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Chemical mediation as a structuring element in marine gastropod predator-prey interactions

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Chemical mediation regulates behavioral interactions between species and thus affects population structure, community organization and ecosystem function. Among marine taxa that have developed chemical mediation strategies, gastropods belong to a diverse group of molluscs found worldwide, including species with a coiled, reduced or absent shell. Most gastropods use natural products to mediate a wide range of behaviors such as defense, prey location or interactions with con- and hetero-geners. Their chemically defended diet, such as cyanobacteria, algae, sponges, bryozoans and tunicates, provides them with a considerable opportunity either as shelter from predators, or as a means to enhance their own chemical defense. In addition to improving their defenses, molluscs also use prey secondary metabolites in complex chemical communication including settlement induction, prey detection and feeding preferences. The assimilation of prey secondary metabolites further provides the opportunity for interactions with conspecifics *via* diet-derived chemical cues or signals. This review intends to provide an overview on the sequestration, detoxification, and biotransformation of diet-derived natural products, as well as the role of these compounds as chemical mediators in gastropod-prey interactions.

1. Introduction

Chemicals are renowned for influencing intra- and inter-specific interactions as well as in shaping the structure of entire ecosystems.[1–3] Chemical communication therefore constitutes one of the most important languages used by nature. Paradoxically, despite their integral role in species interactions, little is known about the nature and structure of these chemicals. Natural selection imposed by predators, pathogens, competitors and epibionts has led to the evolution of chemical, physical/mechanical, and

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phenological defenses in organisms.[4,5] In terms of chemical defenses, an enormous variety of adaptive chemical compounds exist, including those that ward off, inhibit or kill potential consumers, those that are antimicrobial and kill viruses, bacteria, fungi, and still others that are allelopathic and suppress competitors.[6–8] These natural compounds, known as secondary metabolites, are small molecules with no known function in the primary metabolism.[9] While the role of chemicals in structuring marine ecosystems has been less studied than in terrestrial ecosystems, there has been an explosion of marine chemical ecology work in the last 15 years.[10–12]

The aquatic environment is a particularly appropriate medium for the transmission of molecules: it is not surprising if chemical mediation is at the center of species interactions in such an environment. Moreover, the overall seascape is dominated by sessile organisms, protected by chemical defenses. The majority of sessile organisms have evolved adaptive traits in order to protect them from predators, pathogens or competitors. In marine systems, primary producers such as cyanobacteria or algae, as well as other sessile animals such as corals, sponges, bryozoans or tunicates, are known to biosynthesize a broad range of different compounds that have cascading effects across trophic levels and shape communities.[10–12] Certain predators have developed chemical-resistance strategies to circumvent these chemical shields, and even use chemical defenses as cues to locate their sessile prey. For example, while chlorodesmin (in 2.1; Fig. 5) produced by the seaweed *Chlorodesmis fastigiata* deters feeding by most fish species, it strongly stimulates feeding by the specialist crab *Caphyra rotundifrons*.[13] Therefore the use of secondary

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Isabelle Bonnard obtained her PhD (in 1996) from the University of Perpignan after specializing in Marine Chemistry at the University of Brittany. In the continuity of her thesis work on the isolation and structural determination of marine cyclic lipopeptides with a cyanobacterial origin, Isabelle joined the team of Prof. Kenneth Rinehart, at the University of Illinois at Urbana-Champaign (USA), for a postdoctoral fellowship. Isabelle was appointed as a lecturer in 1998 at the University of La Réunion, developing the chemistry of natural products from diverse marine organisms from the Mascareign Area. Isabelle moved to the University of Perpignan in 2004 and joined CRIOBE, focusing her research interests on chemical ecology, chemical mediation in the marine ecosystem and environmental metabolomics.

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metabolites to deter predators, rather than attract them, has important implications for the success of individuals and populations. But, in addition to facilitating escape from predators, secondary metabolites may mediate a wide range of other behaviors, such as finding prey, mating with suitable partners or interacting with congeners.[14] The multi-species interactions in which gobies defend acroporid corals from allelopathic algae, are an example of how chemical communication and defense underlie coral reef resilience. The responses of both the coral and fish are mediated by chemical signals and cues.[15]

Gastropods are one marine taxa that have developed strategies of chemical-resistance, and they use chemical cues to locate their sessile prey.[16] Marine gastropods are slow-moving, often physically unprotected (soft-bodied) benthic molluscs, and as such, strong selection pressures have led to the development of defense mechanisms to increase their chances of survival. Furthermore, in addition to their restricted vision, marine gastropods often live in environments where visual information is limited, but where chemical information abounds and they have evolved to use such information to their advantage. Herbivorous marine gastropods are able to consume chemically defended prey, such as cyanobacteria and macroalgae. Similarly, carnivorous gastropods consume chemically-defended herbivores or chemically-defended filter-feeding sessile invertebrates such as sponges, bryozoans or tunicates. Therefore, within their sphere of perception, marine gastropods must select useful chemical cues from the chemical noise in their surrounding smellscape.

The class Gastropoda is the most diverse class in the phylum Mollusca, with 60 000–80 000 representatives including snails and slugs, and whose taxonomy is still under revision.[16,17] Heterobranchia is a taxonomic clade of snails and slugs, which includes marine, aquatic and terrestrial gastropod molluscs. The Jörger *et al.*[16] classification was recently redefined for the Heterobranchia. This review will use both the Jorger and the classification schematic outlined by Bouchet & Rocroi[17] for non-Heterobranchia gastropods.

Snails, with their coiled shells, represent the ancestral gastropod body plan, whereas slugs in which the shell is strongly reduced or absent, illustrate the trend towards reduction, then internalization, and finally loss of the shell. The evolution of chemical defenses in opistobranch gastropods was addressed initially by Faulkner and Ghiselin,[18] and then by Cimino and Ghiselin.[19–21] Of the two hypotheses proposed to explain the timing of shell reduction and loss in these animals in relation to the appearance of a chemical defense, either shell reduction preceded the evolution of a chemical defense (a post-adaptive scenario) or was made possible by the presence of a chemical defense (a pre-adaptive scenario), the latter is favored as "it seems biologically implausible for an animal to dispense with a protection so effective as a shell without a substitute defense".[18] Moreover, in some groups in which the shell is still relatively well developed, a chemical defense is already present.

In this review we do not intend to exhaustively cover the chemical ecology of all shelled or shell-less marine gastropods. Predatory marine gastropods with large and robust shells, which interact chemically with their prey using harpoons to inject venom into their prey, such as cone snails, are not treated;

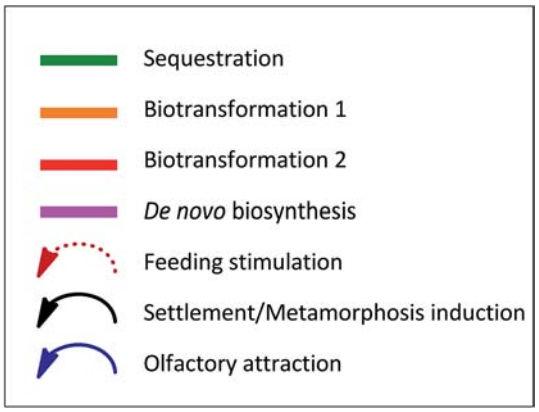

Fig. 1 Color code adopted for all figures.

a rich literature already exists on this subject.[22,23] Within Gastropoda, we focus on the clade Heterobranchia, but we rely, in part 3, on examples taken from the neighboring clades, Caenogastropoda and Vetigastropoda.

Numerous publications have concentrated on the sequestration and biotransformation of diet-derived compounds, or on the role of prey secondary metabolites in foraging or settlement of marine gastropods, but rarely have data on both been synthesized together. In this integrative review we have chosen to address the different issues: (i) sequestration (ii) detoxification and biotransformation, and (iii) role of diet-origin natural products as chemical mediators in gastropod-prey interactions.

For all figures, chemical structures are numbered in order in which they appear in the text. Arrows connect the different trophic levels, the prey organisms to predators that ingest the compounds. We use a color code related to sequestration, biotransformation, feeding stimulation, settlement/metamorphosis induction and olfactory attraction (Fig. 1). Structures are framed in green when isolated from a primary producer (first trophic level) and sequestered by the predator (second trophic level), are framed in orange when the compound is biotransformed by a predator, framed in red when the compound is biotransformed again, and framed in magenta when compounds are biosynthesized *de novo*. The bioaccumulated concentration relative to that found naturally in prey, the natural concentration (NC), are given in red when known (*e.g.* 2× NC refers to twice the natural concentration in prey). The body parts in which the compounds are located are also given in figures when known, with abbreviations as follows: digestive gland (DG), anterior mantle gland (AMG), opaline gland (OG), ink gland (IG).

2. Gastropods capable of sequestering diet-derived chemicals

The role of secondary metabolites as a chemical defense strategy of algae, sponges, bryozoans, tunicates or cyanobacteria, has been widely studied.[24,25] However, many consumers have developed counteradaptations that enable them to feed on such chemically-defended prey without apparent negative effects. This evolutionary adaptation by terrestrial and marine species involves the development of mechanisms to process certain chemicals in order to tolerate prey secondary

metabolites and even use them as an effective defense by sequestering and/or excreting them. Whether or not potential predators are deterred by prey secondary metabolites can be explained by variation in their post-ingestion responses.[26] Both terrestrial and marine species alike show four general mechanisms for processing ingested secondary metabolites: Absorption, Distribution, Metabolism (biotransformation) and Excretion (ADME). Sequestration and concentration or bioaccumulation may be assimilated into absorption and distribution and will be discussed here, whereas metabolism and excretion are discussed in part 3. Sequestration may occur in different parts of the molluscan body; head, foot, digestive gland (hepatopancreas), or mantle. Excretion may be carried out *via* mucus, faeces, ink or opaline secretions.

Here we review how gastropods have become adapted to feeding on a particular chemically-defended diet by storing, concentrating and excreting diet-derived compounds. In addition, where examples exist, *de novo* synthesis of secondary metabolites by gastropods will be described. Biotransformed metabolites are described in detail in part 3.

2.1 Sequestration of diet-derived chemicals by sacoglossans

Sacoglossan mesograzers (class: Gastropoda, clade: Heterobranchia, clade: Euthyneura, clade: Panpulmonata), a group of heterobranch molluscs, have a wide geographical distribution, being present in the majority of shallow tropical and temperate marine environments worldwide. They are generally cryptically colored and have a specific feeding habit: feeding suctorially and almost exclusively on the cell sap of macroalgae from the phylum Chlorophyta.[27] Interestingly, primitive species are shelled (subclade Oxynoacea) and feed only upon the siphonalean green algal genus *Caulerpa*, while the more evolved species are shell-less (subclade Plakobranchacea) and are found to feed on various algal genera.[27–29] Both shelled, as well as the more primitive shell-less, sacoglossans are kleptoplasts, having the ability to sequester functional long-lived chloroplasts from photosynthetic organelles which enables the mollusc to photosynthesize as well as fix carbon.[30,31] *Elysia timida*, *E. chlorotica*, *E. clarki*, *Oxynoe viridis* and *Costasiella ocellifera* are known to store chloroplasts from their algal food *via* selective digestion so that digestive enzymes do not harm the chloroplasts. Furthermore, shelled species appear to acquire additional defense by sequestering secondary metabolites from their algal prey. Some shell-less species also concentrate algal secondary metabolites, and sometimes take this defense one step further by biotransforming them, while others are able to biosynthesize *de novo* toxic polypropionates.[32–34]

The shelled sacoglossan *Oxynoe panamensis*,[35] specialist of the green algae *Caulerpa* sp., is able to sequester four compounds that show toxic activity against mice and rats.[36] Caulerpicin C-24 **1**, palmitic acid **2**, β-sitosterol **3** and caulerpin

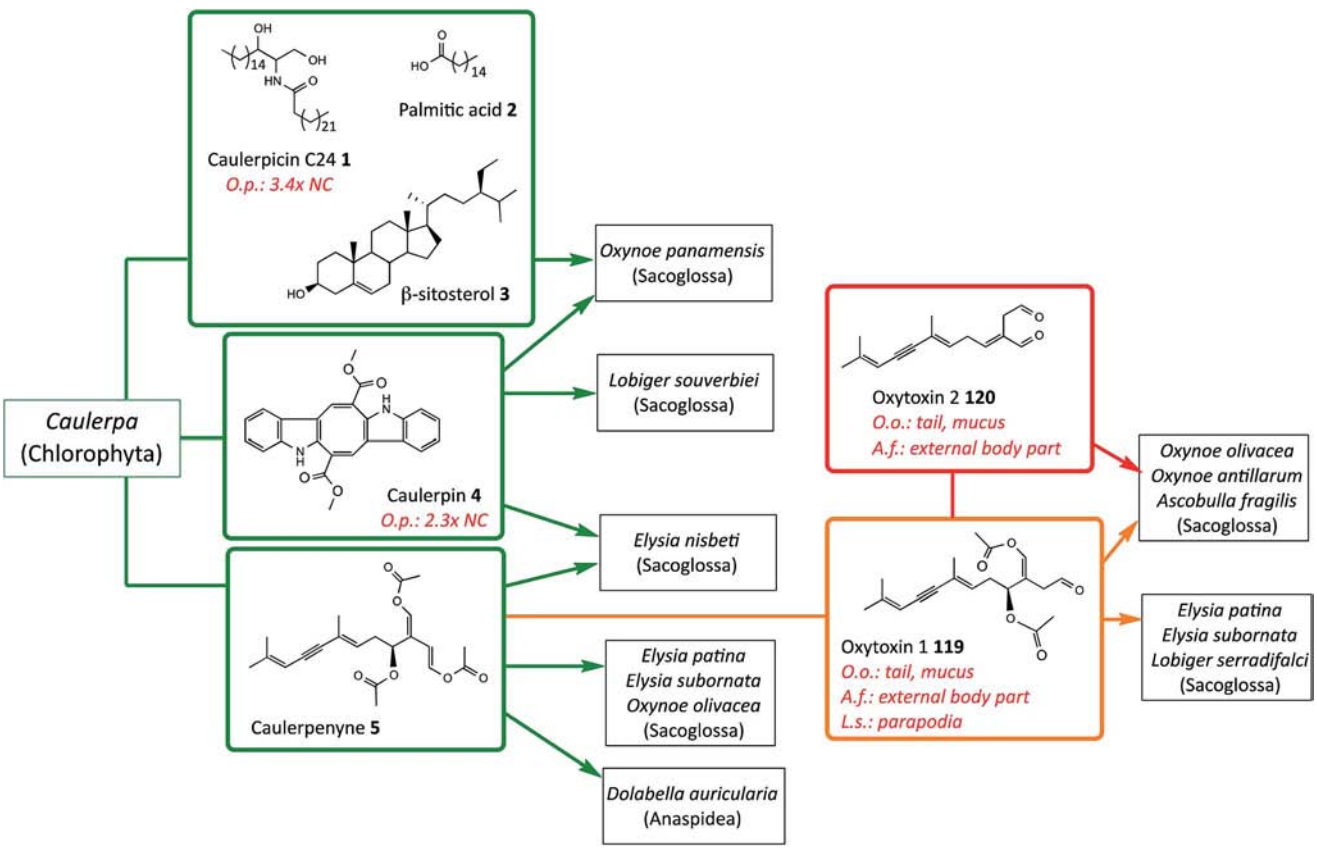

Fig. 2 Sequestration of algal secondary metabolites by *Oxynoe panamensis* (*O.p.*), *Lobiger souverbiei*, *Elysia nisbeti*, *E. patina*, *O. olivacea*, *E. subornata* and *Dolabella auricularia* and biotransformation by *O. olivacea* (*O.o.*), *Ascobulla fragilis* (*A.f.*), *O. antillarum*, *L. serradifalci* (*L.s.*), *E. subornata* and *E. patina*.

4 are detected in the mollusc and caulerpicin C-24 **1** and caulerpin **4** are more concentrated in the mollusc than in the original alga, indicating bioaccumulation (Fig. 2). Although when irritated or attacked the sacoglossan mollusc secretes an astringent milky mucus that is toxic to predatory fish,[37] we do not know if this toxicity is diet-derived as the toxin was neither characterized nor was the presence of the four accumulated algal compounds or their biotransformed compounds examined in this secretion. Other shelled sacoglossans such as *Oxynoe olivacea* (Fig. 6a) found on *Caulerpa prolifera* and *Lobiger souverbiei* found on *C. racemosa* sequester the toxic molecules caulerpin **4** and caulerpenyne **5** (Fig. 2) respectively.[38] Interestingly, **4** and **5** are two compounds from different pathways since the former is an alkaloid, probably a tryptophan dimer, while the latter is a sesquiterpene. However, the presence of these compounds as a potential defense is not confined to shelled species.

Gastropods of the shell-less *Elysia* genera are often specialists of green algae. The shell-less *Elysia patina* and *E. subornata* reared on *C. racemosa* store caulerpenyne, while *E. nisbeti* found on the same species is able to sequester caulerpenyne **5** and caulerpin **4** (Fig. 2) respectively.[18] In these examples, these molecules are stored as a chemical defensive strategy, in particular for *E. subornata* in which caulerpenyne constitutes the main component of the defensive mucus secretion.

The shell-less sacoglossans *Elysia translucens* that feeds upon *Udotea petiolata* and *Bosellia mimetica* upon *Halimeda tuna*, store secondary metabolites from their algal food.[34] *E. translucens* sequesters udoteal **6**, while *B. mimetica* accumulates halimedatetraacetate **7** (Fig. 3), however the compounds do not show any ichthyotoxicity. Shell-less *Elysia* genera are also often found on *Halimeda* species, such as *Elysia tuca* that feeds on *Halimeda incrassata*. Besides the fact that *E. tuca* accumulates the diet-derived fish deterrent halimedatetraacetate **7** (ref. 38 and 39) (Fig. 3), which confers it a chemical defense, the mollusc is also able to acquire chloroplasts from the algae.[40,41] These combined strategies enable *Elysia* both to photosynthesize and be cryptically colored, certainly increasing its chances of survival.

Furthermore, the shell-less *Elysia rufescens* feeds upon *Bryopsis* sp. and accumulates the algal secondary metabolite kahalalide F **8** (Fig. 4). This depsipeptide, present in mucus secretions, acts as a deterrent against reef fish conferring an effective defense to the mollusc.[42,43] Moreover, kahalalide F displays antileishmanial activity as well as antifungal activities against several fungal strains and may play an ecological role for the alga and the mollusc by suppressing or relieving the pressure of fungi and parasites.[44] Kahalalides A **9**, B **10**, G **11** and K **12** are also produced by *Bryopsis* sp. and sequestered by *E. rufescens*, although the ecological functions have not been investigated.[45–47] However, it is noteworthy that kahalalide A inhibits the growth of the bacteria *Mycobacterium tuberculosis* and may be used to suppress pathogenic bacteria of the alga and the mollusc.[44] Similarly, the presence of kahalalide O **13** has been detected both in *Elysia ornata* from Hawaii and *Bryopsis* sp., while kahalalide F **8** is also present in *Elysia grandifolia*[48,49] from Indian Ocean (Fig. 4). However, the origin of kahalalides is unclear since kahalalide F **8** has also been isolated from *Vibrio* sp. and the molluscs could acquire kahalalide-producing bacteria from the surface of *Bryopsis* sp. and retain them as symbionts.[50,51]

The shell-less *Costasiella ocellifera* specifically consumes the chlorophyceae *Avrainvillea longicaulis*[52] (Fig. 5). Avrainvilleol **14**,[38,53] a brominated diphenylmethane, is the main secondary metabolite produced by this green algae. The compound is toxic to reef fish and induces feeding avoidance behavior in the

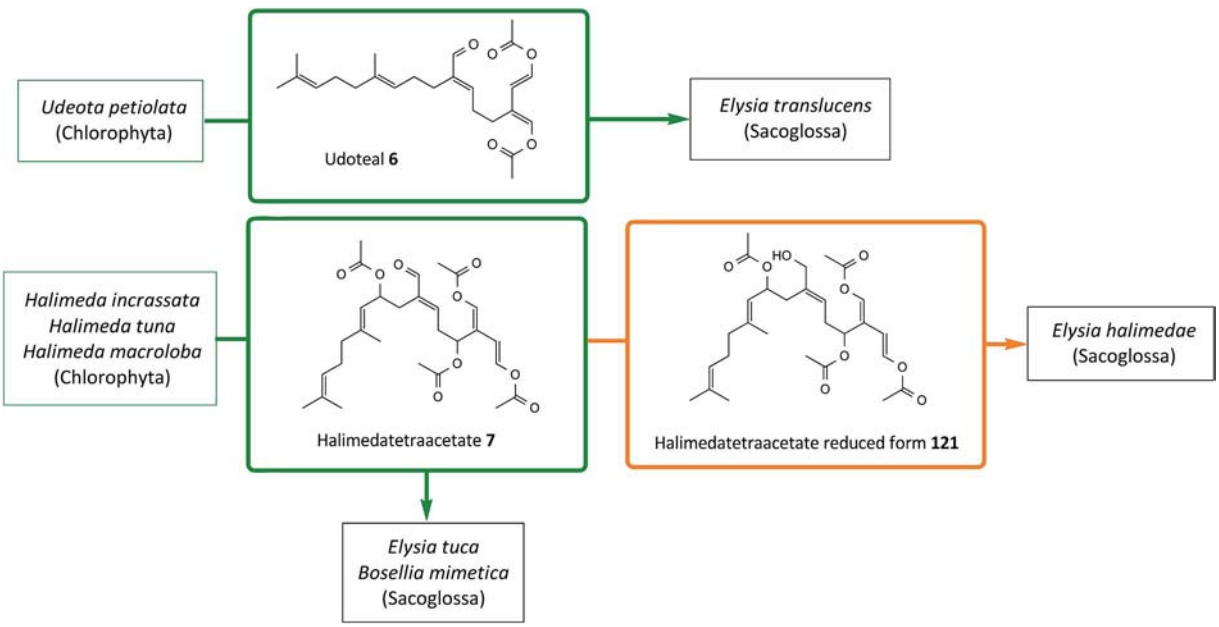

Fig. 3 Sequestration of algal secondary metabolites by *Elysia translucens*, *E. tuca* and *Bosellia mimetica* and biotransformation carried out by *E. halimedae*.

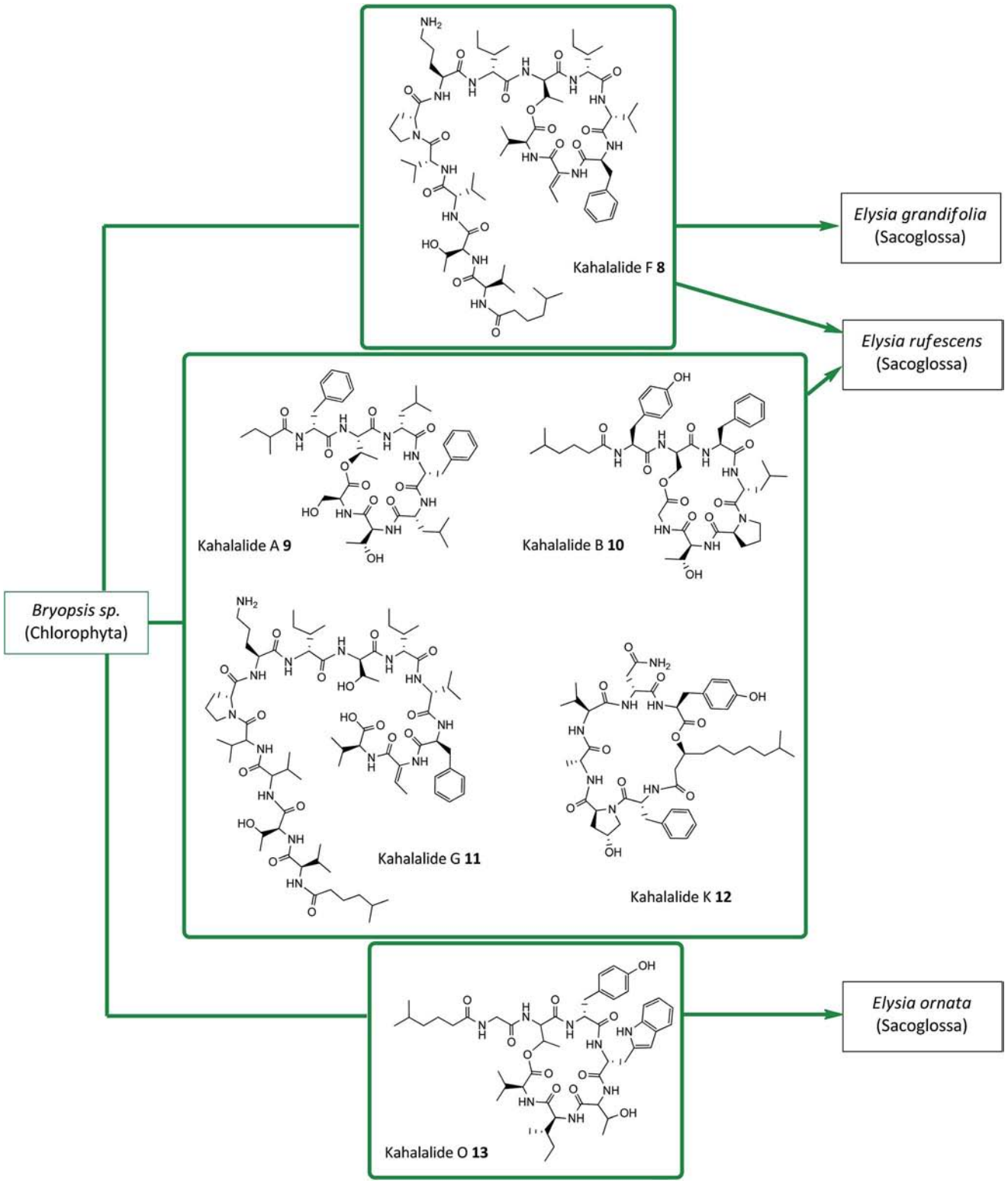

Fig. 4 Sequestration of algal secondary metabolites by *Elysia grandifolia*, *E. rufescens* and *E. ornata*.

herbivorous damselfish, *Pomacentrus coeruleus*. Therefore, *C. ocellifera* may acquire an effective defense against predatory fish by storing avrainvilleol **14** (Fig. 5).

In addition to sequestering secondary metabolites, the shell-less gastropod *Mourgona germaineae* has developed an additional defense mechanism in response to predator aggression.[54] Some heterobranch molluscs possess cerata, dorsal and lateral excrescences on the upper body. *Mourgona germaineae* responds to a predatory attack by autotomising their cerata (the spontaneous casting off of cerata) and secreting a toxic mucus.

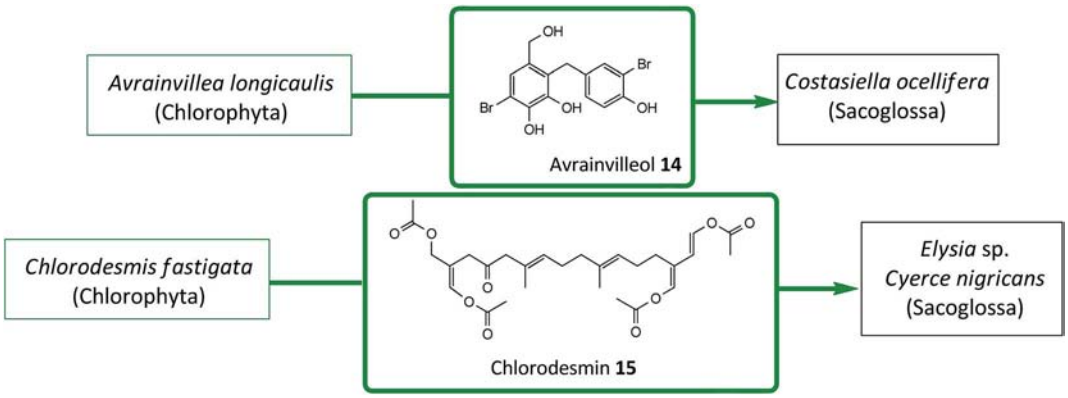

Fig. 5 Sequestration of algal secondary metabolites by *Costasiella ocellifera, Elysia* sp. and *Cyerce nigricans*.

The toxic secretion used in this defense is a non-fully identified water-soluble toxin produced by the algae *Cymopolia barbata* and transferred to the specialist heterobranch during feeding.[54]

The evolution of counteradaptations enabling consumers to feed on chemically-defended prey without apparent negative effects applies as much to sacoglossan herbivores as it does to their own carnivorous predators. The cytotoxic diterpenoid chlorodesmin **15**, which is the major secondary metabolite of the seaweed *Chlorodesmis fastigiata*, is a fish deterrent and confers an effective chemical defense to the algae.[13] However, it does not protect it from herbivory by two specialist herbivores, the shell-less *Elysia* sp. and *Cyerce nigricans*, nor does the sequestered chlorodesmin **15** (Fig. 5) protect *Elysia* sp. and *C. nigricans* from their carnivorous predator the dorid nudibranch, *Gymnodoris* sp. The diterpenoid is only found in small amounts in *C. nigricans*, which uses aposematism (displaying conspicuous colors) and biosynthesizing *de novo* toxic polypropionate compounds[55,56] as alternative defense strategies.

2.2 Sequestration of diet-derived chemicals by nudibranchs

Nudibranchia (class: Gastropoda, clade: Heterobranchia, clade: Euthyneura, clade: Nudipleura) are a group of soft-bodied marine gastropod molluscs that shed their shells after their larval stage. They occur in seas worldwide, and counter to sacoglossans, which are generally herbivorous and cryptically colored, nudibranchs are carnivorous and are well known for their conspicuous colors and use of mimicry. Sacoglossans are generally cryptically camouflaged, rendering them difficult to locate and affording them safety from predation by sharing the same color pattern as their habitat, or their prey[58] (Fig. 6a). On the other hand, most nudibranchs are mimetic, and emit cues of interest to a potential predator. Aposematism is a term given to a phenomenon in which species associate unpalatability with a warning signal, such as conspicuous colors, intended for potential predators (Fig. 6b).[59,60] This strategy is beneficial both for the aposematic species and for its potential predator. Müllerian mimicry is a multi-species generalization of aposematism: Müllerian mimicry complexes are formed by two or more unrelated distasteful species which have come to mimic each other's aposematic signals.[61] In contrast, species emitting a similar visible signal to another species, but lacks toxicity, show Batesian mimicry. This strategy consists of resembling a toxic species, using a similar color pattern and benefitting from reduced predation, without the associated costs of toxicity.[62] Interestingly, recent studies have proposed that secreted compounds may act as both a defensive component and warning signal and be the key element of chemosensory aposematism.[63,64] Considering the reliance of mimicry on toxicity, and their conspicuous colors, the sequestration of secondary metabolites is predicted to be common in Müllerian nudibranchs[65].

Several examples of nudibranchs using conspicuous colors associated with toxicity have been described.[57,66] Predatory

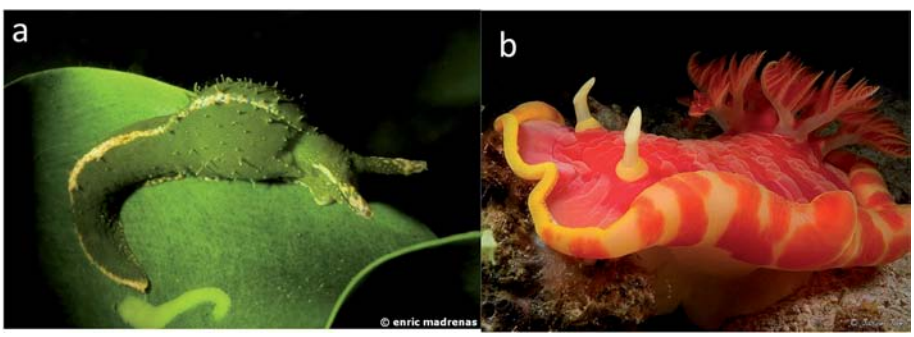

Fig. 6 (a) The cryptically colored sacoglossan *Oxynoe olivacea* (credits: Enric Madrenas). (b) The aposematic nudibranch *Hexabranchus sanguineus* (credits: Jason Jue).

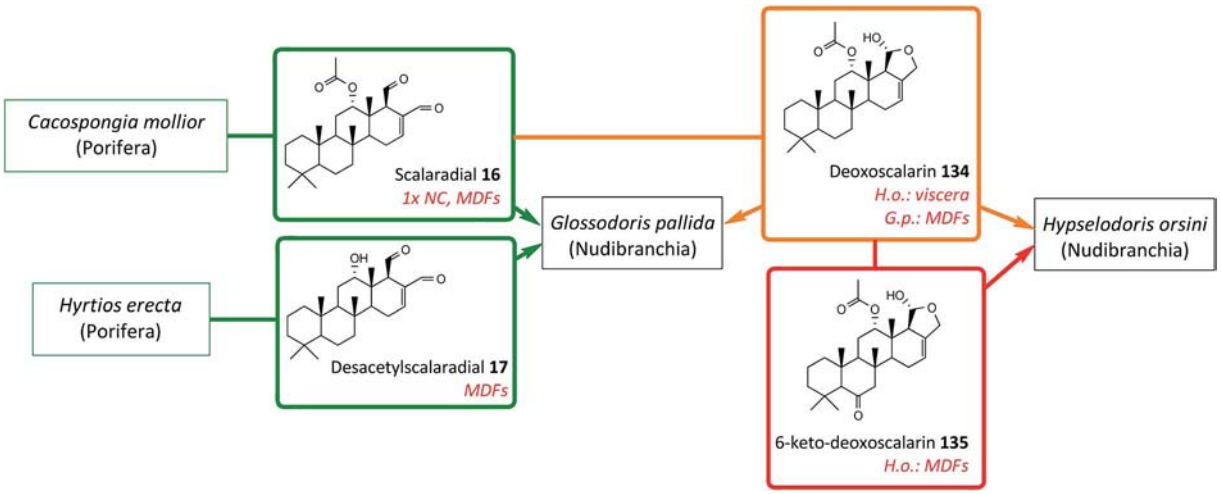

Fig. 7 Sequestration of sponge secondary metabolites by *Glossodoris pallida* and biotransformation carried out by *G. pallida* (*G.p.*) and *Hypselodoris orsini* (*H.o.*).

fishes avoid prey that exhibit visual cues; for example, yellow, purple and green nudibranchs repel the bluehead wrasse, *Thalassoma bifasciatum*.[67] In addition, the mummichog *Fundulus heteroclitus*, avoids unpalatable nudibranchs after tasting only a single individual.[68] In many cases, nudibranchs acquire toxicity by storing diet-derived metabolites. This

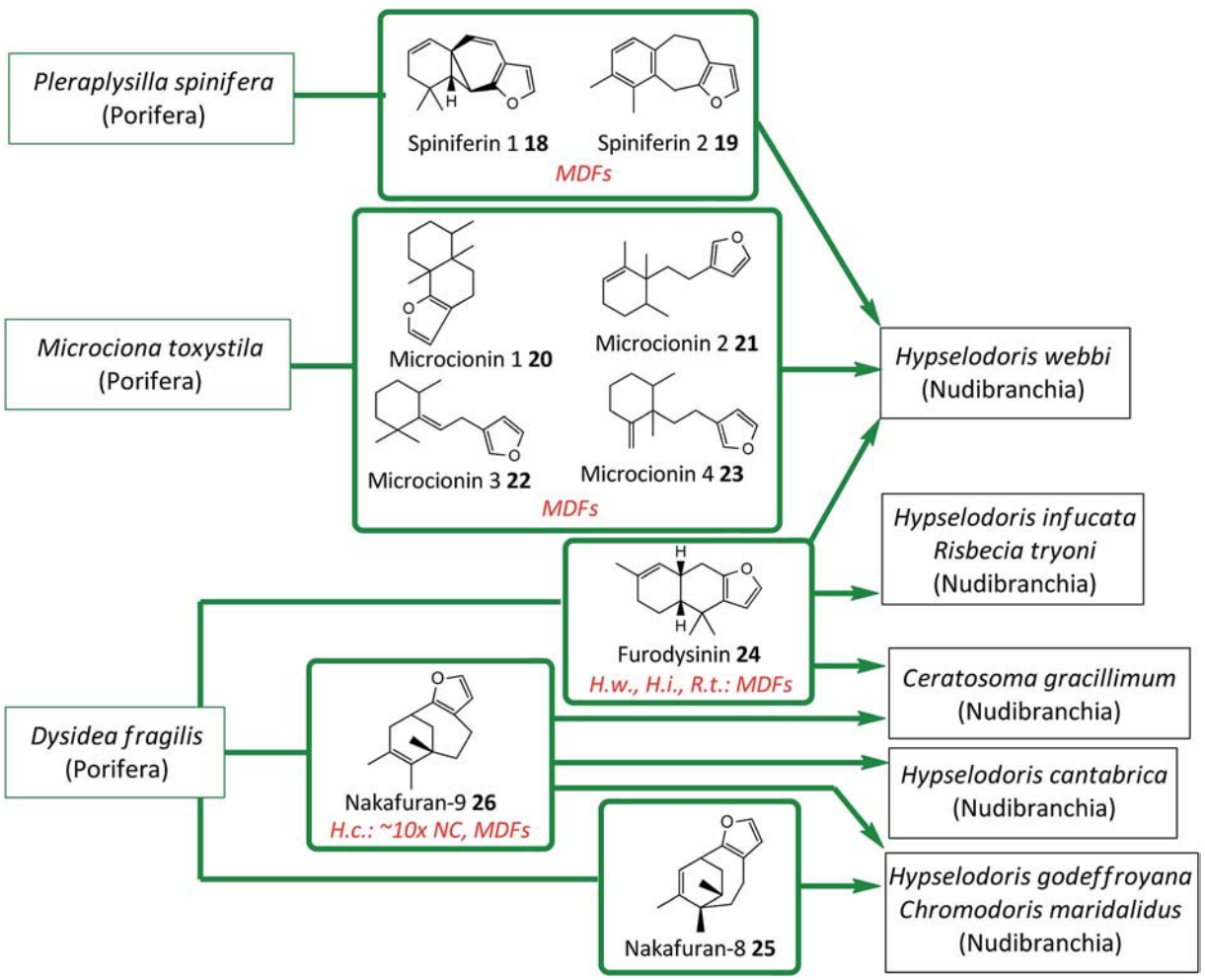

Fig. 8 Sequestration of sponge secondary metabolites by *Hypslodoris webbi* (*H.w*), *H. infucata* (*H.i*), *Risbecia tryoni* (*R.t*), *Ceratosoma gracillimum*, *H. cantabrica* (*H.c*), *H. godeffroyana* and *Chromodoris maridolidus*.

sequestration generally occurs in specialized glands located on exposed parts of the body, such as mantle dermal formations (MDFs) and provides them with an effective chemical defense.[57,69,70] The Chromodorid nudibranch, *Glossodoris pallida*, bioaccumulates two diet-derived diterpenoids, scalaradial **16** and desacetylscalaradial **17** from the sponges *Cacospongia mollior* and *Hyrtios erecta* respectively, and concentrates them in their MDFs[71,72] (Fig. 7).

Similarly, *Hypselodoris webbi* sequesters seven sesquiterpenoids also in its MDFs, from the three sponges, *Pleraplysilla spinifera*, *Microciona toxystila* and *Dysidea fragilis*.[69] *H. webbi* stores spiniferins 1 **18** and 2 **19** from *P. spinifera*, microcionins 1–4 **20–23** from *M. toxystila* and (−)-furodysinin **24** from *D. fragilis* (Fig. 8). *H. webbi* rapidly transfers all of the sesquiterpenoids from the sponges to their MDFs.[69]

Although nakafuran-8 **25** and nakafuran-9 **26** (Fig. 8) produced by the sponge *Dysidea fragilis* show anti-feeding activities, and confer protection against predators, such as the common *Chaetodon* spp. reef fish,[73] the nudibranch *Hypselodoris cantabrica* is able to circumvent this defense and bioaccumulate nakafuran-9 **26** in its MDFs (Fig. 8). Furthermore, these sesquiterpenes are found at a higher concentration than in the sponge, indicating that the heterobranch is more protected than its prey.[74] Two other nudibranchs, *Hypselodoris godeffroyana* and *Chromodoris maridalidus* are also able to store nakafuran-8 **25** and nakafuran-9 **26** from *D. fragilis*[73] (Fig. 8).

In order to determine whether the site of metabolite sequestration is important for nudibranch defense, six species of the chromodorid family were dissected into four parts including inner organs, mantle tissue devoid of MDFs, MDFs and dissection residuals.[70] The deterrent activities of eight diet-derived terpenoids and their crude extracts were then determined for each body part using the generalist brine shrimp *Palaemon elegans*.[70] *P. elegans* is a potential predator of chromodorid nudibranchs, and in trials using artificial, and chemically unprotected nudibranchs sculpted from squid muscle, they preferentially attacked the edges of the model's mantle.[70] These sites correspond to the location of MDFs in live nudibranchs and which, on attack, would release high concentrations of repellent chemicals.[70] Nudibranchs are therefore expected to sequester secondary metabolites in their MDFs, the most accessible and preferred part of the body to predators. Indeed, all six nudibranchs accumulate all but one of the eight terpenoids in their MDFs. *Chromodoris sinensis* and *Hypselodoris* sp. accumulate aplyroseol-2 **27** and the highly deterrent (+)-tetradehydrofurospongin-1 **28** respectively at high concentrations in their MDFs[70] (Fig. 9). *Hypselodoris infucata* and *Risbecia tryoni* sequester (−)-furodysinin **24** in their MDFs (Fig. 8) and these compounds show significant deterrent feeding activity even at concentrations lower than those found in the MDFs. Nakafuran-9 **26** and (−)-furodysinin **24** are also found at high concentrations in the MDFs of *Ceratosoma gracillimum* (Fig. 8). However, interestingly, *Glossodoris atromarginata* accumulates the two deterrent terpenoids spongiatrioltriacetate **29** and spongiatriol-diacetate **30** in MDFs, while spongiatriol **31**, that does not show any deterrent activity,

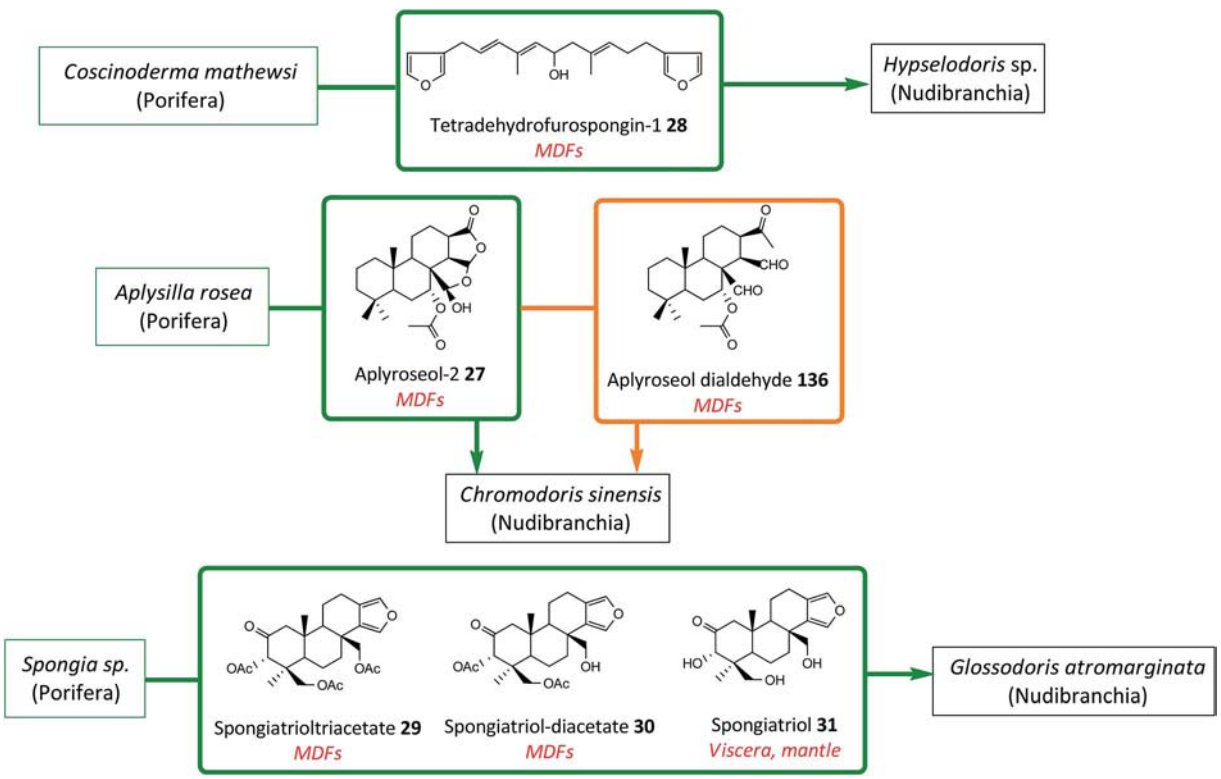

Fig. 9 Sequestration of sponge secondary metabolites by *Chromodoris sinensis*, *Hypslodoris* sp. and *Glossodoris astromarginata* and biotransformation carried out by *C. sinensis*.

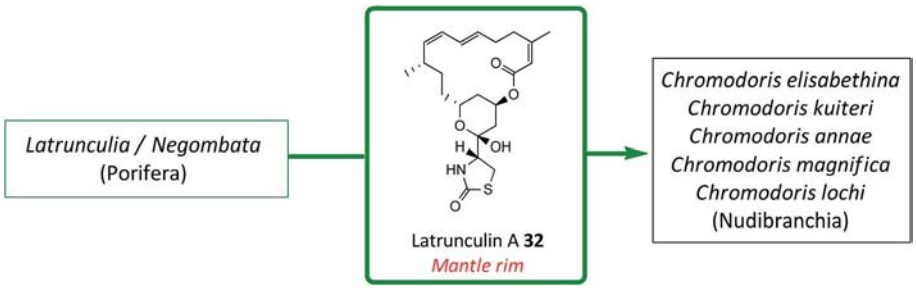

Fig. 10 Sequestration of latrunculin A by *Chromodoris elisabethina*, *C. kuiteri*, *C. annae*, *C. magnifica* and *C. lochi*.

is only found in the mantle and viscera (Fig. 9). This finding suggests that *G. atromarginata* selectively accumulates closely related sponge secondary metabolites in different locations of its body as a function of their capacity as a feeding deterrent.[70]

Five species of the genus *Chromodoris* have been chemically investigated in order to investigate the anatomical distribution of a diet-derived compound.[75] Individuals of each species were dissected into viscera and mantle tissues. The sponge macrolide latrunculin A **32** (Fig. 10), produced by the genera *Latrunculia* and *Negombata*,[76] is stored by the five *Chromodoris* species (*C. elisabethina*, *C. kuiteri*, *C. annae*, *C. magnifica* and *C. lochi*) in their mantle rim, where MDFs are located, while other compounds are only found in the viscera. Interestingly, and similarly to two deterrent terpenoids spongiatrioltriacetate **29** and spongiatriol-diacetate **30**, latrunculin A **32** is more potent than the other compounds found in the viscera, and is toxic and unpalatable against the generalist brine shrimp *Palaemon serenus*. These results highlight the specific sequestration of latrunculin A **32** and the authors proposed that the compound acts both as a chemical defense and as a warning signal for potential predators.[75] Hence, the five nudibranch species may form a visual and chemosensory Müllerian mimicry complex.

However, *Hypselodoris fontandraui* lacks MDFs yet has a similar color pattern to that of other *Hypselodoris* species that possess MDFs.[62] To determine if this species stores secondary metabolites elsewhere or lacks a chemical defense and thus

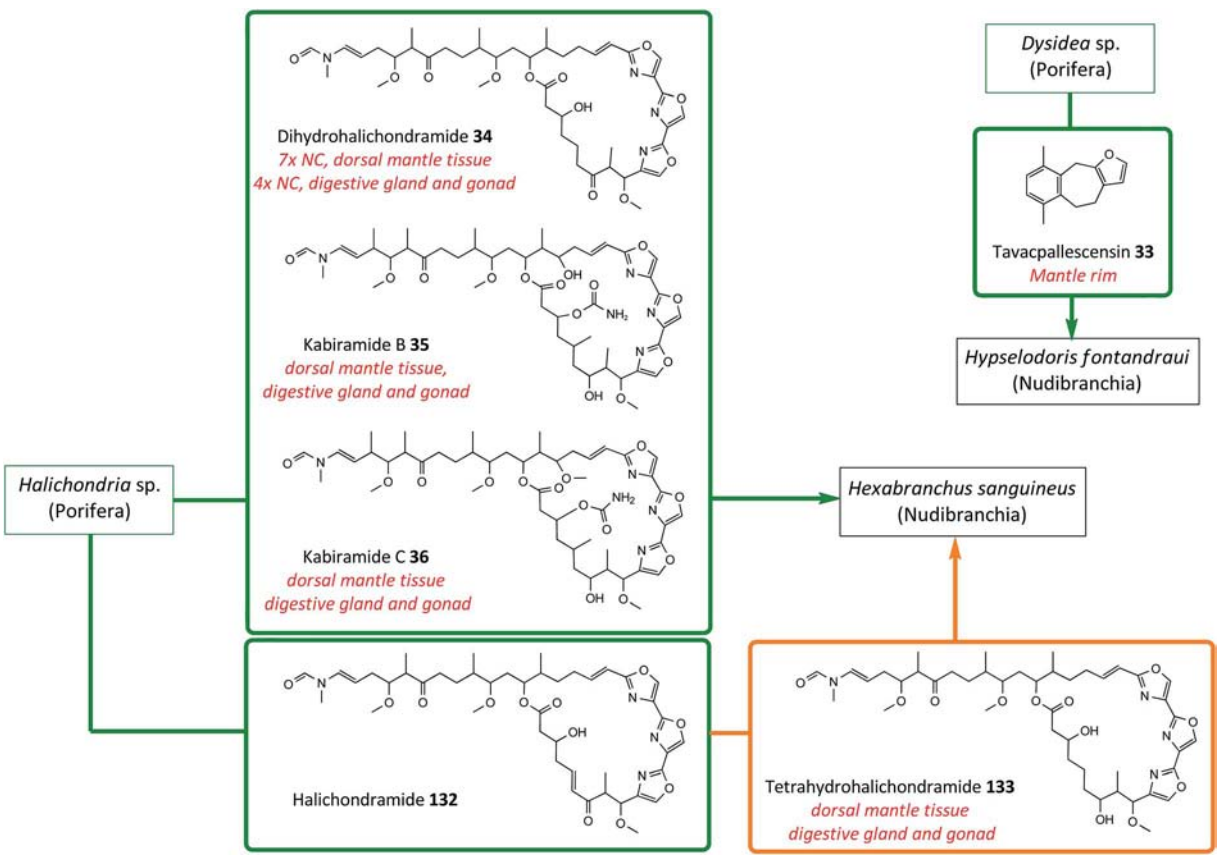

Fig. 11 Sequestration of sponge secondary metabolites by *Hypslodoris fontandraui* and *Hexabranchus sanguineus* and biotransformation carried out by *H. sanguineus*.

uses Batesian mimicry as a defense mechanism, individuals were studied histologically and chemically. The furanosesquiterpenoid tavacpallescensin **33**, the main compound of a *Dysidea* sponge genus, was isolated from *H. fontandraui* and was found to be four times more concentrated along the mantle border than in other external parts and twenty times more concentrated than in inner parts[62] (Fig. 11). This metabolite, also present in mucus secretions, repels the shrimp *P. elegans*.[62] In addition, histological studies revealed structures with a granular component in the body wall, just below the border of the mantle whose function is comparable to those of MDFs.[62] Therefore, *H. fontandraui* is indeed chemically defended and uses structures other than MDFs to store secondary metabolites, proving that it uses aposematic Müllerian mimicry similar to other *Hypselodoris* species.

In addition to compounds from the terpene family, the nudibranch *Hexabranchus sanguineus* (Fig. 6b), sequesters three macrolides dihydrohalichondramide **34**, kabiramides B **35** and C **36** from two *Halichondria* sponges (Fig. 11), which are particularly concentrated in the dorsal mantle tissue and deter predation by the reef fish *Thalassoma lunare*. However, the metabolites are also sequestered in the digestive and gonad glands, which are in turn transferred to the eggs of the sea slug, an example of vertical transmission.[77] As such, the deterrent activity of the metabolites provides both the adult nudibranch and its eggs, with chemical defenses.

Several diet-derived terpenoids have also been isolated from the dorsum of the generalist nudibranch *Cadlina luteomarginata*.[78,79] Microcionin 2 **21**, furodysinin **24**, furodysin **37**, albicanol **38**, albicanyl acetate **39** and luteone **40** are found in the external parts of *C. luteomarginata* (Fig. 12). The first three compounds are certainly produced by sponges, while the origin of albicanol remains unclear. Moreover, further studies reveal that albicanyl acetate **39**, luteone **40** and additionally cadlinaldehyde **41** are biosynthesized by the nudibranch and are found in MDFs and also in the remaining mantle.[80] Interestingly, the concentrations of luteone **40** and cadlinaldehyde **41** are inversely correlated with the availability of structurally similar diet-derived compounds. Therefore, *C. luteomarginata* is able to maintain its chemical defense by sequestering diet-derived secondary metabolites when present and by modulating the production of endogenous compounds according to the presence of chemically-defended prey.

Metabolite sequestration has been found in four other nudibranch species based on chemical studies of the entire body, but the specific location is not known. *Anisodoris nobilis* sequesters *N*-methylnucleoside doridosine **42** originating from the sponge *Tedania digitata*[81] and *Phyllidia varicosa* stores the two isomer terpenoids 2-isocyanopupukeanane **43** and 9-isocyanopupukeanane **44** (ref. 82) from *Hymeniacidon* sponges (Fig. 13). Similarly, *Peltodoris atromaculata* accumulates unnamed polyacetylenes produced by the sponge *Petrosia ficiformis*.[83] Finally, the deterrent secondary metabolite heteronemin **45**, produced by the sponge *Hyrtios erecta*, is accumulated by *Glossodoris hikuerensis* and *G. cincta*[71,84] (Fig. 13).

Nudibranchs not only sequester metabolites from sponges, but also from bryozoans and ascidians. *Tambja abdere* and *T. eliora* feed upon the bryozoan *Sessibugula translucens*[85] (Fig. 14). In turn, *Roboastra tigris*, another nudibranch, preys upon *T. abdere* and *T. eliora* and the alkaloids tambjamines A–D **46–49**, present in the bryozoan are sequestered by all three nudibranchs. As tambjamines deter feeding by the spotted kelpfish *Gibbonsia elegans*, there is evidence for the transmission of both metabolites and the defense mechanism across two trophic levels. During an attack by *R. tigris*, *T. abdere* secretes a distasteful mucus containing deterrent chemicals which may cause the predator to abandon its attack, while *T. eliora* attempts to escape by swimming away. The cytotoxic tambjamine K **50** has also been isolated from the nudibranch *Tambja ceutae*, but appears to originate from the bryozoan *Bugula dentata* which contains this tambjamine in small amounts[86] (Fig. 14). Interestingly, tambjamines are also present in ascidians, as the nudibranch *Nembrotha* spp., acquires tambjamines C **48**, E **51** and F **52** from its diet, the ascidian *Atapozoa* sp.[87] (Fig. 14). Whether tambjamines are biosynthesized by a common microorganism rather than both bryozoans and ascidians is still being investigated.

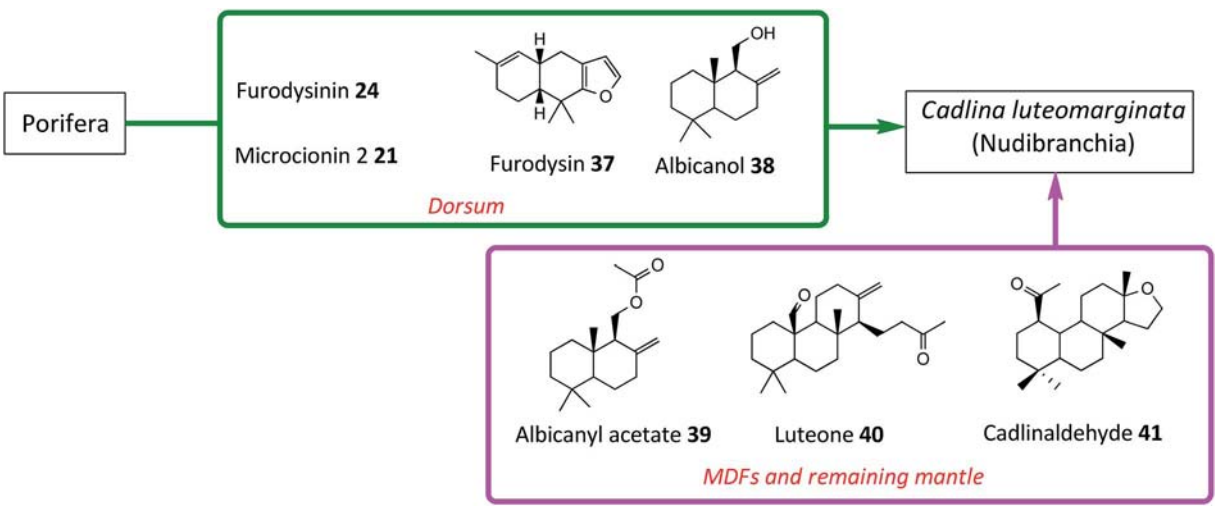

Fig. 12 Sequestration of sponge secondary metabolites and *de novo* biosynthesis by *Cadlina luteomarginata*.

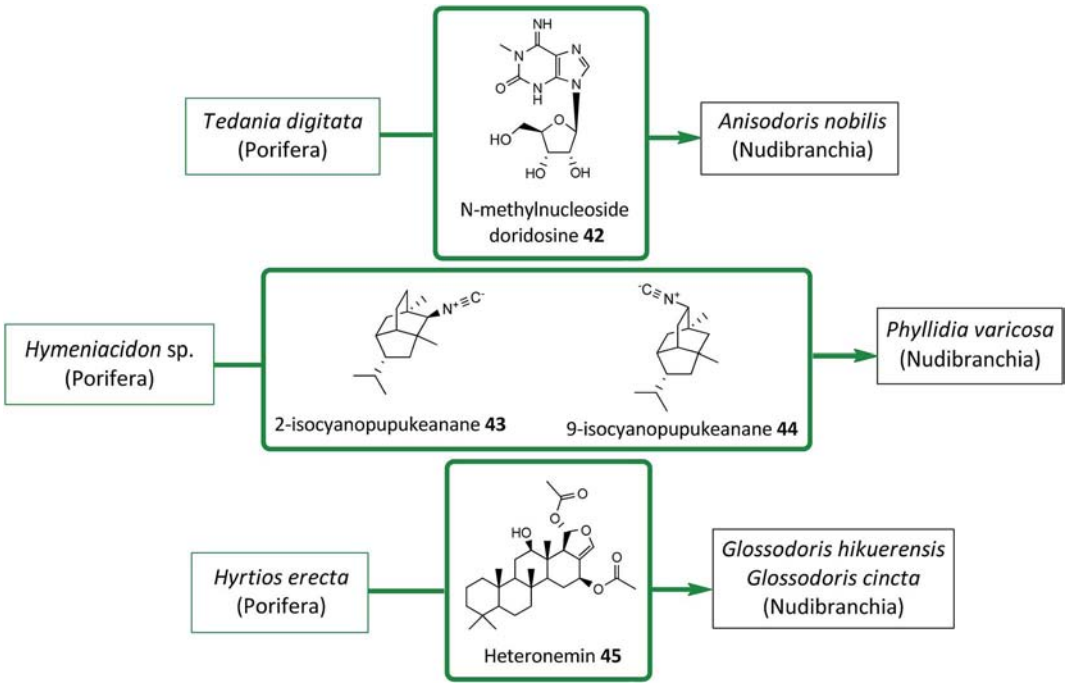

Fig. 13 Sequestration of sponge secondary metabolites by *Phyllidia varicosa*, *Anisodoris nobilis*, *Glossodoris hikuerensis* and *G. cincta*.

Finally nudibranchs, as exemplified by *C. luteomarginata*, in addition to sequestering secondary metabolites are also able to biosynthesize them *de novo*. The omnivorous nudibranch *Bathydoris hodgsoni* likely biosynthesizes *de novo* hodgsonal **53** (Fig. 15), a sesquiterpene found to repel the sea urchin *Odontaster validus*.[88,89] The compound, found at high concentration in the

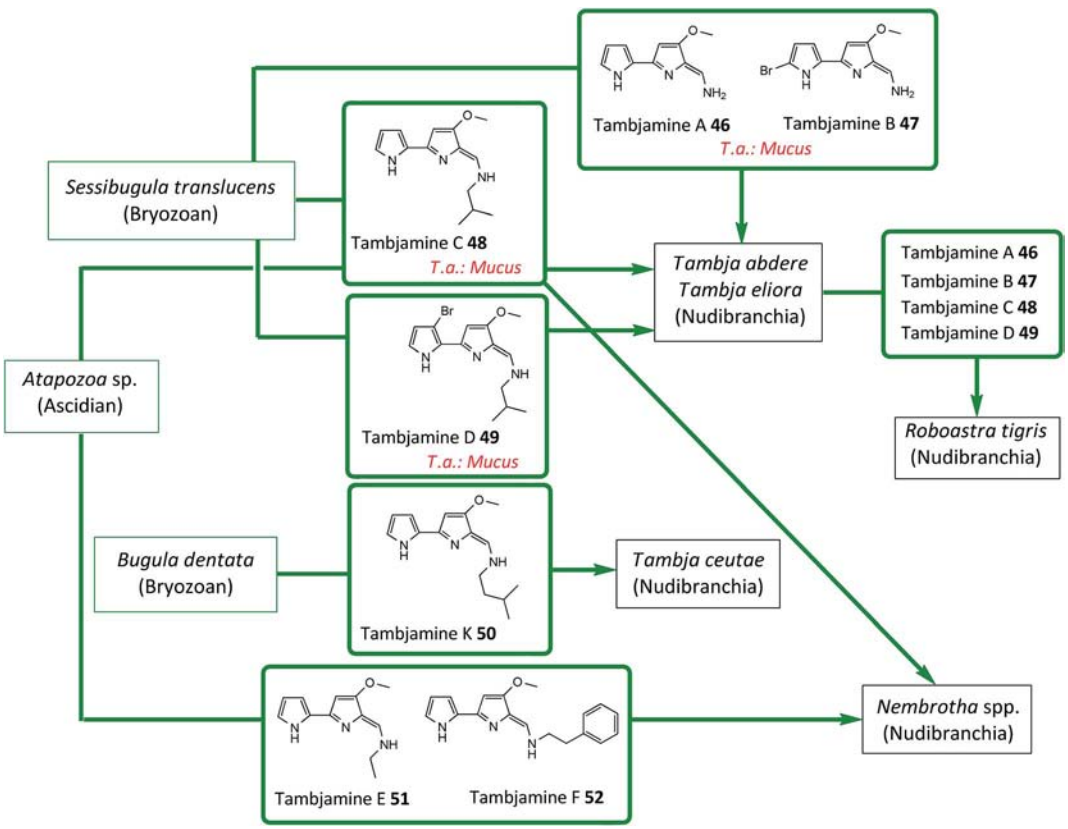

Fig. 14 Sequestration of bryozoan and ascidian secondary metabolites by *Tambja abdere* (*T.a*), *T. eliora*, *Roboastra tigris*, *T. ceutae* and *Nembrotha* spp.

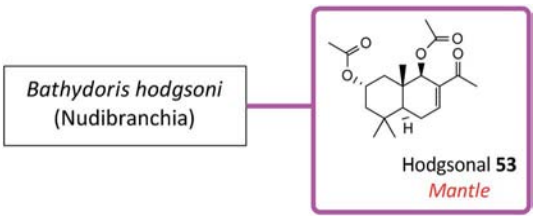

Fig. 15 Potential *de novo* biosynthesis of hodgsonal by *Bathydoris hodgsoni*.

mantle tissue, presumably constitutes an effective form of defense and the authors hypothesize that hogsonal **53** is biosynthetized *de novo* by the nudibranch since it was not detected in the viscera, as it should be in the case of a dietary compound. However, biosynthesis has not been studied in this species and the closely related chemical structures of both hodgsonal **53** and the sponge secondary metabolite albicanol **38** have raised doubts about the origins of hodgsonal.

2.3 Sequestration of diet-derived chemicals by anaspideans (sea hares)

Anaspidea (class: Gastropoda, clade: Heterobranchia, clade: Euthyneura, clade: Euopisthobranchia) have soft bodies with an internal shell. They are herbivorous cryptically colored heterobranchs, similar to most sacoglossans, feeding on rich chemically-defended seaweeds or cyanobacteria and have developed specialized chemical defense strategies. Sea hares secrete an ink mixture which operates as an antipredator mechanism by acting on the olfactory and non-olfactory chemical senses of predators.[90] The ink mixtures elicit aversive behavior in the sea anemone *Anthopleura sola*[91] and inhibits foraging and feeding in the blue crab *Callinectes sapidus*.[92] This ink mixture is composed of both ink released by the ink gland, and opaline secreted by the opaline gland.[93,94] The components of opaline and ink are diet-dependent or can be biosynthesized *de novo*[95] and some of the molecules appear to deter predators. The coloration of this ink mixture is often purple due to aplysioviolin **54** and phycoerythrobilin **55** derived from a light-harvesting protein present in red algae (Rhodophyta) and cyanobacteria and which act as deterrents against *C. sapidus*[92,96–98] (Fig. 16). Furthermore, five mycosporine-like amino acids (MAAs) have been isolated from the opaline secretion of the sea hare *Aplysia californica* including asterina-330 **56**, *N*-isopropanol palythine **57** and *N*-ethyl palythine **58** which act as an intraspecific alarm signal for juveniles warning them of a predatory attack on a conspecific.[99] The other two MAAs, palythine **59** and *N*-methyl palythine **60** are also components of opaline, but do not act as alarm chemical signals. All five of these MAAs are found in the red algae *Gracilaria ferox* and *Agardhiella subulata* (Fig. 16) on which *A. californica* feeds.

Sea hares of the *Aplysia* genus are for the most part generalist herbivores of red algae, with geographical location dictating their preferred species. In Guam, *Aplysia parvula* prefers the red alga *Portieria hornemannii*, which produces the generally unpalatable compounds apakaochtodenes A **61** and B **62** (Fig. 17), but also feeds on the red alga *Acanthophora spicifera* which does not contain any unpalatable compounds.[100] In turn, *A. parvula* fed solely on *A. spicifera* are eaten by the fishes *Abudefduf saxatilis*, *Thalassoma lutescens* and *Arothron manilensis*, unlike *A. parvula* fed on *P. hornemannii*, providing evidence that

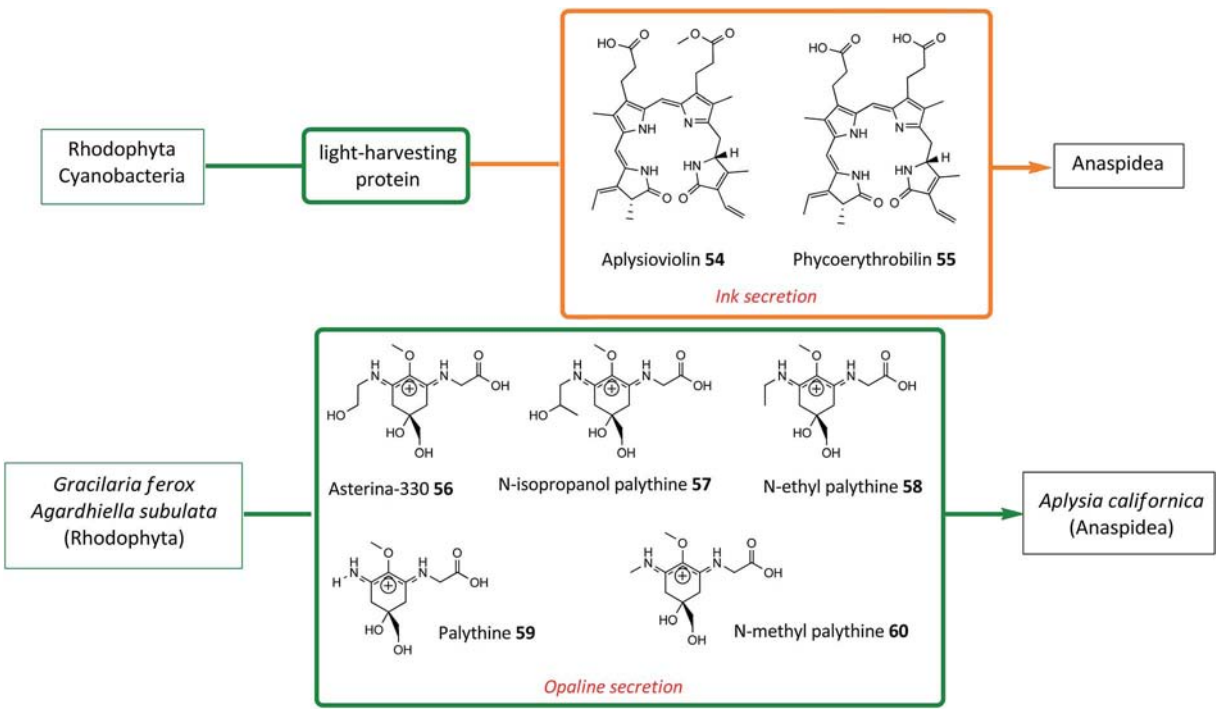

Fig. 16 Biotransformation of algal secondary metabolites by different Anaspidea and sequestration by *Aplysia californica*.

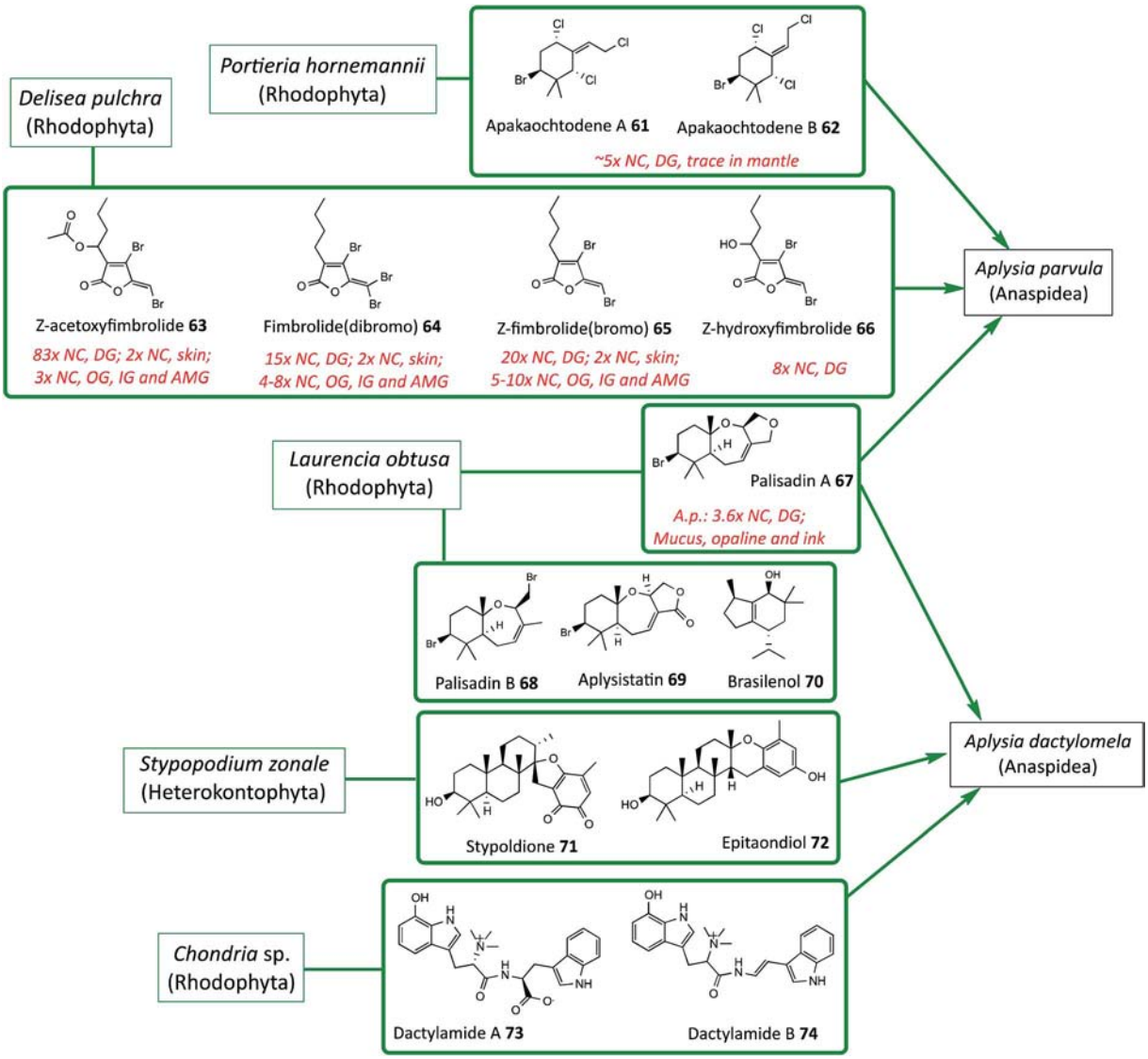

Fig. 17 Sequestration of algal secondary metabolites by *Aplysia parvula* and *A. dactylomela*.

the ingested unpalatable compounds defend the sea hare from predators. The two main algal secondary metabolites ingested, the tetrahalogenated monoterpenes apakaochtodenes A **61** and B **62**, are sequestered in the digestive gland, while small amounts are also detected in the mantle.[100] In contrast, in Australia, *A. parvula* feeds upon two red algae: *Delisea pulchra* and *Laurencia obtusa*.[101] *D. pulchra* produces the halogenated furanone *Z*-acetoxyfimbrolide **63** at high concentrations, which deters reef fish, as well as fimbrolide(dibromo) **64**, *Z*-fimbrolide(bromo) **65** and *Z*-hydroxyfimbrolide **66** at moderate concentrations (Fig. 17). *L. obtusa* produces the deterrent sesquiterpene palisadin A **67** at high concentrations as well as palisadin B **68**, aplysistatin **69** and brasilenol **70** (Fig. 17). Thus, *A. parvula* fed on *D. pulchra* accumulates *Z*-acetoxyfimbrolide **63**, fimbrolide(dibromo) **64**, *Z*-fimbrolide(bromo) **65** and *Z*-hydroxyfimbrolide **66** in their digestive gland and moderate concentrations of *Z*-acetoxyfimbrolide **63**, fimbrolide(dibromo) **64** and *Z*-fimbrolide(bromo) **65** in the skin, the anterior mantle gland as well as in the opaline and ink glands[102] (Fig. 17). On the other hand, *A. parvula* fed on *L. obtusa* sequesters palisadin A **67** in the digestive gland, mucus and opaline secretions. The sequestration of these compounds from both diets in mucus and opaline and ink secretions indicate their use as a defense strategy.[101] However, another sea hare, *Aplysia dactylomela*, sequesters palisadin A from the same red algae in its digestive gland, but not in mucus or opaline secretions.

Similarly, *Aplysia dactylomela* accumulates stypoldione **71** and epitaondiol **72** from the brown alga (Heterokontophyta) *Stypopodium zonale*[103] (Fig. 17). In addition, dactylamides A **73** and B **74** (Fig. 17), as well as isolaurenisol **75** and allolaurinterol **76** (Fig. 18) have been found in *A. dactylomela*.[98] Isolaurenisol **75** and allolaurinterol **76** are sesquiterpenes related to those of the aplysin family and are produced by the red algae *Laurencia distichophylla* and *L. filiformis* respectively (Fig. 18), while the dactylamides A **73** and B **74** could be biosynthetic precursors of chondriamides (bisindolic amides) produced by the red algae genus *Chondria*.

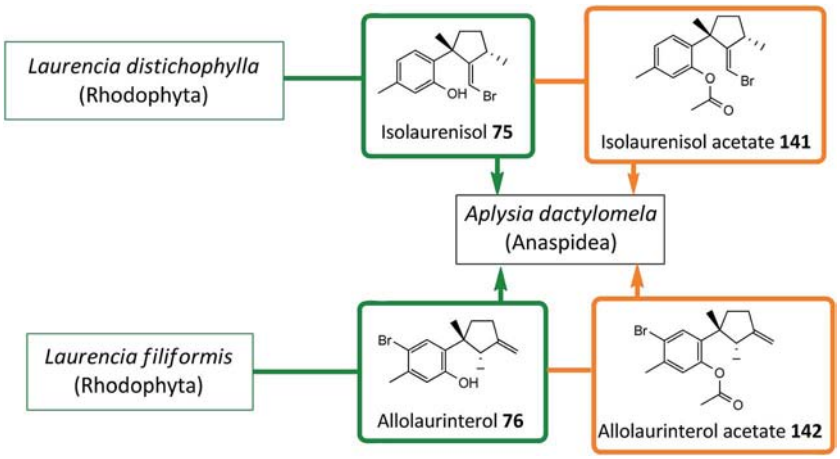

Fig. 18 Sequestration and biotransformation of algal secondary metabolites by *Aplysia dactylomela*.

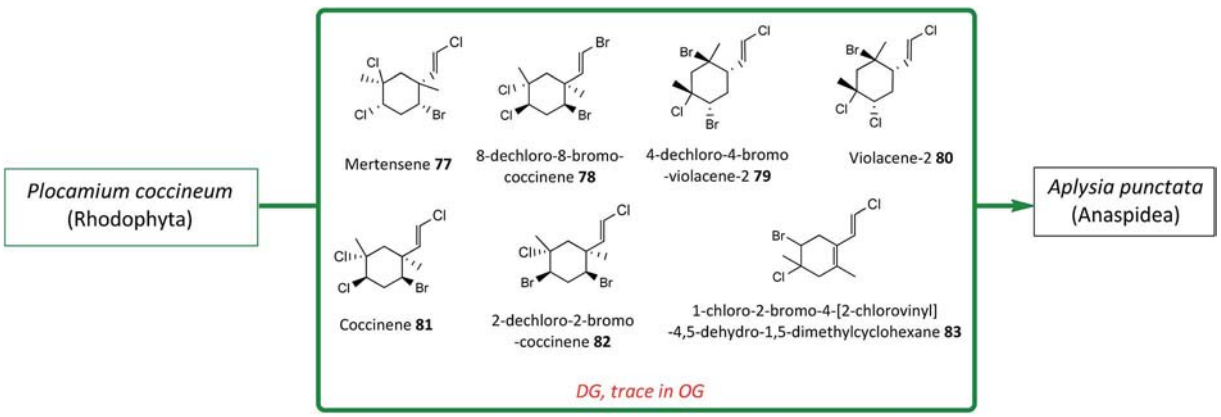

Fig. 19 Sequestration of algal secondary metabolites by *Aplysia punctata*.

In another example of sequestered diet-derived compounds, seven cyclic halogenated monoterpenes are stored in the digestive gland and are found in trace levels in the opaline gland of *Aplysia punctata*[104] (Fig. 19). These monoterpenes, mertensene **77**, 8-dechloro-8-bromo-coccinene **78**, 4-dechloro-4-bromo-violacene-2 **79**, violacene-2 **80**, coccinene **81**, 2-dechloro-2-bromo-coccinene **82** and 1-chloro-2-bromo-4-[2-chlorovinyl]-4,5-dehydro-1,5-dimethylcyclohexane **83** are produced by the red alga *Plocamium coccineum* and transferred to the sea hare during feeding (Fig. 19).

Two other sea hares, *Aplysia juliana* and *A. kurodai*, were studied to determine their ability to sequester different secondary metabolites from various origins in their digestive glands.[105] *A. juliana* sequesters the cyanobacterial compound malyngamide B **84** and the brown alga metabolite pachydictyol A **85**, while *A. kurodai* sequesters pachydictyol A and the sponge secondary metabolite luffariellolide **86** [105] (Fig. 20). Similarly, the secondary metabolites aplysin **87**, debromoaplysin **88**, laurinterol **89**, pacifenol **90**, johnstonol **91** and pacifidiene **92** produced by the alga *Laurencia pacifica* are found in the digestive gland of the generalist *A. californica*[106] (Fig. 20). Thus, multiple studies carried out on species in the *Aplysia* genus conclude that algal and cyanobacterial secondary metabolites are compartmentalized in the digestive gland. However, lower concentrations are also found in secretions, indicating their potential use as defense mechanisms.

The fate of cyanobacterial secondary metabolites in *Stylocheilus striatus*, (previously identified as *Stylocheilus longicauda*), has sparked considerable interest in researchers. *S. striatus* is considered a specialist on the cyanobacterium *Lyngbya majuscula*,[107–110] which appears to be a prolific source of chemicals, although due to misidentification of this cyanobacterium species it may not be as prolific as previously thought because all the chemicals are likely produced by different species considered to be *L. majuscula*. The fate of lyngbyatoxin A (LTA) **93** and debromoaplysiatoxin (DAT) **94** produced by *L. majuscula* collected in Moreton bay, Australia, was investigated in the digestive gland, the foot and the head and also in excretions of the sea hare, *S. striatus* (Fig. 21). The metabolites are present at high concentrations in the digestive gland and at low concentrations in the other sites, while LTA **93** is also present at very low concentrations in faecal matter, eggs and in ink.[111] In addition, the malyngamides A **95** and B **84** isolated from *L. majuscula* collected from Guam are concentrated in the

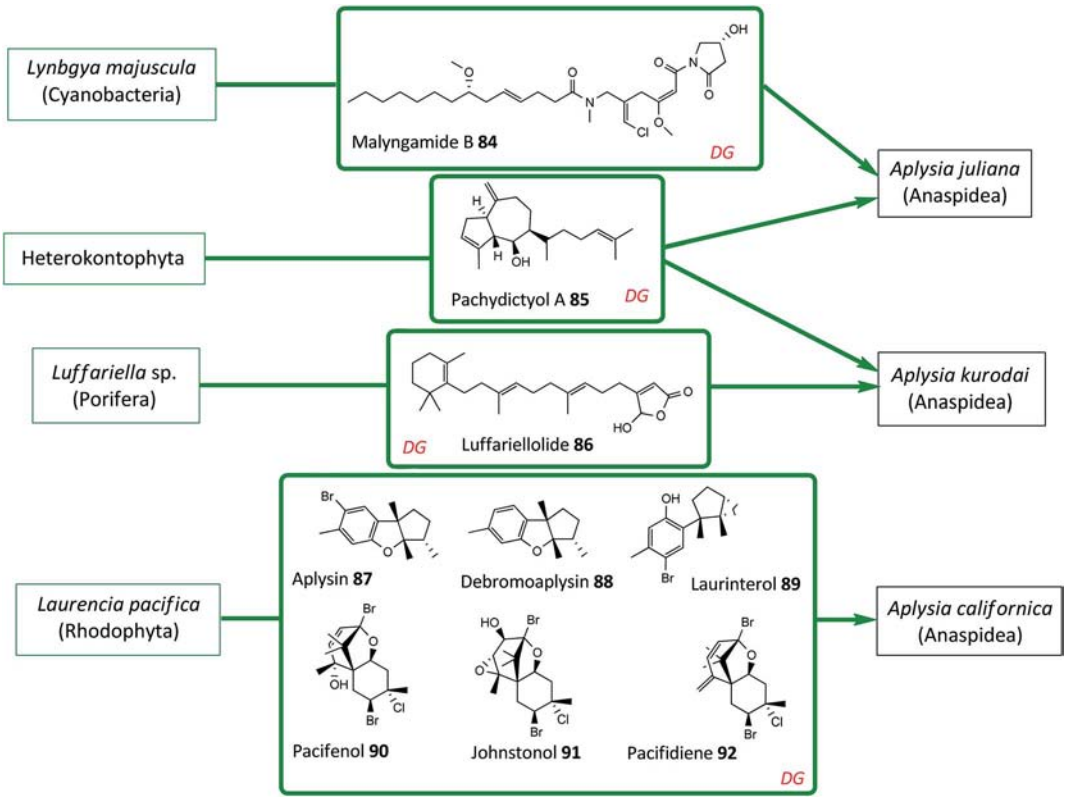

Fig. 20 Sequestration of cyanobacterial, algal and sponge secondary metabolites by *Aplysia juliana*, *A. kurodai* and *A. californica*.

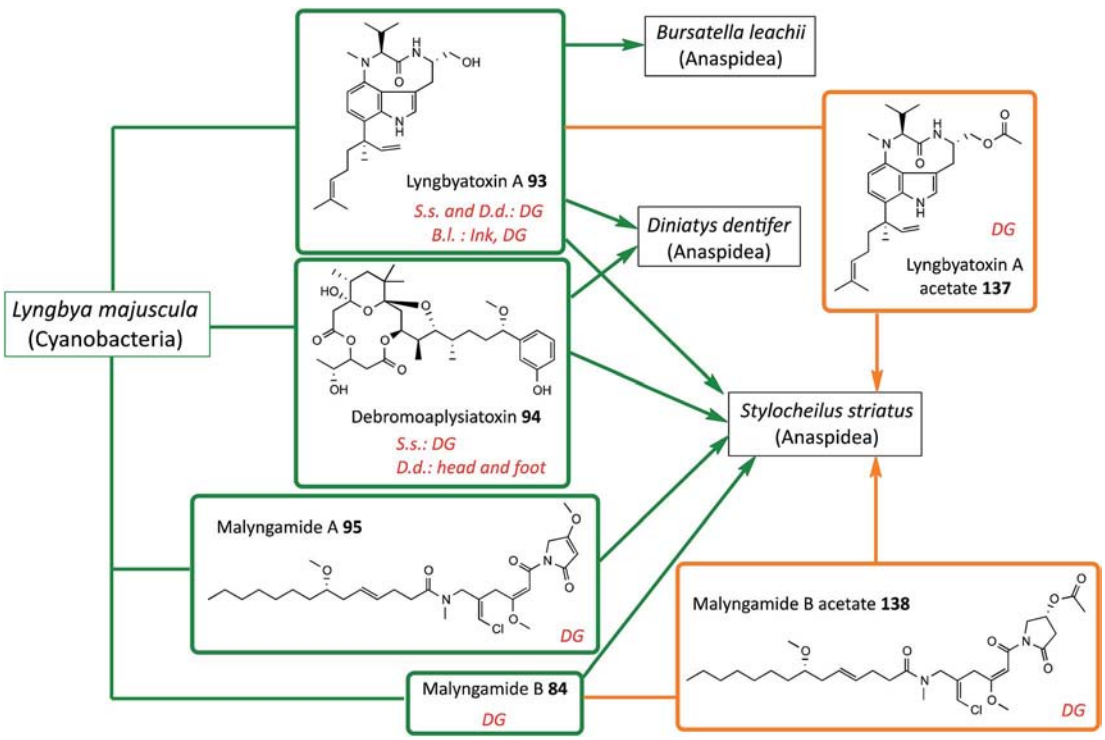

Fig. 21 Sequestration of cyanobacterial secondary metabolites by *Stylocheilus striatus* (*S.s*), *Diniatys dentifer* (*D.d*) and *Bursatella leachii* (*B.l*) and biotransformation carried out by *S. striatus*.

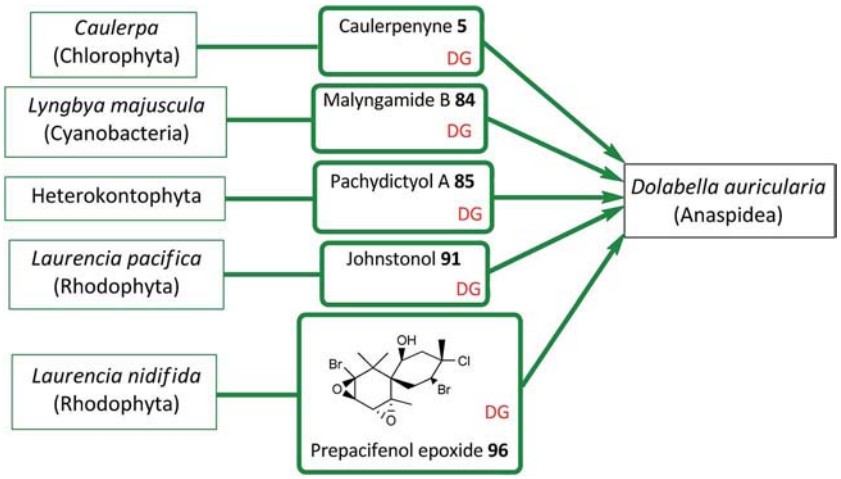

Fig. 22 Sequestration of algal and cyanobacterial secondary metabolites by *Dolabella auricularia*.

digestive gland of *S. striatus*[107,112] and show deterrent activities against the puffer fish *Canthigaster solandri* and the crabs *Leptodius* spp.[109] (Fig. 21).

Furthermore, *Dolabella auricularia*, a generalist sea hare, stores compounds in the same way as *S. striatus* in its digestive gland:[97] caulerpenyne **5** from *Caulerpa* spp., pachydictyol A **85** from brown algae, malyngamide B **84** [112] from *L. majuscula*, johnstonol **91** from *Laurencia pacifica* and prepacifenol epoxide **96** from *L. nidifida* (Fig. 22). The ecological role of sequestered diet-derived metabolites has not yet been defined for either *S. striatus* or *D. auricularia*. In contrast, although *Bursatella leachii*, another sea hare feeding on *L. majuscula*, also sequesters LTA **93** in the digestive gland, higher concentrations are found in ink, highlighting the existence of a potential defense strategy[111] (Fig. 21).

The sea grass *Halophila stipulacea* constitutes the main food item of the sea hare *Syphonota geographica* which is able to store several diet-derived compounds.[113] The macrocyclic diterpenoid syphonoside **97** as well as the flavonoids apigenin **98**, genkwanin **99** and chrisoeriol **100** (Fig. 23) are transmitted from sea grass to the sea hare.[114,115] These compounds are found in the sea hare's viscera (internal organs) but no further studies have investigated their sequestration in other parts of the mollusc.[115] Interestingly, the relative amount of syphonoside is much higher in the mollusc than in its prey indicating a bioaccumulation effect.[114]

2.4 Sequestration of diet-derived chemicals by other gastropods

Other instances of gastropod molluscs bioaccumulating compounds include *Diniatys dentifer* (order Cephalaspidea) which sequesters LTA **93** and DAT **94** from *L. majuscula*[111] (Fig. 21). However, whilst both of these compounds are found in high concentrations in the digestive glands of *S. striatus*, DAT **94** is only found in the head and the foot in *D. dentifer* and at high concentrations and LTA **93** is present in low concentrations in the digestive gland. In addition, the cephalaspidean mollusc *Philinopsis speciosa* contains six cyclic depsipeptides; kulolide-1 **101**, kulolide-2 **102**, kulolide-3 **103**, kulokainalide-1 **104**, kulomo'opunalide-1 **105** and kulomo'opunalide-2 **106**, as well as the

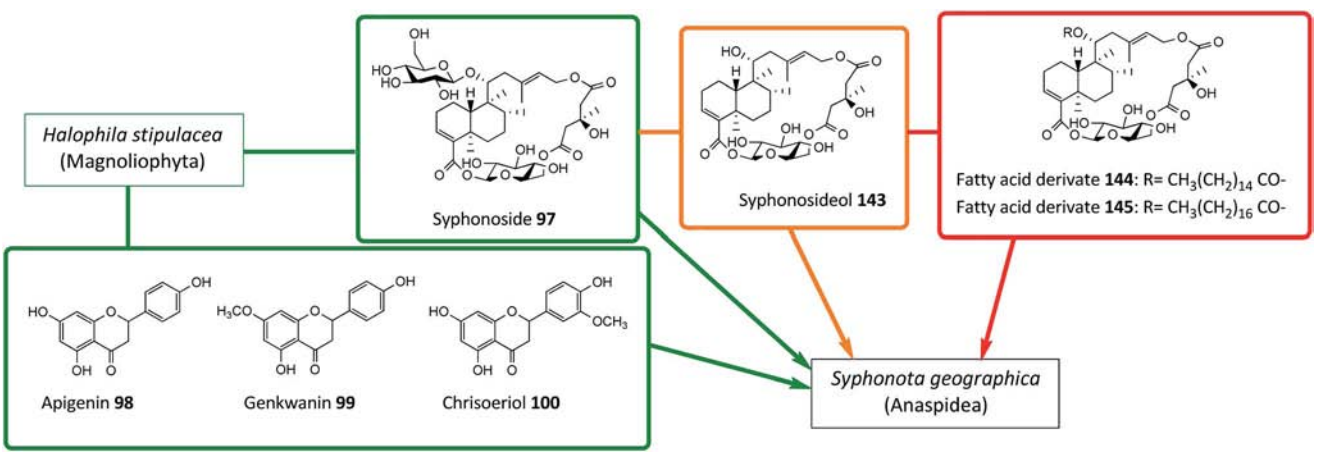

Fig. 23 Sequestration and biotransformation of marine plant secondary metabolites by *Syphonota geographica*.

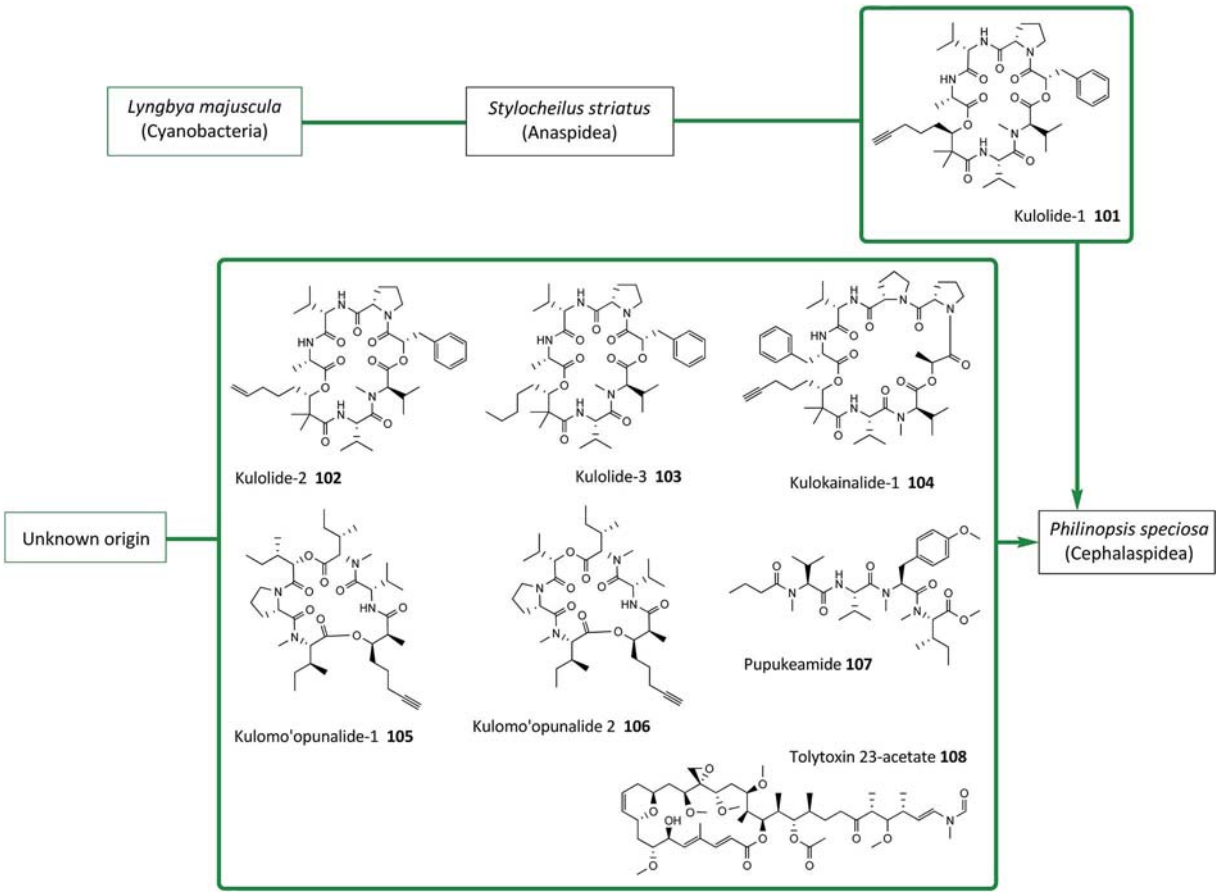

Fig. 24 Sequestration of secondary metabolites originating either from cyanobacteria or from an unknown origin by *Stylocheilus striatus* and *Philinopsis speciosa*.

linear peptide pupukeamide **107** and the macrolide tolytoxin 23-acetate **108** (Fig. 24). *P. speciosa* is a generalist carnivore and consumes sea hares including *S. striatus* and *D. auricularia*. Thus, the above-mentioned diet-derived compounds are bioaccumulated across two trophic levels, as for example, kulolide-1 **101** in *P. speciosa* acquired from *S. striatus* was originally sequestered from the cyanobacterium *L. majuscula*[116] (Fig. 24).

The shelled cephalaspidean, *Bulla gouldiana*, biosynthesizes three polypropionates *de novo* as a defense strategy.[117] 5,6-Dehydroaglajne-3 **109** and isopulo'upone **110**, which show considerable toxicity to mosquito fish, *Gambusia affinis*, are produced by the mollusc and stored in its mantle, and niuhinone-B **111** is also produced but for which no activity has been found (Fig. 25). *Navanax inermis*, another cephalaspidean heterobranch, feeds on *B. gouldiana* and also accumulates these three compounds. Although the presence of the diet-derived polypropionates in external parts of the body has not been determined, their storage could be linked to a chemical defense strategy.[118]

The omnivorous mollusc *Tylodina perversa* (order Umbraculida) provides another clear example of a predator using diet-derived compounds as a defense mechanism. *Aplysina aerophoba* and *A. cavernicola* are two sibling sponge species common in the Mediterranean sea, but *T. perversa* is always associated with *A. aerophoba* and never found on *A. cavernicola*. Sponges in the order Verongida are known to produce brominated isoxazoline

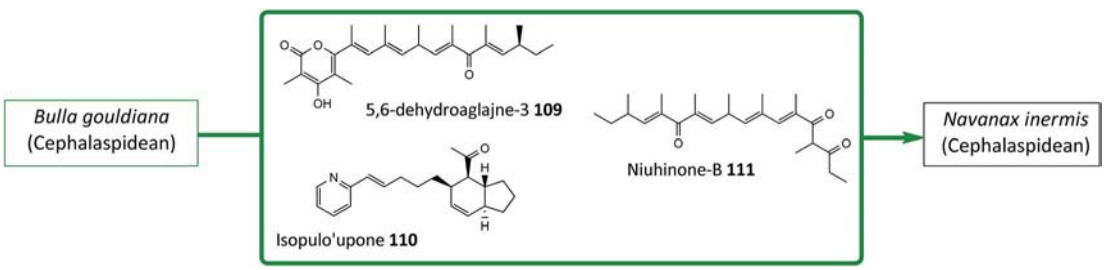

Fig. 25 Sequestration of biosynthesized *de novo* molluscan secondary metabolites by *Navanax inermis*.

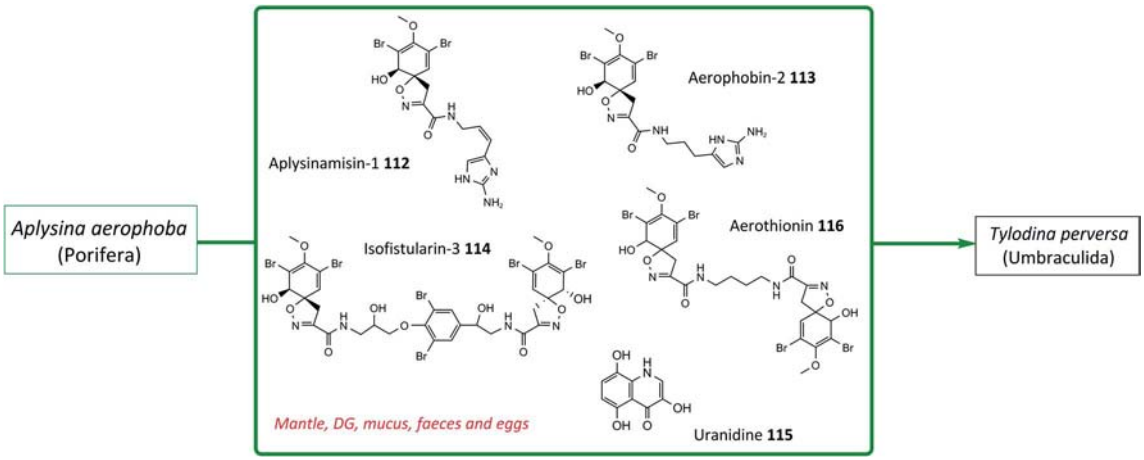

Fig. 26 Sequestration of sponge secondary metabolites by *Tylodina perversa*.

alkaloids, and *A. aerophoba* contains considerable amounts of aplysinamisin-1 **112**, aerophobin-2 **113** and isofistularin-3 **114** [119,120] (Fig. 26). *A. aerophoba* also lives in shallow waters, exposed to solar irradiation, enabling cyanobacteria to develop on its surface, whereas *A. cavernicola* thrives in shady environments such as deep underwater caves.[121] The mollusc mostly consumes the cyanobacteria, only eating a fraction of the sponge.[122] Nevertheless, the sponge-derived brominated alkaloids that act as strong feeding deterrents to the blenny, *Blennius sphinx*, are found in high concentrations in the mantle of the sea slug *T. perversa*, whereas concentrations in the digestive gland, mucus secretions, faeces and eggs are lower.[123] Interestingly, aerophobin-2 **113** is the main compound found in the mantle, indicating differential sequestration of diet-derived compounds within the sea slug. The bioaccumulation of the deterrent alkaloids in external tissues, as well as in eggs, likely demonstrates a defense strategy against predators. In addition, *T. perversa* sequesters the sponge pigment uranidine **115** in order to have the same yellow coloration as its prey, becoming cryptically colored (Fig. 26). Finally, aerothionin **116**, a major metabolite known to be produced only by *A. cavernicola* but also present as a minor compound in *A. aerophoba* (personal observations), is accumulated in the sea slug (Fig. 26).

As we have seen, gastropod molluscs sequester secondary metabolites, mainly from their diet, but also some biosynthesize them *de novo*. The compounds can be concentrated in exposed or internal parts of the molluscan body, with locations varying across either inner organs including gonads, digestive gland, opaline or ink glands, or external tissues such as skin, mantle rim, anterior mantle gland, or MDFs. A few cases demonstrate that secondary metabolites can be transmitted to eggs as an example of vertical transmission.

The compounds found at high concentration in external tissues are located optimally for defense, but without testing every ecological function it is not possible to conclude whether the storage of deterrent compounds in inner organs represents the absence of a defense, a mechanism against auto-intoxication and proximity to enzymes involved in its detoxification and excretion, a mechanism to keep the gut microbiome healthy or as a defense mechanism by acting as a reservoir for exterior tissues. The strategy of storing secondary metabolites in more exposed body parts would indeed be an effective defense mechanism, as preferentially storing them near the surface would facilitate the release of toxic chemicals during a predator attack.[70] Conversely, some researchers have proposed an alternative hypothesis whereby MDFs are located in external tissues to avoid autotoxicity or to remove toxic compounds, which later evolved into a defensive mechanism.[72,124] However, this alternative hypothesis has been subject to criticism because MDFS in almost all chromodorids lack ducts that would allow them to readily release compounds in a purifying manner. Moreover, Carbone *et al.*[70] demonstrate that some closely related compounds are selectively bioaccumulated in nudibranch MDFs, while others are found in viscera. This selective separation of metabolites between those transferred to MDFs and those removed from the body through the normal pathway, occurs during digestion lending support to the hypothesis that MDFs have primarily a defensive function.

3. Biotransformation and excretion of diet-derived secondary metabolites

The general mechanisms for processing ingested secondary metabolites after sequestration and concentration or bioaccumulation (assimilated into absorption and distribution) discussed in part 2, include metabolism and excretion which will be summarised here in part 3. The general mechanism of metabolism and excretion is separated into 3 phases according to their enzymatic architecture: phase I and phase II constitute the biotransformation (including detoxification) steps of xenobiotics and excretion occurs during phase III.[125,126]

3.1 Mechanism of metabolism and excretion: phases I, II and III

Ingested secondary metabolites are functionalized in phase I, during which multiple reactions introduce a functional group that reduces the lipophilicity of the compounds.[126,127] In terrestrial and marine species, these reactions involve multiple

families of enzymes that carry out various biotransformations such as hydroxylations, hydrolysis, reductions, oxidations, dehalogenations, dehydrogenations, heteroatom dealkykations, deaminations or epoxidations.[125,127,128] Among them, cytochrome P450 monooxygenases (CYPs) are an important phase I family of enzymes that add polar groups, such as a hydroxyl group, onto compounds. CYPs mediate the metabolism of endogenous compounds and catalyse the biosynthesis of signal molecules such as steroids, but are also able to functionalize various xenobiotics.[128,129] The total level of P450s shows wide variation among different phyla; crustaceans have high levels compared to molluscs, echinoderms and polycheates.[127] However, the amount of P450 is not correlated with its activity, since coenzymes and cofactors such as P450 reductase, cytochrome b5 and NADPH are also required.[128,130] Cytochrome P450 is able to interact with a wide variety of lipophilic molecules and enables the organism to detoxify a considerable range of chemicals.[131] However, in some cases, reactions carried out by CYPs can lead to more toxic, mutagenic or carcinogenic compounds.[127,131]

Phase II biotransformation reactions occur either with the functional group introduced during phase I, or directly with a functional group already present on xenobiotics. Phase II reactions generally result in a greater increase in hydrophilicity than achieved during phase I, in order to enable excretion in phase III.[126,127] These reactions are controlled by multiple families of enzymes, allowing glucuronidation, sulfonation, acetylation, methylation, conjugation with amino acids and conjugation with glutathione.[127] Glutathione S-transferases (GSTs) form a major phase II family of enzymes which are located in the cell cytosol and are responsible for conjugative reactions by binding the tripeptide glutathione onto endogenous or exogenous electrophilic substrates.[125,132] Furthermore, GSTs appear to play an important role in marine herbivores, as they are involved in allelochemical biotransformations.[126] The wide range of electrophilic xenobiotics, which can undergo reactions catalyzed by GSTs may be explained by the fact that each GST contains two kinds of ligand binding sites: one site shows strong specificity for glutathione, whereas the other is able to bind with a broad array of compounds. GSTs may also be responsible for sequestering exogenous compounds as a protective mechanism.[133–136] Indeed, some human and insect GSTs exert a strong binding affinity for exogenous compounds enabling them to be sequestered in the cytosol.[137,138] In this way, the activity of the toxicant is inhibited by storing it away from target nuclear proteins thus preventing any toxic effects on gene regulation. Many studies have isolated GST activity in the presence of various exogenous compounds and proven that some induce GST activity. In contrast, some exogenous compounds act as GST inhibitors or transcriptional repressors,[139,140] indicating a potential prey defense strategy against consumers.[125] GSTs also play a role in protection against oxidative stress by catalyzing glutathione peroxydase activity.[141] This stress may be caused by exogenous compounds, such as secondary metabolites, increasing the amount of reactive oxygen species (ROS) in cells.[141,142] GSTs are therefore involved in different mechanisms allowing the organism to increase its tolerance to a wide array of exogenous compounds.

Phase III excretion, the final step of ADME, is undertaken by membrane proteins known as ABC transporters (or multixenobiotic transporters) that play a role similar to that of a bouncer, by controlling the absorption, distribution and excretion at the cell gate, the membrane.[143,144] These proteins, encoded by the ABC superfamily of genes, have been well studied in human pharmacology as they are responsible for multidrug resistance.[143,145,146] ABC transporters are composed of seven subfamilies termed ABC-A to G. Among them, ABC-B, ABC-C and ABC-G appear to have a wide array of substrates including phase II conjugates and are responsible for trafficking molecules through cell membranes.[126]

3.2 Examples of detoxification and biotransformation

Most of the studies regarding ADME have been carried out on humans, mammals[127] or insects[147] and research on the enzymatic architecture of marine gastropods is still in its infancy.[148,149] The anatomical organization of digestion is different across gastropod molluscs. Caenogastropods (formerly prosobranchs) and heterobranchs have two main digestion organs: the stomach and the digestive gland.[148] In heterobranchs, the digestive gland constitutes the primary site of enzymatic digestion, and is composed of various cell types, including rhodoplast digestive cells, specialized cells involved in the biotransformation of diet-derived secondary metabolites.[148,150] In caenogastropods, the digestive enzymes are produced in the stomach, while the digestive gland is involved in the absorption and excretion of products.[151,152]

3.2.1 ADME identified in marine gastropods: detoxification. A three phase enzymatic architecture has been identified in a marine gastropod, the generalist consumer *Cyphoma gibbosum* (class: Gastropoda, clade: Caenogastropoda, clade: Littorinomorpha), which feeds on chemically rich gorgonian corals.[149] Prostaglandins constitute the main chemical weapons within the gorgonian genus, *Plexaura*, and deter most predators with the exception of *C. gibbosum* that regularly feeds on this coral species. Firstly, twelve genes encoding for phase I CYP enzymes are present in *C. gibbosum* and appear to be located in digestive gland cells. The gorgonian *Plexaura homomalla* produces deterrent prostaglandin A_2 (PGA_2) **117** analogs as its main chemical defense[149,153] (Fig. 27) which likely induce the expression of *C. gibbosum* genes that produce CYP4BK and CYP4BL transcripts. CYP4BK and CYP4BL are closely related to vertebrate CYP4A and CYP4F that metabolize prostaglandins. Secondly, phase II GST enzymes have been characterized and located in the digestive gland cells of *C. gibbosum*.[154] GST activity remains high when the mollusc is exposed to eight species of gorgonian corals, but is inhibited in the presence of coral crude extracts,[133] indicating that gorgonian extracts contain possible substrates for *Cyphoma* GST enzymes and may explain why GST activity is so high in this molluscan predator. A bioassay-guided fractionation identifies PGA_2 **117** as the most GST inhibiting compound. *Cyphoma* GSTs can be saturated by PGA_2 **117** produced by *P. hormomalla*. The results also reveal that extracts

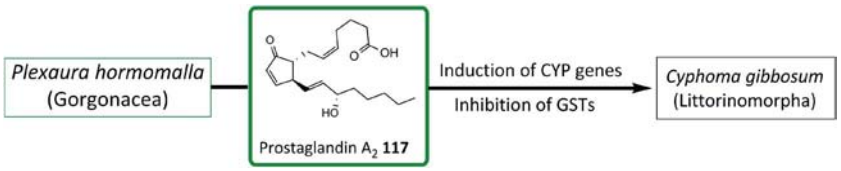

Fig. 27 Induction of CYP genes and inhibition of GSTs in *Cyphoma gibbosum* when exposed to prostaglandin A2 **117**.

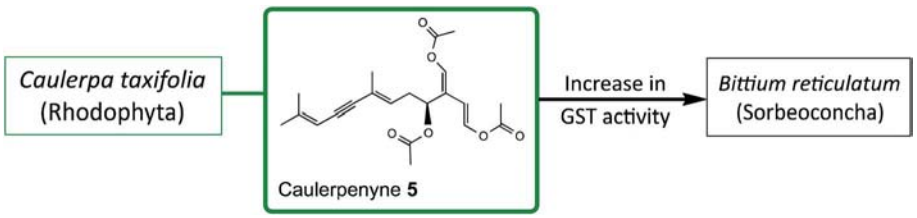

Fig. 28 Gastropoda response to oxidative stress: induction of an antioxidant mechanism in the presence of caulerpenyne 5, a diet-derived chemical.

from all gorgonian species contain putative GST substrate or inhibitors. Thus, having a high GST activity may allow *C. gibbosum* to feed upon a wide range of chemically defended prey. Finally, partial cDNA sequences encoding four ABC proteins, belonging to ABC-B and ABC-C families, have been identified in the digestive gland cells of *C. gibbosum*.[155] These proteins show similarities with vertebrate ABC transporters that are in charge of glutathione conjugate transport through the cell membrane.

The gastropoda *Bittium reticulatum* (class: Gastropoda, clade: Caenogastropoda, clade: Sorbeoconcha) feeds on the toxic alga *Caulerpa taxifolia* as well as the non toxic seagrass *Posidonia oceanica*.[141] Accordingly, the activities of the antioxidant enzymes glutathione peroxidase, GST and glutathione reductase are higher in the case of the herbivore consuming *C. taxifolia*. Thus, antioxidants, produced in response to oxidative stress, are induced in the presence of caulerpenyne **5**, the main defensive alga compound, and represent an adaptive response by the mollusc (Fig. 28).

The mean basal activities of GST in non-gastropod molluscs *Katharina tunicata* (class: Polyplacophora, order: Neoloricata) and *Cryptochiton stelleri* (class: Polyplacophora, order: Neoloricata), which frequently consume red algae containing the feeding deterrent lanosol **118**, increase significantly during feeding.[156] Phase I CYP3As and phase II GST are also found at very high concentrations in the digestive gland of the generalist consumer *Haliotis rufescens* (class: Gastropoda, clade: Vetigastropoda)[157] (Fig. 29). However, the mean basal activity of GST in *H. rufescens* was very high even before feeding on red alga, *i.e.* even before the uptake of lanosol **118**. Thus, continuously high levels of GST activity may enable *H. rufescens* to detoxify a wider range of secondary metabolites, and may represent an adaptive strategy for generalist species. These results may demonstrate that the induction of detoxification enzymes is related to the different feeding behaviors of marine herbivores, specialists *versus* generalists and the tolerance of specialist molluscs to secondary metabolites may be linked to their adaptive increase in GST activity.

Sacoglossans and nudibranchs may also use an enzymatic architecture to biotransform diet-derived compounds and this strategy could enable these molluscs to enhance their chemical defense and/or decrease the toxicity of absorbed molecules.[38,70] Indeed, some compounds may show specific toxicity against certain species, whilst being harmless to others. The converted products could also be inoffensive for the mollusc, while maintaining or increasing their deterrent activities against predators.

3.2.2 Sacoglossans: biotransformation into more toxic forms for defense. Although no study has revealed the presence of a three-phase enzymatic architecture in sacoglossans, some instances of diet-derived compound biotransformation may indicate the presence of a similar process and this is an interesting field for future research. Firstly, the green alga *Caulerpa*

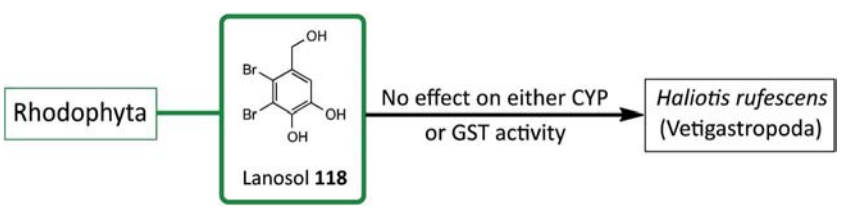

Fig. 29 Effect of lanosol 118 on CYP and GST activity in *Haliotis rufescens*.

prolifera is consumed by three shelled heterobranchs: *Oxynoe olivacea* (Fig. 6a), *Lobiger serradifalci*, and *Ascobulla fragilis*.[38,158] Caulerpenyne **5** constitutes the major component of *C. prolifera* and is biotransformed into oxytoxin 1 **119** by three herbivores. Consequently, monoaldehyde oxytoxin 1 **119** is modified into dialdehyde oxytoxin 2 **120** by *O. olivacea* and *A. fragilis* (Fig. 2). Interestingly, oxytoxins 1 **119** and 2 **120** are compartmentalized respectively in the mantle and in mucus secretions of *O. olivacea*[159] and in external body parts of *A. fragilis*,[158] while oxytoxin 1 **119** is located in the parapodia of *L. serradifalci*.[158] Bioassays indicate than oxytoxins 1 **119** and 2 **120** are more toxic to mosquito fish *Gambusia afinis* than caulerpenyne **5**. In addition, contrary to caulerpenyne **5**, compounds **119** and **120** show antifeedant activities in the wrasse *Thalassoma pavo*, the damselfish *Chromis chromis* and the sea bass *Serranus hepatus*. Thus, biotransformation of caulerpenyne **5** into two deterrent compounds that are then sequestered in external tissues and found in mucus secretions, provide clear examples of defense mechanisms in three shelled heterobranchs. Caulerpenyne **5** is also converted into oxytoxin 1 **119** by *Oxynoe antillarum*, *Elysia subornata* and *Elysia patina* and then to oxytoxin 2 **120** by *O. antillarum*[38] (Fig. 2).

Furthermore, *Elysia halimedae*, a specialist herbivore feeding on *Halimeda macroloba*, converts the algal diterpenoid halimedatetraacetate **7** into a reduced form **121** [160] (Fig. 3). This modified compound is then sequestered in high concentrations and transmitted to eggs. Halimedatetraacetate **7** and its modified form **121** deter several herbivorous fishes and the *Elysia* diterpenoid is also deterrent towards a variety of carnivorous fishes. When attacked, the mollusc releases a mucus secretion containing the deterrent compounds providing evidence of a defense strategy using biotransformation.

Sacoglossans in the genus *Thuridilla* modify epoxylactone **122**, an algal secondary metabolite produced by the green algae *Pseudochlorodesmis furcellata* and *Derbesia tenuissima*[161] (Fig. 30). The Mediterranean sea slug *T. hopei* converts epoxylactone **122** into thuridillins A–C **123–125**,[19,34] *nor*-thuridillonal **126**, dihydro-*nor*-thuridillonal **127** and deacetyl-dihydro-*nor*-thuridillonal **128**.[162] Furthermore, the Australian mollusc, *T. splendens*, converts the algal metabolite into thuridillins A **123**, D **129**, E **130** and F **131** [163] (Fig. 30). Thuridillins A **123** and B **124** might result from an oxidation of epoxylactone, while a reduction in the algal compound might lead to thuridillin C **125** [164] and phase I enzymes may be involved in these biotransformations. Thus, two species of *Thuridilla* genus are able to modify the algal secondary metabolite epoxylactone into nine different compounds, revealing a wide diversity of biotransformation pathways. However, the activity of biotransformed compounds has not been investigated and it is unknown if biotransformation increases their chemical defense.

3.2.3 Nudibranchs: biotransformation into more toxic forms for defense. As mentioned in 2.2, the nudibranch *Hexabranchus sanguineus* (Fig. 6b) sequesters secondary metabolites from its sponge prey, but halichondramide **132**, the most abundant compound in *Halicondria* sp., is not stored by the nudibranch.[77] Rather, the mollusc converts halichondramide **132** into tetrahydrohalichondramide **133** (Fig. 11). This

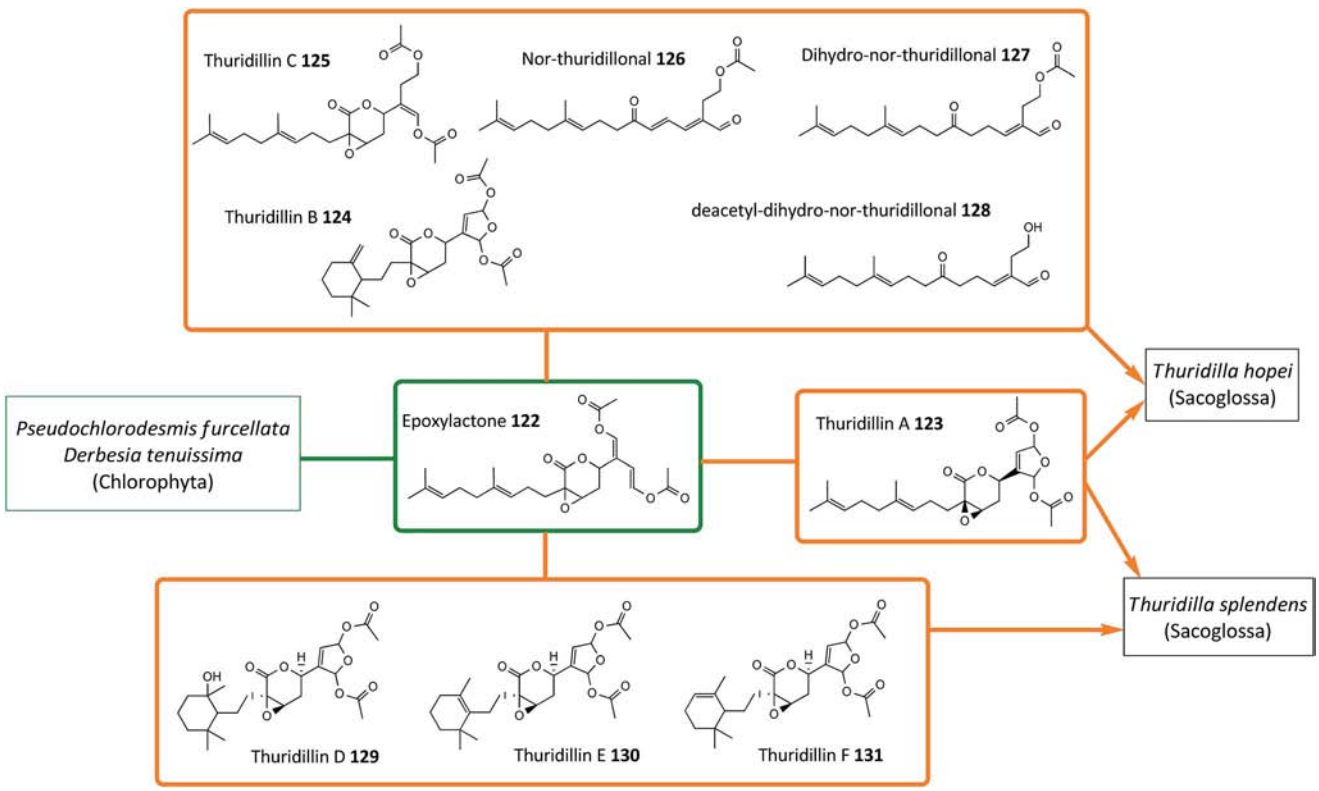

Fig. 30 Biotransformation of the algal secondary metabolite epoxylactone **122** by *Thuridilla hopei* and *Thuridilla splendens*.

biotransformation likely occurs in the digestive system involving two reactions: double bond hydrogenation and carbonyl reduction, reactions that may be carried out by phase I P450 enzymes. The deterrent modified-compound is then sequestered in the mantle, digestive gland, gonads and eggs.

Many terpenoids produced by sponges are sequestered by nudibranchs. Furthermore, some nudibranchs are able to modify these terpenoids, such as *Hypselodoris orsini* that feeds on *Cacospongia mollior*[165] (Fig. 7). The major sponge metabolite scalaradial **16** is converted by selective aldehyde reduction into deoxoscalarin **134** which is found in the viscera and then oxidized into 6-keto-deoxoscalarin **135** which is compartmentalized in MDFs. The previously mentioned *Glossodoris pallida* also converts scalaradial **16** into deoxoscalarin **134** and stores it in MDFs[72] (Fig. 7). Once again, P450 enzymes may be involved in these reactions.

Chromodoris sinensis sequesters the sponge metabolite aplyroseol-2 **27** and biotransforms it into its dialdehyde derivative **136** (ref. 70) (Fig. 9). This modification may involve reductions carried out by phase I enzymes and methylation carried out by phase II enzymes. Aplyroseol-2 **27** and aplyroseol dialdehyde **136** are both concentrated in MDFs and the modified compound appears to be three times more concentrated than the original one. Interestingly, aplyroseol-2 **27** does not show any deterrent activity, while the dialdehyde derivative **136** induces considerable feeding-avoidance behavior. Thus, biotransformation may occur to increase the chemical defense of the sea slug. The specialist nudibranch, *Tritonia hamnerorum*, feeding on the chemically defended gorgonian *Gorgonia ventilina*, remains to date, the only nudibranch for which ABC transporters have been identified.[155]

3.2.4 Anaspideans: biotransformation with loss of toxicity. *Stylocheilus striatus* sequesters diet-derived compounds from *Lyngbya majuscula* in its digestive gland and appears to biotransform some of them. LTA **93** from *S. striatus* collected in Moreton bay, Australia, undergoes acetylation leading to lyngbyatoxin A acetate **137** which is also sequestered in the digestive gland[166] (Fig. 21). In addition, malyngamide B **84** is converted into malyngamide B acetate **138** by *S. striatus* in Guam (Fig. 21). Contrary to the parent molecule, malyngamide B acetate **138** does not repel the pufferfish *Canthigaster solandri* or the crab *Leptodius* spp and its toxicity on both brine shrimp *Artemia franciscana* and the sea urchin *Echinometra mathaei* is diminished. Sea hares in the *Aplysia* genus are also able to carry out acetylations on diet-derived compounds. For example, *Aplysia dactylomela* converts 14-keto epitaondiol **139**, produced by the brown alga *Stypopodium zonale*, into 3-keto epitaondiol **140** [103] (Fig. 31). Similarly, the algal metabolites isolaurenisol **75** and allolaurinterol **76** are converted into isolaurenisol acetate **141** and allolaurinterol acetate **142** respectively[98] (Fig. 18). Acetylation may be carried out by phase II enzymes and biotransformation by an adaptive detoxification mechanism. However, it is unlikely that the modified secondary metabolites are used in chemical defense as they are sequestered in an inner organ without being excreted and they also lose toxicity.

The above-mentioned *Syphonota geographica* sequesters several compounds from its prey *Halophila stipulacea* and may biotransform syphonoside **97** into syphonosideol **143** and then into two fatty acid derivates **144** and **145** [114] (Fig. 23). However, such biotransformations, which significantly increase the lipophilicity of the compounds, have never been described before among sea hares or other heterobranchs.

3.3 Detoxification limitation hypothesis and feeding choice

As vital GSTs can be saturated by diet-derived secondary metabolites during detoxification, there may be a limit to the number of metabolites that can be ingested before toxicity is incurred. Freeland and Janzen[167] introduced the detoxification limitation hypothesis (DLH) to understand generalist herbivore behavior and how secondary metabolites could limit feeding rate.[168] This hypothesis predicts that generalist herbivores would select a mixed diet rather than a single one, to ingest different secondary metabolites with non-overlapping detoxification pathways. Feeding rates would be higher with a mixed diet compared to a single one, enhancing overall consumer performance (growth, survival and/or fecundity).[169,170] However, few studies have confirmed DLH in marine ecosystems, and no study has investigated DLH in marine gastropods. Two marine herbivores, the urchin *Arbacia punctulata* and the amphipod *Amphithoe longimana* decrease feeding rates when their total secondary metabolite concentration increases.[170] Feeding rate may also be influenced by the nutritional value of the prey consumed; a consumer feeding on a nutritionally poor diet would increase foraging, *i.e.* consume more food in order to fulfill their nutritional requirements, but would also consume more secondary metabolites than if they ingested less of a nutritionally rich diet with the same concentration of secondary metabolites. Consumers may thus risk ingesting more secondary metabolites that they can detoxify.[170,171] A mixed diet also offers a nutritional complement to the consumer as found in *Dolabella auricularia*, which grows faster on a mixed rather than on a single diet.[172] Nitrogen concentration remains another factor that may play a role in foraging choice; for instance, the gastropod *Littorina sitkana* prefers to

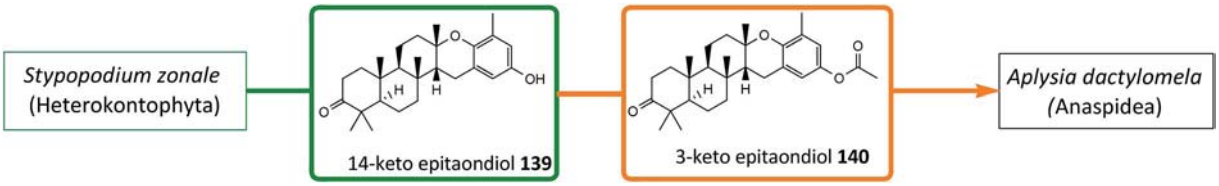

Fig. 31 Biotransformation of the algal secondary metabolites 14-keto epitaondiol 139 by *Aplysia dactylomela*.

consume algae with a high nitrogen concentration regardless of the presence or absence of chemical defenses.[173]

3.4 Induction of chemical defenses

Sedentary species or prey have adapted defense mechanisms to counter attacks by predators, but such defenses are costly.[174,175] Therefore, the production of defenses is often linked to the rate of predation: if high then constitutive defenses are constantly produced even in the rare absence of an attack. On the other hand, when predation rates are spatially or temporally variable then facultative defenses are only induced upon attack.[176] Induced resistance may be an adaptation to minimize costs by keeping defenses low until they are needed.[177,178] Whilst predator-induced morphological defenses have been found in several marine taxa, including barnacles,[179,180] bryozoa,[181,182] and seaweeds,[183,184] there are only a few examples of marine gastropods inducing chemical defences of their prey. The brown alga *Fucus distichus* responds to periwinkle *Littorina sitkana* grazing by increasing the concentration of polyphenolic compounds,[184] to which the herbivores respond by grazing on ungrazed algae. The brown alga *Ascophyllum nodosum* provides another clear example of chemical defenses induced by grazing of marine gastropods.[185] The concentration of phlorotannins is strongly increased in response to an enzyme present in the saliva of *Littorina obtusata* and cause different herbivore behavioral responses.[186] Thus, these chemicals would: (1) increase the frequency of feeding but decrease the amount of food ingested per meal; (2) decrease the overall amount of algae consumed; and (3) elevate *L. obtusata* mobility in order to find ungrazed prey.

An activated defense (short-term inducible defense[187] or dynamic defense[188]) is a chemical defense which involves the rapid conversion of one secondary metabolite into another more potent defensive compound upon attack.[189] This conversion allows an organism to quickly produce potent feeding deterrents that are biologically active but unstable, thus minimising the risk of autotoxicity.[189] For example, upon predator attack, the marine algae, *Halimeda* spp. are able to rapidly convert halimedatetraacetate (Fig. 3) to halimedatrial.

These are all examples of direct defenses that by themselves affect the susceptibility to attack.[190] However, another form of defense, indirect defense, serves to attract natural enemies of the attacking predator and reduce damage to the prey. The only marine example involves gobies defending Acroporid corals from allelopathic algae in response to chemical cues from the coral.[15] However, to date there are no examples with marine gastropods.

4. Chemically mediated interactions

As described by Mark Hay,[14] chemically mediated interactions have major impacts on population structure, community organization, and ecosystem function. Inter- and intra-species communication involves distinctive chemical signals and cues. Chemical signals are emitted intentionally by a sender towards a receiver, and are generally beneficial to the sender.[191] Chemical signaling also occurs in intra-specific communication, for example, by pheromones. In contrast, chemical cues are released unintentionally by a sender and are intercepted by a receiver. Thus, the interception of cues may either be neutral or disadvantageous to the sender. A compound released by prey and intercepted by a predator, allowing it to locate the prey, is considered to be a chemical cue. The difference between these two types of chemical communication, signals and cues, may be flexible over time in which communication could evolve *via* adaptation and exaptation (a change in the function of a trait).[192] For example, senders could originally emit chemicals such as waste products, defensive molecules or by-products with non-communicative functions that could be precursors for the evolution of more complex communication. In addition, chemical cues can evolve into chemical signals; such exaptation is known as "chemical ritualization".[192] Conversely, the evolution of chemical signals into chemical cues can also occur as another type of exaptation.[193,194] Marine gastropods have mastered the art of using chemical cues and signals across a large range of spatial scales for feeding preferences, foraging, mate attraction, larval metamorphosis and settlement. Here the role of secondary metabolites in palatability, olfaction and in mucus trail following is described in marine gastropods.

4.1 Prey chemicals as determinants of feeding preferences

Although a wide range of secondary metabolites produced by cyanobacteria, algae, sponges or tunicates act as feeding deterrents to potential predators, some consumers are able to circumvent these chemical shields, and find putative unpalatable prey palatable. In these cases, secondary metabolites evolve into attractants to predators. However, only a few studies have demonstrated that feeding choice could be related to the palatability of secondary metabolites. Among them, the interaction between the sea hare *Stylocheilus striatus* and *Lyngbya majuscula* is a good predator-prey example, as the cyanobacterium is known to produce a broad range of compounds.[195] A comparison of the palatability of non polar and polar extracts of closely related cyanobacteria belonging to *Lyngbya*, *Moorea* and *Okeana* genera revealed that *S. striatus* is stimulated to feed on non polar and polar extracts of *Lyngbya* spp. as well as on the non polar extracts of *Moorea producens* (formerly *Lyngbya majuscula*).[196] The majusculamides A **146** and B **147** (combined), and the malyngamides A **95** and B **84** are deterrents against the pufferfish *Canthigaster solandri* and the crab *Leptodius* spp., yet appear to be palatable to *S. striatus* at natural concentrations[109,110] (Fig. 32). However, at higher concentrations, these molecules also repel sea hares. Similarly, pitipeptolide A **148** induces *S. striatus* to feed on *L. majuscula*, yet it is repellent to the sea urchin *Echinometra mathaei*, the crab *Menaethius monoceros*, and the amphipods *Parhyale hawaiensi* and *Cymadusa imbroglio*.[197] Furthermore, these cues are conserved geographically, as crude extracts of *L. majuscula* collected from Moreton Bay, Australia, also strongly stimulate feeding in *S. striatus* from Guam.[198,199] Only a few studies have demonstrated the palatability of secondary metabolites since it is difficult to identify

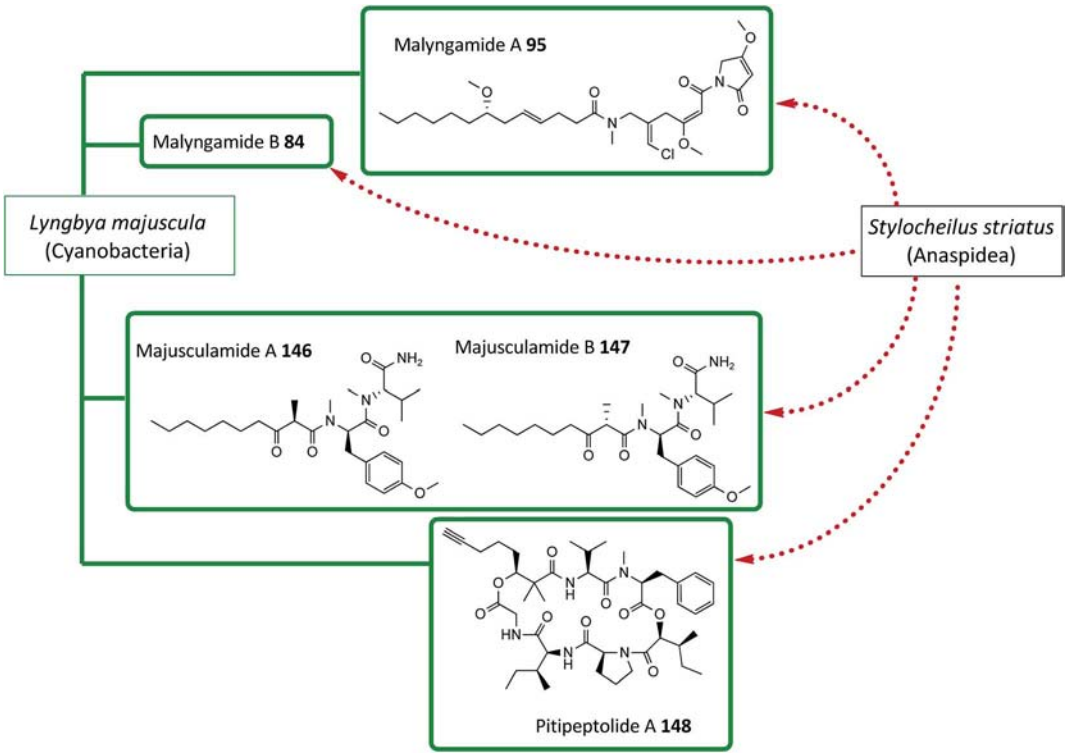

Fig. 32 Cyanobacterial secondary metabolites as determinants of feeding preferences for *Stylocheilus striatus*.

the molecule, or the association of molecules, among the whole metabolome that is responsible for feeding preferences.

4.2 Secondary metabolites and chemoreception

For marine gastropods, olfaction is an essential sense for medium and long distance reception of signals and cues and is mediated through different sensory organs. In aquatic eupulmonates (formerly Pulmonata), the osphradium is the main chemosensory organ, although the cephalic tentacles also play a role in orientation towards olfactory cues.[200] Several organs are implicated in caenogastropod (formerly prosobranch) chemoreception, including the cephalic and metapodial tentacles, the anterior margin of the foot, the siphon tip, osphradia and the bursicles. Similarly, other heterobranch molluscs (formerly Opistobranchia) are able to detect chemical cues mainly using their rhinophores and tentacles; the anterior edge of the oral veil and the osphradium are also implicated to a lesser degree.[29,200–204] These chemosensory organs play a crucial role in intra-specific communication by detecting pheromones. For example, *Aplysia* sea hares detect water-borne proteins from conspecifics, such as attractin, enticin, temptin and seductin,[205–207] sex pheromones involved in mate attraction, as well as in extending the duration of egg-laying. As the first interaction between a predator and its prey involves the detection of chemical cues, chemoreceptors also play an essential role in foraging.[29,203] The presence of chemoreception in marine gastropods, notably sacoglossans, has been proven from the presence of head lifting behavior.[29,208,209] Sea slugs show a specific behavior in the presence of chemical cues from their prey; they lift their head and the anterior part of their body in the direction of the stimulus.[208] Chemoreception has also been described in nudibranchs[201] and rhinophores are active even at the larval stage, helping larvae during settlement by selecting a habitat based on the presence of suitable food.[210] Here, the role of prey secondary metabolites in settlement, metamorphosis and foraging using chemoreception is reviewed in gastropods.

4.2.1 Secondary metabolites as inducers of settlement and metamorphosis of gastropod larvae. Larvae of various benthic species are released into the pelagic zone for the duration of their larval period as either filter-feeding planktotrophs or non-feeding lecithotrophs.[211] Larval dispersal enables benthic species to colonize distant areas that cannot be reached by adult movement alone. Larval recruitment can be induced by physical, biological or chemical cues, but in specialist associations, chemical cues released by prey are often identified as the main factor responsible for the settlement of predatory larvae.[212,213] Most heterobranch larvae need an exogenous cue to induce settlement and metamorphosis.[213]

Among shell-less sacoglossans, *Alderia modesta* is considered a specialist herbivore on the yellow-green alga *Vaucheria longicaulis*.[214] This mollusc lives in temperate estuaries and exhibits a rare polymorphism producing both planktotrophic (feeding) and lecithotrophic (non-feeding) larvae.[215] A percentage of the lecithotrophic larvae are able to metamorphose spontaneously, while the other part needs an exogenous cue - compounds released by *V. longicaulis* in the water. Chemical cues from other algal species do not induce metamorphosis in larval *Alderia*

modesta. The chemical signature of the cue appears to be composed of low molecular weight carbohydrates such as mannitol or glucose and unknown high molecular weight carbohydrates suggesting that polar compounds also play a role in marine chemical ecology.[214,216] The percentage of lecithotrophic larvae that spontaneously metamorphose and those requiring algal induction are variable. The percentage of spontaneous metamorphosis significantly decreases when adult molluscs are starved over 24 h prior to oviposition in order to enhance the dispersal potential of larvae. This example indicates the presence of a variable dispersal strategy.[215]

Settlement of the specialist nudibranch *Phestilla sibogae* has sparked the interest of many researchers as the nudibranch only feeds on *Porites compressa*.[212,217,218] The sea slug produces planktotrophic larvae that settle and metamorphose in response to a small polar compound released by the coral prey.[219] Other nudibranch larvae in the *Phestilla* genus that feed on different coral species as adults also respond to chemical cues of these different prey which induce settlement and metamorphosis. *Phestilla minor* consumes *Porites lutea* and *P. annae*, and metamorphoses in the presence of *P. lutea*, *P. annae* and *P. cylindrical*, while the metamorphosis of *Phestilla* sp. 2, that preferentially feeds on corals in the genus *Goniopora*, is induced in the presence of *G. fruticosa*, *G. minora* and *G. lobata*.[212,218]

Hermissenda crassicornis is a nudibranch with planktotrophic larvae which can metamorphose in response to natural inducers released by the hydroid *Tubularia crocea*.[220,221] The hydroid inducer is water soluble, but is not the only compound that can induce metamorphosis in the sea slug. GABA (γ-aminobutyric acid), choline, serotonin, glutamate or ions such as K^+ and Cs^+ (at low concentration) also induce a high proportion of metamorphosis. In another example, the aposematic nudibranch *Hypselodoris infucata*, a specialist feeder of the sponge *Dysidea* sp., produces planktotrophic larvae that metamorphose and settle in response to chemical-cues released by *Dysidea* sp.[222] However, this phenomena also occurs in the presence of three other sponges *Halichondria coerulea*, *Sigmodocia* sp., and *Tedania macrodactyl* which are sympatric with *Dysidea* sp. Natural inducers may also be produced by mutual microorganisms thriving on the primary biofilm. Larvae of *H. infucata* use non-specific cues to settle on, or close to, specific prey indicating that selection may occur later during the juvenile or adult stages. The larvae of another specialist nudibranch, the sponge feeding *Rostanga pulchra* are able to delay metamorphosis for at least three weeks after becoming competent, and only the presence of a specific prey species, *Ophlitaspongia pennata*, will induce settlement and metamorphosis.[223] Furthermore, the nudibranch *Onchidoris bilamellata* feeds exclusively upon barnacles and its settlement is only triggered in water conditioned with this prey.[224] Lecithotrophic larvae of the nudibranch *Adalaria proxima* metamorphose in the presence of chemicals released by its preferred bryozoan prey *Electra pilosa* and the inducer may be a peptide with low molecular weight (<500 kDa).[225,226]

Aplysia californica, a generalist consumer of the algae *Plocamium cartilagineum* and *Laurencia pacifica*, also requires chemical cues to trigger its settlement.[227] Metamorphosis of *A. californica* larvae occurs in the presence of chemical cues from several algae including *Rhodymenia californica*, *Corallina officinalis*, *P. cartilagineum*, *L. pacifica*, *Callophyllis violacea*, *Dictyopteris undulata*, *Pachydictyon coriaceum*, *Pterocladia capillacea*, *Centroceras clavulatum* and *Chondria californica*. However, only juveniles that settle on *P. cartilagineum* and *L. pacifica* actually consume the alga which induced their metamorphosis, the juveniles that settled on the other eight algal species attempt to find another food source.

The settlement of four other sea hares suggests that generalist consumers may also show a preference for a prey species during settlement.[228] *Aplysia juliana* preferentially settles on *Ulva fusciata* and *U. reticula* and post-larval growth is optimal. On the other hand, metamorphosis is reduced on *Enteromorpha* sp. as is post-larval growth. The settlement of *Aplysia dactylomela* is induced by chemical cues from a range of different genera of red algae such as *Chondrococcus*, *Gelidium*, *Laurencia*, *Martensia*, *Polysiphonia*, and *Spyridia*. However, the percentage of metamorphosis is highest in the presence of *Laurencia* sp., the mollusc preferentially consumes this algal species and it provides the highest growth rate. *Stylocheilus striatus* preferentially settles and feeds on the cyanobacterium *Lyngbya majuscula*, yet the red algae, *Acanthophora spicifera*, *Spyridia filamentosa* and *Laurencia* sp., also induce its settlement, but which result in lower post-larval growth.[228] Finally, larvae of *Dolabella auricularia* settle and metamorphose in the presence of the red algae *Laurencia*, *Amansia*, and *Spyridia*, the brown alga *Sargassum* sp. and an unidentified mat-forming cyanobacterium. However, post-metamorphic *D. auricularia* grows faster on the cyanobacterium, and *Spyridia filamentosa* is the preferred food for older juveniles.

The abalone *Haliotis iris* is a shelled generalist herbivore feeding on various algal foods such as the crustose coralline algae *Phymatolithon repandum*.[229] The biofilm on the surface of the alga formed by cyanobacteria and diatoms barely induces larval metamorphosis, whereas the alga without biofilm induces metamorphosis in nearly 100% of the larvae. Another example in a shelled mollusc, is the queen conch *Strombus gigas*, a generalist consumer feeding on various algae, but whose nursery ground substrata is dominated by *Laurencia poitei* and *Thalassia testudinum*.[230,231] Settlement and metamorphosis are induced by *L. poitei* and the epiphyte *Fosliella* sp. found on the detrital blade of *T. testudinum*. The compounds inducing settlement are water soluble with low molecular size (<1000 Da).

Crepidula fornicata is an invasive shelled gastropod exhibiting gregarious behavior and whose settlement is triggered by chemical signals released into the water by conspecifics.[232,233] Furthermore, metamorphosis also occurs in response to the presence of the halogenated compound dibromomethane **149**, which is released by red algae in the Corallinaceae family (Fig. 33).

In conclusion, settlement and metamorphosis by many marine gastropod species, either generalists or specialists, are driven by chemical cues, although the inducing compounds have rarely been identified. However, in many cases, prey

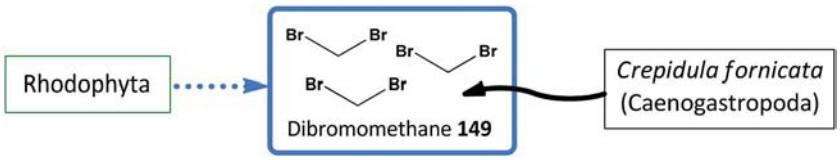

Fig. 33 Settlement and metamorphosis of *Crepidula fornicata* induced by dibromomethane.

chemicals implicated in settlement are water soluble with variable molecular weights, whereas non polar molecules do not seem to trigger such phenomena.[225] The duration of the period during which larval settlement can take place varies among species and larvae with a small competence period are associated with a wide range of chemical cues that induce settlement, whilst larvae with a long competence period can afford to be more specific.[230] However, settlement in some gastropods, such as sea hares, with a relatively long larval stage, occurs on various algal species that are not normally consumed. This would suggest that diet selection occurs later, and that the use of chemoreception for foraging plays a significant role in both juvenile and adult stages. The trend emerging from these studies seems to indicate that settlement may be more efficient, in terms of settlement percentage, for shelled molluscs with low mobility in response to chemical cues from their preferred prey compared to shell-less, more mobile species that colonize and metamorphose on a broad range of species and then may select their preferred food as juveniles or adults.

4.2.2 Secondary metabolites as inducers of foraging in juvenile and adult gastropods. The role of sensory organs in foraging during juvenile and adult stages is essential, especially for mobile, shell-less gastropods. However, only a few studies have demonstrated that marine molluscs use olfaction to find food or identify the chemical compounds that elicit foraging behavior. Among them, sacoglossans use olfaction and exhibit a specific behavior (headlifting) in response to food stimuli. *Elysia subomata* prefers to feed upon *Caulerpa ashmeadii*, but also feeds on other algae of the genus *Caulerpa*.[208] The sea slug is able to detect large polypeptides with a molecular weight of 2000–3500 Da released by algae of the genus *Caulerpa*. Five other species of sacoglossans: *Oxynoe azuropunctata*, *Elysia eoelinae*, *E. papillosa*, *E. tuca*, and *Ercolania fuscata* also respond to food stimuli.[29,209] Indeed, the filtered homogenates, containing proteins, of the preferred algal food induce headlifting behaviors in their respective sea slugs. In addition, most species show a response in the presence of *Caulerpa* homogenates and this may be because this alga is the ancestral food of sacoglossans.

The specific interaction between *Halimeda incrassata* and *Elysia tuca* and the transmission of halimedatetraacetate **7** and chloroplasts from the alga to the sea slug has been discussed in part 2.[41] *H. incrassata* is a chemically defended and calcified seaweed that produces 4-hydroxybenzoic acid **150** and halimedatetraacetate **7**. Despite the fact that reproduction in *Halimeda* remains rare and ephemeral (~36 h), the abundance of sea slugs on alga during the reproductive stage, when it is not mechanically defended with calcified thalli, is 12–18 times higher than on a vegetative individual. This is due to the production of high concentrations of the deterrent compound halimedatetraacetate **7** by reproductive *H. incrassata* in order to compensate for the temporary lack of a mechanical defense, which *E. tuca* intercepts. Vegetative algae produce 4-hydroxybenzoic acid **150** (Fig. 34) and the sea slug is able to chemically distinguish between vegetative and reproductive individuals, showing a preference for uncalcified algae.

We have previously described, in part 2, the transmission of tambjamines in an ecosystem formed by the bryozoan *Sessibugula translucens*, two nudibranch consumers *Tambja abdere* and *T. eliora* and their nudibranch predator *Roboastra* tigris[85] (Fig. 14). *T. abdere* locates *S. translucens* by detecting the presence of tambjamines A **46** and B **47** ($>10^{-10}$ M) in the water. However, a higher concentration of these two compounds ($>10^{-8}$ M) deters *T. abdere*. The tambjamines, in addition to a foraging cue, may also act as alarm pheromones as they are produced by nudibranchs in response to an attack by the predator *R. tigris*. Such an attack triggers the nudibranchs to secrete mucus containing high concentrations of tambjamines (Fig. 35). *T. abdere* is therefore able to detect and differentiate between two concentrations of tambjamines that differ only by orders of magnitude in their concentration. Thus, tambjamines A and B are used as both chemical cues enabling *T. abdere* to find its prey *S. translucens*, as

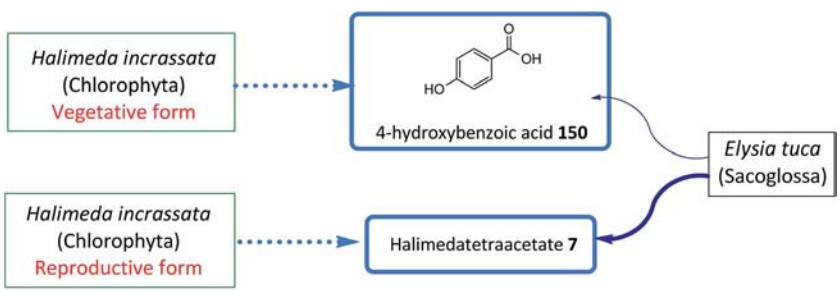

Fig. 34 *Elysia tuca* tracks either the algal metabolites halimedatetraacetate **7** or 4-hydroxybenzoic acid **150** to locate its prey.

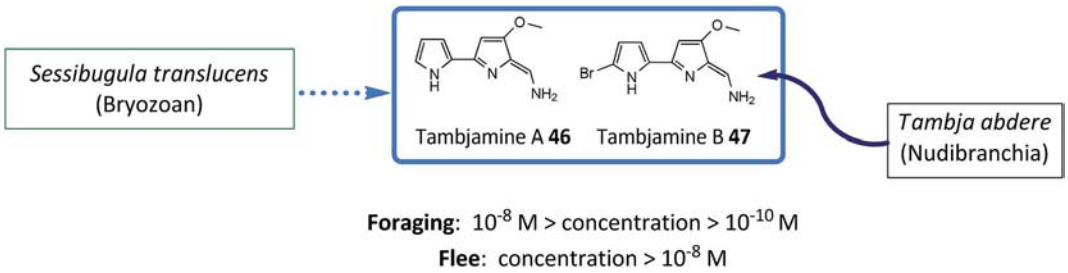

Fig. 35 *Tambja abdere* tracks the bryozoan secondary metabolites tambjamines A and B to locate its prey and flee when the concentration is higher.

well as chemical signals of intraspecific alarm pheromones in the case of a predatory attack.

The remaining examples demonstrate the use of olfaction to find food, but without identifying the chemical compounds involved. The sea anemone *Anthopleura elegantissima* constitutes a source of food for the aeolid nudibranch *Aeolidia papillosa*, and it is detected *via* its chemical cues.[234] However, no compound has been identified and the chemical cue could originate from either the sea anemone or from their endosymbiotic algae. Another example involves the specific interaction between *Phestilla sibogae* and *Porites* spp. for which cues used in settlement were previously discussed. The adult nudibranch is also able to detect chemical cues from *Porites* in water, but no compound has been identified.[201] The hydroid *Tubularia crocea* is a food source for the nudibranch *Hermissenda crassicornis* which detects its prey remotely using chemoreception.[235] Furthermore, *Tylodina perversa* is also able to track chemical cues from the sponges *Aplysina aerophoba* and *A. cavernicola*, as they both contain the same brominated compound family, yet the mollusc only consumes *A. aerophoba*.[120] Contrary to *A. cavernicola* that lives in shady environments, the preferred sponge, *A. aerophoba*, thrives in high solar conditions which enables cyanobacteria to proliferate on its surface. It is these cyanobacteria that *T. perversa* actually consumes, as well as some of the sponge, therefore it appears that *T. perversa* uses chemical cues from the sponge to indirectly locate its preferred food. Feeding preferences are actually influenced by the presence or absence of these photosynthetic organisms, as *T. perversa* prefers to feed upon sponges with high concentrations of cyanobacteria.[122]

Pleurobranchaea californica uses chemical cues to detect previously encountered unpalatable prey.[236] During the first encounter between *P. californica* and the aposematic nudibranch *Flabellina iodinea*, the former attacks the latter, but the toxicity of the prey causes the predator to release it. During subsequent encounters, *P. californica* detects the presence of nudibranch chemical cues and alters its behavior so as not to physically encounter the toxic sea slug, an example of adaptive learned avoidance.

Finally, the scavenging gastropod *Nassarius festivus* spends most of its time resting in sand and uses its siphon to detect chemical cues from carrion and then uses chemoreception to find its prey.[237] However, it has been proven that ocean acidification can influence this behavior. Indeed, a pH of 7.0 has a strongly negative effect on foraging performance, such as reducing travel speed during foraging, foraging success and consumption rate while also increasing feeding time.[237]

4.3 Secondary metabolites as inducers of mucus trail following

Mucus secretion is used by molluscs for several purposes including defense, sliding, as well as prey and conspecific recognition. Indeed, shelled and shell-less marine gastropods such as periwinkles,[238–245] abalone,[246] pulmonates[247,248] and heterobranchs[249,250] are known to use contact chemosensory to detect a conspecific's mucus trail. Most of the mucus trail is polarized enabling the mollusc to follow the trail "in the right direction" which maximizes the chances of encountering a conspecific.[239,250] Some marine gastropods are even able to determine the sex of the congener.[241] This phenomenon enables the mollusc to find a conspecific by following the mucus trail in order to mate. However, only one example of trail following with mucus containing diet-derived compounds has been demonstrated. The above-mentioned nudibranch *Roboastra tigris* is able to detect and follow the mucus secreted by two of its prey species *Tambja abdere* and *T. eliora*.[85] Diet-derived tambjamines A–D **46–49** are present at low concentrations in mucus and could be responsible for trail following.

5. Conclusion

Chemicals are essential for structuring marine gastropod-prey interactions. Marine gastropod molluscs benefit from the chemical defenses of their prey, steal these defenses, and sometimes even biotransform them to enhance their own defenses, use them as intraspecific chemical signals, or as tracking cues to locate their food. Shell-less species, such as nudibranchs, are able to sequester diet-derived defense compounds and create their own chemical shield against predators by concentrating metabolites in external body tissues. Additionally, the excretion of toxic diet-derived compounds *via* mucus or ink remains an efficient defense mechanism used by sacoglossans and sea hares against predators. The biotransformation of diet-derived compounds, driven by a well-organized enzymatic structure, may either further increase the strength of chemical defenses as found in some nudibranchs, or decrease toxicity such as in sea hares or shelled species. Indeed, biotransformation in shelled species is always

carried out for the purpose of detoxification, whereas it has a dual role in shell-less species. It is therefore not unlikely that, for some molluscs, enzymes have evolved from having an initial detoxification role to one enhancing defense by assisting in either the sequestration of toxic compounds or in the modification of parent molecules to enhance their toxicity. Chemical cues and signals are omnipresent in gastropod-prey interactions whether for foraging, settlement or as intraspecific pheromones, but only a few studies have identified the molecules involved in these processes. Therefore, identifying such chemicals constitutes an interesting challenge in coming years. Moreover, understanding such phenomena is essential to further comprehend the impact that our changing environment, such as global warming and ocean acidification, may have on structuring not only marine gastropod-prey interactions, but entire ecosystems.

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