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## Ecology and bioprospecting

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### Abstract

Bioprospecting is the exploration of biodiversity for new resources of social and commercial value. It is carried out by a wide range of established industries such as pharmaceuticals, manufacturing and agriculture as well as a wide range of comparatively new ones such as aquaculture, bioremediation, biomining, biomimetic engineering and nanotechnology. The benefits of bioprospecting have emerged from such a wide range of organisms and environments worldwide that it is not possible to predict what species or habitats will be critical to society, or industry, in the future. The benefits include an unexpected variety of products that include chemicals, genes, metabolic pathways, structures, materials and behaviours. These may provide physical blueprints or inspiration for new designs. Criticism aimed at bioprospecting has been addressed, in part, by international treaties and legal agreements aimed at stopping biopiracy and many activities are now funded by agencies that require capacity-building and economic benefits in host countries. Thus, much contemporary bioprospecting has multiple goals, including the conservation of biodiversity, the sustainable management of natural resources and economic development. Ecologists are involved in three vital ways: first, applying ecological principles to the discovery of new resources. In this context, natural history becomes a vast economic database. Second, carrying out field studies, most of them demographic, to help regulate the harvest of wild species. Third, emphasizing the profound importance of millions of mostly microscopic species to the global economy.

### Keywords

biomimicry; bioprospecting; ecology; intellectual property; pharmaceuticals

## INTRODUCTION

All we have yet discovered is but a trifle in comparison with what lies hid in the great treasury of nature.

Antonie van Leewenhoek (1680)

Bioprospecting is the exploration of biodiversity for new biological resources of social and economic value. It is carried out by a wide variety of industries, the best known being the pharmaceutical industry, but also by a variety of branches of agriculture, manufacturing, engineering, construction and many others (Beattie *et al.* 2005).

Pharmaceutical bioprospecting has been sharply criticized for what has become known as 'biopiracy' (Mgbeoji 2006) in which large international pharmaceutical corporations make use of local medicinal knowledge without acknowledging that it is indigenous intellectual property. Thus, profits have accrued solely to the pharmaceutical companies and indigenous peoples received little or nothing in return (Laird 2002; Finger & Schuler 2004). The Rio Declaration and the Convention on Biological Diversity (1992) clarified the rights of indigenous people and local communities in this setting and a variety of treaties and national laws have been enacted worldwide to control the use of intellectual property and to establish equitable benefit sharing (ten Kate & Laird 1999; Laird 2002; Dutfield & Suthersanen 2008). Their effectiveness varies greatly but some programs, such as the International Cooperative Biodiversity Groups (ICBG) program of the National Institutes of Health, has recognized the issue by insisting bioprospecting: (i) protects rather than degrades the biodiversity resource; (ii) promotes host-country capacity building and intellectual property rights; as well as (iii) seeking novel biological resources for profit. This formula has been adopted by many research groups that emphasize securing economic benefits for host countries or cultures and the promotion of biodiversity conservation (e.g. Kursar *et al.* 2006; INBio 2009). Nevertheless, many issues remain unresolved (Roe & Elliott 2010).

The Millennium Ecosystem Assessment (MEA) (<http://www.millenniumassessment.org>) provided an opportunity to review ecosystem products (as opposed to services) and to place the search for them in the context of bioprospecting. The sponsors of the MEA, notably UNEP, UN-FAO and the World Bank, encouraged the expansion of the term bioprospecting beyond pharmaceuticals to the search for a wide variety of other products including crop pollinators, biological control agents, organisms for biological monitoring, plant genetic resources for agriculture, horticulture and forestry, species for aquaculture, bioremediation, ecological restoration and biological mining and a wide range of organisms that provide products such as new materials and designs, or intellectual inspiration for applications in engineering, manufacturing and construction. Put another way, the MEA placed biodiversity at the core of many major industries worldwide.

The resource for all bioprospecting is biodiversity and the goal of this brief review is to illustrate the extent of the organisms and habitats that are currently the focus of bioprospecting industries. We will also suggest the importance of the various roles that ecologists can adopt in these rapidly expanding sectors of biodiversity use.

## CASE STUDIES

### Exploiting variation in marine organisms

Marine organisms commonly produce greater varieties and quantities of metabolites than is currently known and ecological investigations can be used to both demonstrate the occurrence of this variance and to inform drug discovery. At a geographic scale, studies with seaweeds, salt marsh plants and marine worms indicate that chemical defenses are more potent in tropical than in temperate populations and that these low latitude regions will be richer in diversity of bioactive metabolites. Bolser and Hay (1996) found that temperate populations of the brown alga *Lobophora variegata* were up to 44× more palatable to a sea urchin than tropical populations of this same species (Fig. 1). Across a diverse set of species pairings, this same study found that tropical seaweeds were only 50% as palatable as

temperate seaweeds and that the majority of this difference was due to lipid-soluble chemical defenses. Pennings and co-workers (Siska *et al.* 2002; Pennings *et al.* 2009) demonstrated similar palatability and chemical defense patterns when comparing higher *versus* lower latitude plants from salt marshes. Kicklighter and Hay (2006) found that tropical worms were less palatable than temperate species, but that this relationship varied as a function of the habitat type from which the worms were collected, and could be reversed in a few particular instances.

Dramatic variance in prey defenses also can occur over much smaller scales, such as among local populations, among individuals within a population, among different life history stages or among different portions of a single individual (Hay *et al.* 1988; Paul & Van Alstyne 1988; Cronin & Hay 1996a,b; Taylor *et al.* 2003; Lopanik *et al.* 2006; Hay 2009). In some cases, differences in chemical potency between populations of the same species can exceed differences among different, co-occurring species (Bolser & Hay 1996; Taylor *et al.* 2003). Recent investigations show that many coral reef species rapidly alter their chemical composition in response to damage from natural enemies or physical stress, resulting in greater levels of chemical defenses and, sometimes, different defensive compounds. This rapid biochemical alteration within aquatic organisms has only recently been discovered by ecologists, suggesting that previous methods of collecting and screening for bioactive compounds might have missed these dynamic responses, thus limiting the metabolites discovered. Induction and activation of chemical defenses following attack appear to be common among marine organisms (Paul & Van Alstyne 1992; Cetrulo & Hay 2000; Toth & Pavia 2007). Even prey as simple as seaweeds and phytoplankton not only induce additional defenses, but recognize the attacking species and respond with the correct defense for that particular species of attacker (Toth & Pavia 2000; Long *et al.* 2007). As examples, when copepods consume the phytoplankter *Phaeocystis globosa*, these prey chemically sense both that their conspecifics are being attacked and which predator is attacking. They respond with opposing phenotypic shifts depending on the consumer that is attacking (Long *et al.* 2007), thus lowering their susceptibility to that specific consumer. Variance in the palatability of a single clone of this phytoplankton can change by nearly 100× over only 3 days when grown under different conditions (Long & Hay 2006). In a similar way, waterborne cues from the copepod *Acartia tonsa* induce paralytic shellfish toxin production in the bloom-forming dinoflagellate *Alexandrium minutum* (Selander *et al.* 2006). Induced *A. minutum* contain up to 2.5 times more toxins than controls and are more resistant to copepod grazing.

Similar prey responses to cues emitted by nearby predators also occur in benthic systems. When Toth (2007) exposed 12 species of Swedish seaweeds to effluents from conspecific neighbours being attacked by the herbivorous isopod *Idotea granulose*, four of the 12 species induced greater distastefulness. Similar patterns can occur for other seaweeds being attacked by other herbivores (Toth & Pavia 2007).

Ecological studies such as these may affect the discovery or production of metabolites of biomedical importance. For example, the bryozoan *Bugula neritina* produces a series of some 20 bryostatin compounds, with bryostatin 1 showing activity against various cancer cell lines and being in several phase II and III clinical trials. The compound has also shown promise in rat model systems for countering depression and dementia (Sudek *et al.* 2007). The concentrations and presence of these compounds differs geographically and with depth at numerous sites and appears to be related to the presence of a bacterial symbiont that is the actual producer of the bryostatins – with the bryostatins serving the ecological function of defending bryozoan larvae against consumers (Lopanik *et al.* 2006; Sudek *et al.* 2007). While some of this variance in bioactivity and compound production may result from among population variance, it may also be that *B. neritina* is actually a complex of cryptic, sibling species (Sudek *et al.* 2007); such complexes of cryptic species also constitute a

significant challenge for drug discovery if one treats presently understood Latin binomials with too much respect. As with *Bugula*, the cosmopolitan brown alga *L. variegata* varies in its production of the antifungal cyclic lactone, lobophorolide, with concentrations varying considerably among individuals within a region and being absent from some ocean regions (Kubaneck *et al.* 2003).

The chemical bases for these changes and differences among populations are inadequately documented in most cases and can be complex; in some cases individual parts of plants will induce chemical deterrents in response to attack while other plant portions will not (Taylor *et al.* 2002). However, knowledge of this variance can be exploited for the discovery of novel pharmaceuticals. In these cases, knowing how to induce increased production of metabolites will allow greater discovery of bioactive metabolites, but inducing many species of prey with the many different species of natural enemies that attack them for uncertain periods of time and across the thousands of species combinations that may be envisioned is not tractable.

However, within-species variance in production of bioactive metabolites can be measured in two simpler ways that allow for an initial assessment of the utility of this approach. First, one can collect the same species from multiple locations that may be experiencing differences in local selection pressures or differences in the frequency of stresses (attacks) that upregulate chemical defenses. In ongoing research in Fiji, extracts from multiple collections of the same species are sometimes producing strikingly different activities in bioassays against bacteria, fungi, TB, malaria and cancer (M. Hay, J. Kubaneck, S. Engle work in progress). Such variance is not a result of poor taxonomy; 18S rRNA sequence analyses indicate that the variance is intraspecific, not interspecific. A second approach is to 'activate' collections before extraction and testing. Paul and Van Alstyne (1992) discovered that some species of tropical seaweeds dramatically alter the chemistry of their tissues within seconds of being bitten or physically damaged, with less bioactive metabolites like halimadatetraacetate being converted to the more active halimedatriol within seconds. These rapid conversions of precursors to more active products upon damage were termed 'activated' defenses. Recent investigations demonstrate that this rapid alteration of bioactive compounds can also occur in phytoplankton (Wolfe *et al.* 1997), freshwater macrophytes (Newman *et al.* 1996; Bolser *et al.* 1998), sponges (Ebel *et al.* 1997) and many seaweeds (Cetrulo & Hay 2000).

In these situations, extracting fresh, frozen or freeze-dried organisms may prevent this conversion, resulting in the failure to detect chemical diversity and bioactivity that might have been detected if the organism was 'activated' prior to extraction. The frequency of activated changes in chemistry was unknown until Cetrulo and Hay (2000) evaluated the occurrence of activated chemical changes for 42 species of temperate and tropical seaweeds. They simply ground seaweeds in seawater for 30 s before adding organic solvents (activating samples – as would occur for a fish chewing a seaweed) or ground them first in organic solvents that should prevent enzymes from functioning and then added seawater. Obvious chemical changes following activation occurred in >70% of the species evaluated, and this produced significant alterations of bioactivity against herbivores for >25% of the species (see Fig. 2 for some examples). The frequency of activated defenses among marine macrophytes appears to be greater than among vascular plants found in freshwater ecosystems (Prusak *et al.* 2005), indicating that marine algae may be more promising than vascular plants as sources of novel activated chemistry. Ongoing studies investigating the bioactivity of 376 activated *versus* control extracts from tropical seaweeds and benthic invertebrates against pathogenic microbes indicate that activation increases the frequency of 'hits' by about 40% and increases the potency of many active extracts by 70–500% on average depending on the species of microbe being used in the bioassays (M. Hay, J.

Kubanek, S. Engle, in preparation). However, in some species, activation increased extract potency by as much as 300×. Chemical elucidations are still underway but this simple procedure significantly increased both the frequency of discovering promising drug leads and the potency of the extracts. This is one of several possible examples where ecological insights into how prey respond to stresses from natural enemies can be used to inform and optimize drug discovery.

## Marine biofouling

Biofouling is a complex series of events that result in the settlement and growth of marine organisms on other living surfaces. It is a significant cost to host organisms through reduced photosynthesis, growth, reproduction and even survival and they have evolved a diverse range of mechanical, physical and chemical strategies to prevent or manage biofouling on natural surfaces (de Nys *et al.* 2010; Wahl 2010). Consequently, it is an interesting example for the development of biomimetic and bio-inspired designs as it also occurs on submerged anthropogenic surfaces such as ship hulls, aquaculture nets, oil and offshore industry infrastructure, power stations and desalination plants, resulting in high economic and environmental costs (Durr & Watson 2010; Edyvean 2010; Henderson 2010; Page *et al.* 2010). The economic cost of fouling is produced by increased drag on ships and therefore higher fuel costs, expensive maintenance and repair, and routine replacement of infrastructure. Environmental costs are most commonly incurred through the transport and transfer of marine pest species around the world with subsequent invasions displacing endemic species (Lewis & Coutts 2010).

The most widely used and highly effective preventative treatment has been the application of tributyltin (TBT) on marine surfaces which effectively prevented the settlement and growth of propagules and larvae of marine plants and animals. However, the environmental consequences of what, in chemical terms, was a very elegant solution were disastrous, and the use of TBT is now banned worldwide by the International Maritime Organisation (Cheyne 2010; ten Haller-Tjabbes & Walmsley 2010). Subsequent technologies such as the use of copper-based coatings, while more benign, lack the longer term efficacy of TBT and, in response, there has been a strong interest in developing effective and sustainable antifouling technologies.

Linking marine ecology and biotechnology provides a wealth of biomimetic and bio-inspired approaches to developing new physical, mechanical and chemical controls for biofouling based on natural antifouling mechanisms (de Nys & Steinberg 2002; Ralston & Swain 2009; Webster & Chisholm 2010). Here we show how understanding natural antifouling mechanisms can provide biomimetic templates for the development of new technologies using the example of surface microtopographies from molluscs.

Sessile marine invertebrates should provide an ideal surface for the settlement and growth of fouling organisms; however, these natural surfaces are often not fouled. Two of the most conspicuous examples are the blue mussels *Mytilus galloprovincialis* and *Mytilus edulis*. For the most part, these mussels remain free of fouling and, when fouling occurs, it is normally only located on the older hinge region. This phenomenon was first documented for *M. edulis* (Wahl *et al.* 1998) and extended to *M. galloprovincialis* (Scardino *et al.* 2003) and other mytilid mussel species around the world (Bers *et al.* 2006a, 2010). Ecological studies have shown that this pattern is seen in a diversity of species where fouling is related to the age of the shell (Guenther *et al.* 2006), the position on the shell (Guenther & de Nys 2006) and, most importantly, to the presence of an intact periostracum (the sclerotised protein coating deposited over the shell matrix as it is formed) (Bottjer 1981; Harper & Skelton 1993; Maoche *et al.* 1996; Scardino *et al.* 2003; Guenther & de Nys 2006). The mechanism of action of the periostracum in preventing fouling has been elucidated from studies where



the surface and structure of the mussel is mimicked by the use of resin models that replicate the physical properties of the shell. This determines whether the defence was physical or chemical in nature. The two key model species *M. edulis* and *Mytilus galloprovincialis* and mytilids in general, have a periostracum that, when physically mimicked, prevents the settlement and growth of fouling organisms in the lab, and more importantly in the field (Scardino & de Nys 2004). Mimicking this surface prevents fouling compared with non-textured (smooth) controls but, surprisingly, it is not as effective as the natural surface. These surfaces are shown in Figure 3. The natural surface of the mussel also provides a level of chemical defence against fouling and evidence strongly supports a combined defence strategy using chemical and physical mechanisms against micro and macro fouling organisms (Scardino & de Nys 2004; Bers *et al.* 2006b).

This phenomenon also occurs on other bivalve species but is not ubiquitous, with some molluscs being heavily fouled while others remain remarkably free. Study of the relationship between the periostracum and fouling offers an opportunity to identify its critical physical features, providing a broader understanding of the mechanisms of fouling prevention and resistance, and consequently a platform from which to develop biomimetic and bio-inspired antifouling technologies.

The initial step in determining the mechanism of action of the physical surface is to mimic it and then identify a consistent pattern of effect. This was done for two fouling-resistant bivalve species *M. galloprovincialis* and *Tellina plicata* (Scardino *et al.* 2006) with topographic characteristics of 1–2 mm sinusoidal ripples (*M. galloprovincialis*) and 4 µm projections (*T. plicata*). Biomimics were tested against diatoms of varying sizes. There was stronger deterrence of diatoms that were larger than the modelled topographies, and stronger settlement of diatoms that were smaller than the modeled topographies, confirming the concept of attachment point theory. This concept has also been demonstrated using micro-fabricated surfaces ranging from smooth through to micron, and millimetre scales against algal spores (Callow *et al.* 2002; Schumacher *et al.* 2007a,b), barnacle larvae (Petronis *et al.* 2000; Berntsson *et al.* 2004; Scardino *et al.* 2008), bryozoans (Scardino *et al.* 2008) and tubeworms (Scardino *et al.* 2008).

The successful recruitment of a fouling organism is an outcome of the selection of a settlement site and its subsequent attachment strength. All settling sessile benthic organisms are subject to removal forces, and minimizing the likelihood of removal is critical to recruitment. Therefore, the concept of testing surface features at the scale of microns against the settlement of larvae of a similar scale has been extended to quantifying the attachment strength of settling larvae on a range of surface topographies (Aldred *et al.* 2010). Not surprisingly, strength of attachment is a key factor in the preferential settlement of surfaces by barnacle cypris larvae where they actively chose to settle to the maximum number of attachment points, and subsequent have the strongest attachment strength (Aldred *et al.* 2010). However, it is surprising that larvae can select a site based on the future potential to adhere most strongly, and this even raises the possibility of some form of memory in invertebrate larvae when selecting preferred settlement sites (Aldred *et al.* 2010).

The outcome of these ecologically based biomimetic studies using bivalve micro-structured surfaces as a model provides two options for applications to biofouling. First, by utilizing least preferred topographies with the lowest number of attachment points, the settlement and growth of propagules and larvae of fouling organisms can be minimized (Scardino *et al.* 2008), and the release of fouling from surfaces maximized (Aldred *et al.* 2010). This has obvious application in the prevention of fouling for vessels and infrastructure. Using the opposite approach, by utilizing the most preferred topographies, settlement and attachment strength and therefore recruitment can be maximized. This has direct application in

aquaculture industries where enhancing the settlement of larvae is critical to success (Carton *et al.* 2007). However, these models have one significant failing in that they only apply to one size of larvae, and therefore essentially one species, or group of species with larvae of similar sizes. This provides a tool in situations where one fouling organism dominates the fouling community, as is the case in the Norwegian aquaculture industry where ascidians are the most abundant and problematic fouling organism (Guenther *et al.* 2009). Alternatively, in aquaculture it potentially provides a more powerful tool where single aquaculture species requiring enhanced settlement are targeted, as in the mussel industry (Carton *et al.* 2007).

The development of bio-inspired materials with a broad spectrum of deterrence is hindered by the lack of a framework of physical surface parameters which can be manipulated to provide improved surface-based antifouling technologies. To address this Scardino *et al.* (2009a) quantified the surfaces of thirty six species of marine molluscs and correlated these with the resistance or the removal of fouling (Fig. 4). This combined approach using materials science and surface characterization, field biology, and correlative analyses provided for the biomimetic characterization of surface parameters associated with fouling resistance, and fouling release. The study measured surface parameters (roughness, waviness, skewness, texture aspect ratio, fractal dimension and hydrophobicity) and correlated these with the fouling resistance of shells in field exposure trials, and the release of fouling on these shells after the trials were completed.

The shell surfaces had a wide range of macro and micro scale features (Fig. 4), and specific surface properties correlated significantly with fouling resistance or fouling release. Increased resistance to fouling was hierarchically correlated with lower fractal dimension, higher positive skewness in the roughness and waviness profiles, a higher texture aspect ratio, and lower mean roughness. Increased release of fouling was correlated with higher mean waviness (Scardino *et al.* 2009a). These parameters provide a template for industry to move towards more fouling resistance and improved foul release materials, either through new materials or incorporating surface features into current antifouling coatings.

It is important to note that the bio-inspired models from bivalves are one of a number of bio-inspired concepts that have been developed to the point of providing direction for industry. Another exciting well-developed bio-inspired model is the shark skin. This model also offers new designs for materials development and has followed a similar route where mechanisms of action are understood (Carman *et al.* 2006; Schumacher *et al.* 2007a,b; Schumacher *et al.* 2008). Both examples highlight the value of biomimetic studies but, more importantly, emphasize that determining the underlying mechanisms of action provides a powerful tool for developing even more innovative and effective bio-inspired technologies (Long *et al.* 2007; Scardino *et al.* 2009b).

The final stage is to link the effects of surface modifications with hydrodynamics. Hydrodynamics affect ship performance as well as the settlement preferences of propagules and larvae, and the effect of novel surfaces and fouling on hydrodynamics, and subsequently settlement at slow speed, needs to be addressed (reviewed by Munk *et al.* 2009). There is significant power in modelling the effects of materials and surface topographies on hydrodynamics, before moving to the longer term patch and hull coating trials that act as an industry standard in the development and implementation of coatings (Sánchez & Yebra 2009).

Ecological knowledge leading to bio-inspired designs improve foul resistant and foul release technologies either through new materials, or through incorporating bio-inspired design principles into existing materials.

## Ecology, biomimetics and bio-inspired design

Biomimetics is a general term for the adaptation of organic processes and products for use in technology, originally coined for engineering (*sens. lat.*). Such adaptation ideally occurs as a process of concept and design, as the technical environment is so different from the biotic, and change of context demands change and adaptation of the function. Biomimetics generally involves little harvesting or extraction, as the final product is independent of the source of the concept; however, the industries involved must be able to source, study and use these processes. Recognizing that there was a resource, that it was biodiversity, and valuing that source, was the first task. Since then, engineers have been challenged to reduce the consumption of fossil energy and to harness natural sources; to recycle, to conserve plants and animals and generally look at new ideas for better design of manufactured products and structures.

**Wood**—Strangling figs such as the banyan (*Ficus* sp.) are unusual in that they grow tensile roots which insert themselves into the ground and change from self-straightening tensile to supporting compression structures capable of supporting a branch reaching horizontally up to 20 m (Fig. 5). This generates a question for the engineer: What mechanisms are needed to monitor the diameter and stability of what starts as a slender thread? But first, why is wood such a successful structural material? The answer lies partly in hierarchy, especially in cellular structures, which is still not fully understood (Plomion *et al.* 2001). The helical cellulose fibres reinforce the walls of the tubular cells which form wood. Under tensile load, the tubes collapse inwards and then the fibres straighten, with associated fracture and deformation, dramatically improving strain energy absorption. Weight for weight, biomimetic versions of such hierarchical structures can out-perform homogenous engineering materials by a factor of five (Chaplin *et al.* 1983). Hardwood has more toughening mechanisms (Hepworth *et al.* 2002). How many novel mechanisms might there be in the forests? Hierarchy in biological materials increases toughness through increased strain energy absorption at the many interfaces between the nano-scale assembly of molecules through the micro-scale of cells and fibres to the macro-scale of repeating arrays of fibres and laminae. The controlled separation at these interfaces leads to distortion and blunting of the crack tip and many diverging, convoluted pathways, enhancing toughness Vincent (2001).

**Adhesives**—The search for new adhesives has been prompted by several examples in nature. Of these ‘Frog Glue’, from the Australian holy cross frog *Notaden bennetti*, is based on a sticky secretion that protects the frog’s skin and is being trialed as a surgical glue for meniscal tears. It could also be the next medical adhesive for musculo-skeletal injuries as it is reputed to be stronger than any non-toxic medical adhesive currently on the market (Millar *et al.* 2009). Bioprospecting among the plethora of biological surfaces and secretions from a wide range of organisms has provided the models or inspiration for a wide variety of industrial adhesives (e.g. Barnes 2007). It is worth quoting Shao and Stewart (2010) as the lead sentence in their recent article encapsulates this type of bioprospecting: ‘The challenges of developing medical adhesives for the wet environment of open surgery are analogous to the adhesion problems solved by marine organisms living at the watery interface of land and ocean. These organisms routinely bond dissimilar materials together under seawater with little if any surface preparation.’ For example, in the common mussel *M. edulis*, the byssus attachment mechanism has a cluster of long collagenous threads with a springy shock absorber at one end and an adhesive disk at the other which is attached to the substrate. The disc uses protein-based glues that are secreted and attached under water while achieving strength in excess of 80 MPa which is about twice the strength of the best industrial epoxy resins when under ideal conditions. The entire mechanism is protected from abrasion by a tough yet extensible protein-metal cuticle (Harrington *et al.* 2010). Turning over the coin,



biological surfaces have also provided the raw materials for non-adhesive surfaces, the most successful taking advantage of the hydrophobicity of lotus leaves, marketed as self-cleaning paints (see <http://www.lotus-effekt.de>).

**Photonics**—Natural models are remarkable and replete with non-polluting colours and reflecting surfaces. In the iridescent blue butterfly *Morpho rhetenor* wing scales achieve coherent light via regular multilayers of cuticle and air (Vukusic & Sambles 2003) while the white cuticle in the beetle *Cyphochilus* sp. optimizes the packing density of scattering centres and voids to produce random visible wavelengths and a characteristic white appearance (Hallam *et al.* 2009). Where transmission rather than reflection of light is important, such as transparent wings and insect eyes, rough anti-reflective surfaces make use of sub-micron ‘nipple arrays’ (Bernhard *et al.* 1965). Currently, the visual systems of beetles are the focus for one branch of biophotonics dedicated to imaging in low-light environments (e.g. Galusha *et al.* 2009).

**Cryoprotectants**—Bioprospecting in extreme environments illustrates both the diversity of organisms and ecosystems involved. Antifreeze proteins have been discovered in a variety of plant, invertebrate and vertebrate species from high altitude or latitude ecosystems and are being applied commercially, either in native or modified forms in, amongst others, the food and aquaculture industries (Zbikowska 2003; Yeh *et al.* 2009). Cryo-preserved derived from the extremophile *Gelidibacter algens* are being used in vaccine production (Powell *et al.* 2009) and tardigrades are the focus of much research into cryptobiosis, that is resistance to desiccation, freezing or oxygen deficiency (Neuman 2006). Organisms from high temperature, high salinity or low oxygen habitats are similarly yielding a wide variety of useful industrial chemicals (Beattie & Ehrlich 2004).

**Biomineralization**—The complex mechanisms that produce structure in organisms are under close scrutiny for many different kinds of industrial structures, materials and surfaces, not least the biochemical processes within soft tissues that produce extremely hard materials such as shells and radulae in the process known as biomineralization. It is fair to say that a range of invertebrates that possess either shells or spicules are the envy of materials and structural engineers as the animals manufacture these structures at ambient marine temperatures, with freely available molecules, without the industrial complexity or pollution of the human equivalent (e.g. Aizenberg *et al.* 2005). There are many groups taking these animals apart molecule by molecule so as to simulate the natural biochemistry in industrial processes (e.g. Barnes 2007; Lee *et al.* 2007; Kroger 2009). Biomineralization is just one of a series of molecular/nanotechnological processes such as self-assembly, energy conversion and electronic signalling currently under bio-inspired investigation (Ball 2002; Seeman & Belcher 2002).

## AN IMPORTANT FUTURE DIMENSION: MICROBIAL BIODIVERSITY

Currently, industrial pharmaceutical discovery favours high-throughput synthetic library screening rather than natural products, but recent technological advances such as 454 sequencing and single-molecule real-time sequencing are being deployed to reveal novel molecules harboured by the vast diversity of unculturable microorganisms. Nobody knows how big this resource is, but it has been described as being ‘so vast as to seem unlimited, and these emerging tools will provide exhilarating discoveries leading to new medicines’ (Li & Vederas 2009). Ecology is a powerful tool in drug discovery, especially in identifying the habitats where drugs such as antibiotics are expected to have evolved, for example, microbe-rich sediments and highly specialized mutualisms such as those that involve bacterial symbionts (Clardy *et al.* 2006; Fischbach & Walsh 2009). The symbiont route also has the advantage that the microbial community involved is likely to be relatively simple and its

ecology, evolution and chemical interactions correspondingly easier to understand (Clardy *et al.* 2009).

Microbial ecology in general has developed as a core bioprospecting discipline. For example, recent research into the chemical ecology of soil microorganisms strongly suggests that antibiotics are generally secreted in sub-lethal concentrations, and hence are as least as important for intra and inter-specific communication, as they are for competitive inhibition, providing powerful insights into combating drug resistance (for a brief review see Mlot 2009). Study of the chemical ecology of marine epibiotic bacteria has provided new antibiotic leads (Burgess *et al.* 1999) and the eco-physiology of microbes has revealed new opportunities for bioremediation (Methe *et al.* 2003; Konstantinidis *et al.* 2009) and biological mining (Rawlings & Johnson 2007; Reith *et al.* 2009). Understanding the chemical ecology of bacterial-fungal interactions is proving important to understanding some human diseases (Hogan & Kolter 2002) and microbial ecology is playing an increasing part in fighting malaria (Blanford *et al.* 2005; Kambis *et al.* 2009). Overall, the exploration of microbial diversity, especially through molecular ecology, led Demain (2000) to estimate the generation of billions of dollars of pharmaceutical, clinical, industrial and agricultural products worldwide annually. Crucially, microbial biodiversity is also being intensely explored for a wide range of new energy sources including bioethanol, biobutanol, biodiesel, methane, methanol and electricity-generating microbial fuel cells, each of them capitalizing on the astonishing diversity of enzymes, metabolic pathways and secondary compounds harboured by microorganisms. Other organisms broadly categorized as microbes include single-cell algae which are being sought for end-products as diverse as hydrogen, solar cell electronics and bio-inspired artificial photosynthesis (Demain 2009; Collini, Wong, Wilk, Curmi, Brumer, Scholes, 2010; Ganapathy *et al.* 2009). Finally in this arena, the ecology of bacterial 'predators' – phages – is developing with a strong clinical context (Abedon 2008).

## ROLES FOR ECOLOGISTS IN BIOPROSPECTING

There are at least three roles for ecologists in bio-prospecting: (i) enhancing the science through ecologically driven hypotheses; (ii) enhancing the science of harvest regulation; and (iii) enhancing awareness of biodiversity as an essential economic resource.

### Ecologically driven hypotheses

Much bioprospecting in the past failed to recognize that the products sought were products of evolution by natural selection in the context of ecosystem dynamics. Further, ecological and evolutionary theories were not considered relevant, nor were the emerging scientific methods associated with the concept of biodiversity. Thus, for ecologists, it may come as a surprise that the idea of introducing testable hypotheses into bioprospecting, in association with proper sampling and experimental design, is relatively new (Beattie 1992). Perhaps one of the first champions of this view was Coley and her team (Coley *et al.* 2003) who, working with plant chemical defence theory, revolutionized the way companies should collect plant material for drug discovery. There have been many more examples of the application of theory and hypothesis testing, for example, Helson *et al.* (2009) showed that the presence of aposematic herbivorous insects can be used to facilitate the identification of plants with compounds active against human diseases. Also Stow *et al.* (2007) and Mackintosh *et al.* (1998) showed that when bioprospecting for novel antimicrobials in insects, social species are likely to be more profitable than solitary ones. Benkendorff *et al.* (2001) hypothesized that the egg masses of marine invertebrates would be especially vulnerable to microbial pathogens and demonstrated high levels of antibiotic activity on their surfaces. These examples show that natural history is, in effect, a massive and largely neglected industrial dataset. The importance of the ecological approach to drug discovery is made all the more clear by calls from the pharmaceutical industry for a return to natural products (Paterson &

Anderson 2005). As Chapman (2004) pointed out: 'Natural products offer unsurpassed chemical diversity and novelty, often with better affinity characteristics and evolutionary selection for biological activity.'

### Regulating wild harvests

In general, the impacts on the environment of bio-prospecting are trivial in comparison with fisheries, agriculture, timber production and, for that matter, urbanization. Nevertheless, concerns have been expressed about the effects of collecting and species extinction on human well-being, mostly focusing on plant medicinal species (Farnsworth & Soejarto 1985; Akerele *et al.* 1991; Hawkins 2008). Most bioprospecting activities require the taking of relatively few specimens and, when a particular species is in high demand, it may be domesticated, for example, the forest under-story herb mayapple, *Podophyllum peltatum*, is now widely cultured for drug derivatives of podophyllotoxin (Zheljazkov *et al.* 2009). However, individual species can be in high demand locally, or even regionally, and, in these cases, ecological research can inform the regulation of harvests. Many of these cases involve harvesting medicinal plants and may not be bio-prospecting in the strict sense; nevertheless, input from ecologists is still vital, especially when harvesting takes place in national parks or in highly disturbed areas (Shanley & Luz 2003). Research that generates demographic data and models can be extremely powerful. For example, demographic analyses have been especially useful for regulating harvests in palm populations in Mexico (Martinez-Balleste *et al.* 2005), an understory palm in Colombia (Rodriguez-Buritica *et al.* 2005) and rattan palms in Indonesia (Siebert 2002). Through the application of a linear stage-structured model Ratsirarson *et al.* (1996) generated leaf-harvest limits for a rare Madagascan plant species. Regression models have informed seed harvesting limits for the Brazil nut in Bolivia (Zuidema & Boot 2002; Peres *et al.* 2003). Research such as this is needed for international harvesting standards, as numerous plant species are over-harvested (Kala 2005; Patzold *et al.* 2006) and ecologists have shown that traditional harvesting grounds are being disturbed in various ways including the introduction of invasive species (McNeely 2006). Some marine bioprospecting resources have been over-harvested, for example, cone shells as sources of clinical neuropharmaceuticals; however, these may be cultured (Nelson 2004). In their review of marine pharmaceutical bioprospecting Hunt and Vincent (2006) concluded that, in general, only small samples were taken and industry avoided dependency on small or geographically restricted populations for both economic and ecological reasons. However, there remains a clear need for ecological research into harvested marine organisms (e.g. Beche-de-Mer Information Bulletin 2002; Benkendorff 2002).

### Economic awareness: ecosystem products and services

The fact that a very wide variety of organisms and habitats are the focus of bioprospecting fuels the case for emphasizing the utilitarian (or instrumental) arguments for the conservation of biodiversity (e.g. Pagiola *et al.* 2002; Maguire & Justus 2008). This is emphasized by the overlap between ecosystem products and ecosystem services. For example, it is well known that ecosystems provide crop protection through the provision of biological control. However, bioprospecting for individual control agents is frequently required as, for example, in the CSIRO search for an agent to control invasion by the Brazilian floating fern *Salvinia molesta*. CSIRO ecologists collected potential biological control agents from South America and, after various difficulties, released the weevil *Cyrtobagus singularis* which cleared the problem remarkably quickly and sustainably (Room *et al.* 1981). Prior to the invasion it would have been difficult to predict that this precise weevil species, unknown except to a handful of ecologists and taxonomists, would be of critical economic and social value; it would have been difficult to make a case for the conservation of this insect species. This type of bioprospecting has been repeated thousands of times in the biological control literature (e.g. Bellows & Fisher 1999). Much the same

situation applies to bioprospecting for crop pollinators where the service of crop pollination is well recognized but the dramatic and continuing decline in honeybee populations ([http://www.oie.int/eng/press/en\\_100428.htm](http://www.oie.int/eng/press/en_100428.htm)) has led to bioprospecting for alternatives, especially as there has been, in recent years, a dramatic increase in the fraction of agriculture that depends on animal pollination (Klein *et al.* 2007; Aizen & Harder 2009). There are numerous studies demonstrating the role of ecologists in bioprospecting for non-honeybee, wild pollinator species, such as native bees in Costa Rica (Ricketts *et al.* 2004) and native beetles in northern Australia (Blanche & Cunningham 2005). Staying in the agricultural context, bioprospecting for new food species or varieties, especially plants and insects, remains very active (Vince 2010; Vogel 2010).

A major consequence of this type of bioprospecting, as with all others, is the lack of predictability, across a wide swath of industries, as to which kinds of organisms, and which precise species, are going to be important. This suggests the wisdom of conserving as much biodiversity as possible (e.g. Handel *et al.* 1994). We know that skeptics, wary of utilitarian conservation arguments, say that only a minority of species will ultimately be useful for bioprospecting. To which we answer – true, but which ones?

Finally, some words of caution: bioprospecting has been advocated as a means of biodiversity conservation as it points to the actual and potential commercial values of a wide range of species and products (e.g. Reid *et al.* 1993). This view has been challenged by some economists on several grounds, one of which can be encapsulated with the question: Once a useful species is discovered and exploited, does this maintain or increase the probability that further such discoveries will be made in its environment? Quite often, this does not seem to be the case (Barrett & Lybbert 2000). However, there are bioprospecting programs such as the International Cooperative Biodiversity Groups (National Institutes of Health) that address such economic issues by explicitly funding capacity-building in the host country so that bioprospecting becomes a home-grown industry, no longer characterized as the hit-and-run activities of the past (Kursar *et al.* 2006). Further, our case histories suggest that further discoveries will be made in previously productive environments, especially with the growing awareness of the size and scope of microbial possibilities and the range of organisms involved in the provision of ecosystem services.

### **The politics of integrating ecology and bioprospecting: the Brazilian experience**

In 2004, the Brazilian government initiated the Program for Biodiversity Research (PPBio, <<http://ppbio.inpa.gov.br>>), largely in response to obligations under the Convention on Biological Diversity. One of the principle aims of the programme was to integrate applications, especially bioprospecting, with other more academic components. The experience illustrated many of the impediments to the inclusion of bioprospecting in the agenda of most ecologists. Most previous networks involving systematics or ecology with bioprospecting created horizontal links between like entities, such as museums, ecological research sites and genetics or biochemical laboratories. Fearnside (2003) pointed out that this type of arrangement where highly structured networks were established to obtain a single result, did not have the flexibility to include a social component. This structure contrasted with the highly effective networks set up by nongovernmental organizations, in which the vertical links between components, promoting social interactions, were as strong as the horizontal links between members of professional guilds.

A National Institute for Science and Technology (INCT), the Centre for Biodiversity Research in the Amazon (CENBAM), tackled the problem of trying to formalize the links between the different stakeholders (Fig. 6). The private sector was not expected to fund such an initiative because the costs were specific to each stakeholder, and the benefits diffuse.

Integration of stakeholders was obviously a valid role for government because it was a role that the private sector could not assume. However, government saw bioprospecting not only as a means of generating resources for the community, but also as a means of paying for the infrastructure necessary to maintain the knowledge-production chains (e.g. universities, research institutions). Thus, government was a stakeholder that competed with the private sector and, as a consequence, there was an emphasis on secrecy and it was not obvious who would, or could, promote the collaboration and integration necessary to include the majority of biodiversity workers such as the traditional/indigenous land holders, field assistants, parataxonomists, ecologists and systematists, who were too far from the final product to benefit from financial agreements.

Perhaps the greatest challenge was in data management (Lynch 2008; Nelson 2009). Data secrecy was a significant aspect of the bioprospecting culture, but ecologists and systematists would not collaborate unless they also had access to the data. Even if the data could not be made public, metadata allowed other researchers to know what has been done.

The recent history of bioprospecting in Brazil reveals conflicts between bioprospectors and other stakeholders. In addition, legislation to protect traditional knowledge from inappropriate use by private industry has been blamed for stifling systematic and ecological research (Antonelli & Rodrigues 2009). However, it appears that the greatest impediment to the integration of bioprospecting within the routine activities of ecologists has been a lack of an institutional structure that brings all the stakeholders to the table. It is evident that this is the role of government, and the lack of governance in this area seems to be a problem for both developing and developed countries.

## CONCLUSION

Bioprospecting is now worldwide in most ecosystems but impacts on the environment are mostly trivial, at least compared with other human activities. The industries involved cumulatively seek a wide variety of organisms and derive a wide variety of products from them. The diversity in genetics, form and function present in most target groups of organisms provides a variety of choices or, to put this another way, a wide variety of alternative highly evolved prototypes. Ecologists increasingly lead or participate in the discovery and resource management aspects of bioprospecting and are instrumental in demonstrating the different economic benefits of biodiversity. The many international treaties and laws that have been established to prevent biopiracy have been successful in some sectors but not in others. However, bioprospecting programs are increasingly involved with issues of equity and the ownership of intellectual property. Overall, Antonie van Leeuwenhoek's remark in 1680, quoted at the start of this review, remains as true now as it did then.

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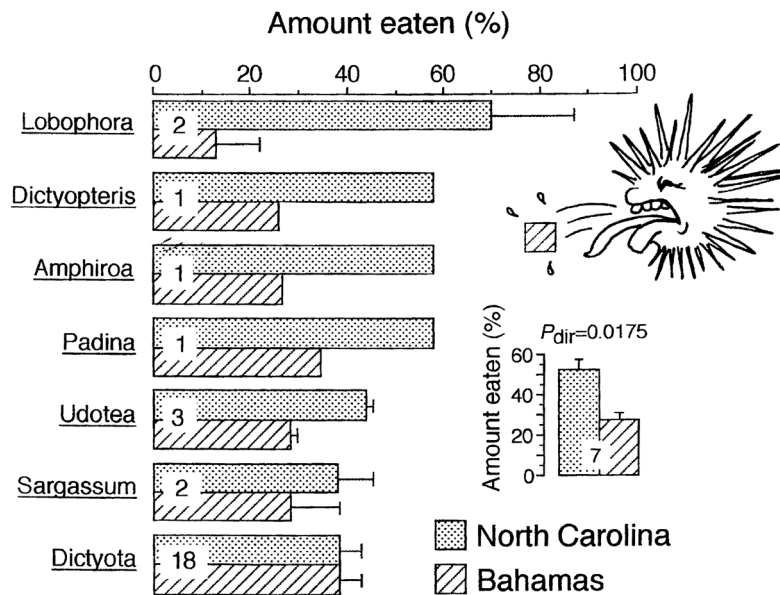
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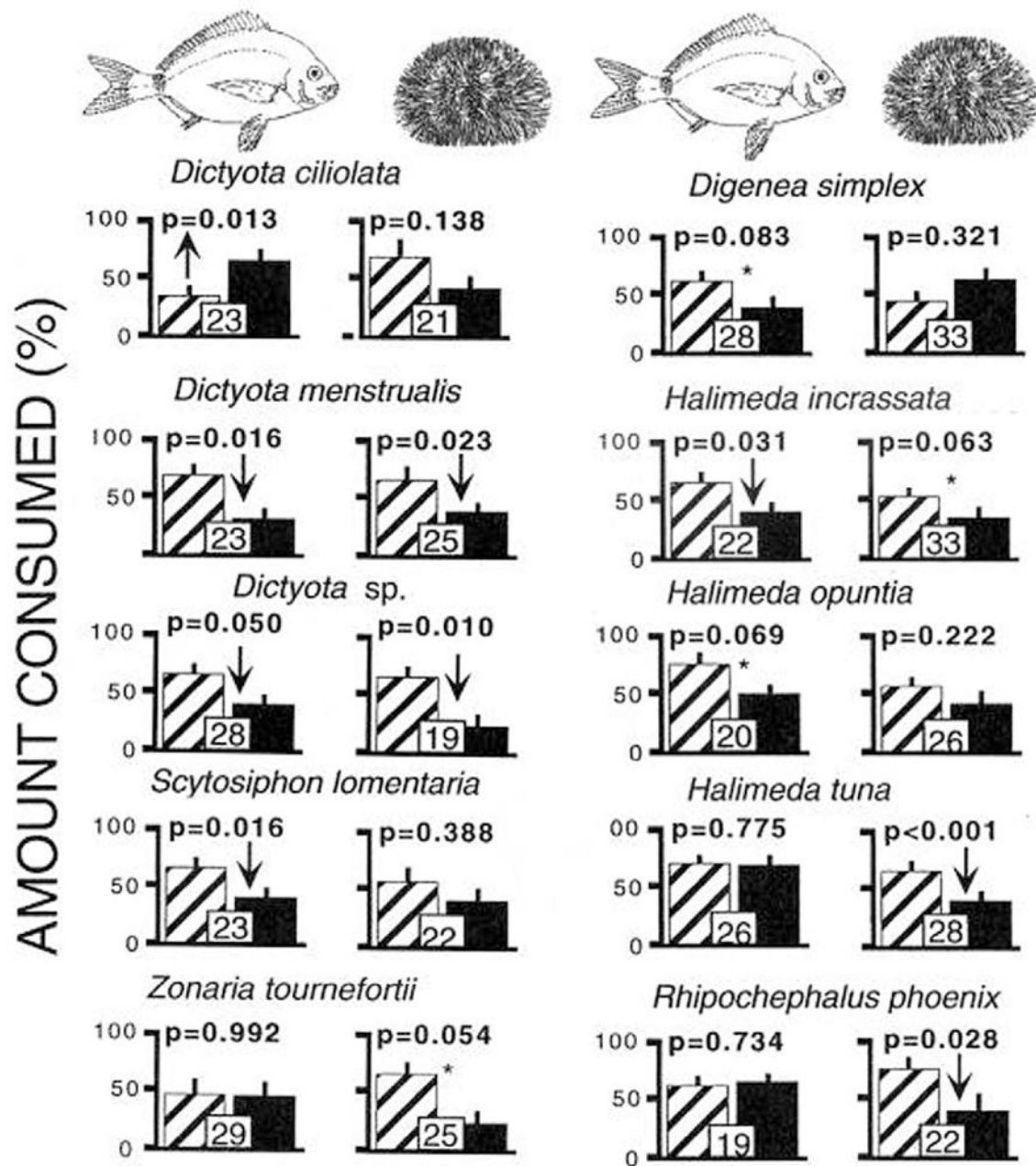


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**Fig. 1.**

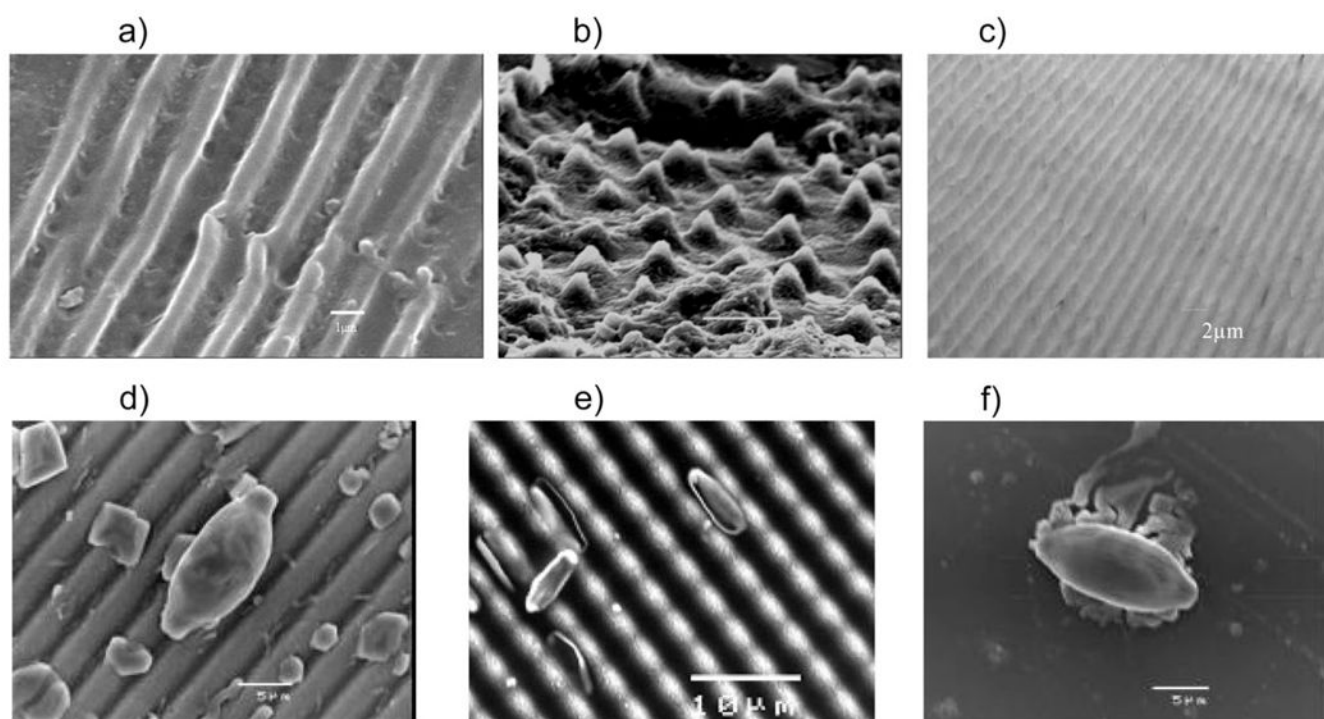
General pattern of sea urchin (*Arbacia*) feeding for seven genera where intrageneric comparisons could be made between temperate and tropical species or collections. For *Dictyopteris*, *Amphiroa* and *Padina*, assays consisted of only one temperate and one tropical species. For the other genera, where contrasts among several species had been assayed, the bars represent mean results ( $\pm 1SE$ ) using each separate entire assay (see Bolser & Hay 1996 for the individual data pairs) as one independent sample. The number in parentheses at the base of each pair of bars indicates the number of separate assays within each genus. Urchins significantly preferred temperate over tropical algae ( $P_{dir} = 0.0175$ , Wilcoxon signed rank test,  $n = 7$ ). Modified from Bolser and Hay (1996).

**Fig. 2.**

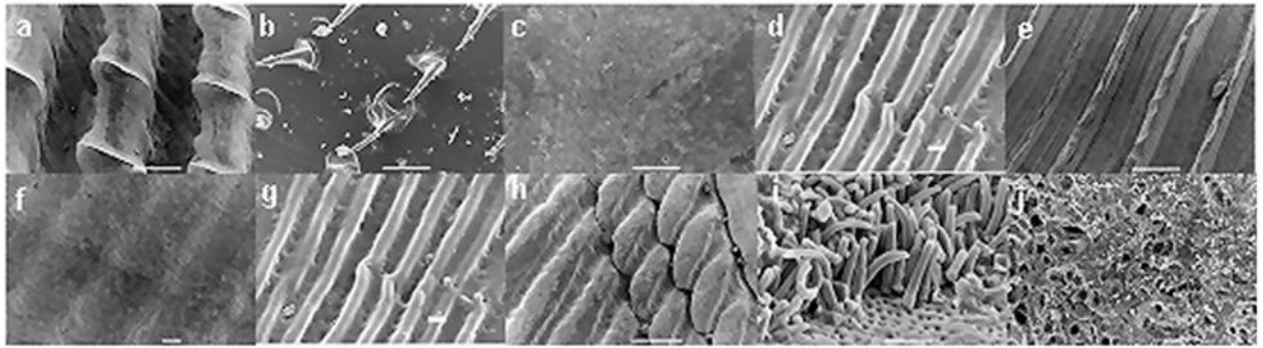
Assays in which local fishes or urchins fed on extracts from control *versus* activated seaweed extracts. Hatched bars show feeding on food treated with extracts from seaweeds that were not damaged before exposure to organic solvents (= controls); solid bars indicate feeding on food treated with extracts from pre-injured seaweeds (= activated extracts). Graphs below fish indicate feeding by fishes (parrotfish for tropical algae and spot-tailed pinfish for temperate algae); graphs below urchins indicate feeding by the sea urchin *Lytechinus variegata*, which occurs in both temperate and tropical sites. Sample size is given at the base of each histogram pair. Error bars indicate 1 SE. *P*-values are from a 2-tailed, paired-sample *t*-test. Lines around a graph highlight significant ( $P < 0.05$ ) effects of activation; The asterisk indicates a nearly significant difference ( $0.05 < P < 0.10$ ). Down-

pointing arrows indicate an activated defense. Figure is modified from data in Cetrulo and Hay (2000).





**Fig. 3.** Scanning Electron Microscopy (SEM) images of (a) natural *Mytilus galloprovincialis* microripples, (b) natural *Tellina plicata* microprojections, (c) modeled 2  $\mu\text{m}$  microripples, (d) modeled 4  $\mu\text{m}$  microripples, (e) modeled 4  $\mu\text{m}$  microprojections, (f) smooth polyimide control. With permission Scardino *et al.* 2006.



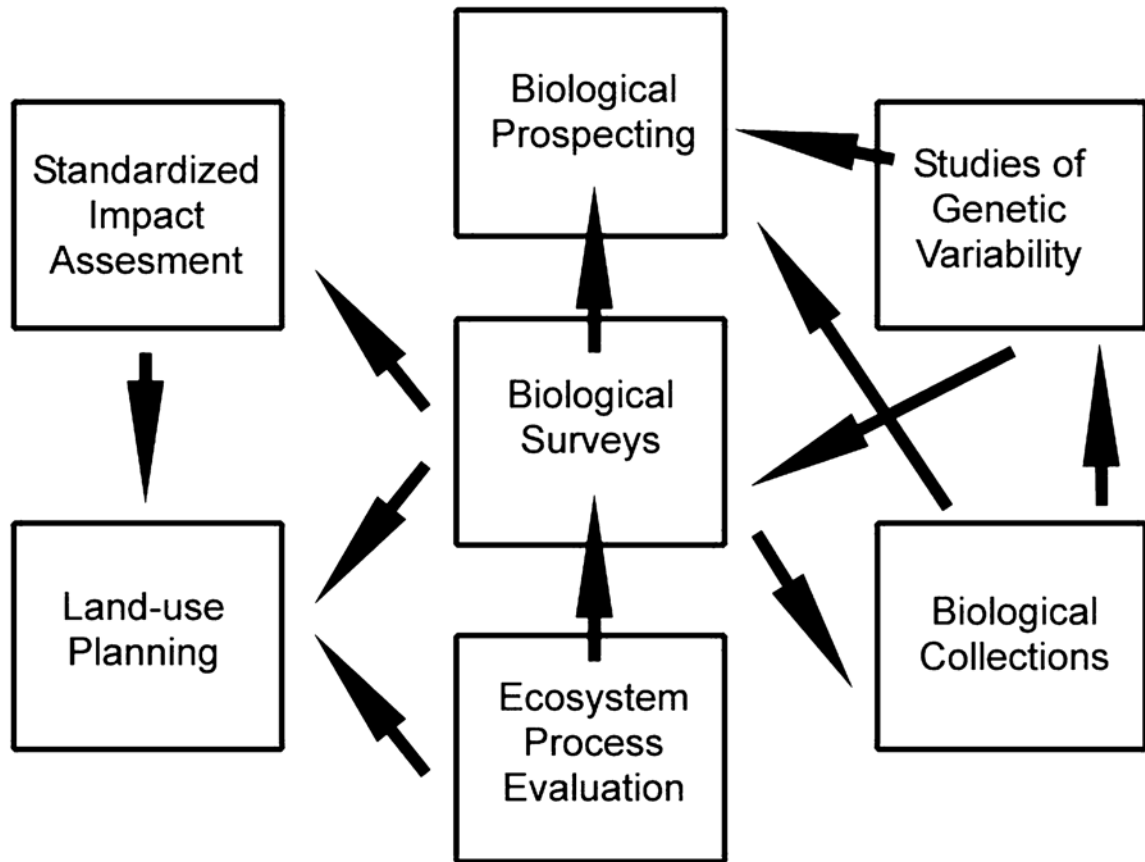
**Fig. 4.**

Scanning electron microscopy images of selected microtopographies for marine bivalve and gastropod shells. Figures a–e are fouling resistant species; Figures f–j are high fouling release. The scale of each picture varies based on the topographical dimensions of the shell (a) *Dosinia juvenilis* ribs 400–500  $\mu\text{m}$  wide (scale 500  $\mu\text{m}$ ), (b) *Tellina inflata* spines 50  $\mu\text{m}$  long, 10  $\mu\text{m}$  wide and spaced 50  $\mu\text{m}$  apart (scale 50  $\mu\text{m}$ ), (c) *Macra olorina* untextured surface (scale 50  $\mu\text{m}$ ), (d) *Mytilus galloprovincialis* ripples 1–2  $\mu\text{m}$  wide (scale 1  $\mu\text{m}$ ), (e) *Acrosterigma reeveanum* ridges, 500  $\mu\text{m}$  wide, 200  $\mu\text{m}$  deep (scale 500  $\mu\text{m}$ ), (f) *Septifer bilocularis* ridges 200  $\mu\text{m}$  wide, ribs 200  $\mu\text{m}$  wide, 100  $\mu\text{m}$  apart (scale 500  $\mu\text{m}$ ), (g) *Mytilus galloprovincialis* (scale 1  $\mu\text{m}$ ), (h) *Hemidonax* sp. ridges 250  $\mu\text{m}$  wide, 250  $\mu\text{m}$  apart, length variable (scale 500  $\mu\text{m}$ ), (i) *Gafrarium tumidum* spines 5  $\mu\text{m}$  long, 1  $\mu\text{m}$  wide (scale 5  $\mu\text{m}$ ), (j) *Divaricella ornate* perforations, 5  $\mu\text{m}$  diameter (scale 10  $\mu\text{m}$ ). With permission Scardino *et al.* 2009.



**Fig. 5.**

This banyan, *Ficus microcarpa*, shows, at the left, young tensile roots, self-inserted into the ground and, to the right, a series of well-established compressive roots. Understanding the transition from tensile to supportive structures informs the design of space frames, struts and joints.



**Fig. 6.**

A simplified diagram of the interactions among different biodiversity studies that indicates the importance of integration among different components of knowledge-production networks (feedbacks not shown).