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SYMPOSIUM

Barnacles and Biofouling

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Synopsis Biofouling, the attachment and growth of organisms on submerged, man-made surfaces, has plagued ship operators for at least 2500 years. Accumulation of biofouling, including barnacles and other sessile marine invertebrates, increases the frictional resistance of ships' hulls, resulting in an increase in power and in fuel consumption required to make speed. Scientists and engineers recognized over 100 years ago that in order to solve the biofouling problem, a deeper understanding of the biology of the organisms involved, particularly with regard to larval settlement and metamorphosis and adhesives and adhesion, would be required. Barnacles have served as an important tool in pursuing this research. Over the past 20 years, the pace of these studies has accelerated, likely driven by the introduction of environmental regulations banning the most effective biofouling control products from the market. Research has largely focused on larval settlement and metamorphosis, the development of new biocides, and materials/surface science. Increased research has so far, however, failed to result in commercial applications. Two recent successes (medetomidine/Selektope®, surface-bound noradrenaline) build on our improving understanding of the role of the larval nervous system in mediating settlement and metamorphosis. New findings with regard to the curing of barnacle adhesives may pave the way to additional successes. Although the development of most current biofouling control technologies remains largely uninfluenced by basic research on, for example, the ability of settling larvae to perceive surface cues, or the nature of the interaction between organismal adhesives and the substrate, newly-developed materials can serve as useful probes to further our understanding of these processes.

Introduction

Biofouling, defined as the accretion of organisms on submerged, man-made surfaces, has been the bane of ship operators for at least 2500 years. Initial attempts to control biofouling, using metal sheathing or various mixtures of waxes, tars, and toxic chemicals, may also have been aimed at making wooden hulls water-tight or at protecting against marine boring organisms [for review, see Woods Hole Oceanographic Institution (WHOI) 1952]. With increased use of metal-hulled ships beginning in the 1800s, however, the rationale for protective treatments shifted from ensuring hull integrity to improving vessels' performance. The effects of biofouling on ships' performance have long been recognized (for review, see WHOI 1952; for early examples, see Atherton 1900; McEntee 1915). Attachment of biofouling organisms, such as macroalgae, hydrozoans, bryozoans, barnacles, polychaete tubeworms, mollusks, and ascidians, increases the frictional resistance of a ship's hull, resulting in an increase in the power and in the fuel consumption required to make speed (WHOI 1952; Townsin 2003). The economic impact of this degradation in performance is enormous. Abbott et al. (2000) calculated that, in 1989, the annual savings in fuel costs to the world's commercial fleet, attributable to the use of tributyltin (TBT)-containing antifouling hull coatings, was approximately \$730 M. Schultz et al. (2011) estimated that the annual cost to the US Navy to combat the effects of biofouling on ships' hulls was between \$180 M and \$260 M. This cost included expenditures for painting and cleaning of hulls, but the vast majority of the cost was due to increased use of fuel (Schultz et al. 2011).

Engineers and scientists realized at least 100 years ago that an improved understanding of the biology of fouling organisms would be required in order to

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solve the problems associated with biofouling (for example, Atherton 1900; Visscher 1927). Up to that time, development of methods to control biofouling consisted of "haphazard experiment and rule-of-thumb procedure" relying on previous experience as opposed to any systematic investigation (Visscher 1927). Crisp (1972) noted that biologists of the 1920s recognized the need to understand both larval settlement and metamorphosis and adhesion of larvae and adults, but that significant progress was not forthcoming due to the absence of an appropriate "conceptual framework" for the necessary experiments. That conceptual framework, in the form of controlled experiments on the settlement behavior of larvae and on the formulation of paints, was just beginning to be constructed (for example, Visscher 1927, 1928; Visscher and Luce 1928). By the time Crisp (1972) wrote his review, important advances had been made not only in understanding larval settlement, but also in adhesion of, for example, adult barnacles (Saroyan et al. 1970). Perhaps the most important development in completing the construction of the conceptual framework was the increasing understanding of the role of the larval nervous system in controlling settlement and metamorphosis of fouling invertebrates (Hadfield 1978; Burke 1983; Rittschof et al. 1986; Yool et al. 1986). The framework guiding current biological research on control of fouling now appears firmly in place, with investigations focusing on the reception and transduction of settlement- and metamorphosis-inducing cues, and on organismal adhesives and their interaction with the substratum (Clare et al. 1992).

Barnacles as a model organism for biofouling research

The community of organisms, both sessile and motile, which occurs as biofouling on ships' hulls is extremely diverse. The Woods Hole review (WHOI 1952) indicated that close to 2000 species had been reported from such communities, including representatives of most of the major groups of organisms. Visscher (1927) retrieved 77 unique taxa (species and genera) from samples of 250 vessels. Over the course of a 2-month voyage, Carlton and Hodder (1995) collected 64 unique taxa from a replica of an early sailing vessel. Davidson et al. (2009) collected 34 unique taxa from 5 containerships. Despite this diversity, when ships' hulls support fouling by macroorganisms (invertebrates and macroalgae), barnacles typically are present. Visscher (1927) found barnacles on 60% of the vessels he sampled. Some 80 years later, Davidson et al. (2009) encountered barnacles on 80% of the 22 containerships they inspected.

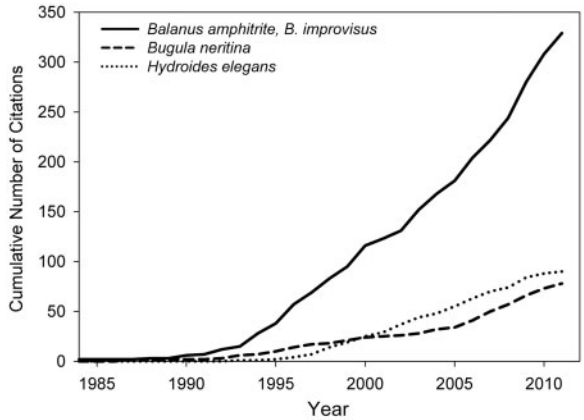

Fig. 1 Cumulative number of citations for biofouling-related research conducted on three different invertebrate model systems. The figure depicts results of searches of the Science Citation Index carried out on August 30, 2011 [*B. amphitrite* (= *Amphibalanus amphitrite*), *B. improvisus*] or September 1, 2011 (*B. neritina* and *H. elegans*) using the species' names as search terms. Results of the search were edited to remove review articles or research that did not address biofouling issues (broadly defined).

Barnacles have become the primary invertebrate model for biofouling-related research (Fig. 1). The Science Citation Index recorded 329 articles between 1984 and September 1, 2011 employing either *Balanus amphitrite* or *Balanus improvisus*, two barnacle models widely used in laboratory or field studies of biofouling (review articles not included). In comparison, 2 other popular invertebrate models for biofouling research, the serpulid polychaete *Hydroides elegans* and the bryozoan *Bugula neritina*, were reported only from 90 and 78 articles, respectively (Fig. 1). Over the past 10 years, the rate of publication of biofouling-related research utilizing barnacles has been four to five times (on average) that of *H. elegans* or *B. neritina*.

Biofouling-related research on barnacles in particular has accelerated since the mid-1990s (Fig. 1). This acceleration does not appear to be due to the further development of the conceptual framework for studying biofouling or due to the introduction of new organismal models, methods, or tools. Instead, the increase in research output appears to be driven by emerging environmental regulations restricting the use of tributyltin in antifouling paints. Self-polishing TBT-based paints were introduced in the 1970s and proved very effective in controlling biofouling on ships' hulls (Abbott et al. 2000; Finnie and Williams 2010); by the mid-1980s, they were employed on over 80% of the commercial fleet (Abbott et al. 2000). The success of these paints was such that research on alternative formulations for coatings was halted, as the biofouling problem was considered solved (Townsin and Anderson 2009). Environmental issues associated with the leaching of TBT from antifouling paints became apparent in the 1980s, and regulations governing TBT-containing coatings began appearing shortly thereafter (for review, see Champ 2000). By 1998, several countries had requested a global ban on the use of these paints (Champ 2000), and within 5 years many manufacturers had withdrawn TBT-containing coatings from the market (Finnie and Williams 2010). The need for a replacement for these paints likely spurred research on, for example, the antifouling effects of natural products (see below) using barnacles as a model organism. The possibility of future regulation of current biocide-incorporating coatings continues to drive biofouling research.

Since the 1990s, research on biofouling using barnacles has focused on larval settlement and metamorphosis, development of new biocides from natural products, materials science, and surface science (Fig. 2). In the past 5 years, study of barnacle adhesives and adhesion has increased, perhaps as a function of an interest in developing more effective nontoxic fouling-release coatings. These coatings obtain their efficacy not by preventing the initial attachment of biofouling organisms, but instead by reducing their strength of adhesion (Swain and Schultz 1996; Schultz et al. 1999). Attached organisms are sloughed from the paint surface as a result of hydrodynamic forces generated as the ship moves through the water during routine operations (Schultz et al. 1999).

Much biofouling-related research continues to make use of relatively simple assays, including field exposure of painted substrates for testing of prevention of attachment of organisms or reduction in strength of adhesion of adult life-history stages (for example, Rittschof et al. 1992a; Swain and Schultz 1996; ASTM 2004, 2011a, 2011b), and laboratory evaluations of biocide toxicity and biocide or surface/materials effects on larval settlement and metamorphosis (for example, Rittschof et al. 1992b).

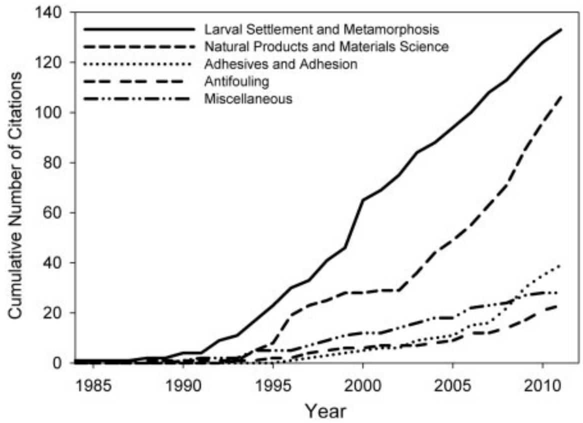

Fig. 2 Cumulative number of citations for biofouling-related research conducted on the barnacles *B. amphitrite* (= *A. amphitrite*) and *B. improvisus*, by research area. The figure depicts results of a search of the Science Citation Index carried out on August 30, 2011 using the species' names as search terms. Results of the search were edited to remove review articles or research that did not address biofouling issues (broadly defined). Abstracts of articles, or the entire article, were then read and the research placed in the appropriate category.

Recently, however, more advanced tools have been brought to bear. These include molecular genetic, transcriptomic, and proteomic approaches to the study of control of larval responses to surfaces, and subsequent metamorphosis (Thiyagarajan and Qian 2008; Thiyagarajan et al. 2009; Li et al. 2010; Zhang et al. 2010; Chen et al. 2011; Bacchetti De Gregoris et al. 2011), and atomic force microscopy, spectroscopy, and other imaging methodologies for examining larval and adult adhesives and their interactions with the substrate (Sun et al. 2004; Phang et al. 2006, 2008, 2010; Barlow et al. 2009; Dickinson et al. 2009; Sullan et al. 2009; Aldred et al. 2011).

Transitioning research to application

Translating the results of current biofouling-related research into commercial products is a complex, time-consuming, and expensive process, requiring interaction between scientists, regulatory entities, and industry (Mårtensson Lindblad 2009). The increased interest in antifouling agents from natural products, for example, had through 2010 failed to yield a commercializable biocide or repellent (Qian et al. 2010). Qian et al. (2010) noted several obstacles to commercializing prospective ''natural'' antifouling biocides, including inability to produce or synthesize the compounds in sufficient quantities to support the coatings industry, and the need to understand the fate and effects of the biocides in order to comply with environmental regulations. Despite the difficulties inherent in the transition or commercialization process, recently there have been successes that derive to some extent from basic research on fouling organisms including barnacles, in particular an improved understanding of the role of the larval nervous system in controlling settlement and metamorphosis (see above).

Dahlström et al. (2000) found that multiple adrenoceptor agonists and antagonists affected attachment of larvae of the barnacle *B. improvisus*. In the case of medetomidine, an adrenoceptor agonist, the potential for development into a commercial product seemed high. Medetomidine inhibited barnacles' settlement even at very low concentrations (1 nM) and the effects were reversible (Dahlström et al. 2000). Self-polishing coatings containing 0.1% added medetomidine were effective in reducing attachment of barnacle larvae in the field (Dahlström 2004). Medetomidine appears to induce swimming in barnacle cyprids, presumably reducing the likelihood that larvae will either encounter or attach to a treated surface (Lind et al. 2010). The receptors that regulate the response to medetomidine have been cloned and characterized (Lind et al. 2010). In 2009, medetomidine, as Selektope®, was submitted for registration as a biocide in the European Union by I-Tech AB (www.i-tech.se) (for review, see Mårtensson Lindblad 2009).

Gohad et al. (2010) bound noradrenaline, an adrenoceptor agonist, to two different polymer surfaces. These surfaces reduced attachment and metamorphosis of barnacle (*B. amphitrite*) and oyster (*Crassostrea virginica*) larvae (Gohad et al. 2010). Larvae settled and metamorphosed normally on adjacent, untreated surfaces, suggesting the inhibitory effect was due to the bound noradrenaline and not unbound compounds in solution (Gohad et al. 2010). The authors noted that this approach to the control of fouling was not suited to commercial application due to the high cost of noradrenaline (Gohad et al. 2010). Their success, however, combined with recent results from studies of barnacle cement (Dickinson et al. 2009; Rittschof et al. 2011), prompted a request for proposals from the US Navy Small Business Innovation Research Program (Solicitation 2011.2, Topic Number N112-166, "Bio-inspired Marine Biofouling-control Coatings") focusing on nontoxic approaches to biofouling control based on current concepts of regulation of larval settlement and metamorphosis and of the composition and curing of adhesives.

Experimental materials as probes of barnacle biology

Despite the accelerated pace of biofouling-related research, the development of most current fouling-control technologies remains largely unaffected by our improving understanding of either larval settlement and metamorphosis or the characteristics of the adhesives of fouling organisms. The initial discovery and development of silicone fouling-release coatings, for example, appears to have been accidental to some extent, and focused mainly on available materials without (apparently) any significant input from biologists (Townsin and Anderson 2009). Similarly, the conceptual basis for some emerging, nontoxic strategies for control of fouling, including biomimetic approaches (Scardino and de Nys 2011) and enzyme-based coatings (Olsen et al. 2007), appears not to arise from research on the responses of the offending organisms themselves. Instead, these approaches take their direction from the study of surfaces or materials that maintain themselves free of fouling (for reviews, see Genzer and Efimenko 2006; Scardino and de Nys 2011), or from a general knowledge of the composition of biological adhesives and the activity of enzymes or inhibitors (Bonaventura et al. 1999; Pettitt et al. 2004). Bonaventura et al. (1999) suggested that it was unnecessary to know the exact composition or curing mechanisms of the adhesives of fouling organisms, because the efficacy of compounds that could either hydrolyze the adhesives or inhibit their curing, could be quantified in simple experiments (for example, Pettitt et al. 2004). In these cases, barnacles or other test organisms in effect serve only as measuring devices.

Whether their development is initiated on the basis of, or otherwise benefits from, any biological knowledge or not, new technologies can, however, provide valuable opportunities to expand our understanding of the biology of fouling organisms. Experimental materials in particular may be useful as probes of the processes of larval attachment or adhesion. Polymer chemists are capable of generating materials with a diversity of well-defined, tightly-controlled chemical, surface, or bulk characteristics that lend themselves to multifactorial investigations of biofouling. Responses of fouling organisms to these materials vary in interesting ways. Settlement of the larvae of *B. amphitrite*, for example, is affected by both surface wettability and surface charge (Gerhart et al. 1992; Petrone et al. 2011). Larvae of *B. improvisus* appear to exhibit different responses than those of *B. amphitrite* (Dahlström et al. 2004), suggesting significant interspecific variation in the ability of barnacles to detect or respond to surface cues at settlement. Barnacles attached to silicone fouling-release coatings occasionally express alternative morphologies of their adhesive plaques (Berglin and Gatenholm 2003; Wiegemann and Watermann 2003). Depending on the coating, plaque morphology can affect strength of adhesion of the barnacle (Sun et al. 2004; Holm et al. 2005; Wendt et al. 2006). Holm et al. (2005) found that the proportion of barnacles expressing an alternative adhesive morphology varied significantly across coatings and maternal families, with the frequency of occurrence ranging from 0% to ~80%. This frequency was positively correlated across the two coatings tested, suggesting that similar genetic or common environmental effects influenced expression on the materials (Holm et al. 2005). The aspect of the fouling-release coatings that causes expression of the alternative plaque morphology is unknown. Barnacle adhesives may be interacting with chemistries available at the coating surface, which affect curing and the formation of structure (Berglin and Gatenholm 1999; Meyer et al. 2006). Rittschof et al. (2011) examined this possibility for four different silicone coatings. Surface extracts of these coatings affected transglutaminase activity in uncured barnacle cement; the effects varied among both the coatings and the six individual barnacles used in the experiment (Rittschof et al. 2011).

Further collaboration between materials scientists, polymer chemists, and biologists could be fruitful in dissecting the processes controlling larval responses to surface cues and the interactions between adhesives and the substrate. The barnacle model is ideal for this type of research. Identification of genetically-influenced phenotypes (see above) associated with particular characteristics of designed surfaces or experimental or commercial coatings, followed by analysis of those phenotypes using current genetic methods (see above) (Yu and Guo 2006; Hedgecock et al. 2007), could reveal the basis for variation in larval responses or strength of adhesion. This information could then be exploited to design new, more effective materials that inhibit settlement or interfere with adhesion.

On biofouling diversity and reliance on the barnacle research model

The community of biofouling organisms is extremely diverse, yet the study of processes associated with biofouling, and the development of new biocides or fouling-resistant materials, relies heavily on the barnacle research model (see above). Studies of metamorphosis and adhesion in other important biofouling invertebrates suggest that, in some respects, this reliance may hinder progress toward a solution. For example, transduction of metamorphic signals in larvae of the serpulid polychaete *H. elegans* (Holm et al. 1998) appears to differ from that in barnacles (Rittschof et al. 1986; Clare et al. 1995; Clare 1996, 1998). Therefore, biocides or bound compounds acting as neuropharmacological agents that target specific sensory pathways may not provide protection against settlement of the full range of fouling invertebrates that a ship may encounter. Similarly, interspecific variation in patterns of strength of adhesion of biofouling on experimental silicone fouling-release coatings indicates that efficacy against barnacles does not necessarily confer efficacy against serpulid tubeworms or oysters (Holm et al. 2006). Importantly, these results do not take into account fouling by bacteria, diatoms, or macroalgae, which also affects frictional resistance of the hull and thus a ship's performance (for example, Schultz 2000, 2007; Schultz and Swain 2000). The diversity of biofouling organisms, and the (presumably) associated diversity in attachment responses and adhesion to painted surfaces, may limit the biocide or coating developer to relatively simple or unfocused strategies that attack only the most broadly-shared processes (Mårtensson Lindblad 2009). A combination of approaches, including novel methods for cleaning hulls ("grooming") (Tribou and Swain 2010), may prove necessary to ensure efficient operation of ships in the face of tightening environmental regulations.

Over 100 years ago, Atherton (1900) noted that "before any substantial advance can be made in the prevention of fouling, a searching investigation is required into the entire subject from all points of view – the biological, the chemical, and the physical." This "searching investigation" is still underway, and studies on barnacles continue to advance the state of the art. A successful resolution to the biofouling problem may not, however, be realized without developing a similar depth of knowledge for other fouling organisms.

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