérôme DELROISSE** (Mons University, Belgium)

Story of a sea cucumber disease: a multidisciplinary approach of the Skin Ulceration Syndrome in Holothuria scabra

14:30-15:00 **Anchana PRATHEP** (Prince of Songkla University, Thailand)

The first observation of Skin Ulceration Disease (SKUD) in Holothuria (Metriatyla) scabra *Jaeger, 1833 from seagrass meadows in Thailand and some metagenomic studies.*

15:00-15:30 **Guillaume CAULIER** (Mons University, Belgium)

From diseases to coelomocytes : the mysterious immune system of sea cucumbers

15:30-16:00 Coffee time

16:00-16:30 **Noé WAMBREUSE** (Mons University, Belgium)

How do sea cucumbers cope with bacterial infection? Let's bring cœlomocytes into the world of omics

16:30-17:00 **Patrick LE CHEVALIER** (University of Bretagne Occidentale, France)

Plasticity and specificity of the bacterial microbiota in the Sea Cucumber Holothuria forskali

Session Economics

17:00-17:30 **Wilson IANONA** (IANONA Wilson, Madagascar)

*Looking for investors for sea cucumber (*Holothuria scabra*) farming project in the Northeastern of Madagascar in the SAVA region*

17:30-18:00 **Agnès JOLY** (Aquaprimeur, IOT, France)

Indian Ocean Trepang, the pioneer and leading private company of the West Indian Ocean producing Holothuria scabra *in aquaculture*

19:00-21:00 - A free drink at the Tri Martolod brewery

(ZA du Colguen, Concarneau)

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Session Fisheries and Aquaculture

Global sea cucumbers fisheries: an update of the past decade

Conand C.[1], Lovatelli A.[2], Shea S.[3] and Wolfe K.[4]

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[3] *BLOOM Association, Suite 2405, 9 Queen's Road, Hong Kong*
[4] *Marine Spatial Ecology Lab, School of Biological Sciences, ARC Centre of Excellence for Coral Reef Studies, The University of Queensland, St. Lucia 4072, Australia*

Sea cucumber fisheries have expanded during the past decade at a faster rate than the implementation of effective managements plans. The exploitation is now qualified as "serial" and "contagious". The most recent trends are shown through the analysis of the last ten years capture data from FAO in addition to detailed information collected from the Indian Ocean countries. Hong Kong remains the most important market for the imports and exports of processed products, mainly the dry "trepang" (or bêche-de-mer), but also for other products as the frozen commodity, which complicates the overall trade analysis and demonstrates expansion of the commercial trade to fishing vessels fitted with refrigeration technology. The data in quantities and values (Hong Kong imports and exports) are analysed for the past six years which includes the Covid-19 period. Globally, the catches have increased compared with previous studies, with new countries developing export fisheries, new species targeted from new regions and new products traded. The continued growth and expansion of the global sea cucumber trade is of concern as the vulnerability of many fished species amplifies.

Community-based emerging fisheries in Nunavut (Canadian Arctic)

Hamel J.F.[1]

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The presentation will outline an ongoing project that builds on interest expressed by Inuit communities in Nunavut (Canada) to assess the status and value of their marine resources and prospects for their sustainable use in the context of food security and climate change. It aims to expand our understanding of nearshore benthic assemblages in the Arctic, with a focus on sea cucumber resources and how they are sustained by winter-spring processes occurring at sea-ice edges. Interlinked segments include fundamental research, practical knowledge transfer and community mobilization. The fieldwork approach centers on the use of a portable remotely operated vehicle (ROV) deployed through holes in the sea ice. The ROV has minimal environmental impact; it captures videos of the benthos and can collect samples for analysis. Videos are used for scientific analyses and to create education material to be shared with end users in the host localities. A complementary segment aims to assess methods best adapted for the collection and handling of sea cucumbers. Benthic life occurring underneath or close to inshore polynyas and landfast ice edges in the Arctic is understudied. Findings thus have the potential to yield novel information while assisting subsistence fisheries and eventual commercial developments. The project brings together academic

investigators (scientists and students), the territorial government, WWF-Canada, the local Hunters and Trappers Organizations (HTOs), and other members of the Inuit communities. Ultimately, it hopes to produce conservation and technical guidelines for benthic species like sea cucumber that could be targeted for premium markets (seafood, nutraceuticals, and pharmaceuticals).

Sea cucumber aquaculture, why not?

Gonzalez-Wanguemert M. [I]

[I] *Wangüemert Fisheries Management & Aquaculture SLU - WANGUMAQUA SLU, Spain*

It is a fact, the sea cucumber demand keeps increasing, and most of their stocks are overexploited around the world, usually following a destructive boom-and-bust pattern with high level of IUU (Illegal, Unreported, Unregulated) fishing.

The fisheries management of sea cucumber is not easy, being highly conditioned by their biological features. Therefore, development of their production under aquaculture conditions seems to be the best option to protect wild stocks, create more efficient management tools (through restocking programmes), and cover the market demand under a sustainable framework.

In this talk, we will walk together along the history and main outcomes which allowed us to develop the aquaculture of two target species (*Holothuria arguinensis* and *Holothuria mammata*) in Portugal and Spain, and their later inclusion on integrated multitrophic aquaculture systems (sea cucumber & sea urchin & algae). Some "brushstrokes" about our ongoing projects focused on sea cucumber in Cabo Verde and other countries from Western Africa in collaboration with FAO, will also be shown.

The uses of European sea cucumbers in Integrated Multitrophic Aquaculture

Slater M.J. [I], Hannon C., Onomu A., Taylor D., MacDonald C., Spreitzenbart S., Stead S.

[I] *Alfred Wegener Institute, I2 Am Handelshafen, 27570 Bremerhaven, Germany*

Recent activities in testing and establishing sea cucumber multitrophic systems in Ireland, Germany and Italy will be presented. The main principles of sea cucumber integration testing; consumption of impacted sediments, survival and growth of sea cucumbers, along with remediation will be presented and discussed with a species overview for further development and identification of key bottlenecks to be addressed.

Future requirements in terms of hatchery, key finfish and shellfish species for targeted integration in Europe and ROW are discussed along with the potential for co-opting RAS systems for hatchery and nursery spaces.

A screening of methods to differentiate wild vs. farmed European sea cucumbers

David F. [1]

[1] *MNHN - Station Marine de Concarneau, Place de la Croix, 29900 Concarneau, France*

Sea cucumbers fisheries are collapsing contagiously all around the world and first signals of natural populations threatening have been revealed in Europe. Meanwhile, holothurians have been considered as the "golden gooses" for ecological aquaculture due to their ability to grow on fish wastes, thus diversifying farmer's income and reducing the environmental impact of monoculture productions. Yet, by raising the interest of Europeans for the aquaculture of sea cucumbers we may also stimulate the demand for fisheries products and possibly open a gateway to illegal fishing. We expect that providing a tool to differentiate wild vs. farmed sea cucumbers before the establishment of a market for farmed individuals in Europe, along with fishing bans, will hinder the development of illegal fishing.

Aquaculture and IMTA applications with mediterranean Sea cucumbers : progress and problems

Rakaj A. [1]

[1] *Laboratory of Experimental Ecology and Aquaculture, Department of biology, University of Rome Tor Vergata, Rome, Italy*

Integrated Multi-Trophic Aquaculture (IMTA) is a new generation aquaculture that allows for the reduction of environmental impacts, higher profits and the diversification of commercial production. This integrative approach, in fact, offers a natural means of encouraging nutritional recycling within aquaculture farms, simulating a natural community through the employment of extractive species with a low trophic level and high market value. In this context, sea cucumbers as deposit feeders species could represent promising candidates for co-culture in IMTA, considering their feeding habits and high market value. However, although their ecological role in many marine habitats and their feeding behaviour seem highly promising and compatible with extensive integration, there are only a few investigations available regarding the co-culture of Mediterranean sea cucumbers in productive systems (Tolon *et al.*, 2017; Neofitou *et al.*, 2019; Grosso *et al.*, 2021; Sadoul *et al.*, 2022; Cutajar *et al.*, 2022).

In this context, aquaculture could offer a sustainable alternative to the exploitation of the wild population, to meet current market

demand and at the same time could promote waste bioremediation nearby productive areas. With regard to Mediterranean sea cucumber species aquaculture, a consistent body of research literature has been produced in the last few years, paving the way for the artificial reproduction of many European species by developing a spawning induction methodology and larval rearing protocol (Domínguez-Godino *et al.*, 2015; Domínguez-Godino *et al.*, 2018; Rakaj *et al.*, 2018; Rakaj *et al.*, 2019; Laguerre *et al.*, 2020; Magdy *et al.*,2021; Schagerström *et al.*, 2022). Nevertheless, there are still some challenges facing sea cucumber aquaculture that need to be overcome for future aquaculture development. Here is presented the up-to-date research progress and problems and future opportunities for tow Mediterranean sea cucumber species *H. tubulosa* and *H.polii*.

Strategic approach and sustainable development of sea cucumber farming adapted to French Polynesia context

Lafille A.[1]

[1] *French Polynesia Marine Ressources Department, BP20, 98713 Papeete, French Polynesia*

Like many Pacific Islands Countries and Territories (PICTs), French Polynesia has experienced over the last thirty years, firstly, a strong sea cucumber fishery for the significant Asian markets demand, and secondly, the arrival of foreign investors proposing hatcheries associated with wild sea cucumbers fishing in order to provide the investments financing, but most often without results visibility. While French Polynesia suffered severe sea cucumbers overfishing between 2008 and 2012, regulations were put in place in 2012 on the fishing and trade, including traceability of all species of sea cucumbers marketed in French Polynesia in order to preserve the resource.

Faced again to foreign investors requests, the French Polynesia Marine Ressources Department (DRM) has been accompanied by expertise from the Pacific Community (SPC) to propose to set a sustainable development strategy for beche-de-mer aquaculture in French Polynesia. This notably involves the need to demonstrate the feasibility of breeding and rearing the targeted species, with sustainable fishery management, genetics and health management

of aquaculture stocks as well as the appropriate regulations establishment.

From 2019, as soon as two species of teatfish sea cucumbers (*H. fuscogilva* and *H. whitmaei*) are listed on the CITES's annexe II, a Research and Development (R&D) program for the sustainable farming of such species is launched. This targeted program for sea ranching is based on a partnership between researchers, developers, management qualified authorities, local authorities, loal fishermen, and above all motivated investors-aquaculturists. Its first particularly promising successes and the next key steps of the project are described with its perspectives.

Development of sustainable sea cucumber farming (*Holothuria fuscogilva*) in French Polynesia

Burgy L.[1]

[1] *Tahiti Marine Products, BP¨62056 Pamatai Hills, 98702 Faaa, French Polynesia*

Sea cucumbers are known to improve the natural productivity of ecosystems, by cleaning the sediments, recycling the nutrients and releasing phosphorus and nitrogen used by algae and corals. The white teatfish (*Holothuria fuscogilva)* and the black teatfish *(Holothuria whitmaei*), have been over exploited for decades for their nutritional and medicinal properties. In order to preserve those species, they have been listed in the Appendix II of CITES (2020).

Since November 2020, Tahiti Marine Products, produces juvenile of sea cucumbers in hatchery, without using any antibiotics or chemical treatments. Developing this aquaculture will help preserve wild stock from being overexploited and repopulate the lagoon with some farmed juveniles. Thus, aquaculture could be a primo solution for a sustainable and durable exploitation of the resource without drawing on the natural stock, and it can also be used as a management tool.

Today, these animals have successfully grown through different stages: nursery, pre-growth and grow-out. As the last phase evolves, TMP begins to focus on strategies to value animals for better profitability.

To date, a range of promising marine substances have been widely applied in the food and pharmaceutical industries. Following the same trend, the search for new bioactive substances within our farmed *Holothuria fuscogilva* is initiated.

To carry out those scientific researches a partnership is setting up between TMP, UPF, IRD, ILM, ToxiPlan, French and Swiss laboratories.

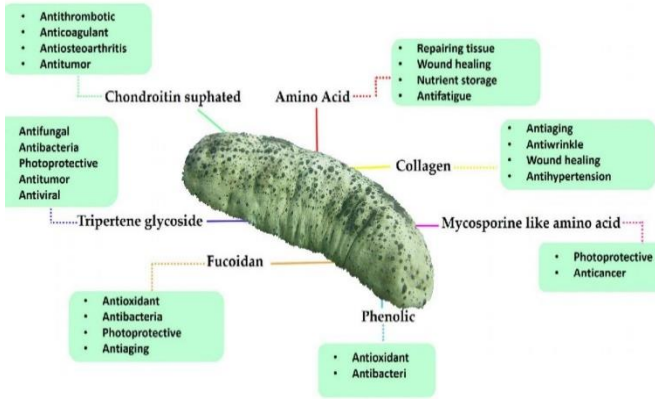

Figure I: Bioactive compounds in sea cucumber and their beneficial actions on human health.

Our goals:

- extract and characterise the collagen and the bioactives molecules present to launch a range of sustainable natural cosmetics (moisturising cream, anti-aging, peeling, scrubs and face masks)
- process (freeze-dry) the whole animal to make food supplements in capsules
- identify and isolate bioactives molecules of interest and/or discover new ones to start pharmacological research

As a conclusion, Tahiti Marine Products (TMP) aims to develop this innovative aquaculture activity in an environmentally friendly manner, in order to sustainably harvest and process the molecules found in these invertebrates for health purposes.

Latest advances in the breeding and rearing of *Holothuria forskali*

Sadoul B.[1], Raymond G.[2], Hérault G.[3], Delaporte-Blanc J.[2], Badou A.[3] and Ameziane N.[4]

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[3] *DGDREVE, National Museum of Natural History, Station Marine de Concarneau, France*
[4] *UMR ISYEB, National Museum of Natural History, Station Marine de Concarneau, France*

Holothuria forskali is widely distributed from the north east Atlantic to the Mediterranean Sea. *H. forskali* has previously been demonstrated to be able to feed on aquaculture waste and is therefore considered as a good candidate for an integrated multitrophic aquaculture (IMTA). Nevertheless, generalization of the use of this species to bioremediate waste of aquaculture production relies on our capacities to reproduce adults, develop larvae and grow juveniles in controlled conditions to supply aquaculture professionals. Work initiated in 2019 has allowed gathering extensive knowledge on these rearing steps of *H. forskali*. We are now able to reproduce individuals collected in the wild, and rear them post metamorphosis with a reasonable total survival rate (11%). Our growth conditions post metamorphosis allow us to expect great survival until the adult stage (69%). Our work also demonstrates the great growth potential of *H. forskali* underneath multiple different primary species produced in aquaculture suggesting a wide range of opportunities for this species to be included in aquaculture productions. This talk will present results at each step required for the extensive use of *H. forskali* in IMTAs; from reproduction to growth potentials.

Initiating reproduction in captivity – emerging species *Parastichopus tremulus*

Christophersen G.[I]

[I] *Møreforsking AS, 5075 PO box, 602I Alesund, Norway*

The red sea cucumber *Parastichopus tremulus* is suggested as a new marine species having potential for aquaculture. *P. tremulus* is a deposit-feeding sea cucumber occurring in the NE Atlantic and all along the Norwegian coast, mainly at some hundred meters depth. Currently the species is only available as bycatch from other commercial fisheries. Scarce knowledge of population dynamics in addition to biological and technological constraints related to a life in captivity, still hinder the development of a sea cucumber industry in Norway. Steps towards closing the life cycle is essential in developing aquaculture, and experience from studies in the Møreforsking lab related to reproduction and early life stages will be presented. Focus will be gonad development, spawning and larval development in captivity. The sea cucumbers used in our studies originate from local stocks in the fjord system close to Ålesund in western Norway (62°N, 6°E). Prospects of future juvenile production and the viability of *P. tremulus* aquaculture in Norway will be discussed.

Latest advances on *Cucumaria frondosa*: biology, fisheries and aquaculture

Mercier A.[1]

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Cucumaria frondosa is widely distributed across the North Atlantic and Arctic oceans, where exploratory and commercial fisheries have developed over that past few decades. The body wall and muscles are being marketed as seafood, but emphasis has lately been placed on developing value-added pharmaceutical and nutraceutical extracts. However, many factors that would allow sustainable exploitation rates to be established remain unknown, including catchability, size at age and natural mortality rates. While a precautionary approach is being followed in most jurisdictions, quotas have nevertheless gone up and some harvesters have already reported decreasing sea cucumber sizes and catch per unit effort for some fishing grounds on the Grand Banks of Newfoundland. Continued research into fisheries-relevant metrics and the potential development of aquaculture methods is therefore crucial.

Challenges emerge because unlike most other commercial sea cucumbers, which are deposit feeders belonging to the order Holothuriida, *C. frondosa* is a slow-growing suspension-feeding Dendrochirotida that ingests phytoplankton and other suspended organic particles. It also exhibits a distinct life-history strategy

involving annual spawning, large vitellus-rich (lecithotrophic) eggs and non-feeding larval stages. This presentation will provide an overview of recent advances in the biology and ecology of *C. frondosa* with the aim to steer its management from a high-volume low-value towards a high-value low-volume model.

Technical feasibility of sea cucumber farming (*Holothuria scabra*) at the pioneer site in North-Eastern of Madagascar

Tsiresy G.[1,2,3], Ianona W.[3] and Fofy N.[3]

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[2] *Ministère de la pêche et de l'économie bleue (MPEB)*
[3] *Société IANONA Wilson, Madagascar*

Aware of the impoverishment of sea cucumber stocks in the natural environment, Madagascar has taken initiatives since the 1990s to improve the management of sea cucumber exploitation. Stock assessment and studies on the biological cycle of certain species have been carried out. Due to the overexploitation of sea cucumbers, the first sea cucumber farm was set up in Toliara (South-West Region of Madagascar) in the early 2000s. This farm was successful and was bought by private companies in 2008. It remained the only hatchery on the Big Island for more than 20 years.

Installing its first equipment in 2018, an aquaculture farm is currently operational in Antalaha, SAVA region, North East of Madagascar. The first spawners were collected between September and November 2018 in the natural environment around Antalaha. Fertilization is caused by thermal shock. The doliolaria stage is obtained from the 14th day, and the rate of metamorphosis varies

between 12 to 18 % of which the total duration of the larval stage varies from 2 to 3 months. The larvae were fed with local strain microalgae. The pre-growth was carried out in liner basins equipped with 10 cm of marine sediment with a density of 20 individuals/m^2 and the growth rate obtained is 0.95 g/d. The enclosures for the enlargement are installed in the grass area beds where the nature of the sediment is sandy-muddy, which allowed to obtain a growth rate of 0.6 to 1.97$g.d^{-1}$ with a density of 5 individuals.m^{-2}. The duration of the breeding cycle is 18 months, until obtaining 450 g individuals size.

The next challenge is to increase growth rates throughout the rearing cycle by changing, for example: (i) the species of microalgae for larvae feed, (ii) reducing the rearing density by pre-growout and growout.

Session Physiology and Ecotoxicology

Ovarian and oocyte maturations of *Holothuria scabra* in the context of aquaculture production

Eeckhaut I.[1,2,*], Sturaro N.[1], Andriantsilonina C.[2], Rasolofonirina R.[3] and Delroisse J.[1]

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Ovarian maturation is by definition the ovarian development until reaching the stage V (*i.e.* the "spawning" stage) and oocyte maturation is the development of oocytes to ootids (the female germ cells ready to be fertilized in sea cucumbers) that are blocked in prophase I of meiosis. These are two processes that must be controlled to ensure the profitability of hatcheries in *H. scabra*. The present work has three objectives: (i) to provide for the first time comprehensive data on the productivity of ootids and embryos in industrial hatchery, (ii) to determine the effectiveness of maturation tanks on ovarian maturation, (iii) to compare the effectiveness of thermal shocks and *in vitro* fertilizations. For that purpose, the analyzes were carried out with *Indian Ocean Trepang* that is a private company producing *H. scabra* in aquaculture. We analyzed data from August 1, 2017 to December 14, 2018 where 291 fertilization trials (thermal shocks and *in vitro* fertilizations) were carried out using 6,154 females and 2,173 males. Various parameters were recorded: the number of sterile individuals, the number of large (>

69 µm) and small (< 69 µm) germ cells in ovaries, the number of ootids, the number of embryos and that of auricularia larvae. Our results show that incubation of the broodstock in maturation tanks for 10 days makes it possible to obtain more suitable ovaries: the number of germ cells <69 um decreases by a factor of 2.5, the proportion of ootids increases from 1.3 to 1.5 x and the fertilization index increases from 1.75 to 2.9 x. The analysis of various parameters also show that thermal shocks and *in vitro* fertilizations have their own advantages and disadvantages. For ease of execution, thermal shocks are advantageous all the more so as they provide greater quantities of embryos. However, during difficult periods, particularly during the cold season or when larval development goes wrong due to infection by copepods, *in vitro* fertilization becomes essential to ensure the profitability of hatcheries.

Sea cucumber aquaculture in a changing world

Dubois P.[1], Cozette J.[1] and Eeckhaut I.[2]

[1] *Université Libre de Bruxelles, Faculté des Sciences, Campus du Solbosch, Bruxelles, Belgium*
[2] *Biology of Marine Organisms and Biomimetics unit, University of Mons, Mons, Belgium*

Sea cucumber aquaculture, either at sea or on land, will have to face the effects of global change, principally ocean warming and acidification. In this presentation, we will review the documented impact of these stressors on the different life stages of sea cucumbers, identifying the most critical factors. We will tentatively assess the costs of manipulating sea water parameters to maintain these at acceptable levels in the case of a tropical aquaculture.

Wild sea cucumber stress: what do enzymatic activities and oxidation protein products tell us about three tropical species (*Holothuria atra*, *Holothuria leucospilota* and *Stichopus chloronotus*)

Frouin P.[1], Pierrat J.[1], Bourdon E.[2] and Forget-Leray J.[3]

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Reunion island hosts about 38 species of sea cucumbers. Among these, *Holothuria atra*, *H. leucospilota* and *Stichopus chloronotus* are remarkable by their high densities on shallow coral reefs (up to 9 individuals per m^2), at some locations. Despite these animals have been studied for few decades, the causes of such aggregations are still to be understood. A current programme studies population structures (including genetics), food sources and stress patterns. Individual stress was assessed by the study of 4 biomarkers for general or more specific stress: Acetylcholinesterase, Glutathione S-transferase, advanced oxidation protein products and proteasome LLVY. Muscle tissues proved to be more informative than tentacles about stress levels. When comparing species, *S. chloronotus* had higher stress level than the two other species. Selected sites included either low or high density (>I individual per m^2) populations. The seven sites were monitored over 3 seasons (I

summer, 2 winters) and results showed no spatial pattern for stress, whatever the species. No significant differences were found between winter and summer. However, interannual winter stress was higher in 2020 than in 2019. Rainfall variability could not fully explain stress variations, except for *S. chloronotus* in one site. When considering the high density populations and the rather steady stress levels beside contaminants inputs, it may be hypothesized that sea cucumbers in shallow lagoons at Reunion island are not subject to stress able to impact population dynamics.

Tube feet and cuvierian tubules: two different adhesive systems from sea cucumbers

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Many marine invertebrates produce adhesives that act in the presence of water, and this has raised growing scientific interest because of the potential to mimic such adhesives for use in biomedicine or dentistry. In our laboratory, we are studying the two different adhesive systems used by sea cucumbers, the tube feet (podia) and Cuvierian tubules. The tube feet are the external appendages of the water-vascular system, a distinctive feature of echinoderms. Tube feet are involved in tasks that require temporary adhesion underwater, such as locomotion, maintenance of position, and feeding. The use of Cuvierian tubules as a specialised defensive mechanism is restricted to a few species of sea cucumber from the family Holothuriidae. When mechanically stimulated, the animal discharges white filaments, the tubules. In seawater, Cuvierian tubules lengthen considerably and readily adhere to any close object and can therefore rapidly entangle and immobilise most potential predators. Tube feet and Cuvierian tubules differ radically in their structure and in their mode of adhesion. Using novel transcriptomic data, we also show they differ in terms of the adhesive proteins involved. Therefore, they turn out to be complementary biological models for the study of adhesion in the marine environment and both could offer novel features or performance characteristics for applications as underwater adhesives.

Towards sea cucumbers as a new model in embryo-larval bioassays in ecotoxicological studies

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Sea cucumbers are widely distributed deposit-feeders that represent an important component of benthic communities worldwide. These echinoderms were recently proposed as candidates in embryo bioassays to provide a new tool in the toxicity assessment of pollutants in marine water and sediments. The aim of this study is to evaluate the usefulness of *Holothuria tubulosa* (Gmelin, 1788), as a model organism for sensitive embryo bioassays, defining the acceptability of controls, minimum sample size, embryo density, and salinity range. Sea cucumber sensitivity to marine sediment elutriates was assessed by comparing their responsiveness in tandem with that of routinely employed sea urchin embryos. The results showed an acceptability threshold of 10% (abnormal embryos), a minimum sample size of 200 embryos, an embryo density of 200 embryos/mL and an optimal salinity range of 36–37‰. The sensitivity to the environmental pollutants and matrices tested revealed values (expressed as EC50) comparable with those of embryos belonging to other marine invertebrates commonly used in bioassays, indicating that this species has a good level of responsiveness. Data demonstrated good discrimination of sample toxicity, with a dose-dependent increase of teratogenic effects for all the tested substances, indicating *H. tubulosa* as a promising species for future assessments of marine pollution.

Session Diseases and Parasites

Story of a sea cucumber disease: a multidisciplinary approach of the Skin Ulceration Syndrome in *Holothuria scabra*

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The set-up of large-scale marine aquacultures is often associated with the emergence of new diseases and disorders. Aquafarmed sea cucumbers from the South-West of Madagascar are no exception to the rule as they suffer from specific disorders called « Skin Ulceration Diseases » (SKUD), more recently described as « Skin Ulceration Syndromes » (SUS) as multicausal origins have been highlighted according to the country or species concerned.

Our Unit uses a multidisciplinary approach to study the biology of the sea cucumber species *Holothuria scabra*, primarily in the context of its aquaculture in southwestern Madagascar. We combine field observations, *in vivo* experiments, morphological descriptions, transcriptomic and metagenomic analyses to better understand the SKUD disease affecting *H. scabra*, its potential causes, and its impact on sea cucumber physiology.

A clear seasonality of the disease is observed with a maximum peak during austral wintertime. Ulcerations are phenotypically characterized by the exposure of the connective tissue that follows the destruction of the cuticle, the epidermis, and the upper part of

the connective tissue. Experimental infection trials with crude ulcer extracts or bacteria isolated from these extracts have not induced skin ulceration. The disease is probably not induced by a pathogen or, at least, the pathogen is not found in the ulcers because the disease is not transmissible by contact. An initial cause of SKUD in Madagascar could be repeated and prolonged exposure to cold temperatures. A large variety of mRNA coding for stress-induced heat shock proteins are globally up-regulated in diseased animals indicating a general stress in SKUD-affected sea cucumbers. In parallel, actors of connective tissue restructuration are locally up-regulated within integument ulcerations. Metagenomic analyses revealed a higher proportion of Vibrionaceae (Gammaproteobacteria) in the ulcerations. Opportunistic bacteria could settle in the dermis of ulcerated individuals and promote ulcer extension. In addition, we compared our shotgun metagenomic results with other results obtained from samples collected in Thailand (Nha Trang, Southern Andaman). Our analyses revealed an extremely variable microbiota associated to sea cucumber healthy integument of various geographic locations. The predicted microbiota was also affected by the conditions (healthy vs ulcerated integument) in both locations. In Madagascar, a higher variation in the microbiota composition of ulcer samples was observed. While Proteobacteria were more abundant in ulcer samples from Madagascar, Actinobacteria were more abundant in those from Thailand. Several bacterial genera were found in ulcer samples of both locations, such as *Brucella* and *Haemophilus*.

The authors thank the company “Indian Ocean Trepang”, based in Toliara, for providing specimens and access to the pens as well as their expertise and advice.

The first observation of Skin Ulceration Disease (SKUD) in *Holothuria* (*Metriatyla*) *scabra* Jaeger, 1833 from seagrass meadows in Thailand and some metagenomic studies

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The skin ulceration disease (SKUD) is a common disease recently reported in sea cucumbers. It is mainly found in aquacultures and has been reported in several countries. We investigated the occurrence of SKUD found in Thai water by examine sea cucumbers in various seagrass meadows in Southern Thailand. There were 7 seagrass meadows of 3 provinces in the southwestern Thailand, Andaman Sea coast examined. The results showed that only 3 meadows (both intertidal and subtidal meadows) in Trang province had SKUD individuals. Thus, intensive monthly monitoring was carried out at Mook island during 2020-2021, to understand *H. scabra* population and a temporal variability on SKUD of sea cucumber. At this site, SKUD individuals tended to be found during monsoon season (July 2020 – December 2021) with 2- 16% of total observed individuals, the length of SKUD individuals was 7.6 – 23.7 cm and they were found both in tide pool and exposed habitats.

However, there was no clear relationship between environmental parameters and percentage of SKUD occurrence. We further our investigation by collected water and soil samples surrounding *H. scabra* with and without the disease, this is to better understand the cause of SKUD using molecular studies. We used metagenomics to assemble bacterial genomes and to compare microbial abundance between SKUD and healthy areas. Yet, there was no significant difference in microbial composition between SKUD and healthy samples in both soil and water. In addition, we reported some novel metagenome-assembled genomes of Alphaproteobacteria, Gammaproteobacteria, and Bacteroidia reconstructed from the datasets. This is the first study of SKUD in Thailand; and to better understand the SKUD phenomenon, which could cause a great loss of sea cucumber population both in the wild and aquaculture industry.

From diseases to coelomocytes: the mysterious immune system of sea cucumbers

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In the wild or in an aquacultivated pond, holothuroids may suffer from a wide variety of parasites and diseases that negatively impact their fitness. These threats may activate their immune system composed of free circulating cells named coelomocytes. These various shaped cells are sometimes better known by their nicknames that were given depending on their supposed activity (e.g. phagocytes, hemocytes, morula cells,...). They can be found in nearly every organ in a holothuroid and their precise roles remain unknown. When detecting a foreign particle, coelomocytes recognise it and aggregate in layers of cells that become, ultimately, a coloured body that will ultimately be ejected out of the holothuroid. This talk will summarize our previous work made on coelomocytes and will investigate how good practices and modern methodologies can help us to better understand the immune system of holothuroids.

How do sea cucumbers cope with bacterial infection? Let's bring cœlomocytes into the world of omics

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With the development of sea cucumber aquaculture worldwide, it is crucial to gain basic knowledge of the sea cucumber immune system to prevent diseases that could limit their production and threaten the sustainability of their aquaculture. Sea cucumber immunity is mediated by circulating cells – coelomocytes. Different coelomocyte types have been distinguished, and it appears that, among echinoderms, sea cucumbers have the greatest diversity. Although these cell types have been described for a relatively long time, their function(s) remain(s) incompletely understood. It may be partly because sea cucumbers are rarely considered as model organisms in comparative immunology, unlike sea urchins for example, and the use of omics tools in their studies is less systematic.

The present research aims to further our knowledge of the coelomocyte immune response to bacterial infection through omics tools, including transcriptomics and metabolomics. We focused on two species of high aquaculture interest: *Holothuria scabra* and *Holothuria forskali*. To investigate their immune response, we

performed injections of lipopolysaccharides – molecules specific to Gram-negative bacteria used to mimic a bacterial infection.

Our results show both differential genes expression and differential metabolites abundance in response to the LPS challenge. Firstly, considering the transcriptomic response, 945 genes were differentially expressed, of which 673 were up-regulated and 272 down-regulated in LPS-treated individuals. Some of these were annotated as immune genes, most of which fall into the category of pathogen recognition receptors. Secondly, concerning the metabolomic response, 6 metabolites, out of 19 identified, increased in abundance while 3 decreased in LPS-treated individuals. Interestingly, some characteristic bacterial metabolites were differentially abundant between the two conditions, suggesting a response of the microbiome to the LPS challenge. In addition, glutamate-glutamine metabolism appeared to be strongly decreased after immunostimulation.

Further research is underway to deepen our understanding of the function of specific types of coelomocytes, notably through the use of single-cell RNA-sequencing. This multi-omics approach should lead to a better understanding of how sea cucumbers cope with bacterial infections and could be useful in combating their diseases. We also hope that the development of these tools will contribute to making sea cucumbers more considered organisms in comparative immunology.

Plasticity and specificity of the bacterial microbiota in the Sea Cucumber *Holothuria forskali*

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In this study, the bacterial communities of the bacterial microbiota were characterized in the deposit-feeder sea Cucumber *Holothuria forskali*. The bacterial microbiota were analysed by both non-culturable approach with V4-I6S Metabarcoding (I) and culturable approach with the isolation of strains and antimicrobial activity assays (2). Different experiments were conducted in this species of echinoderms.

(i) Analysis of the bacterial microbiota in different tissus of animals collected in two remote sites: the Glenan's Archipelago and the Brézellec's end (Brittany, France)

(ii) Analysis of the bacterial microbiota of Holothurian stocked in tanks for few months

(iii) Analysis of the bacterial microbiota in the coelomic fluid of three Echinoderms located in the same area

(iv) Analysis of the bacterial microbiota during the cycle life of *H. forskali* (3)

(v) Analysis of the bacterial microbiota during different food diet

Most of the OTUs of the bacterial microbiota were affiliated to the phylum Proteobacteria and notably five orders: Burkholderiales, Flavobacteriales, Alteromonadales, Vibrionales and Pseudomonadales. Significant differences were observed regarding richness, biodiversity and composition between species, sampling dates and tissus suggesting hence specificity and plasticity in the bacterial microbiota of the sea cucumber.

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Session Economics

Looking for investors for sea cucumber (*Holothuria scabra*) farming project in the Northeastern of Madagascar in the SAVA region

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After carrying out surveys in the northwestern and northeastern of Madagascar in May 2018, Antalaha was chosen as the location for the experimentation and implementation of the project for a simple reason that the promoter is native of this region and have a biologist technician's team able to control the process. We try to make our contribution to the development of the country by bringing in foreign currency and creating other sources of income for the villagers besides their daily activities. The main activity of the population in this region is planting vanilla.

We used our family second home located at 15 km from Antalaha so that we could start sea cucumber farming trials in the district of Antalaha (SAVA Region) located in the northeast of Madagascar. We have adapted this place as temporary hatchery and pre-grow-out in a HDPE tank that we have installed, and the grow-out site to a HDPE enclosure placed at 36 km from Antalaha.

Our source of electricity is based on solar and thermal energy. Currently the total number of staff is 36 and the total investment cost and various operating costs since the start of the project in

2018 to date is around 2,400,000,000 Ar (about 600,000 USD) and we expect to pay it off within 3 years.

There are problems of heavy rain and cyclone in Antalaha but we have found solutions even if the latter prove to be expensive at the beginning. However, the biggest problem that has slowed down our project is finding *Holothuria scabra* spawners around our site. This problem forced us to travel from Vohémar to Cap-Est Antalaha in order to collect a hundred of sea cucumbers. Currently, we manage to secure 100,000 individuals of all sizes from 15 g to 700 g, including 7,000 spawners close to fertilization within 3 months.

Our project has a promising future, because during the 4 years of experimentation most of the problems we encountered have all been resolved and this is why we have set our objective of producing at least 50 t/year in all the grow-out sites. Thousands of households in the northern Madagascar will benefit from this project if investors will be there to support us. We are working together with villagers who could run grow-out farms.

To carry out our project, we are looking for investors for a sum of 10,000,000 USD repayable over 10 years and reimbursement is made from the 4th year to be able to export 50t of trepang, estimated at more than 5 000,000 USD in revenue per year within 3 years.

Indian Ocean Trepang, the pioneer and leading private company of the West Indian Ocean producing *Holothuria scabra* in aquaculture

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Indian Ocean Trepang company was created in Tuléar, South-West of Madagascar in 2011. Pêcherie du Sud and Aquaspark are the main shareholders. A new 500m² hatchery was built on a new site, Ankaloha, located 10 km south of Tulear. Annexed to the hatchery, a life base was created as well as the nursery now equipped with about 50 ponds of 100 m^2 (for juveniles coming from the hatchery) and 50 other ponds of 1000 m^2 (for larger juveniles).

The fattening of *H. scabra* is done in enclosures at sea. It is performed on one hand according to the "company farm" model (company employees paid for the grow-out work) with 100 hectares enclosures on the historical site of "La Mangrove" on the bay of Sarodrano, and 120 hectares since 2018 at Andamilamy, located north of Tulear. On the other hand, growout is also done according to the "village farming" model (village farmers paid for production). Currently, village farming includes 60 hectares of enclosures, insuring revenues to 400 farmers, eq. 400 families.

A trepang factory and the offices of the IOT company are located in the free zone in the port of Tulear. 220 equivalent full-time people are now employed by IOT: hatchery, nursery, sea pens,

factory, logistic, security, maintenance and administration / direction. Security counts for 40% of the company's full-time people.

About 9 tons of dried product (=trepang) are yearly sold to China, corresponding to I4M$ turnover. IOT is currently looking at markets for by-products and other markets than food. The company is also looking at other geographical areas for production outside Madagascar, and is open to other species.