Abstracts and new publications

Taxonomy of the heavily exploited Indo-Pacific sandfish complex (Echinodermata: Holothuriidae)

Claude Massin, Sven Uthicke, Steven W.Purcell, Frank Rowe and Yves Samyn

Source: Zoological Journal of the Linnean Society (2009) 155:40–59

Two commercially valuable holothurians, called the "sandfish" and the "golden sandfish" vary in colour and have a confused taxonomy, lending uncertainty to species identifications. A recent molecular study showed that the putative variety *Holothuria (Metriatyla) scabra* var. versicolor Conand, 1986 ("golden sandfish") is a distinct species from, but could hybridise with *H. (Metriatyla) scabra* Jaeger, 1833 ("sandfish"). Examination of the skeletal elements and external morphology of these species corroborate these findings. The identity of *H. (M.) scabra* has been unambiguously defined through the erection and description of a neotype, and several putative synonyms have been critically re-examined. The nomenclatorially rejected taxon *H. (Metriatyla) timama Lesson*, 1830 and *H. (M.) scabra* var. *versicolor* (a nomen nudum) are herein recognized as conspecific and are allocated to a new species, *Holothuria lessoni* sp. nov., for which type specimens have been described. In addition, the holotype, and only known specimen of *H. aculeata* Semper, 1867, has been relocated and is redescribed. It is considered a valid species. Taxonomic clarification of this heavily exploited species complex should aid its conservation and permit species-specific management of their fisheries.

Effective fluorochrome marking of juvenile sea cucumbers for sea ranching and restocking

Steven W. Purcell and Bernard F. Blockmans

Source: Aquaculture (2009) 296:263–270

Dermal spicules (or 'ossicles') of cultured sea cucumbers can be fluorescently marked with tetracycline and calcein for sea ranching and restocking but optimal immersion conditions are unknown. Lethal and non-lethal effects, and the efficacy of marking spicules in juvenile sandfish (*Holothuria scabra*), were examined under different immersion conditions. Fluorescence brightness and the proportion of marked spicules generally increased with concentration and duration of immersion. Frequency of burial (an indicator of stress) in sandfish increased with both fluorochromes at concentrations above 50 mg L^{-1}. Growth in the two-week post-marking was unaffected at immersion concentrations of 50 and 100 mg L^{-1} compared to controls, but appeared inhibited by immersion in solutions of 200 and 400 mg L^{-1} of tetracycline or calcein. Sequential marking by tetracycline (yellow) and calcein (green), in either order, showed that calcein was deposited in a higher proportion of spicules. Three other fluorochromes with disparate colors, alizarin complexone, calcein blue and xylenol orange, also marked sandfish spicules and expanded the variety of dichromic combinations. Both tetracycline and calcein fluoresced more brightly when juveniles were marked at 26 or 30 °C than at 21 °C, and this low temperature appears also to reduce the proportion of spicules marked by tetracycline. Our findings show that seawater temperature should be regulated for ex situ immersion marking. The behavioral and biological sensitivities of sandfish demand care in administering the fluorochromes. Fluorochrome immersion at 100 mg L^{-1} for 24 h at ≥26 °C provides a practical compromise-between minimizing the fitness of released juveniles and ensuring the efficacy of the markers for studies on the growth and survival of sea cucumbers stocked in the wild.

The ecological role of *Holothuria scabra* (Echinodermata: Holothuroidea) within subtropical seagrass beds

Svea-Mara Wolkenhauer, Sven Uthicke,Charis Burridge,Timothy Skewes and Roland Pitchera

Source: Journal of the Marine Biological Association of the United Kingdom
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Some sea cucumbers species are heavily exploited as bêche-de-mer for the Asian food industry and the global decline of certain highly sought after species has generated an interest in determining the ecological function of those animals within their ecosystem. This study investigated the ecological role of *Holothuria scabra*, a commercially valuable tropical species closely associated with seagrass beds. Seagrass productivity, seagrass and benthic microalgae (BMA) biomass and organic matter (OM) were measured during two exclusion experiments conducted using *in situ* cages deployed for two months both in 2003 and 2004. Density of *H. scabra* was manipulated in caged exclusions (near-zero density, 'EX'), caged controls (natural densities, 'CC') and uncaged controls (natural density, 'NC'). Seagrass growth was lower when holothurians were excluded (5% in 2003, 12% in 2004). Seagrass biomass decreased in all treatments, but reduction was greater in EX than in controls (18% in 2003, 21% in 2004). Both BMA biomass and OM increased in EX

compared to NC/CC (in 2004). From a multivariate perspective, a principal component biplot separated EX from both types of controls in 2004, and multivariate tests based on four attributes supported this separation. These results indicate that seagrass systems may suffer in the absence of holothurians; however, the effect size varied between the two experiments, possibly because experiments were conducted at different times of the year. Nevertheless, our results suggest that holothurian over-fishing could have a negative impact on the productivity of seagrass systems.

A new method to induce oocyte maturation in holothuroids (Echinodermata)

Aline Léonet, Richard Rasolofonirina, Ruddy Wattiez, Michel Jangoux and Igor Eeckhaut

Source: Invertebrate Reproduction and Development (2009) 53(1):13–21

Oocyte maturation in sea cucumbers is stopped during the meiosis at prophase I. Maturation naturally concludes just before spawning leading to a mature oocyte ready to be fertilized. Although thermal shocks applied on mature individuals can induce spawning, it is currently not possible to perform reliably *in vitro fertilization of sea cucumber oocytes. The present paper reports the discovery of* a new and highly effective oocyte maturation inducer (OMI), called MIF (for Maturation Inducing Fractions). MIF's effects on oocytes of the marketable species *Holothuria scabra* were analysed and compared to that of the few OMIs described in literature. MIF induces the maturation and fertilization of more than 90% of oocytes while other OMIs [1-Methyladenine, dithiothreitol (DTT), dimercapto-propanol (BAL) and L-cysteine] induces 28 to 90% of oocytes to mature depending on the tested inducer. Moreover the use of the other OMIs results in fertilization rates that never exceed 40%, and the obtained larvae often present developmental abnormalities. MIF's action on *H. scabra* oocytes is efficient yearlong even outside the spawning period of *H. scabra*. MIF is effective on the oocytes of all aspidochirote species tested so far but only when it is prepared from spawns of regular female sea urchin.

Coral Reef Sea Cucumbers in Malaysia

Kamarul Rahim Kamarudin, Aisyah Mohamed Rehan, Ahmad Lutfi Lukman, Hajar Fauzan Ahmad, Mohd Hanafi Anua, Noor Faizul Hadry Nordin, Ridzwan-Hashim, Rosnah Hussin, and Gires Usup

Source: Malaysian Journal of Science (2009) 28(2):171–186

This study aims to document species presence and distribution of sea cucumbers (Echinodermata: Holothuroidea) in Malaysia. Several coral reef habitats in Peninsular Malaysia, West Malaysia and Sabah, East Malaysia were selected as study sites. In summary, the present data showed the-presence of 50 species of sea cucumbers from three orders and seven genera, with 34 species require further species identification. It was found that Order Aspidochirotida in general and genus *Holothuria* in particular were the major species classes. The most dominant species in Malaysia was *Holothuria leucospilota*. As many as 37 species were found in Sabah, 21 species were recorded in Peninsular Malaysia and 10 species were present in both regions. Of 15 *Actinopyga* species, 14 species recorded were from Sabah. These findings may be due to the extensive distribution of coral reefs and low level of marine pollution. However, the possibility of biogeography factors within and out of the Sunda Platform area cannot be ruled out. In contrast, low level of species diversity was observed in few study sites in Peninsular Malaysia especially in Langkawi Island possibly due to anthropogenic threats. Future studies including more study sites and molecular phylogeny are to be incorporated in order to obtain better view on the presence and distribution of sea cucumbers in Malaysia.

Origins and biomechanical evolution of teeth in echinoids and their relatives

Mike Reich and Andrew B. Smith

Source: Palaeontology (2009) 52(5):1149–1168

Echinoid teeth are without doubt the most complex-and highly specialized skeletal component to have-evolved in echinoderms. They are biomechanically constructed to be resilient and tough while maintaining a self-sharpening point. Based on SEM analysis of isolated tooth elements collected primarily from the Ordovician and Silurian of Gotland, we provide a detailed structural analysis of the earliest echinoderm teeth. Eight distinct constructional designs are recognized encompassing various degrees of-sophistication, from a simple vertical battery of tooth spines-to advanced teeth with multiple tooth plate series and a reinforced core of fibres. These provide key data from which we reconstruct the early stages of tooth evolution. The simplest teeth are composed of stacked rod-like elements with solid calcite tips. More advanced teeth underwent continuous replacement of tooth elements, as a simple self-sharpening mechanism. Within echinoids tooth design was refined by evolving thinner, flatter primary plates with buttressing, allowing maintenance of a sharper and stronger biting edge. Despite the obvious homology between the lanterns of ophiocistioids and echinoids, their teeth are very different in microstructural organization, and they have

evolved different self-sharpening mechanisms. Whereas echinoid teeth evolved from a biseries of mouth spines, ophiocistioid goniodonts evolved from a single series of mouth spines. Rogeriserra represents the most primitive known battery of tooth elements but its taxonomic affinities remain unknown.

Status and management of the sea cucumber fishery of La Grande Terre, New Caledonia

Steven W. Purcell, Hughes Gossuin and Natacha S. Agudo

Source: WorldFish Center Studies and Reviews N° 1901 (2009). The WorldFish Center, Penang, Malaysia. 136 p.

In New Caledonia, the sea cucumber fishery has operated since the 1840s. In 2007, the reported export value of sea cucumbers from New Caledonia (~5.3 million USD) was twice that of tuna – ranking it the second-most valuable marine export commodity after farmed shrimp. This project was conducted by the WorldFish Center from October 2006 to May 2008, under support from the national ZoNéCo programme. We used field population surveys, landing surveys, socio-economic surveys with fishers and processors, and a stakeholder workshop to evaluate the sea cucumber fishery of the main island, La Grande Terre, and provide recommendations for its management.

Sea cucumbers, trochus and giant clams were surveyed on 50 lagoon and barrier reef sites using stratified, replicate, belt transects that were geo-referenced using GPS technology. More than 6,000 sea cucumbers were counted. Additionally, we measured and weighed 1,724 sea cucumbers, of medium or high value, collected along the 1,475 transects. We used structured questionnaire-based interviews of 26 fishers and seven processors to describe the social context of the fishery. We measured and weighed 2,433 individual sea cucumbers from a total of 54 landings from fishers among the six study regions.

About 12 sea cucumber species of high and medium value are harvested. Distributions were quite patchy for most species. On average, we observed 8 sea cucumber species per site. Species richness was similar between the two Provinces and between reefs in reserves and those open to fishing. Populations of a few commercial species appear depleted, namely *Holothuria fuscogilva*, *Holothuria lessoni* and *Actinopyga lecanora*. Several other species were relatively sparse, namely *A. mauritiana*, *A. miliaris* and *H. scabra*. Most of the other commercial species were relatively common and have breeding populations at some sites that should allow for some further recruitment.

A comparison of size-frequencies of sea cucumbers in landing and those from field surveys suggest that there was some selection by fishers for larger individuals, but not in all regions. Most of the sea cucumber fishers are men aged 30–50 years with many years of fishing experience. Sea cucumbers were the most important source of income for most of the fishers and many of them only spent a couple of days fishing each week. The catch-per-unit-effort (CPUE) of fishers varied among regions. Fishers in Province Nord processed their own catches more often than fishers in Province Sud because they are further from processing centres. Compared to perceived historical CPUE, estimates of current CPUE from landing and interviews with fishers indicate that catch rates have declined in some regions. The CPUE of fishers has increased near Nouméa and further south, but the catch has broadened to include many low-value species that can dominate the catch volume.

Some stocks of sea cucumbers in New Caledonia can probably sustain further fishing impact, at modest levels. Stocks for some other species are low or depleted and management regulations should be brought in to ensure their breeding populations do not decline further. Fishers in some areas are still harvesting sea cucumbers intensely even though the average sizes of animals have declined and even though they believe the abundances have declined. The capture of some small animals and responses from questionnaires shows that more education of fishers is needed through regular visits by fisheries officers.

We propose and discuss 13 recommendations for actions to be taken by the fisheries services and fishery regulations to be imposed on fishers. In particular, we propose fishing closures of several species and advise regulations to limit industrial-type fishing. A management plan needs to be rapidly established in the Provinces of New Caledonia that will safeguard the reproductive potential of sea cucumber populations and their biodiversity on reefs. We recommend an adaptive management approach, whereby the management plan can be changed over time through new information from the social-ecological system.

Elucidation of molecular diversity and body distribution of saponins in the sea cucumber *Holothuria forskali* (Echinodermata) by mass spectrometry

Séverine Van Dyck, Pascal Gerbaux, Patrick Flammang

Source: Comparative Biochemistry and Physiology (2009), Part B 152:124–134

Sea cucumbers contain triterpene glycosides called saponins. We investigated the complex saponin mixture extracted from the common Mediterranean species *Holothuria forskali*. Two different body components were

analyzed separately: the body wall (which protects the animal and is moreover the most important organ in terms of surface and weight) and the Cuvierian tubules (a defensive organ that can be expelled on predators in response to an attack). MALDI/MS and MALDI/MS/MS were used to detect saponins and describe their molecular structures. As isomers have been found in the Cuvierian tubules, LC/MS and LC/MS/MS were performed to identify each saponin separately. Twelve saponins have been detected in the body wall and 26 in the Cuvierian tubules. All the saponins from the body wall are also present in the Cuvierian tubules but the latter also contain 14 specific saponins. The presence of isomeric saponins complicated structure elucidation for the whole set but 16 saponins have been described tentatively. Among these, 3 had already been reported in the literature as holothurinosides A and C, and desholothurin A. Molecular structures have been proposed for the 13 others which, in the present work, have been provisionally named holothurinosides E, F, G, H, I, A1, C1, E1, F1, G1, H1 and I1 and desholothurin A1. The diversity and organ specificity of the saponins described here are much higher than what had been reported to date in any sea cucumber species.

Reproductive biology of *Actinopyga echinites* and other sea cucumbers from La Réunion (Western Indian Ocean): Implications for fishery management.

Sophie Kohler, Sylvie Gaudron and Chantal Conand.

Source: Western Indian Ocean Journal of Marine Science (in press)

The sea cucumber fishery is important in several countries of the western Indian Ocean (WIO) but is generally not adequately managed. A regional MASMA programme (Marine Science for Management) granted by WIOMSA (Western Indian Ocean Marine Sciences Association) is providing data on the reproduction of some commercial species. In La Réunion, the 2 target species are *Actinopyga echinites* and *Holothuria leucospilota*. These sea cucumbers are very abundant on the fringing reefs and were sampled monthly during 2005–2006. Data on the population structure and on the reproductive cycle of *Actinopyga echinites* are presented here. The main results are: 1) gutted weight (EW) distribution of individuals within the population of Planch'Alizés site is plurimodal with a main mode at 85–95g, 2) sex-ratio is skewed toward females, 3) anatomy of gonads is described in 5 maturity stages, 4) a seasonal reproductive cycle with a major spawning event in December–January and a minor spawning event in April, 5) size at first sexual maturity EW_{50} equal to 45 g is determined from another site (a sea grass bed with juveniles). These results are integrated with data from other holothurian species such as *H. leucospilota*, *H. atra* and *Stichopus chloronotus* previously studied in La Réunion and will be useful for research on the reproductive biology of sea cucumbers conducted in the other countries of WIO. 'Seasonal closure' using results on the spawning season during the warm waters period and 'minimum size' using size at first sexual maturity are tools for enhancing sustainable management of the fisheries.

PhD THESIS ABSTRACTS

Phylogeny, systematics, population dynamics and nutrition of aspidochirote holothuroids (Echinodermata) in an Algerian *Posidonia oceanica* meadow

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The aspidochirotid holothurians commonly named "sea cucumbers" are major component of the *Posidonia oceanica* meadow. They play an important role in recycling the organic matter and the oxygenation of the bottom sediment.

The systematic study of Mediterranean aspidochirotid holothurians was established in the 19th century, when all these species were described on the morphological and anatomical level. The identity, the validity, and limits of these species has been the subject of considerable debates through the 20th century, mostly because there is substantial intraspecific variations, and limited interspecific differentiations among them. Until now species limits and relationships in this assemblage have not been explored with molecular techniques. Thus, our study proposes to describe and determine the phylogeny, the systematic, the population's dynamics and the nutrition of this class of Echinodermata in the Algerian coasts.

The systematic study was carried out, by using modern molecular systematic and morphological methods (clustering). These two methods enabled us to differentiate six sampled holothurians forms [*Holothuria (Holothuria) tubulosa*, *Holothuria (Roweothuria) polii*, *Holothuria (Holothuria) stellati*, *Holothuria (Panningothuria) forskali* and both morphotypes of *Holothuria (Platyperona) sanctori*]. Many individuals were sampled for each species in various localities of the Algerian coast in particular in the two studied stations: Sidi-Fredj and Tamentefoust. These individuals were photographed alive, their DNA was analyzed starting from fraction of gene (rRNA 16S) and the morphology of their endoskeleton was described and measured. This study has led to a definitive test of species boundaries in the Mediterranean species and showed that:

- The two color morphs of *Holothuria (P.) sanctori* that have been debated in some of the literature are genetically identical and thus represent the same species;
- *Holothuria (H.) stellati* whose confusion was always admitted, is genetically distinct and well defined species and present characteristics which characterize it as well on the morphological and genetic levels;
- *Holothuria (H.) tubulosa* the most common species, and "best known" species in the Mediterranean Sea, is not one species, but two cryptic species that have not been previously recognized or even suspected. We determined their distinctiveness on genetic, morphologic, anatomic and endoskeleton levels;
- However few specimens of holothurians analyzed in our collection, have given unusual sequence data. It remains to confirm them by analyzing other sequences data using other molecular markers to interpret these specimens. However, it is clear that one specimen will probably represent either another species previously unknown, or a hybrid between the two known species [i.e. *H.* (*R.*) *polii* and *H.* (*H.*) *stellati* for the non identified specimen *Holothuria* (*R.*) *polii* B].

The seasonal evolution of biomass/ density ratio exhibited a maximum in summer and a minimum in fall for both species [*H.* (*H.*) *tubulosa* and *H.* (*R.*) *polii*]. The minimum value of biomass/ density ratio may be interpreted as an indication of recruitment. The mean abundance of *H.* (*R.*) *polii* was significantly lower in the polluted station (Tamentefoust) than in unpolluted station (Sidi-Fredj). The collected data confirm the importance of *H.* (*R.*) *polii* as an indicator of pollution.

The analysis of digestive contents of the various species illustrates the alimentary specificity of each species: The holothurians, which ingest the coarse and fine sediment [*H.* (*H.*) *tubulosa*, *H.* (*L.*) *polii* and *H.* (*H.*) *stellati*]; the holothurians that select fine and very fine sediment [*H.* (*P.*) *forskali* and *H.* (*P.*) *sanctori*]. Concerning the selectivity of organic matter, *H.* (*P.*) *forskali* is the most selective species followed by *H.* (*P.*) *sanctori*, *H.* (*H.*) *tubulosa*, *H.* (*H.*) *stellati* and *H.* (L.) *polii.*

Characterisation of an agent that induces oocyte maturation in aspidochirote sea cucumbers including *Holothuria scabra* (Jaeger, 1833), a high market-value species

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(Developer: Igor Eeckhaut)

Certain sea cucumbers such as *Holothuria scabra* are considered highly nutritional food resources by Asian communities. Intensive harvest of these animals has brought about a sharp drop in their populations over recent decades, particularly along the coasts of Madagascar. This very alarming situation gave rise to an inter-university project involving the marine biology laboratories of the U.L.B. (*Université libre de Bruxelles*) and the University of Mons along with the Tulear Fisheries and Marine Sciences Institute in Madagascar. This project led to the creation of a company, "Madagascar Holothurie SA", the first of its kind in the western Indian Ocean. The company's goal is to sell farm-raised sea cucumbers. One of the major hurdles that have to be overcome in order for this kind of company to be profitable is getting a maximum number of specimens to sell in as short a time as possible. So, at the beginning of the farming process, you need to have as many fertilised eggs as possible. However, when sea cucumber oocytes are removed by dissection, they remained blocked at prophase I of meiosis, a blockage that is only naturally removed during spawning. There are, of course, various techniques to induce spawning but these methods are very spotty and can only be used during the sea cucumber's reproduction period, which in Madagascar goes from November to March. This Belgian-Malagasy project has developed a very unusual patented procedure to make it possible to have millions of fertilised eggs on a continuous basis, i.e. anytime of the year. This process is based on *in vitro* maturation and fertilisation of oocytes removed from genitors. It involves the use of a new extract called nirine. Nirine induces maturation in sea cucumber oocytes that are blocked in prophase I of meiosis and would only naturally be unblocked by spawning. Our work is designed to understand the action mechanism behind nirine's active ingredient, determine its nature, isolate and identify it. The sea cucumbers used for this experiment were *Holoturia scabra*, a high market-value Indo-Pacific species found in Madagascar, and *Holothuria tubulosa*, a Mediterranean species tested in our laboratory in Belgium.

Chemical defences in marine invertebrates: Body distribution, abundance and eco-physiological role of saponins in holothuroids (Echinodermata)

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(Supervisor: Patrick Flammang)

Holothuroids (sea cucumbers) are benthic marine invertebrates belonging to the phylum Echinodermata. The skeleton of these organisms is strongly reduced making the body wall flexible but also more vulnerable when facing predation. Among their anti-predatory mechanisms, two defence systems have been the subject of numerous studies: the Cuvierian tubules, a physical system only present in some species belonging to

the family Hothuriidae, and the saponins, a chemical system common to all holothuroid species. Cuvierian tubules are little caeca, attached to the basal part of the left respiratory tree, which can be expelled outside of the animal in response to a potential aggression. Following their expulsion, tubules lengthen and become sticky when they enter in contact with their target. They can therefore entangle the predator, giving the holothuroid the opportunity to crawl away. As for saponins, they are triterpene glycosides contained in the body wall and the viscera of holothuroids. These secondary metabolites seem to be deterrent to all non-specific predators. Saponins appear to be particularly concentrated in the Cuvierian tubules, suggesting a particular relationship between tubules and saponins, a relationship probably linked to the defensive function. However, until now, studies performed on the saponins of the Holothuriidae have rarely made the distinction between the body wall and the Cuvierian tubules. The present work reports the study of saponins in these two body compartments for six holothuroid species from the Mediterranean Sea (*Holothuria forskali*) and from the Indian Ocean (*Actinopyga echinites*, *Bohadschia subrubra*, *Holothuria atra*, *Holothuria leucospilota* and *Pearsonothuria graeffei*). This study focuses on the detection, the description and the quantification of the saponins in all these species, as well as on the tissue localisation and the biological effects of the saponins of *H. forskali*.

Several mass spectrometry techniques were used to make a complete study of the body wall and Cuvierian tubules saponins of *H. forskali*. Matrix Assisted Laser Desorption/Ionisation (MALDI) and Electrospray Ionisation (ESI) methods were used to produce ions corresponding to the different saponins in gaseous phase. Information allowing the complete characterisation of these different ions (molecules) was obtained by performing tandem mass spectrometry experiments. Twelve saponins were then detected in the body wall and 26 in the Cuvierian tubules. All the body wall saponins are common to both tissues, 14 saponins are thus specific of the tubules. The presence of isomeric saponins detected by LC-ESI/MS complicated the elucidation of molecular structures. However, sixteen saponins were described including 3 previously described congeners known as holothurinosides A, C and desolothurin A. The 13 new ones were named holothurinosides E, F, G, H, I, A_1, C_1, E_1, F_1, G_1, H_1, I_1 and desholothruin A_1. This last one has recently been detected in the species *Bohadschia argus* in which it was named arguside E.

The saponin extraction and analysis methods that were established for *H. forskali*, allowed the realisation of a complete comparative study of the body wall and Cuvierian tubules saponins of the 5 tropical species (except for *H. atra* which lacks tubules). These saponins were classified into two categories; non-sulfated saponins detected in *B. subrubra*, *H. leucospilota* and *P. graeffei* and sulfated ones detected in *A. echinites*, *H. atra*, *H. leucospilota* and *P. graeffei*. The total number of saponins differs among species but there is also an important intra-species variation, between the body wall and the Cuvierian tubules. A semi-quantitative study, using two complementary methods (the measurement of the hemolytic activity and the orcinol reaction) allowed to show that the concentrations of saponins are always higher in the Cuvierian tubules than in the body wall, and also that the different species enclose saponin quantities varying in a ratio of 1 to 8.

The precise localisation of saponins in the holothuroid tissues had never been studied until now. The first method we used, a histochemical labelling on sections using lectins, did not yield convincing results. On the other hand, the use of Imaging Mass Spectrometry (IMS) led to interesting results and, moreover, highlighted differences between the tissues of stressed and relaxed individuals. In the body wall of relaxed individuals, saponins are mainly localised at the level of the epidermis, except the saponins detected at *m/z* 1287 and 1303 which are rather localised at the level of the mesothelium. In stressed individuals, these last ones are no longer detectable in the body wall, moreover, the epidermal localisation of the other saponins is more pronounced. These results have been confirmed by classical mass spectrometry analyses (MALDI) on saponins extracts of both internal and external fragments from the body wall. It is interesting to note that relaxed individuals of *H. forskali* seem to release only one saponin in their close surroundings (holothurinoside G) while stressed individuals secrete 6 saponins: 4 known congeners (holothurinosides C, F, G and desolothurin A) and 2 new ones detected at *m/z* 1301 and 1317. Eco-physiological experiments were realised to investigate the effect of saponins secreted by the body wall on potential fish predators. Saponins have been either added directly in the seawater or presented in pellets as food. Only the highest doses of saponins seem to have an effect on fish (deterrence to highly concentrated pellets or even death at high level of saponins in seawater). The different observations suggest that saponins of *H. forskali* could act as olfactory aposematic signals.

The whole body of results indicates that there are important qualitative and quantitative variations in the saponin content of the different species of Holothuriidae, but also between the different body compartments of a given species. These differences could be linked to the evolutionary history of the family. The various mass spectrometry techniques were well adapted to the study of saponins in holothuroids for their easy use as well as for their rapidity of execution.

NEW PhD THESIS

PHD student: Guillaume Caulier

(Supervisors: Patrick Flammang, Igor Eeckhaut)

Symbiotic relationships are vital to ecosystem structure. These interspecific relations require a means for the host and symbiont to recognise each other. The type of communication that makes this host selection possible is generally chemical and involves olfactory signals, known as kairomones, that have the power to attract a commensal or parasite host. Although the existence of kairomones has already been proven by a large number of studies, the exact chemical nature of these metabolites has never been revealed in marine symbiotic relations. This project will mainly concentrate on identifying kairomones and on their effects, which allow sea cucumbers and Crinoidae species to be recognised by their respective hosts (i.e. the sea cucumber crab *Lisocarcinus orbicularis* (Dana 1952) and the shrimp *Synalpheus stimpsoni* (De Man 1888). Behavioural experiments will be carried out to study the ability various kairomones have to attract symbionts. These compounds will be identified through mass spectrometry and chemoreceptors will be studied by electron microscope and IMS (Imaging Mass Spectrometry).

PhD THESIS UNDERWAY

Biology of symbiotic relationships in the marine setting: Characterising the epidermal and gill adaptations of Carapidae fish associated with echinoderms

Maité Todesco

(Supervisor: Igor Eeckhaut)

Biological diversity in coral reefs is extremely rich. Very close associations between interspecific organisms, known as symbiotic relationships, are very diverse there and involve a wide range of species. Some lesser-known fish, such as *Onuxodon*, *Carapus* and *Encheliophis* from the Carapidae family (Ophidiiformes), have lifestyles that are very similar to clownfish, which are acclimated to their anemones. These anguilliform fish do, in fact, have the ability to penetrate and remain inside various invertebrate hosts such as ascidians, bivalves, starfish and, more commonly, sea cucumbers. They live in their pharynxes, mantle cavities, body cavities or respiratory trees (i.e. the sea cucumber respiratory system). Only a dozen species from the genera *Carapus* and *Encheliophis* have the ability to live with echinoderms.

Recently, numerous aspects of symbiotic Carapidae biology have been elucidated. The main points of their biology, which have now been clearly established, can be summarised as follows: 1) *Carapus* are commensals that only use their hosts for shelter, while *Encheliophis* are parasites that mainly feed on the gonads of echinoderms; 2) their life cycles include a free larval stage; 3) these fish are opportunistic and most often infest more than one host species; 4) a single echinoderm very rarely hosts several Carapidae species at one time; and 5) these fish can communicate by emitting sounds.

Carapidea that are associated with echinoderms seem to be unaffected by their hosts' means of defence. In particular, those associated with sea cucumbers are not harmed by the Cuvierian tubules, defence organs consisting of several hundred tubules that attach to the base of the left respiratory tree. These tubules, which are expulsed when a specimen is disturbed, are sticky and toxic. Their toxins (also found in the epidermis of asteroids and sea cucumber) are saponins, strongly hemolytic triterpenoid derivatives. In the natural setting, these toxins mainly have a dissuasive effect on predators such as crabs and fish but they are lethal in aquariums. Carapidae are remarkable in that they do not become sticky when they come into contact with the Cuvierian tubules. In addition, preliminary observations have shown that when Carapidae are placed in aquariums with other fish and Cuvierian tubules, all the fish die within three minutes except for the Carapidae. In any event, Carapidae must have some remarkable adaptation in terms of their epidermis, mucus and gills as is the case with clownfish that are acclimated to their anemones. My work concentrates on these adaptations. In particular, it covers microscopic and biochemical characterisation of these three structures in those Carapidae, which are echinoderm symbionts. It is composed of three complementary sections (i.e. a physical-behavioural study, a microscopic analysis of the structures mentioned above and a biochemical analysis of the composition of their membrane mucus and sterols).