Commercially-traded Fibrous Proteins of Animal Origin: the Physical, Biological and Social Science

Introduction

The proposed program investigates three specific fibrous proteins derived from animals and establishes means to invite students to meaningful scholarship and research about them. Fibrous proteins were selected because of their past and present value in human commerce. The goal is a modern program in which undergraduate students construct new bridges of understanding between the Sciences, the Engineering and the Technologies that support human commerce in the fibrous proteins of animals.

The scope is limited to fibrous proteins that have commercial value and serve a principal biological function outside the animal body which produces them. Mammalian keratins, arthropod silk fibroins and molluscan byssal are all within the scope of the program. Actin, myosin, fibrin and elastin are outside the scope because the latter serve their primary function inside the animal body. Collagen, the most abundant protein of vertebrates, is excluded despite the commercial value of tanned animal hides. Invertebrate preCols, collagen-like and a principal component of byssal threads of marine mussels, are included because these fibers function outside the animal body.

Keratin and fibroin have a long history in textile manufacturing and commerce. Keratin is the principal component of wool fiber and fibroin is the principal component of silk fiber. The third fiber type, mussel byssus, is not a commodity. Indeed, when this type of clam is prepared as food the bundle of byssi ("the beard") is removed and discarded. "Sea silk", a cloth made from spun byssi of the large Mediterranean bivalve mollusk *Pinna nobilis* has likely never been a commodity, even though some small articles of clothing were made from it in the 18th Century. This establishes unquestionably, in contrast to wool and silk, that knowledge about molluscan byssal fiber only has commercial value in an information-based commerce. Hopefully, the students of mussel byssi can contribute to a knowledge-based commerce for the 21st Century.

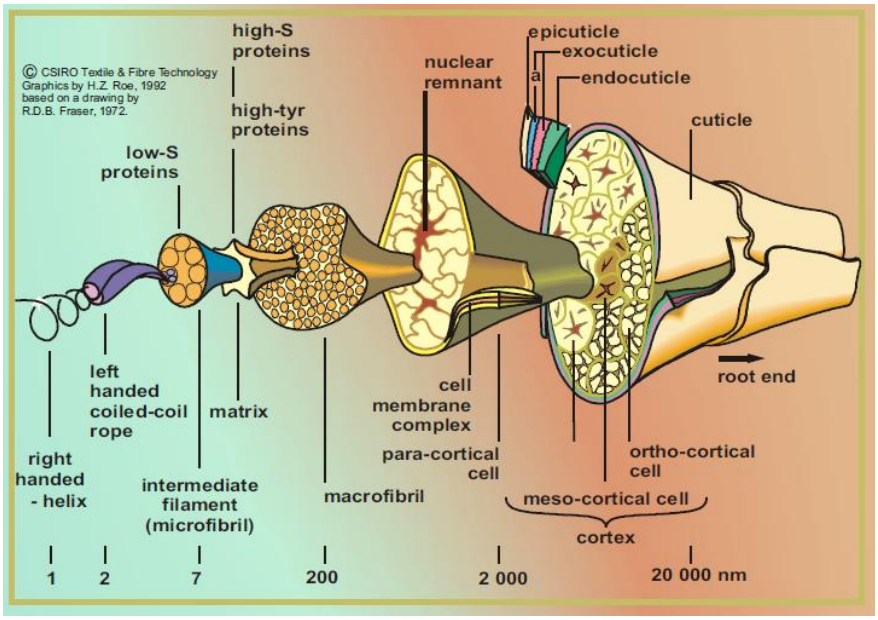
I have three plans for projects. All support direct participation of undergraduates. Establishment of collaborations is another key aspect of each project. The first project stands in an intersection of biochemical, agricultural and artistic aspects of mammalian wool. The second project is located where biochemical, materials science and engineering aspects of arthropod silk meet. The third project investigates ecological, biological and engineering aspects of mussel byssal.

Wool Research in Massachusetts – a Cottage Laboratory Industry for the 21st Century?

Before the creation of synthetic fibers like nylon, rayon, polyacrylonitrile, polyester and polyaramide, wool and cotton were the common man's fibers of choice for clothing and other textiles. Standard biochemistry textbooks report that keratin, a coiled-coil protein is the major component of hair and wool but those textbooks do not provide the nuanced description found in a short web document (CSIRO 2008) about merino wool. Briefly, the diversity of proteins found in wool fibers is reported to span about 170 amino acid sequences which are not distributed evenly along the protein fiber. In the older literature (Powell, et al. 1983 for example) one reads about Low Sulfur, High Sulfur and High Glycine + Tyrosine Keratins which are located in the fiber cortex (low sulfur) orthocortex (high sulfur) and paracortex (high glycine + tyrosine). Those names are rarely used today but the location of the protein types is shown, conceptually, in Figure 1.

Keratinization (the formation of disulfide crosslinks between polypeptide chains), isopeptide bonds (formed between carboxylate and amine-containing side chains), hydrophobic interactions (between hydrocarbon side groups) and salt bridges between acidic and basic side chains all contribute to the chemical structure and reactivity of keratin fibers.

Figure 1. Cartoon of Wool Fiber Structure



Copied from (CSIRO 2008)

Interestingly intermediate filament proteins, a major component of the cytoskeletal matrix are a specialized form of keratin or, perhaps more correctly, the fibrous keratin supramolecular assembly that is wool is the secretory product of a specialized gland in ovine skin called the wool follicle. The wool of Merino sheep is highly desirable for spinning into yarn because its fibers demonstrate a natural tendency to "crimp" that helps assemble a continuous yarn from shorter fibers by hand or machine spinning. The crimp of merino wool is illustrated in Figure 2, taken from a recent comparative analysis (Li, et al. 2008) of sheep that are wild-type with respect to wool crimp and genetically crimp-deficient mutant individuals. The mutant phenotype is called felting luster (FL).

Figure 2. Wild Type and Felting-Lustre Merino Wool Morphology

Photographs, copied from Li, et al. 2008, illustrate the crimp of wool samples from wild type (a) and three different FL mutant merino sheep (b-d).

Since 1983 it has become clear that the proteins originally called Low Sulfur Keratins are actually Keratin Intermediate Filament proteins (KIFs), localized primarily in the fiber matrix (see Figure 1). High and ultra-high sulfur proteins are Keratin-associated proteins (KAPs) localized in the paracortical cells of a wool fiber (Figure 1) and the glycine and tyrosine-rich keratins are actually glycine and tyrosine-rich KAPs localized in the orthocortical

cells of a wool fiber (Figure 1). Indeed the central thesis supported by Li's data is that the FL mutant phenotype is an inverse trichothiodystrophy (brittle, sulfur-deficient hair) syndrome. Specifically, Li and colleagues's data show that the absence of specific glycine and tyrosine-rich KAPs (KAP2.12 and KAP4.2) from FL wools leads to decreased orthocortical character of the fibers. This implies the existence of torsion within wild type fiber that is caused by the interplay of orthocortical and paracortical cell types. Since that torsion is absent from FL mutant wool fibers the role that KAP2.12 and KAP4.2 play in causing orthocortical character is implicated in creating the crimp of wild type merino wool fibers.

The study of wool and wool biochemistry will provide opportunities for a variety of students to engage in research. I hope to establish relationships with faculty here and at local colleges and universities whose research has an intersection with this project, with veterinary specialists in ovine medicine at the Cummings School of Veterinary Medicine at Tufts University, with artisans working in textiles at the Worcester Center for Crafts (http://www.worcester.edu/WCC/default.aspx) and with sheep farmers in Central Massachusetts (http://worcestersheep.com/).

The commercial value of wool defined the role of sheep farmers in an agricultural society. An average sheep provides ~ 3 kg of wool (enough to manufacture two large modern men's suits) annually. Thus, a farmer keeping twenty sheep could provide the raw material to clothe several local families and trade this commodity for other goods of equal value. Cloth produced from wool fiber retains a unique place in the textile industry because of the unique combination of "wearability" properties illustrated in Table 1. The data in Table 1 were taken from a recent review article (Rogers 2006). At present global wool production is about 1.3 million tons per year with 60% of that used for clothing.

Table 1. Clothing Fibers' Characteristics, Compared on a 1-5 Scale of Performance

Fiber Property	Wool	Polyester	Nylon	Acrylic	Cotton
Insulation	1	4*	4	4	1-2
Moisture absorption	1	5	4	5	2
Elasticity	1-2	2	2	3	3-4
Fire resistance	1-2*	3	3	5*	5*
Anti-static	1	5*	4*	5*	2
Liquid water repellancy	1-2	2	2	3	4-5
Shrinkage resistance	5*	1	1	1	2

1, excellent; 2, very good; 3, good; 4, moderate; 5, poor demonstration of the property; *, the property can be improved by applying a special treatment

More than 50% of all wool is produced in one of three countries: Australia (25%), New Zealand (11%) and China (18%). The UK ranks seventh in wool production (2%) and the United States eleventh (0.77%). However this still means that the United States contributes a little over ten thousand tons of wool, annually, to the world market. Given the recent recognition of a need to develop sustainable strategies for growth and economic development it is not farfetched to envision opportunities for small farms in first world countries to start up under a fair trade premise. However, social change may be required to enable that.

Nearly two hundred years of industrialization resulted in a U.S. countryside dominated by large agribusiness farms. There are social divides between city and country dwellers that will not be conducive to a new wave of migration from cities to the country. Education may be one means to promote the development of a population who choose sustainable agriculture as a way of life. Clearly, not everyone who chooses to do research about wool will want to become a shepherd. However, an undergraduate research project on wool might open other avenues of interest to a student, in Chemistry, Biochemistry, Engineering or Business theretofore unimagined and unconsidered.

It is unlikely that wool shall ever compete with polyester and polyester blends in the woven textiles and nonwovens (felts, filters and disposable diapers) market. It is also unlikely that the niche market for wool will disappear completely. However, there is a need for basic research on wool-related topics. The Australian Commonwealth Science and Industrial Research Organization (CSIRO), a major driver of wool research, shut down a number of labs over the last 15-20 years and focused its resources on a narrow set of goals centered on increased quantity, increased quality and biological harvesting of merino sheep wool. Merino sheep are closely related to the Rambouillet breed most commonly raised in the United States. Therefore it should be possible to take what good work has come out of Australia and build on it in the context of American strengths and opportunities.

Silk – the Web of Remorse or a Biomedical Business Opportunity?

In this project students investigate the interplay between biomedical research and commerce. The natural collaborators sought are local faculty in Chemistry, Physics, Engineering and Biology whose research intersects technical aspects of the project. Additional collaborators at this school are sought in the Departments of Economics and Business Administration. At other schools we would like to open lines of communication with Professor D. Kaplan (Tufts University), Professor C. Hayashi (University of California, Riverside), Professor T. Scheibel (University of Bayruth, Germany) and Professor F. Vollrath (University of Oxford, UK).

From Ancient through Medieval times the silk trade created wealth for merchants and made the "road" they traveled famous. The silk trade created early social ties between Europe and Asia. The fiber of Silk Road fame is secreted from labial glands of silkworms, the larvae of a moth *Bombyx mori* (Sehnal and Sutherland 2008) indigenous to the Far East. A fifth instar *B. Mori* larva spins the silk to form a protective cocoon in which it will undergo metamorphosis. Reported estimates vary but suggest that three to five thousand silkworm cocoons are consumed to harvest one kg of silk fiber (roughly the raw material in a pure silk kimono). One reason silk became the fiber of European royalty is this difference in scale between silk production and the production process for wool.

Transportation was another contributor to the cost differential, in Europe, between wool and silk. Trading along the Silk Road, while glamorous, was dangerous and a natural monopoly existed because the means of silk production was in China. Only later were silkworms and their food plant (mulberry) imported to Europe and the Middle East. A near-monopoly on insect silk production persists, even today. In 2005 commodity silk production totaled about 453,000 tons. China contributed 70% to that total and Japan, the tenth-highest silk producer, contributed 0.14% of the total. Four of the top-ten silk producing countries: Uzbekistan (4.2%) Iran, Romania and Brazil are currently outside the Far East.

Fineness is one characteristic that makes silk a desirable fiber for textiles. The average diameter of insect silk fiber is 12.8 μ ($\pm$ 25%), about twice a fine as a Merino wool fiber (Range 12-24 μ). Silk cloth demonstrates a unique luster and drape as well as good thermal properties.

Insect silk fibers consist of fibroin protein nanofibrils held together by accessory proteins called sericins. The strength and durability of silk led to its use as a non-absorbable surgical suture that must be removed or remain in place. However, insect silk for surgical sutures must be processed by a "degumming" treatment to remove sericins, which are immunogenic (Huang, et al. 2003), from the fibers' surfaces. Common approaches to degumming insect silk include 1) extraction with water at high temperatures, (2) extraction with dilute aqueous alkali or soap solutions,

or (3) proteolytic digestion (Becker, Willman and Tuross, 1995). Research on silk, as a biomaterial, is also seeking applications in a wide variety of non-medical fields including photonics, adhesives, electronics and the manufacturing of microfluidic devices (Omenetto and Kaplan, 2010).

Another Arthropod group, the Arachnids, also produce silk. The literature on spider silk was recently reviewed (Romer and Scheibel, 2008). Spiders demonstrate a variety of silk glands whose spun secretions serve different functions including egg case protection, prey entrapment and offspring dispersion. Every fiber is made from one or two unique types of silk structural proteins (fibroins). Spider fibroins (spidroins) have very high molecular weights, estimated at 200–350 kiloDaltons with 10,000 base pair (or larger) transcripts. Evidence from cDNA sequencing indicates that spidroin proteins are modular. The polypeptide associated with a particular gland is primarily composed of an uninterrupted block of repetitive sequence that is flanked on both sides by about 100 amino acids (aa) of non-repetitive amino- (N-) and carboxyl- (C-) termini (Ayoub, et al. 2007).

The most famous spider silks are construction material in the webs of orb-spinning spider species, such as *Araneus didadematus* and *Nephila clavipes*. Dragline silk (found in the web frame, radial spokes and the tether by which a spider hangs from its web) and viscid silk (forming the catching spiral) are two major types of spider-silk fiber produced by the orb-spinners. Dragline silk is secreted by the Major Ampullate (MA) gland and catching (or viscid) silk is secreted by the flagelliform gland (FL). An Araneid spider like *A. diadematus* or *N. clavipes* produces additional spidroins in up to five other glands (Dicko, et al. 2008) that differ from one another with respect to the repeating amino acid sequence motif.

Long of interest to zoologists and naturalists, spider silk recently became a hotbed of research in the field of Regenerative Medicine. There, spidroin proteins, along with natural and recombinant *B. mori* silk fibroin, are under consideration as a biomaterial in tissue engineering and other projects that require stem, or differentiated, cells to be cultured *in vitro* and implanted in an animal to promote wound healing or another organ-reparative procedure (Meinel, et al. 2005).

Silks are a particularly attractive biomaterial for such purposes because of chemical plasticity, slow biodegradability, low immunogenicity and remarkable tensile properties of the fibrous form. In nature, the dragline silk of an Araneid spider web demonstrates extremely high values (See Figure 3) for the common tensile properties stiffness[1], strength[2], extensibility[3] and toughness[4]. Hysteresis[5] is a prominent feature of spider silk loading and unloading curves and it reflects the ratio of energy dissipated to energy absorbed as shown in Figure 3. This observation led to the recognition that spider silk behaves like a viscoelastic material (Gosline, et al. 1999).

Taking advantage of amino acid composition and gene sequence data Gosline et al. (1999) modeled spider silk as a semi crystalline biopolymer comprising two major parts: a β-sheet crystalline part that contains hydrophobic polyalanine sequences and an amorphous part composed of amino acid residues, linked via hydrogen bonds. Somewhat later workers at the Universityof Oxford Zoology Department proposed two models for the assembly of spidroins that are summarized in Figure 4. The native structure and assembly of spider silks is an active research area, particularly when new physical measurement techniques present the opportunity to readdress partially-resolved questions.

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[1] Stiffness is defined as the initial ratio of stress to strain in a fiber loading experiment where stress = Force/x-sectional Area (GPa) and strain = $\Delta L/L$ (Length and change in length are both measured in meters bu extensibility is a pure number)

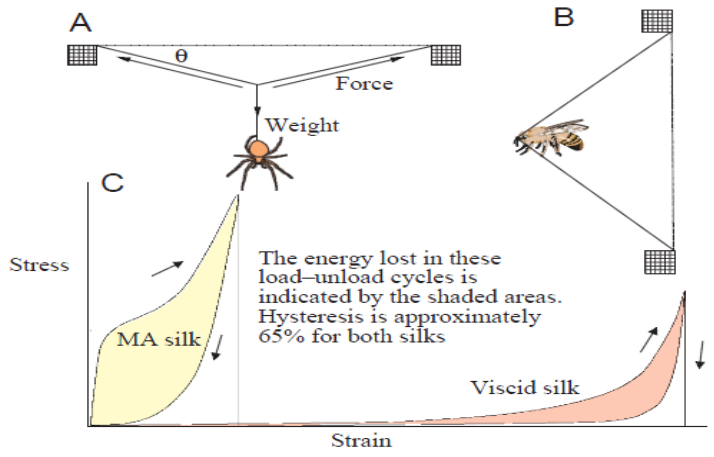
[2] Strength is the maximum loading force carried before fiber failure (breakage)

[3] Extensibility is the maximum $\Delta L/L$ carried before fiber failure (breakage)

[4] Toughness is area under the stress-strain curve

[5] Hysteresis is anisotropy in any cyclical process. In this case it means different values of strain are observed at the same stress during loading and unloading of the same fiber

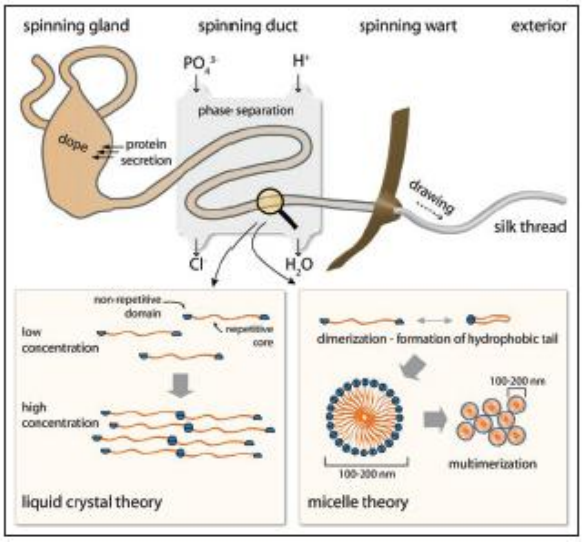
Figure 3. Stress-strain of *A. Diadematus* Silks and Tensile Properties Comparison



Material	Stiffness, E_{init} (GPa)	Strength, σ_{max} (GPa)	Extensibility, ε_{max}	Toughness (MJ m^{-3})	Hysteresis (%)
Araneus MA silk	10	1.1	0.27	160	65
Araneus viscid silk	0.003	0.5	2.7	150	65
Bombyx mori cocoon silk	7	0.6	0.18	70	
Tendon collagen	1.5	0.15	0.12	7.5	7
Bone	20	0.16	0.03	4	
Wool, 100% RH	0.5	0.2	0.5	60	
Elastin	0.001	0.002	1.5	2	10
Resilin	0.002	0.003	1.9	4	6
Synthetic rubber	0.001	0.05	8.5	100	
Nylon fibre	5	0.95	0.18	80	
Kevlar 49 fibre	130	3.6	0.027	50	
Carbon fibre	300	4	0.013	25	
High-tensile steel	200	1.5	0.008	6	

Upper panel: A, Dragline silk stress-strain diagram; B, Viscid silk stress-strain diagram; C, Stress-strain curves of dragline and viscid silk showing hysteresis. Lowe Panel: Table displaying *A. diadematus* tensile properties in the context of other relevant fibers. Copied from (Gosline, et al. 1999)

Figure 4. Model Silk Gland and Theories of Fiber Assembly



Top panel, conceptual drawing that summarizes the assembly of major ampullate (dragline) silk by *A. diadematus*. Protein "dope" is secreted by the glandular epithelium and accumulated to high concentration in the lumen. The region identified as "spinning duct" is reminiscent of a countercurrent multiplier, such as found in the vertebrate kidney's loop of Henle. There protein undergoing assembly is dehydrated and ultimately pulled through the spinning wart as a silk thread. The lowe r panels depict two current theories of silk nanofibril assembly. It must be noted that another figure in the same paper (Romer and Scheibel 2008) set a precedent by representing crystalline (β–sheet) structures in blue. Thus in this cartoon depicting the liquid crystal theory of fiber assembly the crystallites of alanine-rich amino acid sequence arranged in antiparallel β-sheets appear to be represented as blue circles while hydrophilic amorphous or elastomeric structure is represented by orange lines. Clearly, this cannot be the case in the micelle theory cartoon where blue circles represent a hydrophilic "Head" and the orange lines implicate a "core" of hydrophobic tails that are deformed into threads by laminar forces on the micelles exerted during drawing.

Drawing copied from Romer and Scheibel 2008.

In the commercial arena Professor Scheibel of the University of Bayruth filed a U.S. patent application in 2004 covering the genes, production methods and applications of recombinant spider dragline silk (USPTO Application

#20070214520 - Class: 800288000). In 2010, Professor Kaplan's group at Tufts University independently published a thorough report on the construction, expression and characterizaton of spider dragline silk of large size (285 kDa) in *E. coli*. That recombinant spider silk fiber demonstrates tensile properties close to those of native dragline fiber (Xia et al., 2010a, Xia, et al., 2010b). Professor Kaplan's group is also deeply involved with *B. mori* silk as a tissue engineering biomaterial (Omenetto and Kaplan 2010). Given the evidence that two candidate recombinant materials mimicing biology (spider silk and insect silk) are becoming available, a competition between universities and companies to exploit potential markets for recombinant silk is likely to erupt. The question is, how will entrepreneurial 21st century students doing research in both, business and biochemistry, judge the contest? At this time the tradeoffs necessary to make tissue engineering and other biomimetic silk applications a scientific and commercial success remain to be defined.

In Greek Mythology Arachne was a girl, renowned for needlework, who thought her skill exceeded that of the goddess Minerva (Pallas Athena). She challenged Pallas Athena and the goddess agreed to participate in a weaving demonstration. Minerva and Arachne each sewed a tapestry. When finished the competitors turned to view one another's products. On seeing the goddess's tapestry Arachne despaired because she understood, at last, that Minerva's work was more beautiful than her own. Distraught, the girl hung herself. Minerva transformed the dying girl into a spider and condemned her to weave and spin forever as a warning to conceited mortals. Perhaps a biomedical advance built on spider silk can redeem Arachne, whose real problem was unwillingness to lose a competition gracefully and move on to compete another day, or in another league.

Zebra Mussel Control and Appreciation

The aim of this project is to characterize and understand the biological and biochemical differences between byssal of marine and fresh water mussels. To achieve that we need to study an animal easily perceived as reprehensible, *Dreissena polymorpha*, the Zebra mussel. The extensive literature on marine mussels' byssal fiber structure and function was recently reviewed (Silverman and Roberto 2007) and continues to grow rapidly (Kausik, et al. 2009, Hwang, et al. 2010, Zeng, et al. 2010, Harrington, Masic, et al. 2010). A much smaller corpus is readily available on the characterization and analysis of *D. polymorpha* byssal fiber biochemistry (Anderson and Waite, 1998, Anderson and Waite, 2000, Xu and Faisal, 2010). A specific objective of the project is to determine how differences in gene regulation during bivalve metamorphosis may (or may not) relate to the life cycle difference between marine and fresh water bivalves that utilize byssal fiber attachment.

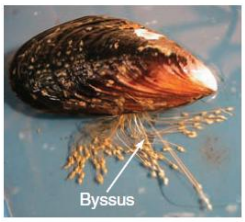
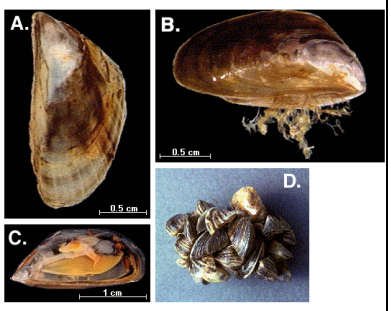
Adult zebra mussels, like marine mussels, live attached to solid substrata by byssal fibers but they inhabit fresh water. Zebra mussels are smaller than common marine mussels like *Mytilus edulis*, prodigious filter feeders and have a very high reproductive rate. Since accidentally introduced in North America in 1988 zebra mussels have become a pest species at hydroelectric and water-treatment plants. At present, coatings that contain cupronickel alloys are a reasonably effective means to control biofouling of industrial sites by zebra mussels. However there is no way, but vigilance, by which to defend the niches of indigenous bivalves.

Indigenous bivalve species are in crisis in North America and Western Europe. The crisis is largely due to decreased habitat. The spread of zebra mussels is a biological threat to indigenous species' continuance because their high rate of reproduction and physiological flexibility make zebra mussels a formidable competitor in most ecosystems. *Elasmidonta undulata* is an example of an at-risk indigenous species whose life cycle is quite different from the life cycles of zebra mussels and other mussels that attach by means of byssal, like the marine *Mytilidae*. Although sometimes called "triangle floaters" adult *E. undulata* neither float nor typically move more than a few

meters. Rather, each spends its adult life burrowed in sediment near where it first settled. *E. undulata* is common in rivers and streams of Maine and Rhode Island[6] and listed as a "species of concern" in Massachusetts.

The noted life cycle difference is illustrated in Table 2 where the data reveal that *Mytilidae* and *D. polymorpha*, who produce and use byssal to attach to substrata in adult life, take the larval form of planktonic veligers. In contrast *E. undulatua*, whose larval form is a fish parasite, employs no holdfast mechanism in adult life. There are remarkable micrographs that demonstrate active glochidium encapsulation by the epithelial cells of a fish fin (Rogers-Lowery and Dimock Jr., 2006). These authors suggested two possible mechanisms for the remarkable behavior. On one hand a specific interaction, possibly between integrins on the fish epithelial surface and molecules in the glochidium exoskeleton, could guide the keratocytes' movements. On the other, the small wound created when a glochidium atttaches affects ion fluxes. Thus the disrupted electrochemical potential of the affected fin or gill epithelium could facilitate keratocyte rounding which would promote migration onto and across the glochidium. The fact that fish experimentally parasitized multiple times by glochidia develop a resistance to additional "infection" argues in favor of the first mechanism but does not rule out participation of the second.

Table 2. Life Cycle Comparison Among Three Types of Mussels

Mussel Type	***Mytilid***	***Dreissena polymorpha***	***Elasmidonta undulata***
Larval Dispersal Mode	**Planktonic veliger**	**Planktonic veliger**	**Parasitic glochidium**
Adult niche	**Marine Intertidal Zone**	**Lakes, Streams and Rivers, top or bottom of water column**	**Lakes, Streams and River bottom**
Retention mechanism	**byssal**	**byssal**	**none**
Image[7,8,9], animal size and lifespan	 Typically 2-2.5" in 7-12 y	 Typically 0.2 -1.5" in 5 y	 Typically <3" in 8-20y

[6]Nedeau, E/ (2007) *Elasmidonta Undulata* Fact Sheet. Natural Heritage Endangered Species Program of the Massachusetts Division of Fisheries and Wildlife, Westborough MA.

[7]Image of a Mytilid, likely M. galloprovincialis , published in Harrington, MJ et al., 2010 and Inserted from http://www.sciencemag.org/cgi/content/full/328/5975/216/F1

[8] Inserted from <http://el.erdc.usace.army.mil/zebra/zmis/zmishelp4/dreissena_polymorpha_the_zebra_mussel.htm>

[9] http://www.nae.usace.army.mil/recreati/hel/ContoocookRiverWebsite/UnderwaterLife/TriangleFloater/FTriangleF.html
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The first hypothesis to be tested is that genes expressed in planktonic veliger larvae of marine bivalves and Dreissena polymorpha are downregulated or absent in glochidial larvae. A second hypothesis to be tested is that those genes are directly or indirectly implicated in byssogenesis. The third challenge will be to identify and characterize the differentially regulated genes and gene-products. An opportunity for innovation will exist if new understanding leads to novelty in the design of a zebra mussel control strategy. In this way knowledge about Zebra mussel biology, chemistry and behavior can advance Science and create value by combating the Zebra mussel infestation of North America's rivers and lakes. Another application for knowledge about zebra mussel byssal may be to design and development of biomimetic adhesive materials with the tenacity of byssi.

This project will directly engage undergraduates and seek to establish collaboration with local faculty in all departments whose research interests intersect any aspect of the project. Outside this school we would like to open lines of communication with Professor J.H. Waite (University of California San Diego), Professor W. Xu (College of Veterinary Medicine, Michigan State University), Professor M. Faisal (College of Agriculture and Natural Resources, Michigan State University) and Professor M.C. Barnhart (Missouri State University). This project will interface with the Commonwealth of Massachusetts Division of Fisheries and Wildlife whose oversight is engaged because zebra mussels are a nuisance species. Therefore it will be necessary to ensure that the laboratory population remains contained therein and that wastewater from the lab is free of live zebra mussel larvae (Massachusetts Department of Conservation and Recreation and Department of Fish and Game 2009).

Research Requirements

- Laboratory space, including a culture room equipped with two 40-50 gallon aquaria (one for adults, one for juveniles), circulating temperature-controlled water baths, programmable actinic light source and air temperature control.
- Dissecting microscope and compound microscope (inverted) equipped with phase contrast objectives.
- Photographic and imaging equipment
- Laboratory supplies, permanent and consumable.
- Travel funds
- New computer and bibliographic software

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