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The Marine Environment: An Uncharted Resource for Drugs

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ABSTRACT

There is a strong "eco-bio-chemo-diversity" rationale to search the marine environment with its unique abiotic and biotic habitats for potential drug leads for serious pathological conditions such as cancer, infectious diseases, neurodegeneration and pain. Compounds produced by marine invertebrate organisms and their associated microorganisms that thrive successfully in five unique types of marine ecosystems are presented here as promising candidates for drug development. Marine organisms have evolved these chemicals to mediate specific biological and ecological interactions for their growth, development, defense and survival.

Keywords:
Philippine blue economy, marine drug discovery, eco-bio-chemo-diversity

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INTRODUCTION

Evolutionary biology is driven by natural selection for ecological systems that emerge as successfully adapted to a particular geo-physico-chemical or abiotic environment. Competitive and cooperative interactions among organisms in the ecosystem ensure the success of the ecosystem, wherein ecological interactions are mediated by biomolecular interactions of small, medium-sized and large biomolecules, between or among macro- and micro-organisms. Ecology drives the evolution

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of organisms through adaptive biology, physiology and biochemistry.

Underlying these adaptations are genetic mutations that result in new genotypes that encode new functional biomolecules (new chemotypes), that define new phenotypes over the long term. These in turn drive the creation of renewed, adapting ecosystems, including ecological niches. Ecology drives biology, and biology drives chemodiversity; likewise, biomolecular mutations drive biology and ecological adaptation and evolution. This is the underlying hypothesis of our research which provides models for marine drug discovery, delivery and development; in particular, to address the problem of drug resistance of cancer cells and infectious microorganisms, and the need to develop new drugs.

In the past two decades, combinatorial chemical libraries produced by large pharmaceutical companies have not fulfilled the promise of producing new drugs for serious diseases or pathological conditions. This is due to the lack of chemical diversity of these libraries which were produced mostly based on peptide structure libraries, and therefore lacked the 3D-strcutural diversity of a wide range of chemical classes of compounds. On the other hand, marine natural products or marine secondary metabolites that have evolved in marine ecosystems to mediate ecological and biological interactions, and therefore have already been successfully field-tested throughout evolution, provide the extreme breadth of chemical structural diversity required to interact with unique pharmacological, physiological and molecular targets. It is thus time to return to creating field-tested eco-bio-chemo-diversity libraries as sources of new probes and drug leads for human diseases.

Over the years, we have focused on several marine macro-organisms and their associated microorganisms as the subject of our research. In the early years, we worked on sponges, the earliest metazoans (multicellular animals) found at the bottom of the phylogenetic tree. Sponges, being sedentary and physically defenseless without a hard covering, evolved diverse secondary metabolic pathways that produce an arsenal of chemicals with growth inhibitory, regulatory and cytotoxic properties, required for their defense and survival, in the presence of other organisms which prey on them or compete with them for space in coral reefs. There is thus a strong eco-rationale for discovering anticancer and anti-infective drug leads from sponges.

We expanded our interest to a more phylogenetically advanced, megadiverse group of marine organisms called mollusks, initially focusing on predatory, venomous gastropods, for which a strong eco-rationale exists for discovering neuroactive drugs for pain and neurodegeneration. These snails produce venom consisting of neuroactive peptides that are used to hunt, pacify and paralyze prey, to defend against predators, and to deter competitors. More recently, we have been studying bivalve mollusks called shipworms (Teredinidae). Locally known as the edible *tamilok*, shipworms are known to harbor bacteria that break down cellulose from wood where the shipworms live, as a source of food and energy.

Further, we are isolating communities of bacteria associated with tissues (unique microhabitats) of sponges, gastropods and shipworms, pursuing the hypothesis that these macro-organisms evolved successfully with associated bacteria. The strong eco-rationale for discovering new drugs from these bacteria is that they produce small, bioactive molecules that significantly contribute to the defense, survival and success of host macro-organisms in a particular marine ecosystem (Figure 1).

Based on our hypothesis, here we present five marine ecosystems that we have been studying, from which we have discovered bioactive compounds as drug leads, and derived insights relating to the mechanism of action of a drug, its delivery and development.

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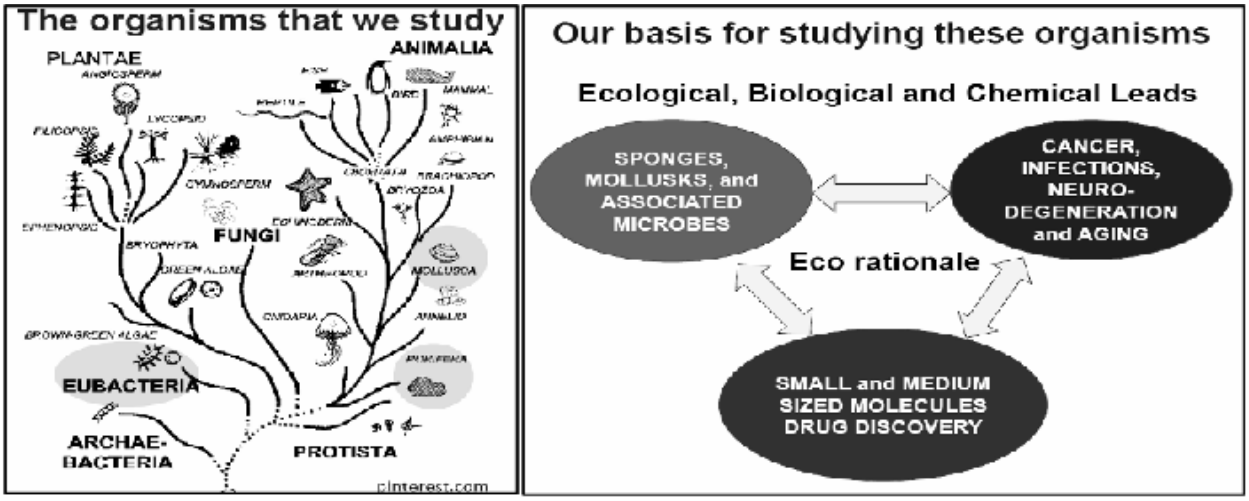

Figure 1. Evolutionary tree showing some organisms of special interest for drug discovery and basis for studying these organisms.

MARINE ECOSYSTEM 1: SPONGES IN CORAL REEFS

Philippine coral reefs are found to possess a high biodiversity and low biomass of organisms. Coral reefs, with unique 3D spaces, i.e., hard, rough rocks and light-and-dark niches, and with generally limited light, oxygen, and nutrients, represent a highly eco-biodiverse environment. They serve as unique habitats for intimate, unique competition-cooperation interactions between diverse macro-organisms such as sponges that abound in and around them. These interactions are mediated by a combination of cytotoxic, growth-regulatory (-modulatory, -inhibitory, -stimulatory) compounds many of which have been isolated from sponges and other coral reef organisms. The dynamic, finely-tuned balancing of competitive-cooperative interactions among organisms limits the growth and reduces the biomass of each organism, and further drives up the "niche" bio-chemo-diversity of organisms.

Bacteria or microbiomes (communities of bacteria) associated with macro-organisms play an important role in mediating these interactions. Genetic regulation to produce and secrete growth-regulatory compounds, as needed, is simpler, faster, and more efficient in bacteria than a sponge. Switching off the genes that produce the compounds is also easier. Further, bacteria are motile or mobile, unlike macro-organisms. Thus, it is hypothesized that bacteria play a significant role in growth regulation and dynamic balancing of organisms in an intimate ecosystem such as a coral reef, serving as "bioactive compound transporters or carriers", much like ATP is an energy carrier molecule. It is suggested further that such a marine ecosystem could serve as a model for the combinatorial synergy of compounds, or what industry calls synthetic lethality combinations, to control the growth of cancer cells or infectious agents (Figure 2).

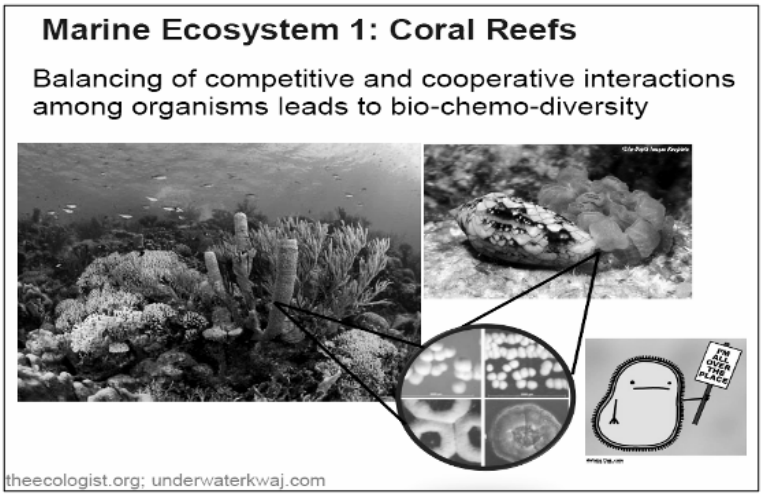

Figure 2. Coral reef ecosystems exemplify the balance of competitive and cooperative interactions among organisms that lead to bio-chemo-diversity.

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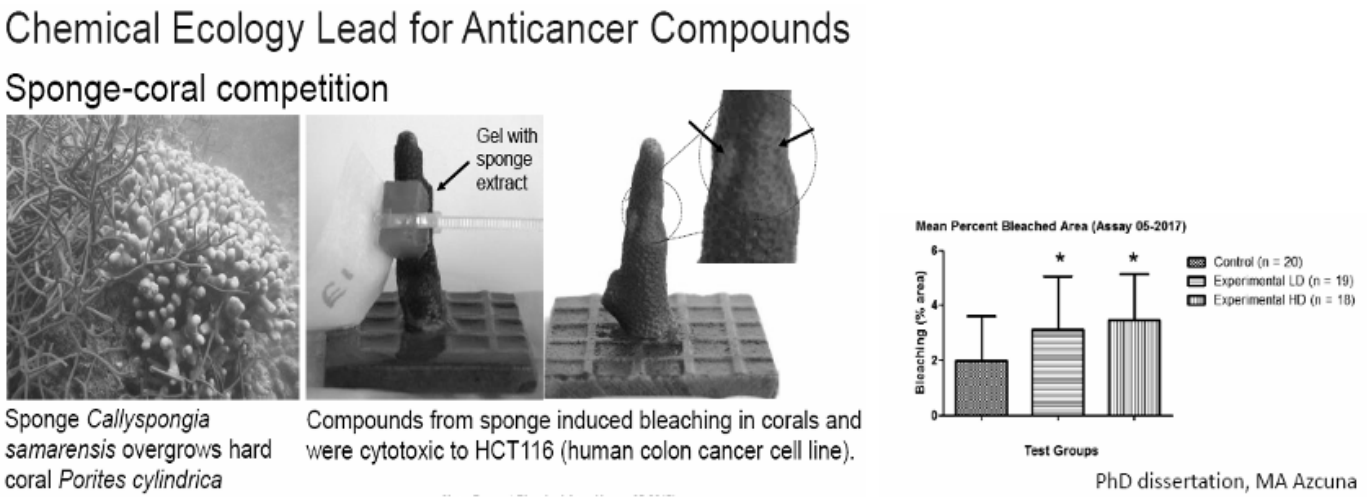

Figure 3. Sponge-coral competition served as chemical ecology lead for anticancer compounds.

An example of sponge-coral competition serving as a chemical ecology lead for anticancer compounds can be seen in the interaction of the "spaghetti" sponge *Callyspongia samarensis* and the hard coral *Porites cylindrica* (Figure 3). Field observations have shown *C. samarensis* overgrowing *P. cylindrica*. When gels containing the sponge extract were placed on pieces of corals in a tank, the compounds from the sponge induced bleaching in the corals at the area of contact. These same compounds also inhibited the growth of HCT 116 human colon cancer cells in MTT (cell viability) assays (Azcuna, Yap and Concepcion, 2017, personal communication, PhD dissertation, unpublished manuscript).

Several compounds isolated from Philippine coral reef marine organisms, principally sponges, act on the major cancer pathways described by Hanahan and Weinberg (2011), as shown in Figure 4. Carteriosulfates and chondropsins act on motility circuits by inhibiting the Wnt/β-catenin signaling pathway. Carteriosulfates, which are sulfated acids, were isolated from a *Carteriospongia* sp. sponge from Sorsogon and were found to inhibit GSK-3β, which phosphorylates and marks β-catenin for degradation in the Wnt/ β-catenin signaling pathway (Doble and Woodgett 2003; Concepcion et al. 2014). Chondropsins, macrolides which were also isolated from a marine sponge (*Irsinia* sp. from Sorsogon), target V-ATPase, thereby blocking vesicular acidification and inhibiting Wnt/planar cell polarity, Akt activation and mTor activity (Coombs et al. 2010; Concepcion et al. 2014).

The adociaquinones, neoamphimedine, deoxyamphimedine and stelletins all work on cytostasis and differentiation circuits (Concepcion et al. 2014). Adociaquinones, quinones isolated from a *Xestospongia* sp. sponge in Bolinao, Pangasinan, inhibit topoisomerase II (Concepcion et al. 1995). Neoamphimedine, a pyridoacridine which was first isolated from a *Xestospongia* sp. sponge in Surigao, and the structurally related deoxyamphimedine both intercalate with and catenate DNA in the presence of topoisomerase II (De Guzman et al. 1999, Marshall et al. 2009). The stelletins, triterpenes isolated from a *Rhabastrella globostellata* sponge in Guimputlan, Mindanao, target the p21 and p53 signaling pathways (Concepcion et al. 2014; Tasdemir et al. 2002).

Aldisines, alkaloids isolated from the sponge *Stylissa massa* from Surigao, disrupt proliferation circuits such as the growth signaling MEK pathway (Tasdemir et al. 2001, Concepcion et al. 2014). Topsentiasterol sulfate E and other fibrosterols are sulfated sterols isolated from a *Spheciospongia* sp. sponge from Cagayan de Oro that target the PKCζ pathway (Whitson et al. 2008, Concepcion et al. 2014). Lissoclinotoxins E and F, alkaloids which were

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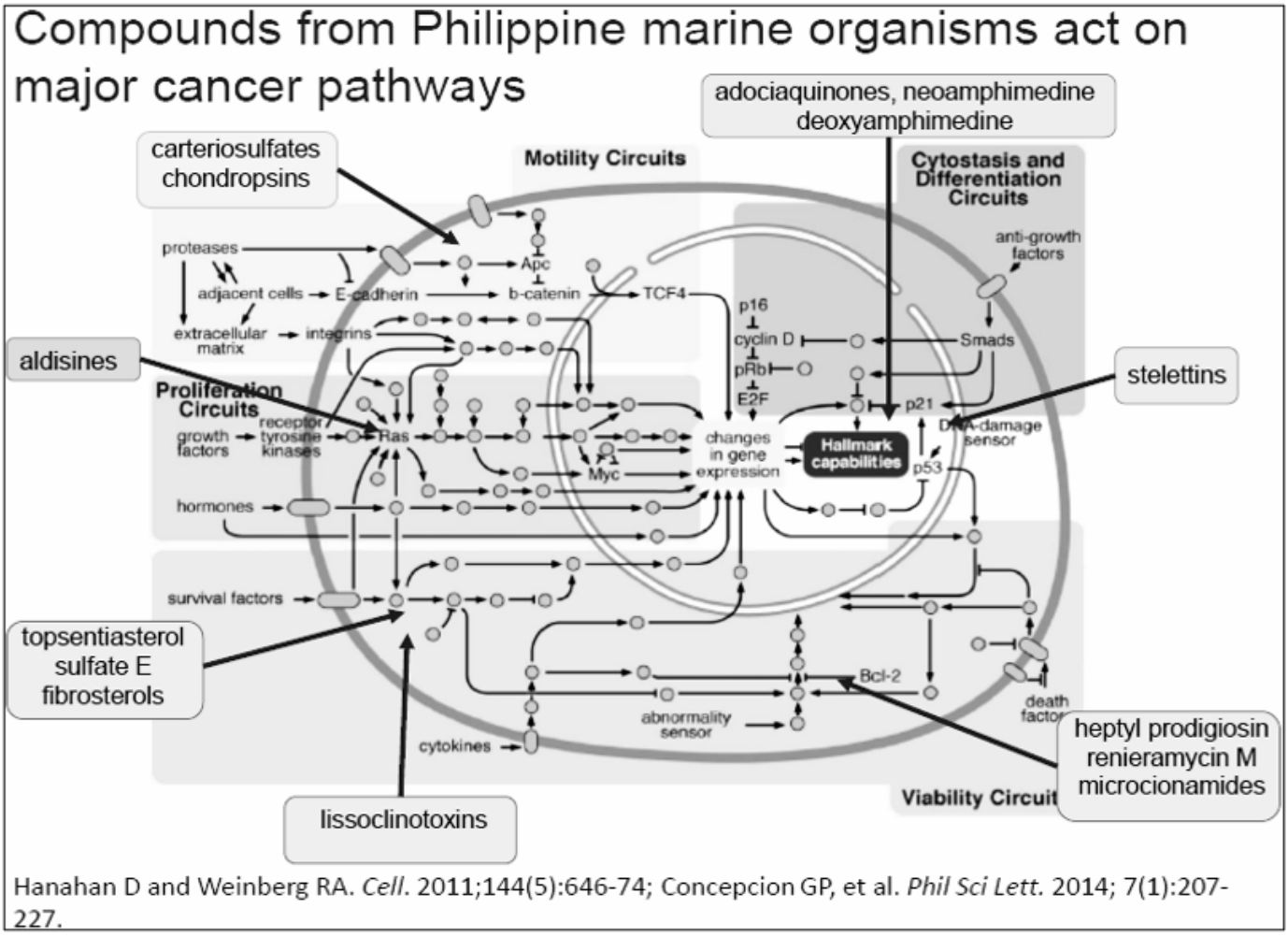

Figure 4. Compounds from Philippine marine organisms act on the major cancer pathways described by Hanahan and Weinberg (2011), from whose paper this figure was adapted.

isolated from an unidentified Philippine didemnid ascidian in the Batanes islands, act on the PI3K/AKT/mTOR pathway (Davis et al. 2003, Concepcion et al. 2014).

Heptylprodigiosin, microcionamides and renieramycins all target the cancer cell's viability circuits (Concepcion et al. 2014). Heptylprodigiosin, a tripyrrole with an aliphatic tail produced by a *Pseudovibrio denitrificans* bacterium isolated from an unidentified tunicate from Zamboanga del Norte caused caspase-3-dependent and CD95-mediated apoptosis and inhibited anti-apoptotic proteins Bcl2 and Bcl-xl (Ranches et al. 2003, Sertan-de Guzman et al. 2007, Concepcion et al. 2014). Microcionamides, modified cyclic octapeptides isolated from a *Clathria (Thalysias) abietana* sponge from Zamboanga target the apoptosis and cell death pathway (Davis et al. 2004, Concepcion et al. 2014). Renieramycins, tetrahydroisoquinolines isolated from the *Reneira* sp. and *Xestospongia* sp. Sponges, downregulate protein tyrosine phosphatase receptor type K (PTPRK) (Suwanborirux et al. 2005, Frincke and Faulkner 1992, Charupant et al. 2009, Concepcion et al. 2014).

While these compounds were isolated from different sponges in different coral reef systems in the Philippines, clearly a strategy being taken by organisms for competition with neighboring

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organisms through growth inhibition and cytotoxicity is to target different mechanistic and molecular pathways which would ensure a "100% kill". This serves as a basis for the combinatorial synergy or synthetic lethality strategy of cancer drug therapy.

Renieramycin M in particular was found to act synergistically with the anticancer compound doxorubicin (widely used in the clinics) when applied to MCF-7 breast cancer cell lines [ER (Estrogen Receptor)-positive cell line], which represents the most common breast cancer phenotype in the clinics (Figure 5). Real-time cell viability assays using a real-time cell analyzer (RTCA) showed increased cytotoxicity and flow cytometry showed increased apoptosis of cells with combined renieramycin M and doxorubicin treatment compared to cells treated with only a single drug (Tun and Concepcion 2016, personal communication, Masters thesis, unpublished manuscript).

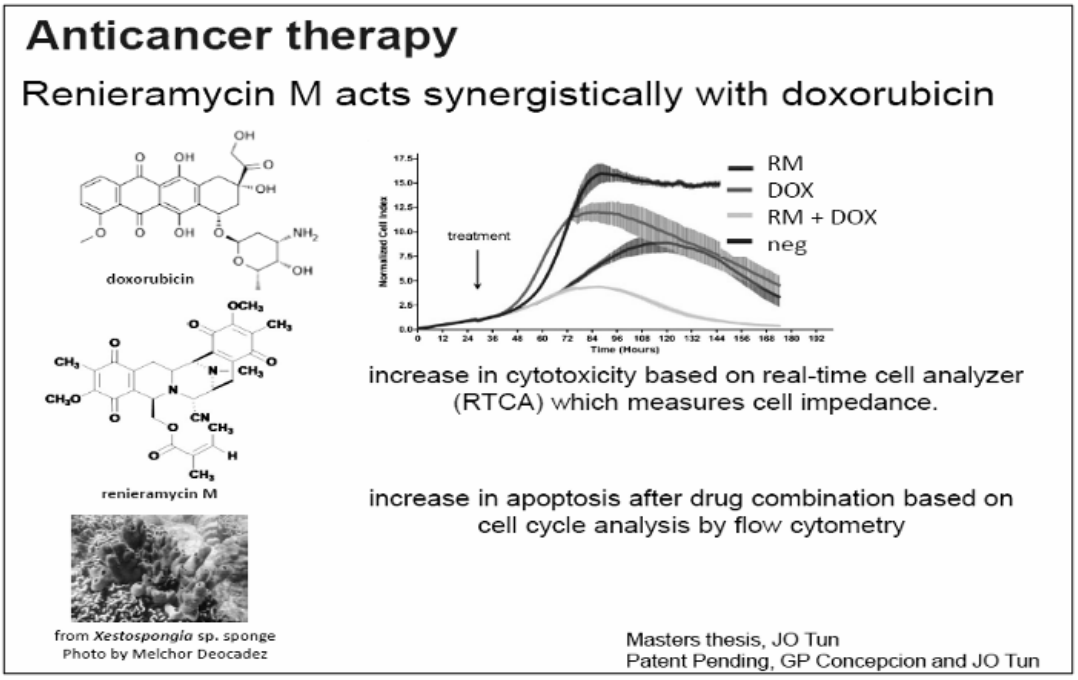

Figure 5. Renieramycin M acts synergistically with doxorubicin.

To recapitulate, several growth and proliferation modulators have been isolated from organisms in coral reef ecosystems that serve as anticancer drug leads. This marine ecosystem presents several strategies for cancer therapeutics. First, employing a synergistic combination of compounds, with each compound acting on a different molecular target, improves the chance of a 100% kill of cancer cells, reduces toxicity to normal cells, and reduces drug resistance. Second, combination therapy can be done with existing drugs to delay the development of resistance to an existing drug and therefore extend the utility lifespan of the drug. Third, leads can be found among bacterial compounds, which can be intermittently released at low doses and have a modulatory or regulatory function to delay or avert drug resistance. Fourth, based on a simple recombinant bacterial structure model for synthetic biology, a synthetic bacterium could be designed to contain and regulate gene clusters expressing and then secreting two or more classes of anticancer compounds acting in synergistic combination on

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different molecular pathways, also making use of the bacterium-host association model as an efficient, *in situ* drug delivery model.

MARINE ECOSYSTEM 2: GASTROPODS IN SPLASH ZONE AND MARINE ECOSYSTEM 3: SHIPWORMS IN MANGROVE

Other marine ecosystems of interest are the splash zone and the mangrove. The splash zone is home to gastropod mollusks such as *Truncatella guerinii*, while the bivalve shipworm *Lyrodus pedicellatus* was found in a mangrove area. Both the splash zone and the mangroves are areas where land and sea interface, with the rocky-sandy substrate of the splash zone, and the brackish waters and unique wood substrates of mangroves, providing an ecologically challenged environment. Mollusks and their harbored microorganisms would survive under these rather harsh, nutrient-limited conditions by producing bioactive compounds (Torres, Tun and Concepcion 2016, personal communication, Masters thesis, unpublished manuscript) (Figure 6).

Antibiotic resistance is one of the world's most serious health problems. It could be countered using the same principles and strategies derived from Nature's strategies that we proposed for treating cancer. For instance, 7,8-dideoxygriseorhodin C, a polyketide which is produced by a *Streptomyces* sp. isolated from the gastropod *Truncatella guerinii*, works synergistically with oxacillin against methicillin-resistant *Staphylococcus aureus* (MRSA). Oxacillin, which belongs to the same beta-lactam antibiotic class as methicillin and penicillin, is a clinically approved drug for susceptible strains of *Staphylococcus aureus*, but eventually it became ineffective due to the emergence of MRSA and ORSA (oxacillin-resistant *S. aureus*). MRSA can be countered by using compounds such as the existing drug oxacillin and the marine drug lead 7,8-dideoxygriseorhodin C which combined show synergistic anti-MRSA activity. Essentially, this

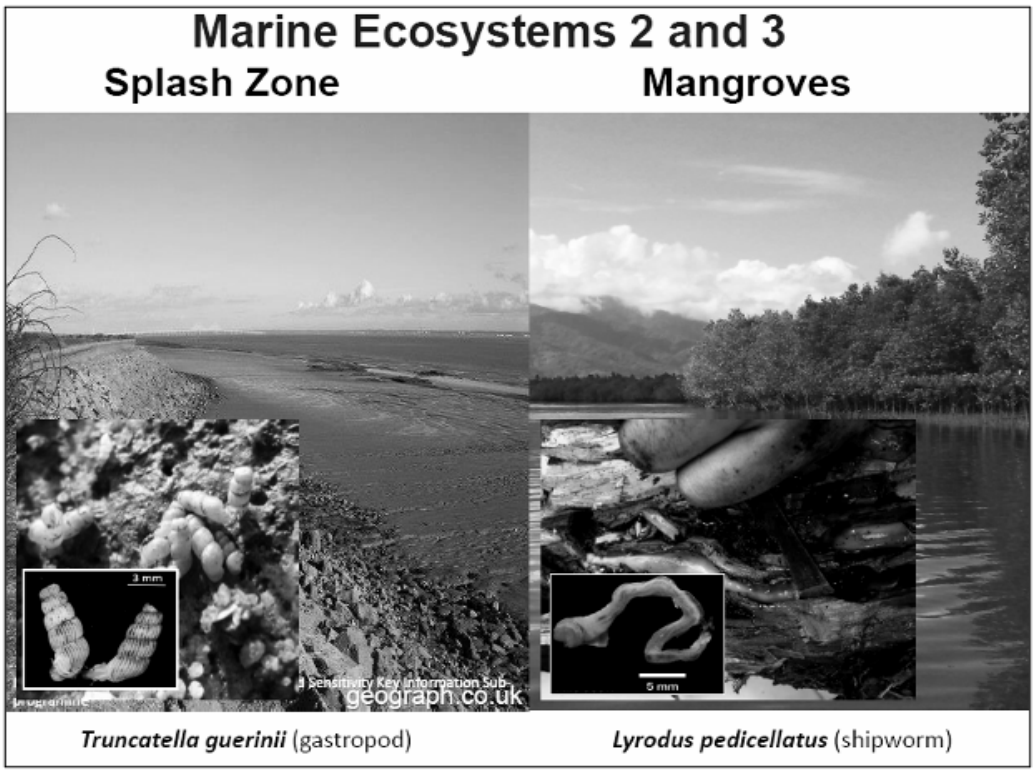

Figure 6. The splash zone and mangroves, marine ecosystems home to bioactive compound-producing gastropods and shipworms.

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brings the antibiotic oxacillin back to the treatment armamentarium. Notably, 7,8-dideoxygriseorhodin C is not cytotoxic to mammalian kidney and ovarian cell lines (Figure 7). This is a model for the possible application of combinatorial synergy involving the revival of out-of-use drugs, which is important at a time when the emergence of antibiotic resistance necessitates the identification of new compounds and new strategies to treat bacterial infections. Significantly, the combination treatment could substitute for vancomycin treatment of MRSA, and prevent more *S. aureus* strains from becoming methicillin- and vancomycin-resistant.

In some cases, antibiotic resistance is attributed to the formation of a biofilm layer that shields the pathogen and prevents penetration and entry of a cytotoxic antibiotic. Such quorum sensing-based biofilm formation is also observed in the marine environment. In the shipworm *Lyrodus pedicellatus*, its versatile, predominant gill bacterial symbiont *Teredinibacter turnerae* not only produces a suite of cellulases that breaks down wood cellulose to provide food and energy to its host, but it also produces two types of antibiotic compounds, tartrolons (macrodioloide polyketides) and oxylipins (oxygenated fatty acids), as hypothesized, to keep the shipworm's gut or caecum sterile, free of nuisance bacteria that would compete with it.

Bacteria generally undergo a cycle of planktonic and biofilm stages and both stages can be regulated by small molecules. Using the biofilm former *Staphylococcus epidermidis* as a model pathogen, we demonstrated that tartrolons inhibit the growth of free-living, planktonic *S. epidermidis*, while oxylipins inhibit quorum sensing by preventing the differentiation of planktonic *S. epidermidis* into biofilm cells, thus disrupting the development cycle (Figure 8). Inhibiting multiple points in the biofilm formation cycle serves as a good model for the treatment of MRSA and other biofilm-forming bacterial pathogens responsible for nosocomial (hospital-acquired) infections, pneumonia, cystic fibrosis, biofilm-covered catheters, and toxic shock syndrome (Elshahawi et al. 2013; Lacerna II, Robes et al. 2017, personal communication, Masters thesis, unpublished manuscript).

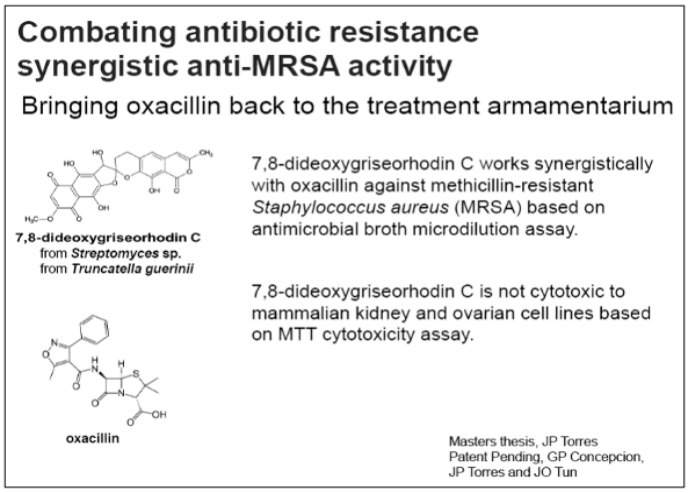

Figure 7. The 7,8-dideoxygriseorhodin C story: bringing oxacillin back to the treatment armamentarium.

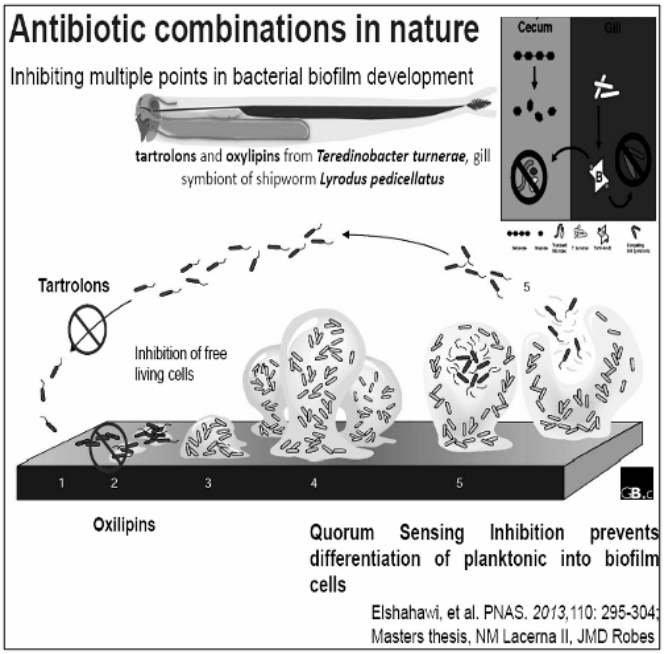

Figure 8. Inhibition of multiple points in bacterial biofilm development, an example of an antimicrobial strategy in nature that provide a good model for treatment for biofilm-forming pathogens.

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As human migration and travel, and intimate, interactive human activities continue to increase significantly, this accompanied by marked climate change manifested as extremes of weather and temperature, and causing many natural disasters in different parts of the world, expectedly, new diseases, especially communicable ones, such as bacterial, fungal and viral infections are emerging. To address these global health problems, we are undertaking a massive screening of bacterial extracts for activity against the emergent ESKAPE pathogens *(Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter sp.)*, TB (*Mycobacterium tuberculosis*), ARCA (amphotericin-resistant *Candida albicans*) and in HIV-1 (human immunodeficiency virus) cytoprotection and latency reactivation assays.

Taking leads from microbial ecology, bacterial communities living in association with marine macro-organisms are being studied based on the working hypothesis that the dominant bacterial endosymbiont protects the host by warding off opportunistic bacteria found in the bacterial community. In the laboratory, an isolated shipworm bacterial endosymbiont challenged with isolated environmental opportunist bacteria led to the elicitation and production of antimicrobial compounds in the endosymbiont. Such competitive interactions are known to induce antimicrobial compound production (Tyc et al. 2014).

A co-cultivation experiment was performed using a well-insert set-up similar to that previously described by Carlson et al. (2015). The presence of the compounds produced by the opportunist microbe or field–based model of pathogen (elicitor) induced the production of several compounds by the shipworm endosymbiont isolate (elicited) not present when the said isolate was cultured alone, as monitored by HPLC (Figure 9). Resazurin-based antimicrobial microdilution assay also showed that antimicrobial activity was significantly greater in extracts from co-cultures of the shipworm isolate with either of two model pathogens, than in the isolates or pathogens in monoculture.

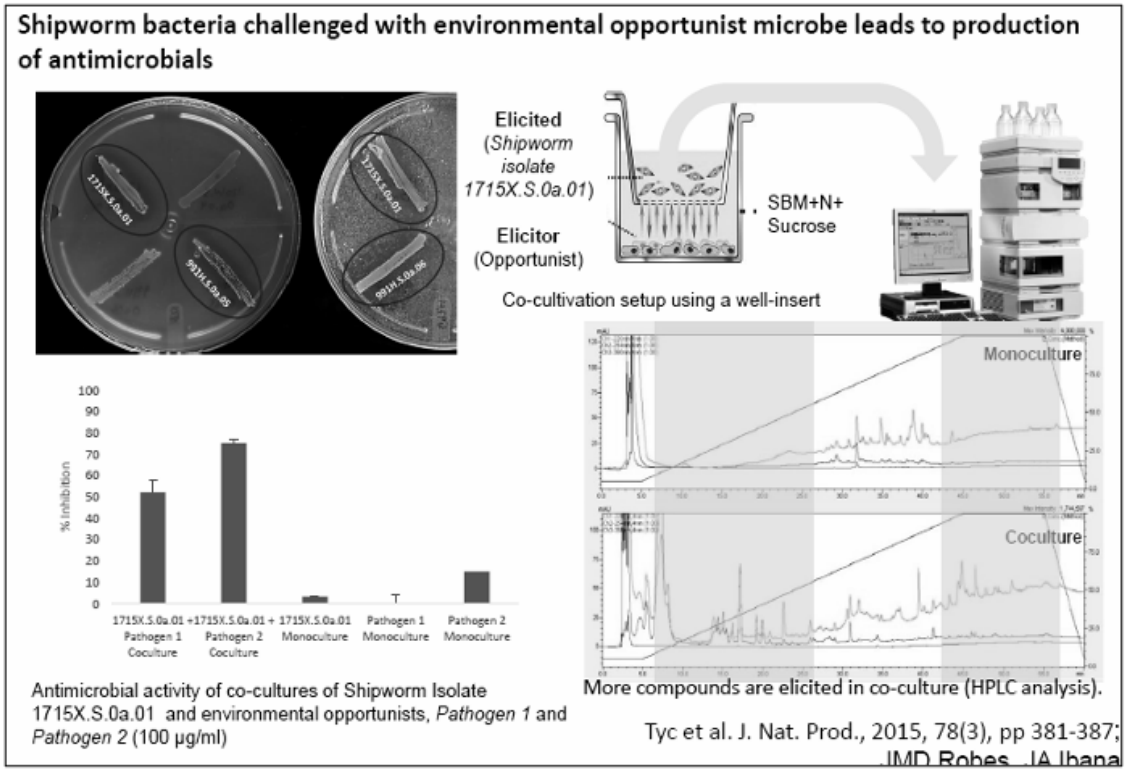

Figure 9. Shipworm bacteria challenged with environmental opportunist microbe leads to production of antimicrobials.

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In summary, antimicrobial bacterial compounds protect mollusks in splash zones and mangroves, and three strategies for finding antimicrobial drug leads can be learned from these marine ecosystems. First, synergistic combination of compounds can be employed, as in the case of the inhibitory compounds targeting the planktonic and biofilm stages of the pathogen. This allows greater activity of the compounds at lower concentrations and thus would delay the development of drug resistance. The second is to combine a new drug with a drug no longer in use. This allows the revived use of "retired" drugs – drugs that were previously effective but were later abandoned when the target pathogen developed resistance. As with the previous anticancer synergy strategy, this also addresses the problem of eventual resistance to the drug. Lastly, since compounds produced by the bacteria themselves in communities can have modulatory or regulatory functions for the bacteria, this can be taken advantage of or mimicked through their elicitation by a test pathogen.

The concept of synthetic lethality in anticancer therapy applies likewise to antibiotic combination therapy. And through synthetic biology, synthetic bacteria or virus-like particles can be designed to incorporate gene clusters that would biosynthesize a combination of compounds targeting different pathways to ensure a 100% kill (synthetic lethality), with sensitive-response gene elements that would up- or down- regulate the production and delivery of compounds as required, much like what exists in a dynamic marine ecosystem where mutable and mobile bacteria are the most efficient carriers or producers of regulatory compounds.

MARINE ECOSYSTEM 4: CONOIDEANS IN SEDIMENT

Marine sediment at various depths and coral reefs rich in small fish are home to predatory, mostly venomous Conoidean gastropod snails (superfamily consisting of cones, turrids and augers). In this unique marine ecosystem, venomous snails evolved an advanced anatomy, i.e., hard protective shell, highly specialized glands and organs, including a venom apparatus and an accessory hunting apparatus. This enables them to burrow, hide, lurk, and attack, pacify and consume prey, making use of strategic cabals of venom peptides which are paralytic, excitotoxic, and hypnotic or anaesthetic (Figure 10) (Olivera 2002, Olivera et al. 2015). With its unique armamentarium, it is not surprising that such a successful, megadiverse superfamily of mollusks has evolved and thrived successfully for tens of millions of years.

More recently, Conoideans have also been shown to harbor microorganisms in its organs and tissues. The seminal work of Olivier Peraud et al. (2009) on three *Conus* species showed microbiomes containing diverse actinobacteria, some of which produce compounds active in the DRG assay. However, while some bacteria have been isolated, purified and cultured in the laboratory, many are "uncultivable" using laboratory culture media and conditions, and so metagenomics is used to identify these bacteria.

Snails engaging in fierce predation, competition and defense with great success, making use of venom peptides and bacterial compounds, are a remarkable model for developing and delivering neuroactive drugs, for which there is a growing demand. Longer human lifespans come with an increased risk and incidence of cancer, neurodegeneration such as Parkinson's Disease, epilepsy, bipolar disorders, depression, Alzheimer Disease, autism, as well as cardiac disease. Cancer-related pain, neuropathic pain, and other types of chronic pain also need to be addressed with new drugs. Notably, one conopeptide, ω-MVIIA first isolated from *Conus magus* is a voltage-gated Ca^{2+}-channel blocker is a FDA-approved anti-pain drug known as Ziconitide or Prialt® (Figure 11) (Olivera et al. 1999, Olivera et al. 2007).

Aside from cone snails (~600 species), we are studying the phylogenetically older, more speciose cousins of the cones known as turrids (~12,000 species). These smaller snails (shell lengths ranging from centimeter to micrometer) that live in deeper waters than cones are collected using *lumun-lumun* nets, a method developed by local fisher folk (Figure 12). As an example, the P-conotoxin-

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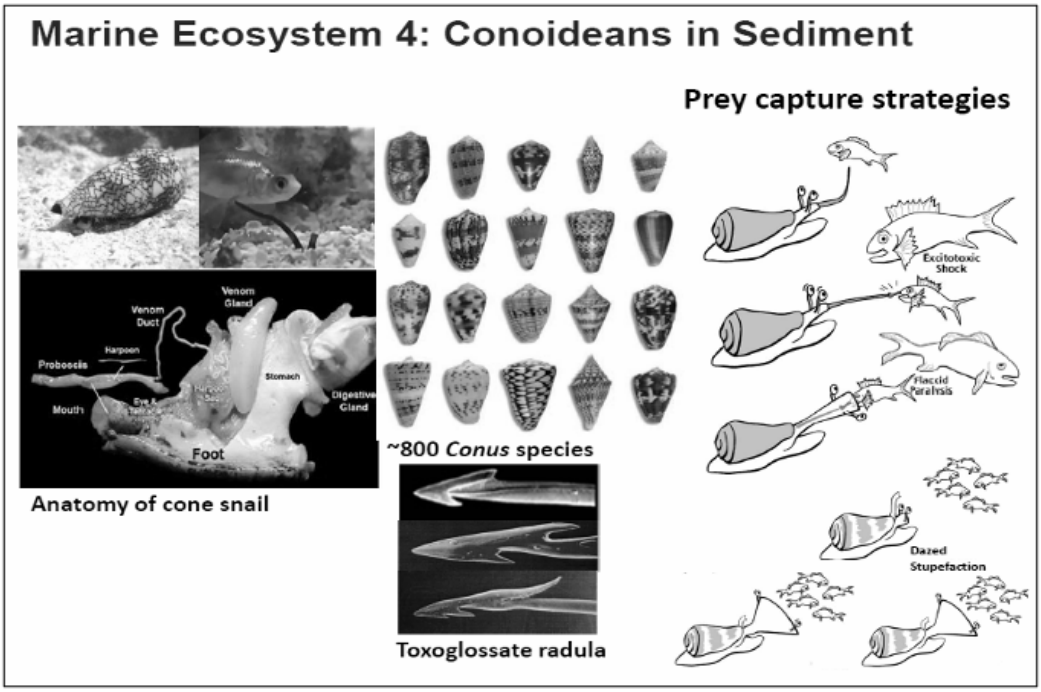

Figure 10. Conoideans in sediment evolved to make use of protective shells and specialized organs for different prey capture strategies, leading to the high diversity of this superfamily.

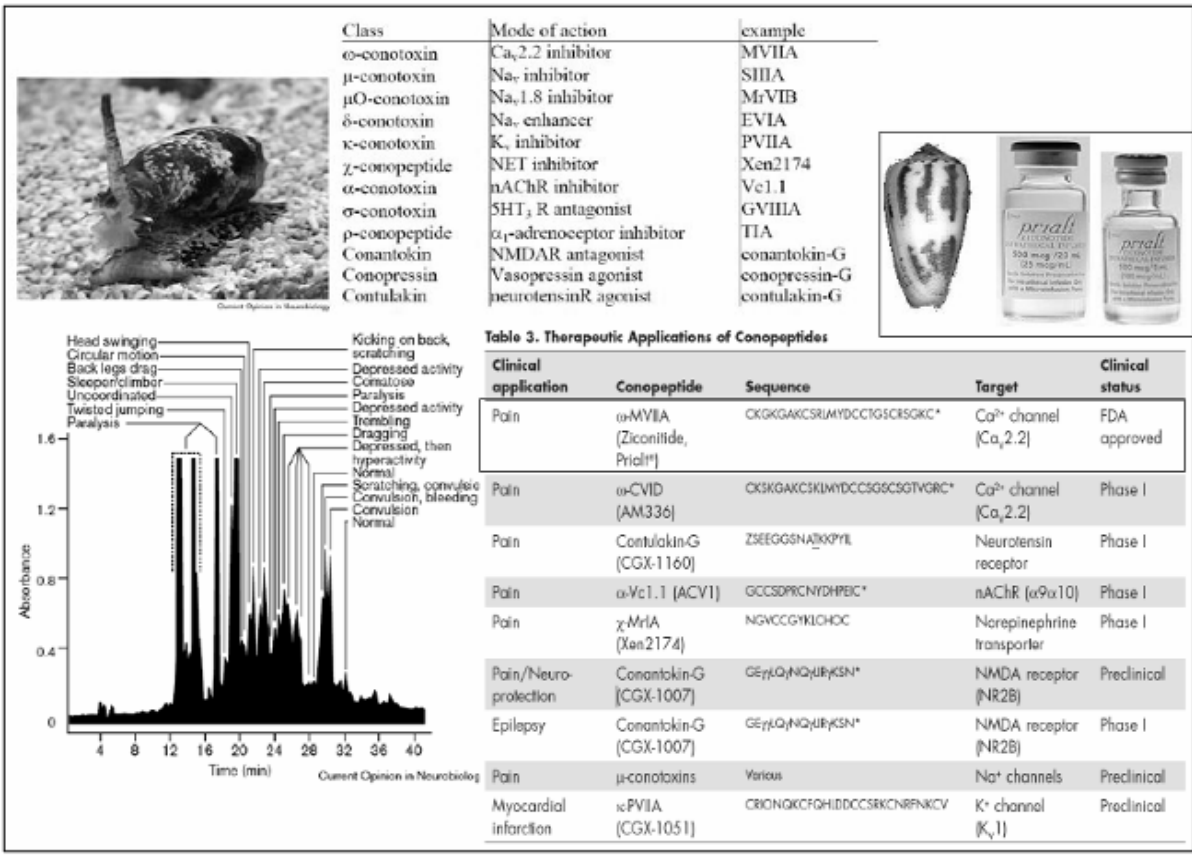

Figure 11. Venom peptides and antibacterial compounds used by cone snails in predation, competition and defense provide leads for neuroactive drugs.

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like crassipeptide (cce9a) isolated from the turrid *Crassispira cerithina* caused age-dependent effects in the intracranial mouse bioassay: lethargy and delayed response to stimuli in 12-14 - day old mice, and hyperactivity in 16-day old mice (Cabang et al. 2011). It elicited and amplified responses in a subset of small-diameter capsaicin-sensitive DRG (dorsal root ganglion) neurons also affected by κJ-conotoxin pl14a, a known potassium (subtype $K_V1.6$) channel-blocker, indicating that cce9a crassipeptide may be a potential analgesic or anti-pain agent since the $K_V1.6$ potassium channel is implicated in pain-sensing (Imperial et al. 2014) (Figure 12).

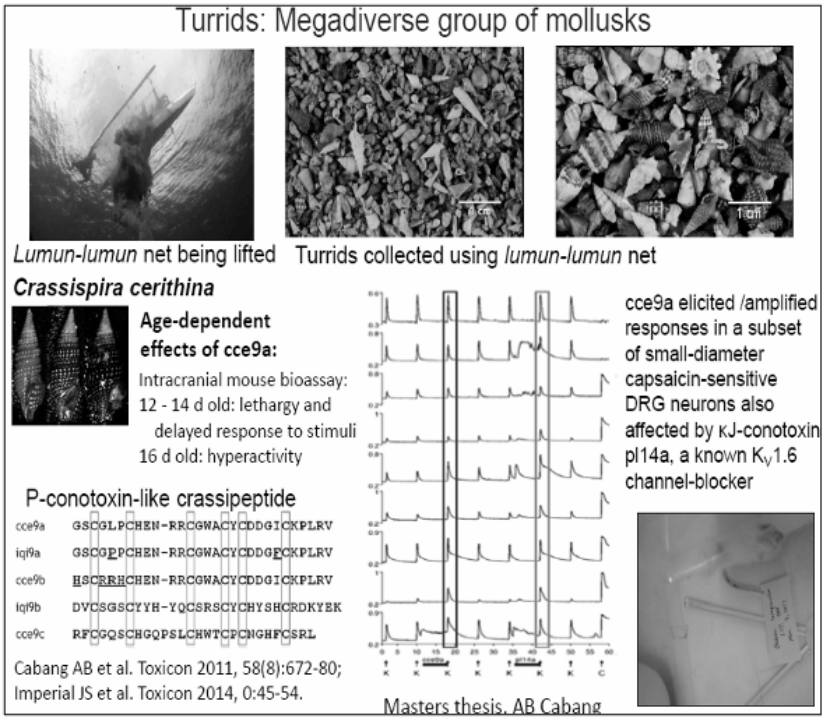

Figure 12. Turrids collected using lumun-lumun nets produce contoxin-like peptides with potential anti-pain application.

But why do these highly defended, highly aggressive, predatory snails still harbor bacteria that produce bioactive small molecules? What purpose do these bacterial compounds serve its host? Perhaps when released to the water, these neuroactive organic compounds, smaller in size than peptides, serve as signals to defend against predators and deter competitors.

The following are three examples of neuroactive compounds isolated from gastropod-associated bacteria. (Figure 13). Pulicatins are thiazoline compounds produced by a Streptomyces sp. CP 32 isolated from *Conus pulicarius*, one of which (compound 6 at 505 nM) inhibits the $5\text{-}HT_{2B}$ serotonin receptor, a subtype relevant in pain and other neurological conditions (Lin et al. 2010). The nobilamides are modified peptides produced by microbial symbionts of *Chicoreus nobilis* and *Conus tribblei*. Two of these (compounds 2 and 5) are long-acting antagonists of mouse and human TRPV1 channels that are implicated in pain (Lin et al. 2011). Nocapyrones are

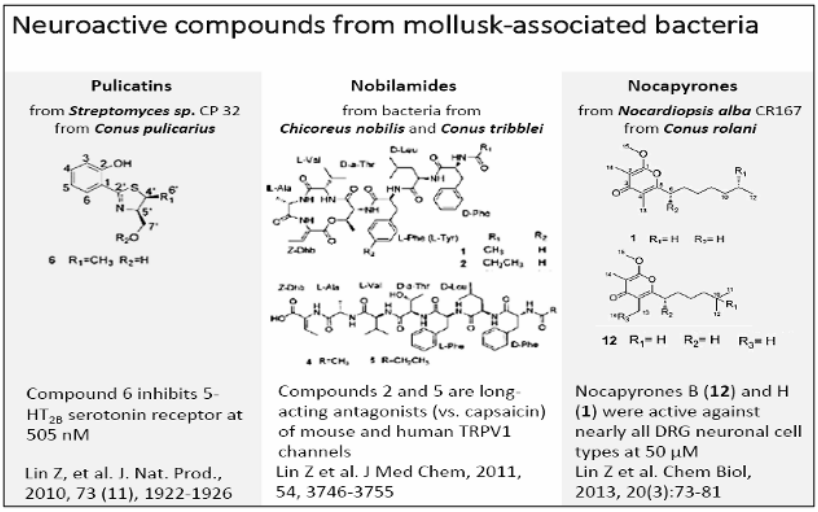

Figure 13. Examples of neuroactive compounds isolated from mollusk-associated bacteria.

polyketides produced by *Nocardiopsis alba* isolated from the foot mucus of *Conus rolani*. Nocapyrones B and H were active against all DRG neuronal cell types at 50 µM (Lin et al. 2013).

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In summary, the well-studied Conoidean strategy for predation is a classic model for successful combination therapy (with venom containing cabals of peptides used for catching specific types of prey) and drug delivery (with disposable hollow harpoons stinging the prey and delivering the venom much like a hypodermic needle). The second gastropod strategy is to make use of microbial symbionts to contribute neuroactive peptides and small organic compounds and other protective compounds for prey predation, warding off competitors and predators.

MARINE ECOSYSTEM 5: GIANT SHIPWORM IN EUTROPHIC HABITAT

A muddy marine lagoon with decaying matter, hydrogen sulfide-rich, oxygen-poor, is where the phenomenal giant shipworm *Kuphus polythalamia* was found, the "creature" first featured in the TV show *Kapuso Mo: Jessica Soho* and spotted serendipitously by one of our researchers involved in the shipworm project.

Kuphus polythalamia is a peculiar shipworm being the only known non-wood boring shipworm, burrowing instead on sulfidic marine sediments. It is the world's largest shipworm and longest bivalve (Figure 14). Unlike its shipworm relatives, it does not partner with cellulolytic symbionts and it has a reduced visceral mass (which includes the digestive organ) which is consistent with not taking in wood or making use of cellulose. Instead it relies on sulfur-oxidizing chemoautotrophic (thioautotrophic) endosymbiotic bacteria that use hydrogen sulfide to fix carbon dioxide into a biomass form that can be utilized by the host. This endosymbiont is the first cultivable sulfur-oxidizing bacteria to be reported in the scientific literature (Figure 15) (Distel et al. 2017, Altamia et al. 2017, personal communication).

The characteristics of its symbionts, its phylogenetic position within the shipworm family (Teredinidae), the reduction of its digestive system by comparison with other family members, and the loss of morphological features associated with wood digestion indicate that in *K. polythalamia*, chemoautotrophic symbiosis arose by displacement of an ancestral heterotrophic symbiosis, and *K. polythalamia* is a chemoautotrophic bivalve descended wood-feeding (xylotrophic) ancestors (Distel et al. 2017).

The *Kuphus polythalamia* story continues to unfold. Much remains to be understood about this enigmatic animal living in a unique marine ecosystem. Intensive studies on its endosymbionts, anatomy, metabolism and mode of nutrition,

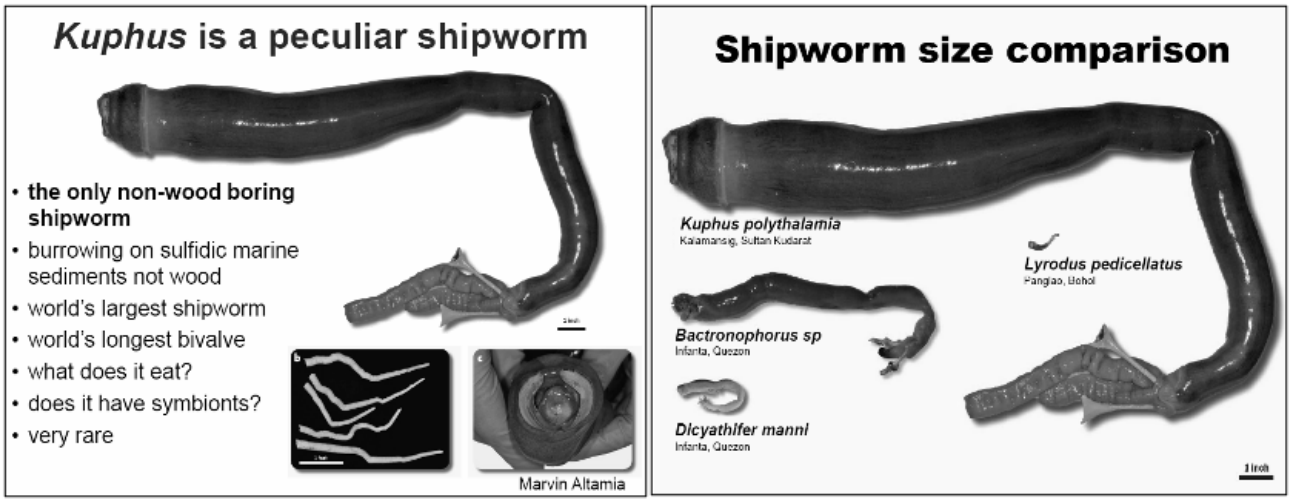

Figure 14. The *Kuphus polythalamia*, shipworm with several peculiar characteristics.

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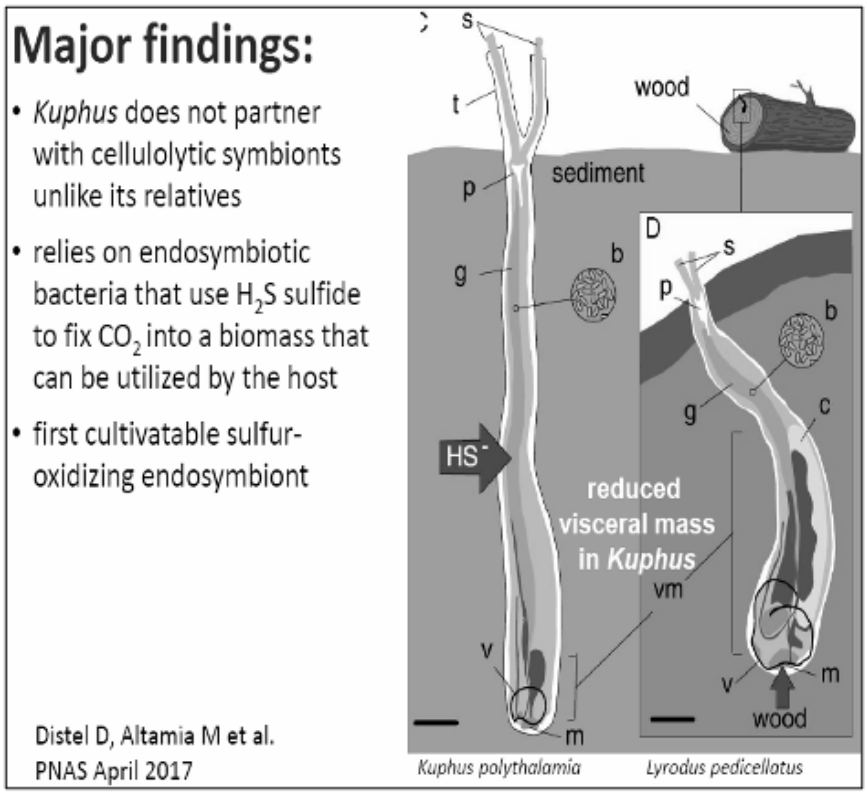

Figure 15. Anatomical comparison of the *Kuphus polythalamia* with *Lyrodus pedicellatus*, another shipworm, showing the loss of features associated with wood digestion in *K. polythalamia*.

life history, reproduction, behavior, habitat and bio-geo-evolution, could lead to opportunities for biotechnological and industrial applications. A fundamental understanding of its growth, metabolism, food and energy production (how it grows to magnificent proportions), correlated with anecdotal reports from local folk on its energizing, nutritional, healing, aphrodisiac, anti-goiter, antimicrobial and other medicinal properties, could provide prospects for developing nutritional, nutraceutical and therapeutic products.

The *Kuphus* story points to interesting links between primary and secondary metabolism provided by microbial symbionts. Growth to a giant size would indicate a superior, efficient primary metabolism. The dominant bacteria infect the host, the host adapts to tolerate them and even benefits from them. Mechanisms of bacterial infection and disease, host defense and tolerance could be better understood by studying this system. Since bacteria produce antimicrobial secondary metabolites, it is possible that these are being used to ward off other bacterial competitors and also to protect the host from harmful bacterial infections; this aside from contributing the basic building blocks for primary metabolism. Indeed, "Big is small", small is versatile and can do all, and the host benefits to become even bigger. The giant *Kuphus* appears to owe its success to its endosymbiont bacteria.

SUMMARY

The rich marine eco-bio-chemo-diversity of the Philippines provides a pharmacological treasure trove that should be conserved, explored and developed further to contribute to the Philippines' Blue Economy and address important diseases afflicting humankind.

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REFERENCES

Cabang AB, Imperial JS, Gajewiak J, Watkins M, Corneli PS, Olivera BM, Concepcion GP. 2011. Characterization of a venom peptide from a crassispirid gastropod. Toxicon 58(8):672-680.

Carlson S, Tanouye U, Omarsdottir S, Murphy BT. 2015. Phylum-specific regulation of resistomycin production in a *Streptomyces* sp. via microbial coculture. J Nat Prod 78(3), 381-387. doi:10.1021/np500767u

Charupant K, Suwanborirux K, Daikuhara N, Yokoya M, Ushijima-Sugano R, Kawai T, Owa T, Saito N. 2009. Microarray-based transcriptional profiling of renieramycin M and jorunnamycin C, isolated from Thai marine organisms. Mar Drugs 7: 483-494.

Concepcion GP, Anas ARJ, Azcuna MA. 2014. Anticancer compounds from Philippine marine organisms act on major pathways in cancer. Phil Sci Lett 7(1):207-227.

Concepcion GP, Foderaro TA, Eldredge GS, Lobkovsky E, Clardy J, Barrows LR, Ireland CM. Topoisomerase II-mediated DNA cleavage by adocia- and xestoquinones from the Philippine sponge *Xestospongia* sp. 1995. J Med Chem 38: 4503-4508.

Coombs GS, Yu J, Canning CA, Veltri CA, Covey TM, Cheong JK, Utomo V, Banerjee N, Zhang ZH, Jadulco RC, Concepcion GP, Bugni TS, Harper MK, Mihalek I, Jones CM, Ireland CM, Virshup DM. 2010. WLS-dependent secretion of WNT3A requires Ser209 acylation and vacuolar acidification. J Cell Sci 123:3357-3368.

Transactions NAST PHL 39 No. 2 (2017)
doi.org/10.57043/transnastphl.2017.1069

Davis RA, Mangalindan GC, Bojo ZP, Antemano RR, Rodriguez NO, Concepcion GP, Samson SC, de Guzman D, Cruz LJ, Tasdemir D, Harper MK, Feng X, Carter GT, Ireland CM. 2004. Microcionamides A and B, bioactive peptides from the Philippine sponge *Clathria (Thalysias) abietina*. J Org Chem 69: 4170-4176.

Davis RA, Sandoval IT, Concepcion GP, Da Rocha RM, Ireland CM. 2003. Lissoclinotoxins E and F, novel cytotoxic alkaloids from a Philippine didemnid ascidian. Tetrahedron 59: 2855-2859.

De Guzman FS, Carte B, Troupe N, Faulkner DJ, Harper MK, Concepcion GP, Mangalindan GC, Matsumoto SS, Barrows LR, Ireland CM. 1999. Neoamphimedine: a new pyridoacridine topoisomerase II inhibitor which catenates DNA. J Org Chem 64: 1400-1402.

Distel DL, Altamia MA, Linc Z, Shipway JR, Han A, Forteza I, Antemano R, Peñaflor LimbacoMGJ, Tebo AG, Dechavez R, Albano J, Rosenberg G, Concepcion GP, Schmidt EW, and Haygood MG. 2017. Discovery of chemoautotrophic symbiosis in the giant shipworm *Kuphus polythalamia* (Bivalvia: Teredinidae) extends wooden-steps theory. Proc Natl Acad Sci USA E3652-E3658.

Doble BW, Woodgett JR. 2003. GSK-3: tricks of the trade for a multi-tasking kinase. J Cell Sci 116: 1175-1186.

Elshahawi SI, Trindade-Silva AE, Hanora A, Han AW, Flores MS, Vizzoni V, Schrago CG, Soares CA, Concepcion GP, Distel DL, Schmidt EW, Haygood MG. 2013. Boronated tartrolon antibiotic produced by symbiotic cellulose-degrading bacteria in shipworm gills. Proc Natl Acad Sci USA 110(4): E295-304.

Frincke JM, Faulkner DJ. 1992. Antimicrobial metabolites of the sponge *Reniera* sp. J Am Chem Soc 104: 265-269.

Hanahan D, Weinberg RA. 2011. Hallmarks of Cancer: The Next Generation. Cell 144(5):646-74.

Imperial JS, Cabang AB, Song J, Raghuraman S, Gajewiak J, Watkins M, Showers-Corneli P, Fedosov A, Concepcion GP, Terlai H, Teichert RW, Olivera BM. 2014. A family of excitatory peptide toxins from venomous crassispirine snails: Using constellation pharmacology to assess bioactivity. Toxicon 89:45-54.

Lazaro JEH, Nitcheu J, Predicala RZ, Mangalindan GC, Ness-lany F, Marzin D, Concepcion GP, Diquet B. Heptylpro-digiosin, a bacterial metabolite, is antimalarial in vivo and non-mutagenic in vitro. J Nat Toxins 2002; 11: 367-377.

Lin Z, Antemano RR, Hughen RW, Tianero MDB, Peraud O, Haygood MG, Concepcion GP, Olivera BM, Light A, Schmidt EW. 2010. Pulicatins A– E, neuroactive thiazoline metabolites from cone snail-associated bacteria. J Nat Prod 73(11), 1922-1926.

Lin Z, Reilly CA, Antemano R, Hughen RW, Marett L, Concepcion GP, Haygood MG, Olivera BM, Light A, Schmidt EW. 2011. Nobilamides A–H, long-acting transient receptor potential vanilloid-1 (TRPV1) antagonists from mollusk-associated bacteria. J Med Chem 54, 3746-3755.

Lin Z, Torres JP, Ammon MA, Marett L, Teichert RW, Reilly CA, Kwan JC, Highen RW, Flores M, Tianero MD, Peraud O, Cox JE, Light AR, Villaraza AJL, Haygood MG, Concepcion GP, Olivera BM, Schmidt EW. 2013. A bacterial source for mollusk pyrone polyketides. Chem Biol 20(3):73-81.

Marshall KM, Andjelic CD, Tasdemir D, Concepcion GP, Ireland CM, Barrows LR. 2009. Deoxyamphimedine, a pyridoacridine alkaloid, damages DNA via the production of reactive oxygen species. Mar Drugs 7: 196-209.

Olivera BM. 2002. Conus venom peptides: reflections from the biology of clades and species. Annu. Rev. Ecol. Syst. 33: 25-47

Olivera BM. 2006. Conus peptides: biodiversity-based discovery and exogenomics. The Journal of Biological Chemistry 281(42): 31173-31177

Transactions NAST PHL 39 No. 2 (2017)
doi.org/10.57043/transnastphl.2017.1069

Olivera BM, Corneli PS, Watkins M, Fedosov A. 2014. Biodiversity of cone snails and other venomous marine gastropods: evolutionary success through neuropharmacology. Annual Review of Animal Biosciences 2: 487-513

Olivera BM, Cruz LJ, Yoshikami D. 1999. Effects of Conus peptides on the behavior of mice. Current Opinion in Neurobiology 9: 772-777.

Olivera BM, Seger J, Horvath MP, Fedosov AE. 2015. Prey-capture strategies of fish-hunting cone snails: behavior, neurobiology, and evolution. Brain, Behavior, and Evolution 86: 58-74

Olivera BM and Teichert RW. 2007. Diversity of the neurotoxic *Conus* peptides: A model for concerted pharmacological discovery. Molecular Interventions 7(5): 251-260

Peraud O, Biggs JS, Hughen RW, Light AR, Concepcion GP, Olivera BM, Schmidt EW. 2009. Microhabitats within venomous cone snails contain diverse actinobacteria. Appl Environ Microbiol 75(21):6820-6826.

Ranches GD, Ruda A, Vollmar A, Tun JO, Concepcion GP. 2013. Heptylprodigiosin induces apoptosis in Jurkat leukemia T cells via CD95 death receptor and evades anti-apoptotic Bcl-2 and Bcl-x proteins. Phil Sci Lett 6: 153-167.

Sertan-de Guzman A, Predicala RZ, Bernardo EB, Neilan BA, Elardo SP, Mangalindan GC, Tasdemir D, Ireland CM, Barraquio WL, Concepcion GP. 2007. *Pseudovibrio denitrificans* strain Z143-1, a heptylprodigiosin producing bacterium isolated from a Philippine tunicate. FEMS Microbiol Lett 277: 188–196.

Suwanborirux K, Amnuoypol S, Plubrukarn A, Pummangura S, Kubo A, Tanaka C, Saito N. 2003. Chemistry of renieramycins. Part 3. Isolation and structure of stabilized renieramycin type derivatives possessing antitumor activity from Thai sponge *Xestospongia* species, pretreated with potassium cyanide. J Nat Prod 66: 1441-1446.

Tasdemir D, Mallon R, Greenstein M, Feldberg LR, Kim SK, Collins K, Wojciechowicz D, Mangalindan GC, Concepcion GP, Harper MK, Ireland CM. 2001. Aldisine alkaloids from the Philippine sponge *Stylissa massa* are potent inhibitors of mitogen-activated protein kinase kinase-1 (MEK-1). J Med Chem 45: 529-532.

Tasdemir D, Mangalindan GC, Concepcion GP, Verbitski SM, Rabindran S, Miranda M, Greenstein M, Hooper JNA, Harper MK, Ireland CM. 2002. Bioactive isomalabaricane triterpenes from the marine sponge Rhabdastrella globos-tellata. J Nat Prod 65: 210-214.

Tyc O, Van den Berg M, Gerards S, Van Veen JA, Raaijmakers JM, De Boer W, Garbeva P. 2014. Impact of interspecific interactions on antimicrobial activity among soil bacteria. Frontiers in Microbiology, 5. doi:10.3389/fmicb.2014.00567

Whitson EL, Bugni TS, Chockalingam PS, Concepcion GP, Harper MK, He M, Hooper JNA, Mangalindan GC, Ritacco F, Ireland CM. 2008. Spheciosterol sulfates, PKCζ inhibitors from a Philippine sponge *Spheciospongia* sp. J Nat Prod 71: 1213-1217.

Whitson EL, Bugni TS, Prdiya S, Chockalingam PS, Concep-cion GP, Feng X, Jin G, Harper MK, Mangalindan GC, McDonald LA, Ireland CM. Fibrosterol sulfates from the Philippine sponge Lissodendoryx (Acanthodoryx) fibrosa: sterol dimers that inhibit PKCζ. J Org Chem 2009; 74: 5902-5908.