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EDITOR'S NOTES

Besides the many past issues on important contemporary education topics it has put forth, the *Journal for the Liberal Arts and Sciences* has also produced a number of special topic editions focusing on pertinent subjects in such academic areas as labor history, English studies (2), military history, religious studies, Indiana history, philosophy, popular culture and recreational studies. The spring 2014 issue of the *JLAS* will feature yet another such special topic edition. This issue will look at recent scholarly research in two related fields, those of science and math, including science and math education topics. Researchers in this issue represent a number of universities including Ball State University, American University of the Caribbean, American Military University, Indiana University-Purdue University at Indianapolis, University of Southern Indiana, Black Hills State University, the Ross School, Oakland City University and a member of the research component of one major national chemical company, Dow AgroSciences.

An important practical aspect of marine chemistry is discussed in our first article, the availability of natural products derived from our seas and oceans. The researchers, after an extensive literature review and study, offer practical examples of several "natural products from the ocean from over the past several years, as well as their current and potential commercial, industrial, biomedical and pharmaceutical applications." In a time of a growing global population and dwindling resources, such information may be of great use.

Bacillus cereus, a common cause of gastroenteritis, is discussed by three microbiologists from a less well known angle in this issue's second research presentation. While *B. cereus* is very much understood in terms of its part in causing gastroenteritis, in rarer cases it can also cause ocular infections which can lead to blindness. The article discusses the dynamics of the latter event.

The third article of our spring 2014 issue shifts our attention from science to math education. Two researchers examined a

group of elementary teachers' perceptions regarding the possible impact of intervention factors implemented during a grant-funded mathematics education initiative. Such knowledge was deemed valuable as it might be useful in increasing the effectiveness of intervention procedures in future math reform projects.

In the world of instructional science, a number of studies have been conducted comparing a face-to-face training environment to an online training environment. Our fourth offering examined whether or not the level of engagement of participants in a training course for new staff and interns with one particular organization would be increased by conducting training online instead of face-to-face and by utilizing multiple forms of media. The findings offer important information to the ongoing conversation regarding which types of online learning work best. Science education in the lower grades was the concern of our next research piece. The study involved an analysis of a state (Indiana) sponsored K-2 reading list of fiction books to check for scientific misrepresentations, the presence of science content and gender representation. Strategies to accompany science and literature connections and more appropriate grade level curricular selections were also offered.

In our fifth article, two microbiology researchers explain how *Bacillus* spp. is normally considered a nonpathogenic soilborne saprophyte species of bacteria that may occasionally contaminate food. Subsequent ingestion of enterotoxigenic *Bacillus* spp. may cause emesis and/or gastroenteritis. In their experiment, repetitive element palindromic polymerase chain reaction (rep-PCR) was applied to differentiate *Bacillus* spp. in artificially contaminated reconstituted nonfat dry milk (NFDM) to see its effectiveness in screening novel isolates of *Bacillus* spp. This study represents significant research, as the application of rep-PCR in the food industry as a quality control measure to detect and/or differentiating pathogens from food would be a major step in thwarting *Bacillus* spp. Continuing the microbiology emphasis, two researchers explain the fascinating possibility of how microbiology might inform the science of criminal justice in our sixth presentation. The study offers an in-depth review of the potential of ribonucleic acid (RNA) methods for biological evidence analysis.

Our next research report takes us back to the arena of science education. This study provides insights into elementary teachers' perceptions of classroom environments spanning the launch of a major state science initiative in Indiana. The initiative promoted science literacy through inquiry-based science kits.

The last offering of the *JLAS* science and math special issue is perhaps the most intriguing. This essay offers the experiences of a female mathematics educator taking an inquiry-based undergraduate physics course as a student. The researcher explains that the essay portrays "the struggles of female students in science classes" and that these struggles "are told from the viewpoint of a credentialed, extroverted insider." Several issues regarding gender bias are brought to light.

I hope you will find this special issue to be an interesting set of academic investigations and I certainly wish to thank the researchers whose hard work made this issue possible.

Randy Mills, Editor
Journal for the Liberal Arts and Sciences

Bioprospecting in New Frontiers: Examples and Applications of Marine Natural Products Chemistry

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Liberal arts students can often struggle to draw a direct and immediate connection between scientific research and its practical applications and relevance to daily life. This is especially true of marine science research. But 75% of our planet is comprised of the oceans, which contain a myriad of species which have no terrestrial counterparts. So it seems obvious that our oceans represent a very attractive reservoir of natural chemical products, yet they have been largely unexplored until the last few decades. The interest among marine scientists in these unusual and biologically active natural compounds and their potential applications seems to be growing exponentially, as evidenced by the growing list of compounds and their often corresponding patents. This paper, though by no means an exhaustive list, discusses just a few examples of such natural products that have been isolated over the past several years, as well as their current and potential commercial, industrial, biomedical, and pharmaceutical applications.

Introduction

It has long been known that our waters are our *largest* ecosystem and resource, but perhaps only now have we realized it is likely our *most important*. Of the recently approximated 8 million species on Earth, estimates project there are 2-2.5 times as many phyla represented in our oceans than can be found in terrestrial environments (Mora et al., 2011; Fenical, 2009). Of all

animal phyla described so far, only phylum Onychophora is not recorded in marine waters, while 15 phyla occur exclusively in the world's oceans (McGinn, 1999). Recent research suggests that over 90% of marine species have yet to be discovered, described, and catalogued (Mora et al., 2011). This much genetic diversity would almost certainly lend itself to an immense amount of chemical diversity, given that genes themselves are codes for chemical compounds, and the current rate of scientific discovery is proof of such (Whitehead, 1999). For example, prior to 1995, ~6500 marine natural products had been identified (Whitehead, 1999), and by 1999, the number had increased to 10,000 (Jaspars, 1999). From 1990 to the present, almost 100,000 new marine natural products have been discovered from invertebrates alone, with a pronounced increase between decades (Leal et al., 2012). From 1990 to 2005, about 800 novel marine natural products were discovered each year (Blunt & Munro, 2008). Most of the marine-derived natural products seem to be of biomedical or pharmaceutical benefit (~50% of all drugs are of natural product origin) (Chin et al., 2006; Fenical, 2009), but they have also been applied commercially in cosmetic products, food additives, biomaterials, and general industrial materials, just to name a few examples (Paul et al., 2011; Molinski et al., 2009; Wijffels et al., 2008).

Health and Medical Applications

Thus far it seems that one of the best marine sources for these novel products of potential medical use are the soft-bodied and mostly sessile organisms that would be at an increased susceptibility to predation, as well as the microbes that symbiotically inhabit them (Haefner, 2003; Faulkner, 2000). An interesting example is a series of tridentol compounds (Fig. 1) isolated (and patented) from *Tridentata marginata* that have been shown to absorb UVA and UVB radiation via their chromophores, thus providing the potential for a natural sunscreen (Lindquist et al., 1996; Lindquist 1998).

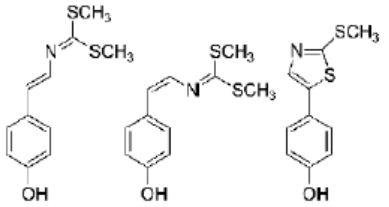


Figure 1.—Tridentatols A (left), B (center), and C (right) isolated from *Tridentata marginata*.

The glue found in common mussels (*Mytilus edulis*), which can stick even to wet surfaces, is now being tried as a biological or surgical glue that would minimize infection and post-operative cellular "clogs" and even blood clots (Huang et al., 2002). The key to its adhesiveness is a unique compound called mussel adhesive protein, which contains a high concentration of an amino acid, DOPA (dihydroxyphenylalanine, Fig. 2), which can cling to wet surfaces with extraordinary strength. When researchers attach the sticky

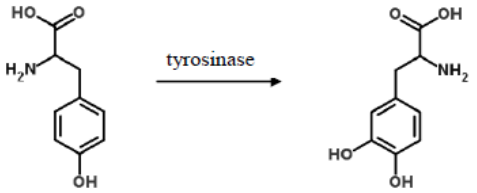


Figure 2.—Synthesis of dihydroxyphenylalanine (DOPA), the key amino acid in mussel adhesive protein.

DOPA molecule to a well-known repellant molecule like polyethylene glycol (PEG), the result is a two-sided compound whose sticky side attaches to internal surfaces, but whose nonstick side can resist protein and cell attachment, such as that encountered by implanted medical devices (Huang et al., 2002).

Commercial, Consumer, and Industrial Applications

In the sponge *Geodia barretti*, a dibrominated cyclopeptide (bromobenzisoxazolone barettin, Fig. 3) was found that acts as an antifouling agent (Hedner et al., 2008). Given that marine growth on vessels and underwater structures costs the shipping industry millions of dollars annually (Braithwaite & McEvoy, 2005), and current additives that are used contain heavy metals

that are toxic to organisms that are not being targeted and that accumulate in the food chain (Braithwaite & McEvoy, 2005; Bhattarai et al., 2007), natural products offer a potentially safer and more selective means of control. The aforementioned cyclopeptide inhibits the settlement of barnacle larvae, and unlike its alternatives, it is biodegradable and works at very low concentrations (de Nys & Steinberg, 1999).

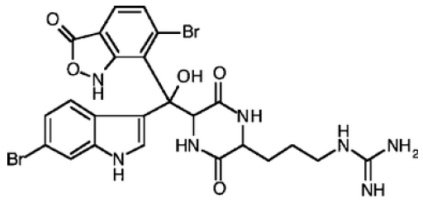


Figure 3.—The antifouling-agent bromobenzisoxazolone barettin (cyclo[(6-bromo-8-(6-bromobenzioxazol-3(1*H*)-one)-8-hydroxy)tryptophan)]arginine) from the sponge *Geodia barrette*.

Natural products have also made their way into the cosmetics sector. Marine Moisturizing Factor 1 (MMF 1) is a multiple compound extract from Coral Seaweed (*Corallina officinalis alba*). It appears that the natural role of this product is to establish new settlement of pieces of the algae that break off due to wave action. However, these compounds also resemble the water soluble moisturizing factors found in human skin. Some of the individual isolates of this extract (Fig. 4) used for skin treatment include 2-pyrrolidone-5-carboxylic acid (PCA) (well known for water bridging properties and widely used in cosmetics formulations for this moisturizing effect), 12-epi-scalaradial (a potent inhibitor of toxins), and spermine and spermidine (polyamines that protect replicating DNA against oxidative injury) (Hamana & Matsuzaki, 1982). In addition, a group of diterpene glycosides known as pseudopterosins from the sea whip *Pseudopterogorgia elisabethae* have shown general anti-inflammatory and analgesic effects, most notably of the group being pseudopterosin E (Look et al., 1986; Roussis et al. 1990). These compounds (in the form of an extract) have actually been used by Estee Lauder in their product Resilience® (Faulkner, 1995).

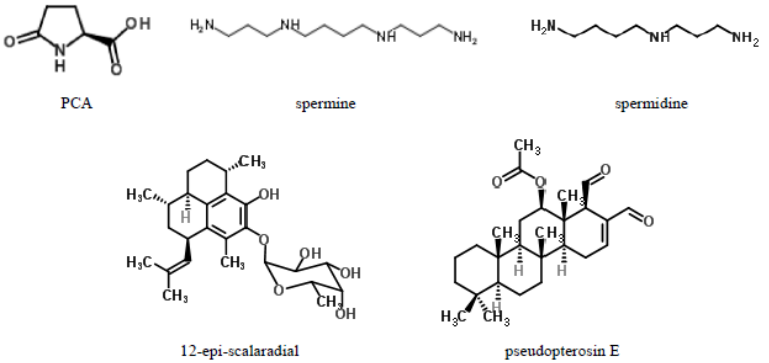


Figure 4.—Constituents of Moisturizing Factor I (MMF 1) extract from coral seaweed (*Corallina officinalis alba*).

Yet another application of marine natural products can be found in the food industry. Chefs use the green dye chlorophyll (a) E140 (Fig. 5) from chlorella algae (who use it to harness energy from sunlight) to color certain foods and beverages green (i.e. pasta and absinthe) (Watanabe, 2005). Since chlorophyll is not soluble in water, it is first mixed with oil. Due to its instability, methods have been achieved now that allow for lyophilization for preservation in powder form. Also, the protein known as phycoerythrin (Fig. 6) that gives the algae *Porphyridium cruentum* its strawberry color is beginning to be used as a natural additive to preserve the reddish coloring of many commercial foods and beverages (Spolaore et al., 2006).

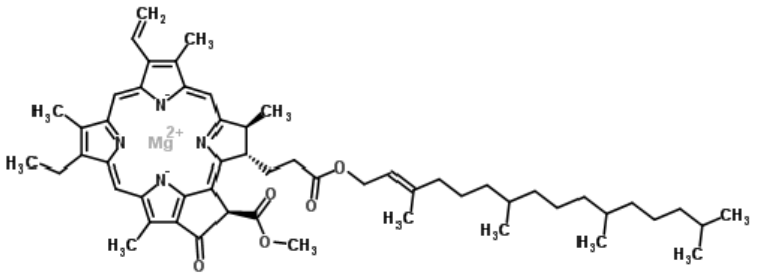


Figure 5.—Chlorophyll (a) E140, as found in chlorella algae.

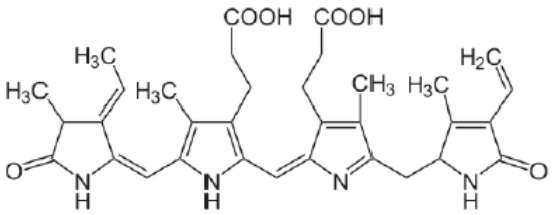
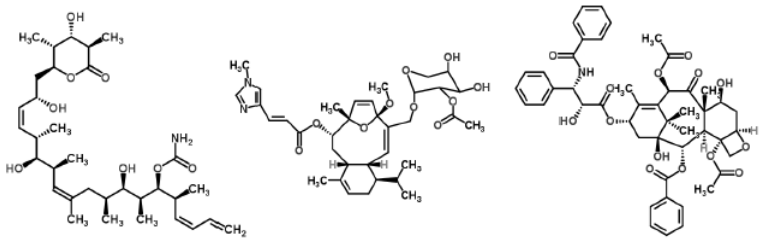


Figure 6.—Phycoerythrobilin, the typical chromophore in phycoerythrin., the pigment protein found in *Porphyridium cruentum*.

Pharmaceutical Applications

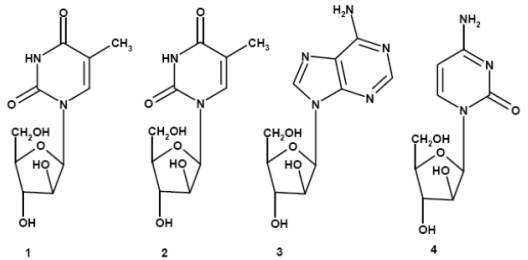
By far the biggest applications of marine natural products seem to be pharmaceutical ones. Marine sponges are especially noteworthy sources of these natural products, not only because of their own biosynthesis, but because of the fact that as much as 60% of their body mass can be due to symbiotic microbes who themselves yield natural products (Lee et al., 2001). As a matter of fact, Porifera and Cnidaria are by far the most dominant sources of new marine products throughout the world (except in polar areas, where Echinodermata have dominated) (Leal et al., 2012). From sponges and related organisms, researchers have isolated many compounds with anti-bacterial, anti-tumor, anti-viral, anti-inflammatory, and analgesic properties. For example, the polyketide discodermolide (Fig. 7) isolated from the sponge *Discodermia dissoluta* inhibits tumor cell growth (Florence et al., 2007). Eleutherobin (Fig. 7), from the coral *Eleutherobia* sp., also inhibits cancerous cells (Long et al., 1998). These two chemicals are very similar in terms of anti-mitotic mode of action to the ovarian cancer drug Taxol (Fig. 7), a terrestrial natural product.



discodermolide (*D. dissolute*) eleutherobin (*Eleutherobia* sp.) taxol (*T. brevifolia*)

Figure 7.—Two cancer- and tumor-inhibiting drugs isolated from marine natural sources. Taxol is a terrestrial natural product, but has a similar mode of action.

Two nucleosides isolated back in the 1950's from the Caribbean sponge *Tethya crypta* contained a rare arabinose sugar rather than ribose. These two chemicals (1 and 2 in Fig. 8 below) led to synthetic analogues (3 and 4 in Fig. 8 below), and currently these 4 chemicals are the only marine related compounds out of clinical trials (Scheuer, 1996). However, in recent years, many marine natural products are showing promise as new drugs and are either in testing or will be soon.



1 spongothymidine 2 spongouridine 3 Ara-A (Vidarabine®, Vidarabin Thilo®) 4 Ara-C (Cytarabine, Alexan®, Udicil®

Figure 8.—Two isolated nucleosides (1 and 2) and their synthetic analogues (3 and 4) from the Caribbean sponge *Tethya crypta*.

Some of the more prominent natural products with therapeutic promise include anti-cancer agents such as Bryostatin 1, dolastin 10, and ecteinascidin 743. Bryostatin 1

(Fig. 9) is a lactone isolated from the marine bryozoan *Bugula neritina* that works at very low concentrations (nanomolar) to combat leukemia (Pettit et al., 1982).

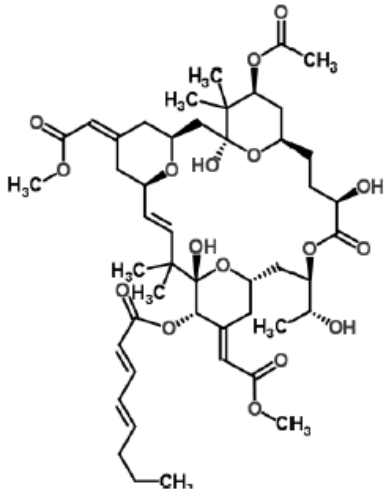


Figure 9.—The anti-cancer agent Bryostatin 1, a lactone isolated from *Bugula neritina*.

Its mode of action is bonding with protein kinase C (de Vries & Beart, 1995). As it nears the end of clinical trials, it seems that alone it is not an effective treatment, but it is a useful synergist to other chemotherapeutic agents.

Dolastatin 10 (Fig. 10) is a linear peptide isolated from the sea hare *Dollabella auricularia* from the Indian Ocean and is a good anti-tumor agent (Pettit et al., 1987). Dolastatin 10 is in clinical trials as an anticancer agent for use in the treatment of breast and liver cancers, solid tumors and leukemia (Yamada et al., 2000).

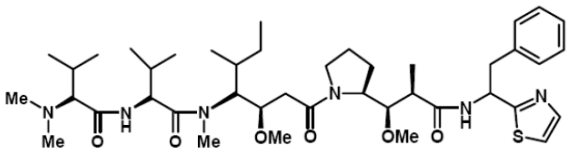


Figure 10—The anti-cancer agent Dolastatin 10, a pepetide isolated from *Dollabella auricularia*.

Ecteinascidin-743 (Fig. 11) is a tetrahydroisoquinoline alkaloid derived from the colonial tunicate *Ecteinascidia turbinata*, a sea squirt that lives in clusters in the Caribbean and Mediterranean seas. Early on, the compound demonstrated very potent activity against a broad spectrum of tumor types in animal models (Rinehart, 2000). The initial sets of clinical trials for this compound were completed in 1998, with the objective of finding the maximum tolerated dose and studying any possible toxicities. The studies identified a safe, tolerable dose and demonstrated the feasibility of applying it in multiple cycles, and early trial results have shown promising activity of Ecteinascidin-743 in the treatment of advanced soft tissue sarcoma, osteosarcoma and metastatic breast cancers (Rinehart, 2000).

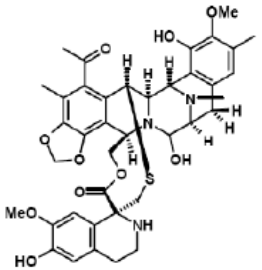


Figure 11—Ecteinascidin-743, the anti-tumor drug isolated from colonial tunicate *Ecteinascidia turbinate*.

Curacin A (Fig. 12), from the blue-green algae *Lyngbya majuscula* has also shown anti-cancer properties (Gerwick et al., 1994).

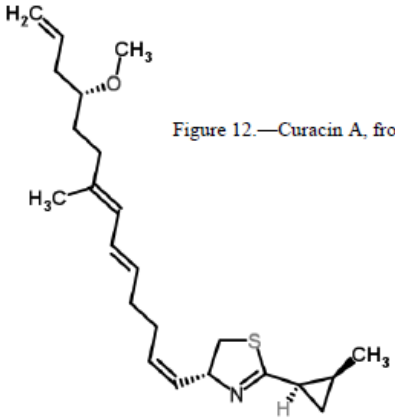


Figure 12.—Curacin A, from the blue-green algae *Lyngbya majuscule*.

Marine organisms are also proving to be a great source of antibiotics. The peptide known as jaspamide (Fig. 13), derived from the *Jaspis* sponges from Fiji, displayed antimicrobial properties against *Candida albicans* yeast and insecticidal properties agains *Heliothis virescens* (Zabriskie et al., 1986). However, it had no effect on a wide variety of Gram positive and Gram negative bacteria.

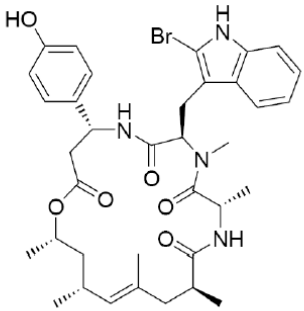


Figure 13—Jaspamide, an antibiotic found in the *Jaspis* sponges from Fiji.

Extracts from the Australian sponge *Cymbastella hooperi* (Fig. 14) showed in vitro anti-malarial activity against the parasite *Plasmodium falciparum* (Konig et al., 1996; Wright et al., 1996).

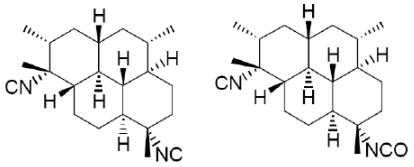


Figure 14—Major anti-malarial compound (left) and a related compound (right) found in *Cymbastella hooperi* extract.

Even if some marine natural products are found to be unsuitable for human pharmaceutical development, they can still prove immensely useful for medical research. For example, Green Fluorescent Protein (Fig. 15), or GFP, is a molecular probe isolated from the jellyfish *Aqueoria victoria*. It absorbs UV light and glows green, and can be molecularly linked to other drugs and proteins, and thus allows tracking and visualization of the biochemical reactions associated with cell biology (Southward & Surette, 2002).

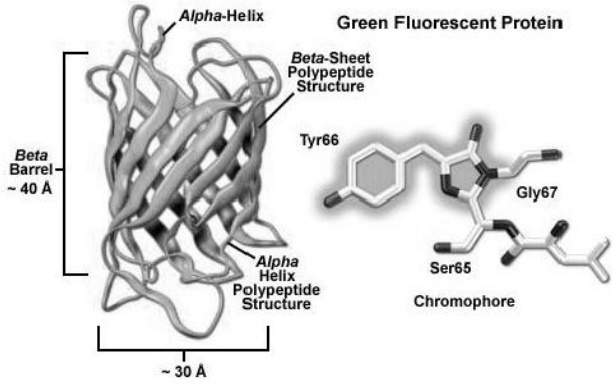


Figure 15.—Green fluorescent protein, found in the jellyfish *Aqueoria victoria*. Image courtesy of Zeiss Microscopy.

Conclusion

The aforementioned are only a relatively recent few of the *many* examples of how marine natural products have been investigated by researchers. Several excellent and recent marine chemical ecology reviews exist that provide countless other examples, and this project does not attempt to provide a

comprehensive or up-to-date overview of all marine natural products (Paul et al., 2011; Ianora et al., 2006; Skropeta, 2008). The point of this work, however, is to illustrate that the search for novel biologically active molecules in our oceans is a relatively new frontier, but it is a very promising one. One of the features that make these products so useful is that they are far less eco-toxic than the alternatives. They are biologically present in the environment and are biodegradable, and thus do not bioaccumulate in the food chain. In addition, because they are of natural origin, they are very target specific, so commercial utilization and exploitation of these chemicals can minimize collateral damage compared to usage of their synthetic counterparts. Since evolution has honed them to work maximally at minimum concentrations, it is also useful that they work very efficiently in small amounts.

This could also be a drawback, however, because they are also present in small amounts in nature, and thus it is very difficult to rely on pure extraction to gain sufficiently therapeutic amounts. To make matters worse, they are incredibly difficult to synthesize should we become dependent on their usage. It is also likely that wide usage of marine products could also have unintended consequences. These products are chemicals that have been refined through the evolutionary process, and as such, they tend to have significant and potent effects on other organisms and ecosystems. They may be natural chemicals, but we are still using them as chemical agents, and as with any chemical agent, they must be used cautiously so as to minimize biological and ecological impact. Predator-prey interactions, population dynamics, and reproductive effects should be monitored, as should natural resistance to these agents due to overexposure at the population level. Impact assessment of the sampling site of these products is also a major concern that is essential when monitoring chemical diversity, as the loss of biodiversity through over-exploitation and habitat degradation are currently primary issues in marine conservation (Costello et al., 2010).

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Bacillus cereus: a bacterial species of environmental and clinical significance

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The Gram-positive spore-forming bacterium Bacillus cereus is considered by most microbiologists to be of importance to the food industry as an enterotoxigenic and emetic toxin-producer, eliciting gastroenteritis and vomiting if ingested in high numbers by susceptible individuals. While these virulence factors, as well as a range of additional extracellular products warrant attention by food safety experts, this species has also become important in recent years through its ability to cause ocular infections. Endophthalmitis is a rare but very serious eye condition where the bacterium (often B. cereus) enters the eye through blunt trauma, intraocular contamination through surgery, or a systemic infection as a result of the breakdown of the blood-ocular barrier. The hemolysin BL (Hbl) protein from B. cereus can cause irreversible tissue damage to the photoreceptors of the retina in less than 24h causing blindness in the affected eye. In most instances of B. cereus induced endophthalmitis, vision loss occurs regardless of the type of therapeutic or surgical intervention utilized. This review discusses some of the well-characterized factors produced by B. cereus which elevate its importance in a variety of industrial and clinical settings.

B. cereus ubiquity and food contamination:

Foodborne illness from a variety of microorganisms effects on average 76 million individuals in the U.S. each year resulting

in some 5,000 deaths (Mead *et al*., 1999). Worldwide statistics on *Bacillus cereus* foodborne illness are underestimated due to a variety of factors, including emetic symptoms similar to *Staphylococcus aureus* intoxication and diarrheal symptoms similar to those elicited by *Clostridium perfringens* type A. Most affected individuals do not seek medical attention due to the short duration of signs and symptoms. *B. cereus* seems to account for between 1.4-12% of foodborne illness outbreaks worldwide (Stenfors *et al*., 2008).

B. cereus is a large (1.0-1.2 μm by 3.0-5.0 μm) Gram-positive aerobic-to-facultative spore-forming rod-shaped bacterium. The word bacillus in Latin translates to small rod, while cereus translates to wax-like. The genus *Bacillus* can be split into two groups: *B. subtilis* and *B. cereus*. The *B. cereus* group consists of *B. cereus*, *B. thuringiensis, B. mycoides, B. anthracis,* and *B. weihenstephanensis*. The members of this group produce lecithinase, but do not produce acid from mannitol, distinguishing them from other *Bacillus* species. As the flagship pathogen of this group, *B. cereus* is ubiquitous in soil and freshwater environments in all temperate zones of the world (Gilbert & Kramer, 1986; Kotiranta *et al*. 2000; Kramer & Gilbert, 1989; Schoeni & Wong, 2005; vonStetten *et al*., 1999). This bacterial genus is capable of contaminating a wide range of food products, including rice, chicken, vegetables, spices, and dairy products. Contamination in the dairy industry may occur when *B. cereus* spores come in contact with the udders of cows (Andersson *et al*. 1995), if the spores colonize feed or bedding, or if the spores survive pasteurization (Claus & Berkley, 1986; Sneath, 1986). This is a serious problem in the food industry because *B. cereus* endospores are in many instances partially resistant to the heat of pasteurization, dehydration, gamma radiation, and other physical stresses. This resistance is due to the ultrastructure of the endospore of course, but also in part to the hydrophobic nature of the spores that allows them to adhere strongly to surfaces and develop biofilm-like properties (Mattson *et al*., 2000; Ronner *et al*., 1990). For example, an irradiation dose of 1.25-4 kGy needs to be administered to reduce spores by 90% (De Lara *et al*., 2002). Also, pasteurization may result in the activation and germination of spores (Hanson *et al*., 2005). In addition, *B. cereus* endospores germinate in response to

particular nutrients such as glycine or in response to physical stress such as temperature (spore germination can occur over 5-50°C in cooked rice) (Granum, 1994) and high pressures (i.e. 500 MPa) (Black *et al*., 2007). Thus foods need to be cooked at least at a temperature of 100°C (212°F) or above to kill most of the endospores (Griffiths & Shraft, 2002).

B. cereus toxin production

B. cereus produces several types of toxins, including four hemolysins (Granum, 1994), three distinct phospholipases, a heat/acid stable emetic toxin called cereulide (a plasmid encoded cyclic peptide) that causes vomiting in infected individuals, and several heat-labile enterotoxins [hemolysin BL (Hbl), nonhemolytic enterotoxin (Nhe), and cytotoxin K] (Lund *et al.*, 2000) that all cause diarrhea. Cereulide has an incubation period of 0.5-6h while the total duration of the emetic syndrome is 6-24h (Ehling-Schulz *et al.*, 2004). The incubation time for the Hbl and/or Nhe-mediated gastroenteritis is on average 12h and the duration of signs and symptoms is between 12-24h (Kramer & Gilbert, 1989). An infectious dose ranging between 10^5-10^8 viable cells or spores is necessary to elicit symptoms, while the concentration of cereulide necessary to elicit disease, has not yet been conclusively determined (Gilbert & Kramer, 1986). While total duration of the emetic syndrome is less than 24h and is usually self-limiting, two rare cases in children have been documented where the cereulide toxin was responsible for inhibiting hepatic mitochondrial fatty-acid oxidation which lead to liver failure and resulted in the death of both children (Dierick *et al*., 2005; Mahler *et al.*, 1997).

The cereulide toxin is encoded by the cereulide synthetase (*ces*) gene located on a 208-kb megaplasmid (Ehling-Schulz *et al.*, 2006). Cereulide exerts its toxic effects by binding to 5-HT_3 receptors on the vagus afferent nerve, which induces an imbalance of cellular potassium leading to mitochondrial swelling (Agata *et al*., 194;Mikkola *et al*., 1999; Sakurai *et al*., 1994). A 2 nM concentration of cereulide causes inhibition of RNA synthesis and the above mentioned cellular cytoxicity events. At high doses of cereulide, massive degeneration of hepatocytes was observed (Yokoyama *et al*., 1999).

The hemolysin BL (Hbl) consists of three proteins termed B, L_1, and L_2 (Beecher & Wong, 1994, 1997; Ryan *et al*., 1997). These toxins are produced from the Hbl operon that codes for HblC, HblD, and HblA toxins, which bind to the membrane of eukaryotes where they oligomerize to form pores allowing fluid accumulation into the cell. In addition, the B and L_1 components of the Hbl enterotoxin complex produce a unique discontinuous beta-hemolysis pattern on blood agar (Beecher & MacMillan, 1990). The nonhemolytic enterotoxin (Nhe) is also composed of three protein components (39, 45, and 105 kDa) that demonstrate homology with each other and the Hbl protein components (Schoeni & Wong, 2005). These proteins produced from the Nhe operon are called NheA, NheB, and NheC. The exact mode of action of how all three proteins work together to act as pore –forming cytotoxins remains to be fully elucidated. All three proteins seem to be required to achieve a cytotoxic effect on their host cell (Lindback *et al*., 2004). While the function of NheC is not yet understood, it is theorized to possibly act as a catalyst to cause NheA to bind to NheB that has attached itself to the host cell membrane leading to cell lysis. Thus due to similar structural and functional properties, both the Hbl and Nhe toxins are believed to belong to a superfamily of pore-forming cytotoxins (Fagerlund *et al*., 2008). There have been limited studies on the exact mode of action for *Bacillus* enterotoxins. These toxins, such as Nhe, form pores in lipid bilayers and that a reverse absorption of fluid, Na^+, and Cl^- by epithelial cells, causing a malabsorption of glucose and amino acids. This causes mucosal damage leading to necrosis. Adenylate cyclase is believed to contribute to the process of reverse absorption of fluid in epithelial cells (Kramer & Gilbert, 1989).

Sources for *B. cereus* contamination in clinical settings

Through biofilm production, *B. cereus* has been implicated in contaminating intravenous catheters (Hernaiz *et al.*, 2003) resulting in *B. cereus*-mediated sepsis (Kuroki *et al*., 2009;Ozkocaman *et al*., 2006). The formation of biofilms also allows the release of planktonic bacteria that produce additional biofilms increasing the severity of the infection (Costerton *et al*., 1999).

In addition to catheter contamination, *B. cereus* and its endospores have been shown to contaminate air filtration and ventilation equipment (Bryce *et al*., 1993), fiber optic bronchoscopy equipment (Goldstein & Abrutyn, 1985; Richardson *et al*., 1986), linens (Barrie *et al*., 1994), gloves (York, 1990), specimen collection tubes and balloons used in manual ventilation (VanDerZwet et al., 2000), alcohol-based hand wash solutions (Hsueh *et al*., 1999), plaster-impregnated gauze (Rutala *et al*., 1986), and many antiseptics such as chlorhexidine and povodone iodine (Dubuoix *et al*., 2005). The most common types of infections *B. cereus* causes, other than foodborne illness, include fulminant bacteremia, central nervous system (CNS) involvement (meningitis and brain abscesses), pneumonia, gas gangrene-like cutaneous infections and endophthalmitis.

B. cereus-mediated endophthalmitis

B. cereus is not only capable of causing food-associated toxicoinfections, but can cause endophthalmitis as well (Davey & Tauber, 1987; Hermandy *et al*., 1990; Ullman *et al*., 1987). *B. cereus* is not the only pathogen capable of causing endophthalmitis, but is considered the most aggressive pathogen causing this condition. Because there is a limited immune response when a pathogen enters the eye, a wide spectrum of pathogens can enter and elicit a wide array of effects. Symptoms can range from a relatively painless anterior chamber inflammation (Aaberg et al., 1998), to an explosive ocular and periorbital infection caused by *B. cereus* (Schemmer & Drebe, 1987). Specific toxin production by a particular microorganism is theorized to account for the difference in symptoms. *B. cereus* induced endophthalmitis is characterized by a corneal ring abscess followed by increased pain, chemosis, proptosis, retinal hemorrhage, and perivasculitis (Callegan *et al*., 1999b). Fever, leukocytosis, and general malaise often appear as the systemic manifestations of this condition (Martinez et al., 2007).

B. cereus induced endophthalmitis can be divided into two categories: exogenous and endogenous. An exogenous source is due to blunt trauma that penetrates the eye, which may occur due to occupation (for example, metal workers), in an agricultural setting (David *et al*., 1994) or infection resulting from unsterile

instruments during cataract surgery. In one example in Rome, an ophthalmologist had four of his cataract patients lose vision in their treated eye one day after their cataract surgery (Simini, 1998). *B. cereus* is ranked second behind *Staphylococcus aureus* which is responsible for about 70% of post-cataract surgery endophthalmitis (Han *et al*., 1996). The three main risk factors surgeons need to be aware of to reduce posttraumatic endophthalmitis are the presence of an intraocular foreign body, delay in closure of the globe, and the location/extent of the laceration of the globe (Jonas *et al*., 2000).

Endogenous sources represent about 2-8% of all endophthalmitis cases (Romero *et al*., 1999) and are due to bacteria entering the posterior segment of the eye. *B. cereus* can accomplish this route of entry through blood transfusion, contaminated needles/illicit drug injection paraphernalia (Grossniklaus *et al*., 1985; Masi, 1978), or by iatrogenic administration of medications such as B vitamins or insulin (Bouza et al., 1979; Motoi et al., 1997). Bouza *et al* (1979) reported a case of *B. cereus* induced endophthalmitis due to contaminated B vitamins that were administered intravenously. When cultured, *B. cereus* was found in the B vitamins that were administered leading to endophthalmitis in the 43 year-old male patient. The patient was administered intravenously B vitamins twice a week for several weeks before developing symptoms, which ultimately resulted in severe vision loss of the right eye.

Moyer *et al* (2009) demonstrated that *B. cereus* is capable of disrupting tight junctions between endothelial cells and the basement membrane of retinal capillaries and retinal pericytes as early as 4h post-infection. Such changes are hypothesized to be responsible for causing the loss of retinal structure and function (Kopel et al., 2008; Moyer *et al*., 2009). The exact toxins from *B. cereus* responsible for causing this breakdown of the blood retinal barrier are unknown but are theorized to consist of the following molecules that may be working individually or in concert to achieve this effect: the Hbl enterotoxin, the Nhe enterotoxin, a crude exotoxin (CET) derived from cell-free *B. cereus* culture filtrates, phosphatidylcholine-preferring phospholipase C (PC-PLC), collagenase, cereolysin O (Shany *et al*., 1974), or cereolysin AB (Scott *et al*., 1996). However, only the Hbl enterotoxin protein has been identified for its role in

endophthalmitis (Callegan et al., 1999a). Hbl enterotoxin has been shown to cause irreversible tissue damage to the photoreceptors of the retina in less than 12-24h causing blindness in the infected eye (Beecher *et al*., 1995; Davey & Tauber, 1987).

B. cereus is capable of disrupting the blood retinal barrier as early as 4h in retinal tissues, 6h postinfection in aqueous humor, and in all other ocular tissues 12h postinfection (Callegan et al., 1999b). *B. cereus* has been shown to be a more rapid and virulent enphthalmitis pathogen compared to *S. aureus* and *Enterococcus faecalis*. Additionally, *B. cereus* seems to exhibit an almost immediate inflammatory response despite low numbers of the organism present at the early stages of infection.

Limited research exists addressing the exact role the immune system plays in endophthalmitis, but the eye is known to be an immunoprivileged site as was first described by Medawar in 1948 (Cunha-Vaz, 1997). The eye restricts both the adaptive and innate immune systems in such a way to balance the challenge of pathogen infection against inflammation-induced vision loss (Streilien, 2003).

In most instances of *B. cereus* induced endophthalmitis, vision loss occurs regardless of the type of therapeutic or surgical intervention utilized because the severity of the disease has progressed to such a condition, that too many toxins have been released by *B. cereus* and many bacteria will have migrated in the eye out of the reach of antibiotics (Callegan *et al*., 2006). Thus within a 12-18h time frame, massive tissue destruction occurs to the retina and surrounding ocular tissues resulting in antibiotics no longer being maximally effective (Callegan *et al*., 2002).

Although outside the scope of this mini-review, a great deal of ongoing work by investigators is centered on elucidating the types of virulence gene regulation occurring in *B. cereus* when grown in varying conditions, such as stressors, or in the presence of factors thought to influence transcription of some of the well-characterized virulence operons previously mentioned. What is clear is that a complex hierarchy of regulatory events play out *via* quorum sensing that are only partially understood at present. Thus, our laboratory and others are centered on dissecting this system of virulence gene regulation to better

understand the *in vitro*- and *in vivo*-specific factors which make *B. cereus* so adaptable.

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Elementary Teachers' Perceptions of Factors Impacting Change during a Mathematical Reform Initiative[1]

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This study examined teacher perceptions of the impact of intervention factors implemented during a grant-funded mathematics education initiative. The following intervention factors were studied: district-level classes, building-level classes, classroom coaching, day-to-day use of research-based instructional materials, district-level administrative support, building-level administrative support, student success as measured informally by teachers during the day-to-day practice of teaching, student success as measured formally by the high-stakes state assessment for South Dakota, and student success as measured by a conceptual performance assessment. Overall, the teachers participating in this study reported that the grant had considerable impact on mathematics instruction; interventions were highly valued, and led to a high level of implementation of standards-based mathematics.

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Introduction

For much of this century, mathematics in this country has been taught in a manner that can be characterized by passive behaviors of students and a teacher lecturing. The Standards 2000 Project developed by the National Council of Teachers of Mathematics (NCTM) called for changes in instructional practice described by the following vision:

> Imagine a classroom, a school or a school district where all students have access to high-quality, engaging mathematics instruction. There are ambitious expectations for all, with accommodations for those who need it. Knowledgeable teachers have adequate resources to support their work and are continually growing as professionals. The curriculum is mathematically rich, offering students opportunities to learn important mathematical concepts and procedures with understanding. Students confidently engage in complex mathematical tasks chosen carefully by teachers…Students are flexible and resourceful problem solvers. Alone or in groups and with access to technology, they work productively and reflectively with the skilled guidance of their teachers. Orally and in writing, students communicate their ideas and results effectively. They value mathematics and engage actively in learning it (2000, p. 5).

Despite years of advocating for this shift in practice, however, little systemic reform of mathematics instruction can be observed in this country (Stigler & Hiebert, 1999).

According to Finn (2008), “nothing in education reform is easy” (p. 36). Fundamental reforms, asserted Cuban (1993), permanently alter structures because those structures are flawed at their core and need complete overhaul. The kind of overhaul that Cuban suggested is rarely found in schools today.

According to Smith (2008):

> Although change can be tangible and obvious when we hold a cell phone or iPod in our hands, it is also intangible and elusive when policies and practices are changed. Change involves emotions and often defies logic. It is complicated and complex, yet much of the time most of us, as individuals,

> manage to deal with it. Organizations, such as schools, are not so fortunate; few are able to significantly change. Consequently, we need a better appreciation of change as it plays out in schools (p. 13).

Building a better appreciation begins with understanding the nature and ramifications of educational change. Fullan (2007) stated "the difficulty is that educational change is not a single entity, even if we keep the analysis at the simplest level of an innovation in a classroom" (p. 129). Fullan (2007) maintained that there are at least three dimensions at stake when implementing a new program: new materials, new teaching approaches, and new beliefs, and that changes in all three are essential if the intended outcomes are to be achieved (2007). The bulk of the responsibility for educational change along these three dimensions rests with teachers (Fullan, 2007). If that is true, then considerable attention must be paid to building the capacity of classroom teachers.

The success of systemic change efforts can be directly linked to the professional development that is provided to the teachers. The need for emphasis on professional development of teachers is supported by the National Commission on Teaching and America's Future in a 1996 report, *What Matters Most: Teaching for America's Future:*

> On the whole, the school reform movement has ignored the obvious: what teachers know and can do makes crucial differences in what children learn. Policies can only improve schools if the people in them are armed with the knowledge, skills, and support they need. Student learning in this country will improve only when we focus our efforts on improving teaching (p. 5).

Currently, little is known about kinds of professional development activities and support that are necessary to produce teacher change in mathematics instructional practice.

Several supports for change in mathematics instruction are being considered in the current research. "Although textbooks and other curriculum materials are ubiquitous in American schools, researchers are just beginning to investigate the

contribution of curriculum materials designed to support teacher learning (Collopy, 2003, p. 287). School-based support is another area of support emerging in the current literature. “A common element of potentially effective PD discussed across the literature is the need for sustained support for teachers as they return to their schools to implement the PD objectives” (Rogers, Abell, Lannin, Wang, Musikul, & Dingman, 2007, p. 510). School-based support could be provided by a teacher leader, another teacher, or the building administrator. The impact of student learning as a motivating factor in teacher change is another emerging area of study. Both Guskey (2003) and Loucks-Horsley, Hewson, Love, and Stiles (2003) identified the use of student learning as a key component to teacher change but claim that it is rarely mentioned in the literature.

Statement of the Problem

In 2002, a National Science Foundation (NSF) grant was awarded to a partnership of education agencies in South Dakota. This grant, entitled Promoting Reflective Inquiry in Mathematics Education (*PRIME*), was a professional development grant designed to improve student achievement in mathematics for the Rapid City Area Schools (RCAS) district in South Dakota and to be a mechanism for systemic teacher change in mathematics instruction. The RCAS K-12 school district has 12,000 students. The student population is 17% Native American and 36% of the students qualify for free or reduced lunch. The district houses 15 elementary schools with approximately 6,000 students.

The professional development provided was a combination of district-wide professional development, which was offered after the end of the duty day to teachers on an invitational basis; and building-based professional development, which was offered primarily during the duty day often with some expectation that teachers participate. The district-wide professional development focused primarily on building teacher content knowledge, exploring the pedagogy of standards-based mathematics, and general training on using standards-based instructional materials. The building-based professional development primarily involved classroom coaching to promote teacher change in practice and building-level classes with specific training on using the standards-based instructional materials. By

the end of 2007, *PRIME* teachers had received more than 120,000 hours of professional development. About 50% of those hours were earned by attending district-level classes; building-level classes accounted for 25% of the hours, and the rest of the hours were earned during classroom coaching.

In addition to professional development for teachers, each building principal and many district administrators were provided with at least 15 hours of *Lenses on Learning* training. This professional development program, developed by the Education Development Center, is specifically designed to build the capacity of school administrators to support standards-based mathematics instruction.

The last major intervention provided to teachers during the life of this grant was the introduction of standards-based instructional materials. During the 2001-2002 school year, just as the *PRIME* grant was being written, the district adopted *Investigations in Number, Data, and Space* developed by Technical Education Research Center (TERC) as one of two sets of instructional materials that could be used to teach elementary mathematics. By the third year of the grant, *Investigations in Number, Data, and Space* had become the dominant instructional materials, used exclusively in most elementary buildings. The intention of this suite of interventions and professional development experiences was that over time, teachers would change their instructional practice in mathematics to be more in line with the inquiry-based learning, active student engagement and learner-constructed knowledge that is called for in the research on standards-based reform in mathematics.

Research Questions

The following research questions guided the research:

1. To what extent did *PRIME* classes, classroom coaching, day-to-day use of tested, research-based, standards-based instructional materials, external support (from building-level administrators, district level administrators and/or building level peers), and student success as measured formally by the DSTEP and MARS assessments, and as measured informally by teachers during the day-to-day practice of teaching, contribute to

changes that elementary classroom teachers made in their mathematics instruction over the course of the *PRIME* grant?

2. What factors from among professional development, exposure to materials, external support, and/or student success were cited as most helpful to prompt the initial change to standards-based mathematics and which factors helped most to sustain the change?

Background

Standards-Based Reform in School Mathematics

The NCTM (2000) emphasized the need to shift instruction from a model in which teachers transmit information to a model in which teachers develop mathematical understanding through exploration and discourse. Teachers implementing reform practices face many challenges, the first of which is to envision a different classroom environment and a new role for themselves in that classroom. *The Principles and Standards for School Mathematics* (2000) provided numerous vignettes to help teachers reconstruct their image of what it means to teach and learn mathematics in a standards-based classroom. Even with these images, teachers had difficulty translating the vision into practice (Anderson & Piazza, 1996).

To depict standards-based reform classrooms, researchers (McDougall, Ross, & LeSage, 2001) described characteristics of standards-based practice by interpreting and classifying actions of elementary and middle school teachers. They identified the dimensions that describe the reform actions of teachers in their classrooms, including students' tasks, discovery, teacher's role, manipulatives, student-to-student interactions, and student assessments.

Steffe and Wiegel (1992) described reform mathematics as a practice based on interactions between teachers and students while engaging in problem solving. Teachers exemplifying reform practice learned about their students' thinking through classroom interactions. Based on the information learned about students' thinking, teachers were then able to create learning trajectories, make instructional decisions, and set new goals within the context of teaching. This conception of reform practice placed primacy on student/teacher interaction, engagement in solving

problems, and using assessments to make instructional decisions, echoing in part the findings of McDougall, Ross, and LeSage (2001). These research studies provide a framework for defining standards-based reform and describing the changes in teacher practices that would be inherent to implementing standards-based reforms.

Several studies have documented that standards-based classrooms effectively change student attitudes toward mathematics. One study that analyzed standards-based classrooms was conducted by NCTM. This large-scale study investigated the effects of implementing the NCTM standards. Researchers found that most students enjoyed working with their peers in group projects and discussions. The importance of communication became evident for these teachers as they watched their students become more confident about their mathematical ability. By using small-group activities, students were able to share their experiences with one another. The researchers also noted that the shift in pedagogy offered more interesting mathematical experiences to students, which they believed to be a contributing factor to positive student attitudes in mathematics (Johnson & Mills, 1998). Gay, Bruening, and Bruce (2000), through the Teacher Development Coalition, conducted a meta-analysis of the current literature about the impact of standards-based instruction on student attitudes. Their comprehensive literature review of various studies and journal articles is designed to provide guidance to teachers as they change their instruction. The common theme through the report is that students learn mathematics best when they construct their own mathematical understanding. The pedagogy that supports this learning is characterized by students working in groups, engaging in discussion, and taking charge of their own learning (Gay, Bruening, & Bruce, 2000).

The literature also strongly supports the conclusion that standards-based reform contributes to higher student achievement and increased conceptual understanding across all grades and demographics. A large-scale review of 154 studies from 1993-2000 found that standards-based teaching provided by teachers who were committed to standards-based reform led to higher achievement in “problem solving and conceptual understanding and no less achievement on objectives

emphasized by traditional programs such as computational efficiency" (Ross, Hogaboam-Gray, & McDougall, 2002, p. 129). Hiebert (1999), in his review of the literature, concluded that standards-based mathematics provides the opportunity for students to gain a conceptual understanding of key mathematical processes *and* to demonstrate skill proficiency.

Some of the most compelling evidence of the value of standards-based teaching comes from qualitative, longitudinal studies following teachers over several years of practice (Hiebert, Carpenter, Fennema, Fuson, Human, Murray, Olivier, & Wearne, 1996; Villasenor & Kepner, 1993). One classic study, conducted by Boaler (1998), compared the performance of students in two secondary schools in England. At one school, teachers were committed to the implementation of mathematics reform. Students worked in cooperative groups on long-term projects, asked their teachers when they wanted help with mathematics concepts, and classroom discussion emphasized student construction of their own thinking. The other school continued traditional teaching methods with emphasis on workbooks and textbooks, finding correct answers, individual work and the transmission of algorithms and procedures. Students in the standards-based classroom were found to perform better than the students who were taught with the more teacher-centered methods. On short, closed-question exams, consistent with the questions that the students in the traditional school had routinely seen, 71% of the students from the traditional school passed compared to 88% of the reform students—despite the fact that the questions were markedly different from the tasks that the reform students had been doing in their classrooms. Furthermore, the students taught by reform methods tended to use intuitive methods to solve the problems such as using contextual clues and prompts from the structure of the problems. In sharp contrast, students in traditional classes were often distracted by the contextual features of the problems.

Another large-scale study, conducted by Balfanz, MacIver, and Byrnes (2006) during the first four years of the implementation of a comprehensive standards-based reform effort in high poverty middle schools in the United States clearly supports the positive impact on student achievement resulting from standards-based reform. The finding from this study

showed "significant and substantial achievement gains across multiple classrooms in multiple schools over multiple years" (p. 57). These research studies firmly establish the benefits for students of the implementation of standards-based reform mathematics.

Professional Development

Broadly defined, professional development refers to the development of a person in his or her professional role. Specifically, "teacher development is the professional growth a teacher achieves as a result of gaining increased experiences and examining his or her teaching" (Glatthorn & Fox, 1996, p. 41). Professional development includes formal experiences such as attending workshops, professional meetings, and mentoring, and informal experiences such as reading professional journals, talking to peers, and watching other teachers practice (Ganser, 2000). This conception of professional development is broader than the staff development or in-service training opportunities that were available to teachers in the past (Glatthorn & Fox, 1996).

Historically, professional development for teachers has been a skill-oriented process using top-down driven methods. As described by Clark (1992), professional development often has a negative connotation because of the way teachers have experienced professional development in the past. Frequently, professional development has meant "a process done to teachers; that teachers need to be forced into developing; that teachers have deficits in knowledge and skills that can be fixed by training and that teachers are pretty much alike" (Clark, 1992, p. 75). Loucks-Horsley (1997) characterized these experiences as "one shot, good bye, God bless you workshops" (p. 134). Teachers left these workshops to return to their classrooms supposedly to implement the new skill. No consideration was given to difference among teachers, students, or classroom situations (Loucks-Horsley, 1997). Many of the traditional professional development opportunities focused on a narrow aspect of skills for effective teaching. No time was devoted to helping teachers develop flexibility in listening to their students, understanding students' prior knowledge, or in developing flexibility in their own beliefs about teaching, all of which are

required for teachers to learn how to provide opportunities for students to learn mathematics well (National Council of Teachers of Mathematics, 2000). The kinds of changes being asked of teachers are not just skill-oriented. In addition to asking teachers to add to their skills, the current reform movement is asking teachers to make changes that are transformative, changing the teachers, and improving their professional practice (Smith, 2001).

Learning from professional development is not sufficient if it does not result in improved classroom instruction. Therefore, one essential goal of professional development is to influence teachers' classroom practices. Sparks (2002) suggested that such a change occurs after a change in teacher beliefs, thus emphasizing the importance of teacher buy-in to the professional development content and processes. Linda Darling-Hammond (1997) asserted that teachers who know a great deal about teaching and learning, and who work in an environment that allows them to know their students well have the most successful classroom practice. This assertion implies that teachers need to be provided with powerful professional learning opportunities that are focused on effective teaching practices and how students learn.

Teacher expertise is one of the most important variables affecting classroom practice and student achievement. Quality teaching in the classroom does not happen by accident. In *How Teaching Matters: Bringing the Classroom Back Into Discussion of Teacher Quality*, Harold Wenglinsky (2000) maps out ways in which three aspects of teacher quality—teacher inputs, professional development, and classroom practice—influence students' academic performance. His study found that professional development is closely linked to classroom practice and that particular types of professional development encourage effective practice. In addition, Wenglinsky found that more extended professional development opportunities were particularly effective at encouraging changes in classroom practice. He concluded that rich, sustained professional development helps teachers become more effective at helping students meet high academic standards.

Joyce and Showers (2002), when examining the impact of professional development on student achievement, defined four

conditions that must be present for staff development to impact teacher practice and significantly affect student achievement. Those conditions are (1) a community of professionals who study together, put into practice what they are learning and share the results, (2) the content of the professional development centers on instructional strategies that have been selected because they have been shown to affect student learning, (3) the magnitude of the teacher change generated must be sufficient to result in palpable gains in knowledge and skills for students, and (4) the process of professional development must enable educators to develop the skills to implement what they are learning.

Guskey (2000) proposed a theoretical model of the multi-dimensional relationship between professional development activities for educators and improvements in student learning. The premise of the model is that the quality of professional development, or what Cohen and Hill (2000) refer to as teachers' opportunities to learn, is influenced by many complex factors. The factors believed to have the most immediate and direct influence on teacher change can be classified into three major categories: content characteristics - the "what" of professional development; process variables - the "how" of professional development; and context characteristics - or the "who, when, where, and why" of the professional development. This theoretical model also yielded three noteworthy implications. First, while the relationship between professional development and improvement in student learning is complex and multi-faceted, it is not random or chaotic. Second, the model offered guidance to those interested in determining what makes professional development effective. Third, the model confirmed the importance of a systemic approach to professional development.

While it is the goal of professional development to help teachers learn new roles and teaching strategies, the root of professional development lies in its efforts to improve student achievement. However, most researchers of professional development do not evaluate the impact on students because of the complexity of the relationship between professional development and student learning. Instead, studies define effectiveness as different kinds of teacher engagement, or at best, teacher change (Guskey, 2005; Huffman & Lawrenz, 2003;

Loucks-Horsley, 1997). Guskey (2000) contended that presently we know far more about professional development processes that fail than we do about those that succeed. Evidence from past failures indicates that neglecting teacher change issues will limit success and, at worst, will result in professional development trainings that fail to bring about significant or enduring change (Guskey, 2000).

Research indicates that professional development is essential if teachers are being asked to make significant change in their instructional practice. According to Fullan (2007), the probability of a teacher implementing a new program or innovation depends largely on their judgment of the magnitude of change required for implementation. The next section will examine the research on professional development to promote change in classroom practice.

Professional Development to Promote Change

Successful professional development trainings are ones that approach change in a gradual and incremental manner. Sparks (1983) recommended that professional development needs to illustrate how the new practices can be implemented in ways that are not too disruptive or require a great deal of extra time and effort. Fullan and Miles (1992) stated that if a new program does require major changes be made, it is best to ease into its use rather than expecting full immersion into the implementation process. One way to sabotage change efforts is to take on too much at one time; however, McLaughlin (1991) argued that while changes intended by professional development efforts should not be too ambitious and require too much too soon, they need to be sufficient in scope to challenge professional thinking and to kindle interest in the change. One approach to kindle interest for those engaging in implementing new instructional strategies is to offer a purpose for the change as well as support for the implementation (Fullan & Miles, 1992; Guskey, 2000).

Researchers have confirmed (Fullan, 2007; Loucks-Horsley, 1997) that the most successful implementation of professional development strategies and methods occurs when teachers are provided with regular opportunities to share perspectives and seek solutions to common problems in an atmosphere of collegiality and professional respect. The same research

confirms that the anxiety that accompanies the implementation of professional development ideas is magnified if the teachers perceive they have no say in the process, or they feel isolated and detached in their implementation efforts (Fullan, 2007; Loucks-Horsley, 1997). For this reason, it is essential that professional development be designed in such a way to allow individuals to support each other and to work together. This nature of support permits those engaging in the difficult change process to tolerate the anxiety of occasional failures and promote continuation of the process.

In order for newly implemented knowledge and instructional practices to be sustained and for the change to endure over time, teachers involved in the implementation need to receive regular feedback on their efforts. According to Guskey (2000), it is well known that successful actions are reinforced and likely to be repeated, while those that are unsuccessful tend to diminish. Practices that are new or unfamiliar, but which are perceived as increasing one's competence and effectiveness, will be accepted and retained. This is especially true for teachers whose primary affective rewards come from feeling certain about their capacity to affect student growth. In the absence of any evidence of their positive effects, new practices are likely to be abandoned (Guskey, 2000). Because of this important finding, specific procedures to provide feedback on results are essential to the success of any professional development effort.

Research indicates that professional development is an integral part of current reform efforts. Ongoing support for gradual change, establishing the need for change, teacher collaboration opportunities, regular feedback on the implementation process, and involving teachers in the planning process are all professional development practices that have significant impact on teacher change.

Factors Impacting Teacher Change

Before teachers are willing to invest their time and energy in an innovation, they must believe the change is significant and necessary to improve their practice (Fullan & Hargreaves, 1992; Hord, Stiegelbauer, Hall, & George, 2006). Once they have made the decision to embark on the change process, several factors have been identified in the research as contributing to

teachers' motivation to change. These factors include improvements in student learning, the use of new instructional materials, understanding children's mathematical thinking, support from a building level administrator, growth in teacher content knowledge, and ongoing support in the form of classroom coaching.

The first of these motivating factors is improvements in student learning. The research confirmed that when teachers observe an improvement in student learning or a positive change in students' attitudes that the innovation is personalized and teacher change can occur (Goldsmith & Schifter, 1997; Guskey, 1986). Udall and Rugen (1997) reported that "completely adopting a practice or innovation is likely to occur only after a teacher has seen evidence of student learning as a result" (p. 83).

Ferrini-Mundy (1997) and Guskey (1986) suggested that teacher change in practice can also be motivated by experiencing different instructional methods and materials. Moskal (2000) and Stein and Smith (1998) gave teachers rich mathematical tasks to utilize in their classrooms. The teachers in their studies noticed new interactions within their classrooms and these observations supported a change in beliefs and classroom practice. Collopy's (2003) in-depth study of elementary teachers supports this finding and adds that new instructional materials can be the impetus for teacher learning as well as teacher change.

A third body of research suggests that knowledge about the evolution of children's mathematical thinking supports teacher change in both practice and beliefs. Carpenter, Fennema, Peterson, and Carey (1988) found that teachers' practice changed when they acquired knowledge about children's problem-solving strategies and organized that knowledge into a framework to guide their instructional decisions. By building on their pedagogical content knowledge, teachers were able to make informed decisions that anticipated and utilized student thinking (Franke, Carpenter, Fennema, Ansell, & Behrend, 1998). Their beliefs about teaching and learning changed as they developed their ability to critically analyze student discourse. Pedagogical knowledge and practical inquiry allowed teachers to

change simultaneously both their beliefs and practices (Franke & Kazemi, 2001).

The importance of the role of building-level leadership in the change process is another factor that has been examined by research. Hall and Hord (2006) conducted a two-year extensive study of teachers' implementation of a very innovative science curriculum in a large (80 schools) suburban school district. Because the interventions and instructional support provided to these schools were identical, the researchers expected that at the end of two years most teachers would be at the same point in terms of implementation. However, a very distinct variation was discovered that appeared to represent a school-by-school difference. That school difference was directly correlated with the level of support and encouragement provided by the building principal. The schools sorted out into three levels of implementation. In the first group of schools where the implementation was very high, the principals were very active and supportive of teachers using the new curriculum. The second group where implementation was moderate, the principals were well organized but they did not expect their teachers to go beyond the minimum. In the third group where implementation was low, the building principals did not help or support their teachers in any way (Hall & Hord, 2006). Andrews and Grogan (2002), in their investigation of the conditions characterizing high performing schools, highlighted the influence of principals in developing and nurturing a collegial community that provided a high level of support for teachers working to change their professional practice. This research provides strong evidence that supportive building principals with high expectations for teacher change can be a factor in motivating teacher change.

The area of teachers' mathematical content knowledge, another factor that can motivate teacher change, has also been examined in order to test the impact of content knowledge on teachers' willingness to change their instructional practice. Ball, Hill, and Bass (2005) contended that the quality of mathematics teaching is dependent on teachers' knowledge of the content. Their research indicates that many teachers in the U.S. lack sound mathematical understanding. Sherin's (2002) study of the role of teachers' content knowledge during the implementation of

mathematics education reform reported that teachers often do not bring enough content knowledge to the work to successfully implement the mathematics reform they were asked to implement. Teachers who were successful at changing their mathematics teaching reported that they needed to learn new content knowledge before they could make effective changes in instruction.

It is well-documented that teachers learn by doing, researching, reflecting, collaborating, analyzing student work, and sharing as they increase their theoretical knowledge (Etchberger & Shaw, 1992; Franke & Kazemi, 2001; Fullan & Hargreaves, 1992; Mills & Pollak, 1993; Oshima, Horino, Oshima, Yamamoto, Inagaki, & Takenaka, 2006). Further research indicates that teacher learning can be another factor that motivates teacher change when the learning is sustained and supported over time (Fullan, 2007; Richardson, 2003). One common way of supporting teacher learning and change over time is through classroom coaching.

Joyce and Showers (2002) defined the concept of coaching as prolonged follow-up and support provided by someone with expertise. They "found that continuing technical assistance, whether provided by an outside expert or by peer experts, resulted in much greater classroom implementation than was achieved by teachers who share initial training but did not have the long-term support of coaching" (Joyce & Showers, 2002, p. 84). Their research identified peer coaching as the most effective professional development strategy for promoting teacher transfer of knowledge into practice with an outcome of 95% transfer (Joyce & Showers, 2002).

Professional development is identified as the key to reforming classroom practice and promoting teacher change. The changes in professional development over time are reviewed with attention to the current model of professional development that focuses much more directly on the transfer of learning to classroom practice in mathematics. The comprehensive research base on educational change now allows for some known patterns and principles to be used to understand any change effort. These change principles are fully applicable to the mathematics effort that is the target of this study. All of the interventions that are identified as motivating

factors in a teacher change effort have been implemented in *Project PRIME*. The existing research looks at each of these factors in isolation while this study will examine the actual interaction of these factors in a comprehensive reform effort.

Results

Implementation Level and Response Rate

All of the 199 elementary classroom teachers from Rapid City Area Schools (RCAS) who had been teaching in the district for at least one full school year were surveyed. The implementation level (non-user, basic user, or advanced user) of standards-based mathematics for all of the teachers was determined by the elementary building-based teacher leaders who have daily interaction with the classroom teachers as well as an advanced knowledge of standards-based mathematics. Whenever possible, multiple leaders rated each teacher to check for inter-rater reliability.

In addition, as part of the survey given to teacher respondents to complete, the "Stages of Concern Questionnaire" developed and researched by George, Hall, and Stiegelbauer (2006), was used to validate the teacher leaders' implementation level classifications for each respondent. In 97% of cases, the implementation level gathered from the survey was a direct match for the implementation level assigned to the respondent by their building-based teacher leaders. Non-users are teachers who are not using standards-based mathematics, basic users are newer to using standards-based mathematics and use it at a mechanical level, and advanced users are more experienced users who are concerned with consequences, collaboration, and refinement.

There were 110 surveys returned for a response rate of 55% for the total population. Roughly one-tenth of the respondents are non-users of standards-based mathematics. Just over half of the respondents are basic users and roughly one-third of the respondents are advanced users. Chi-squared testing was done to determine if the population group was significantly different from the respondent group, revealing that the proportion of non-users, basic users, and advanced users in the respondent group was not significantly different from the population, so the

respondent group is a representative sample in terms of implementation level.

Demographic Data

Regarding gender, respondents indicated that 9% were males and 91% were female. Regarding grade level taught, respondents indicated that 7% taught pre-kindergarten or kindergarten, 36% taught first or second grade, and 57% taught third, fourth, or fifth grade. Regarding years of teaching experience, respondents indicated that 17% had 0-3 years of teaching experience, 15% had 4-7 years of teaching experience, 24% had 8-15 years of teaching experience, and 44% had 16 or more years of teaching experience. Regarding years of involvement with the *PRIME* grant, respondents indicated that 19% had 1-2 years of involvement, 17% had 3-4 years of involvement, and 64% had 5 or more years of involvement.

Support and Impact

To add to the demographic picture, the survey included three questions regarding the perceived level of support the respondents felt they have received from the district administration, the building administration, and the *PRIME* grant as they were working to improve their mathematics instruction. Regarding district support for teachers during the *PRIME* grant, 5% of respondents felt that the district was not supportive, 20% felt the district was modestly supportive, 47% felt the district was supportive, and 28% felt the district was very supportive. Regarding building principal support for teachers during the *PRIME* grant, 2% of respondents felt that their principal was not supportive, 5% felt their principal was modestly supportive, 30% felt their principal was supportive, and 63% felt their principal was very supportive. Regarding *PRIME* grant support for teachers, 5% of respondents felt that the *PRIME* grant was not supportive, 26% felt the grant was modestly supportive, 41% felt the grant was supportive, and 28% felt the grant was very supportive.

Respondents were also asked to rate the overall impact of the *PRIME* grant on their mathematics instruction. Four percent of respondents indicated that the *PRIME* grant had no impact, 9% indicated that the *PRIME* grant had slight impact, 14% indicated that the *PRIME* grant had some impact, 45% indicated that the

PRIME grant had considerable impact, and 28% indicated that the *PRIME* grant had very great impact on their mathematics teaching. On average, the respondents felt that overall, the *PRIME* grant had a considerable impact on their mathematics instruction.

Impact of PRIME Classes

Data was collected on two different types of *PRIME* classes, district-level classes and building-level classes. An analysis of the means and standard deviations of the impact scores for *PRIME* district-level and building-level classes was used to determine the level of impact of these two types of classes. On average, the respondents indicated that district-level classes had considerable impact and building-level classes had some impact on their mathematics teaching.

Impact of Classroom Coaching

An analysis of the mean and standard deviation of the impact scores for classroom coaching was used to determine the level of impact of classroom coaching. On average, the respondents indicated that classroom coaching had some impact on their mathematics teaching.

Impact of Standards-Based Instructional Materials

On average, the respondents indicated that research-based, standards-based instructional materials had considerable impact on their mathematics teaching. Research-based, standards-based instructional materials received a highest impact of all of the interventions.

Impact of External Support

Data was collected on two different types of external support, district-level administrative support and building-level administrative support. On average, the respondents indicated that both district-level administrative support and building-level administrative support had some impact on their mathematics teaching.

Impact of Assessments of Student Learning

Data was collected on three different measures of student learning: two formal measures, DSTEP and MARS assessments (which were administered in the spring of each school year) and levels of informal student learning as perceived by teachers during the day-to-day practice of teaching. An analysis of the means and standard deviations of the impact scores for all three student learning types was used to determine the level of impact of these three factors. On average, the respondents indicated that both DSTEP, the high stakes state assessment for South Dakota, and day-to-day student learning had considerable impact on their mathematics teaching, while MARS, a performance assessment added by the district for life of the *PRIME* grant, had some impact on their mathematics teaching.

Relating Demographics and Impact of Intervention Factor

The intervention factors were examined across implementation level and demographic subgroups to determine if significant differences in the level of importance of each intervention factor existed in either implementation levels or demographic subgroups. It was determined that the mean impact scores of the intervention factors did not show a significant difference when the respondents were grouped by gender, years of teaching experience, or years involved with the *PRIME* grant.

Using *t* testing, it was determined that the mean impact scores of the intervention factors in grade level taught subgroups revealed a significant difference in the mean impact scores of both the MARS, a conceptual performance assessment used during *PRIME*, and the DSTEP, the high-stakes assessment used in South Dakota, assessments. Teachers of pre-kindergarten through second grade had significantly lower mean impact scores on both MARS and DSTEP, than their third- through fifth-grade teacher counterparts.

When respondents were grouped by implementation level (non-users, basic users, and advanced users of standards-based mathematics) there was a significant difference in the mean impact scores on four intervention factors: classroom coaching, building-level classes, DSTEP assessment, and instructional materials. The mean classroom coaching impact scores for both basic and advanced users were significantly higher than the

mean classroom coaching impact score for non-users. The mean building-level classes impact score for basic users was significantly higher than the mean building-level classes impact score for non-users. The mean student success as measured by DSTEP impact score for non-users was significantly higher than the mean DSTEP impact score for basic users. The mean instructional materials impact scores for both basic and advanced users were significantly higher than the mean instructional materials impact score for non-users. No other intervention factors had significantly different mean impact scores when grouped by implementation level.

Ranking the Impact of the Intervention Factors

Respondents were asked to specify the level of impact of each of the nine intervention factors on their mathematics teaching. The intervention factors were ranked by the mean score of that level of impact to determine importance of each intervention in the change process.

When the intervention factors were ranked for the entire population, Instructional Materials, Student Learning, *PRIME* District Classes, and DSTEP were found to be the top four interventions by this group. All four of those interventions were rated as having considerable impact on mathematics teaching. To determine which interventions helped most prompt initial change, the basic users were examined in the same manner. The basic users were used in this case because by definition the basic users are the users who have just made a change in their mathematics instruction. When the intervention factors were ranked for the basic users, Instructional Materials, Student Learning, *PRIME* District Classes, and District Administrative Support were founded to be the top four interventions, in that order, by this group. All four of those interventions were rated as having considerable impact on mathematics teaching.

To determine which interventions helped most to sustain change, the advanced users were examined in the same manner. The advanced users were used in this case because by definition the advanced users had changed their mathematics instruction some time ago and were currently refining their practice based on their experiences. When the intervention factors were ranked for the advanced users, Instructional

Materials, Student Learning, *PRIME* District Classes, and Classroom Coaching were found to be the top four interventions, in that order, by this group. All four of those interventions were rated as having considerable impact on mathematics teaching. An examination of all demographic subgroups (gender, years of experience, years of involvement with the *PRIME* grant and grade-level taught) revealed that Instructional Materials and Student Learning were top two intervention factors in all cases.

Discussion

The purpose of this survey research was to gain a better understanding of the teacher change in mathematics instruction that has occurred because of *Project PRIME*. The first observation from this study is that there has been considerable change in teachers' instructional practice since the beginning of *PRIME*. The findings, based on both teacher leader observations and classroom teacher self-report, suggest that the majority of classroom teachers are now implementing standards-based mathematics instruction to some extent in their classrooms. In addition, the majority of respondents reported that the *PRIME* grant had considerable or very great impact on the way they teach mathematics. Those two findings together strongly suggest that elementary mathematics instruction has changed as a result of the work of *Project PRIME*.

All of the interventions factors had at least some impact on the mathematics instructions of the classroom teachers. This is consistent with the research findings of Joyce and Showers (2002) regarding classroom coaching, Collopy (2003) and Ball and Cohen (1996) regarding of instructional materials, Udall and Rugen (1997) regarding student success, and Hall and Hord (2006) regarding external support.

Implementation level (non-user, basic user, or advanced user of standards-based mathematics) affected the perceived impact of some of the intervention factors. Advanced users of standards-based mathematics reported that seven of the nine intervention factors had considerable impact on their mathematics instruction, while basic users of standards-based mathematics cited four intervention factors as having considerable impact and non-users of standards-based mathematics reported only two intervention factors at the considerable impact level. Since

advanced users have made the most change in their classroom practice, some interventions could become more valuable later in the change process. For example, classroom coaching had considerable impact only on advanced users. Advanced users are more reflective about the effectiveness of their new classroom practice, so having a classroom coach as a sounding board for that reflection becomes valuable and important. These findings are particularly important because research findings on the wisdom of practice for leaders engaged in implementing and sustaining improvement projects in mathematics education cites "using available resources efficiently and effectively" (Weiss, Heck, Miller, & Cress, 2004, p. 2) as a key idea in strategic leadership of these projects.

The research findings yielded four intervention factors with specific results for discussion. These four intervention factors are day-to-day use of tested, research-based instructional materials; student success as measured informally by teachers during the day-to-day practice of teaching; student success as measured formally by DSTEP (the high-stakes assessment used in South Dakota) and MARS (a performance assessment used during *Project PRIME*); and building-level classes.

The first intervention, day-to-day use of tested, research-based instructional materials was the number one intervention factor for the entire respondent group as well as the subgroups of basic and advanced users of standards-based mathematics. This finding underscores the importance of having instructional materials that support the change in instruction. This finding needs to be paired with the fact that *PRIME* district-level classes were the number three intervention for the entire respondent group. This finding corroborates the research findings of Loucks-Horsley, Hewson, Love, and Stiles (2003) regarding the importance of professional development and Ball and Cohen (1996) about the potential for instructional materials to help scale change in classroom practice. Having research-based instructional materials, paired with professional development designed to build content knowledge and good instructional pedagogy, promotes changes in classroom practice.

The second intervention, student success as measured informally by teachers during the day-to-day practice of teaching was the number two intervention for the entire respondent group

and for teachers at each of the three implementation levels (non-users, basic users, and advanced users) of standards-based mathematics. It is interesting to note that all teachers, even non-users of standards-based mathematics, report that student learning has considerable impact on their mathematics teaching.

The third intervention, student success as measured formally by DSTEP (the high-stakes assessment used in South Dakota) and MARS (a conceptual performance assessment used during *Project PRIME*) had significantly more impact on teachers of grades three and above than on teachers of grades two and below. This finding is logical given the fact that neither of these formal assessments are given to students before third grade. Despite efforts by school leaders to make all teachers feel responsible for testing results, the impact of formal assessments was not as high for teachers whose students are not tested at their grade level.

The fourth intervention, building-level classes, had significantly more impact on basic users of standards-based mathematics than on non-users. Building-level classes are typically geared specifically to train teachers on how to use the research-based instructional materials. Often these classes provide a good introduction and summary of one or two units of the instructional materials. Basic users are those users who are just beginning to use standard-based instructional materials, so these classes are very timely in meeting their immediate needs.

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The Utilization of Rasch Measurement to Evaluate a Survey for Online Training

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Many studies have been conducted where a face-to-face training environment is compared to an online training environment. While some research has been conducted with online training in not-for-profit organizations, little to no research has investigated the engagement of the participants. The purpose of this study was to determine if the level of engagement of participants in a training course for new staff and interns with Cru would be increased by conducting training online instead of face-to-face and by utilizing multiple forms of media. The survey that was utilized by Cru included questions adapted from the Student Course Engagement Questionnaire (Handelsman et al., 2005) and was analyzed utilizing the Rasch measurement model to understand whether the survey successfully met the requirements for measuring engagement. The Rasch measurement analysis revealed that the survey was weak and did not measure engagement, thus the results of the survey revealed no significant differences in the level of engagement. The results of this study did reveal a significant need to understand how to formulate questions that garner measurable results. Further research is recommended with new questions being added to the survey that are considered to have a greater level of difficulty.

Introduction

Corporate trainers take on many different roles in the instructional design process. One of these roles may be that of evaluator, where they are seeking to understand how to improve upon an existing course. One of the most common ways to evaluate is the utilization of a survey. Surveys are used to elicit feedback for multiple reasons with the goal of improvement.

Surveys may be presented any time during a course but for evaluative purposes they are often presented at the completion of a module or the entire course. Questions may have multiple intentions for their inclusion in the survey. Questions that are for memory recall may transpire as true or false, fill in the blank, multiple choice, or matching. The trainer desires to know whether the individual retained the information that was provided during the training. A second intention is to have a greater understanding as to whether the participant had a particular emotional response based on the module or course that was just taken. While the trainer may have intentions for asking a particular question, the question may not be clear and thus the quality of the responses are poor.

Background

Cru, a faith-based organization, utilized a survey to evaluate the level of engagement between participants in a face-to-face course they had historically operated and an online training course they were conducting a test pilot on. Cru recruits over 600 new staff and interns each year. Training is required for interns and full-time staff members. Currently, Cru staff and interns are trained at specific locations where they are assigned. Upon arriving at that location, each trainee is paired with a coach with whom they meet on a weekly basis for 2 years. During each of these training sessions, a coach will teach the materials and engage with the trainee in hands-on experiences, as well as discuss readings. Trainees receive these materials by downloading Portable Document Format (PDF) files from a wiki and printing them to read in preparation for meeting with the coach. Wikis are online collaborative editing tools that allow participants to become editors of a topic at any time and from any location (Leuf & Cunningham, 2001).

The national training director developed a new form of this training in an online format. The online environment utilized the Moodle learning management system and was asynchronous, meaning that participants had the flexibility to access course materials when it was convenient and go through each course module at their own pace with the same deadlines for ending each module as the face-to-face training participants. The online environment also had 18 modules and utilized multiple media to

present the materials including videos, audio, and discussion boards. The face-to-face training environment only offered text-based materials through downloadable PDFs. The intention was that by utilizing a different course environment and by presenting materials with the use of multiple media-related tools, the level of engagement of the participants would increase. In order to understand whether the engagement level of the participants increased, a review of how engagement is measured was conducted.

Purpose and Importance of the Study

The purpose of this study was to determine if the level of engagement of participants in a training course for new staff and interns at Cru would increase by conducting training online instead of face-to-face and by utilizing multiple forms of media. This study enabled Cru to understand whether the utilization of an online training course would increase the level of engagement of participants. If this were proven to be true, Cru would increase their use of the online training by offering it in other regions of the United States.

Research Questions

1. Did the survey successfully meet the requirements for measuring engagement according to the Rasch model?
2. What were the differences in level of engagement with the course materials between participants in Cru's traditional face-to-face training with a coach and participants in a strictly online environment?

Participants and Data Collection

While Cru is a national organization, the pilot was limited to 70 participants in the Great Lakes Region, which includes Ohio, Michigan, Indiana, and Illinois. Participants in this study were selected with a random selection and sample assignment from the target population. Group 1 participated in a face-to-face training environment with support from a mentor/coach for 1 year (January to December) and consisted of 30 members. Group 2 participated in an online training environment with support from an online mentor/coach for 1 year (January to December) and

consisted of 36 members. Each group participated in the study during the same academic year.

Measurement of Engagement

Specific task participation on the part of the participant can often signify engagement. This includes participation in online discussion boards, providing feedback, solving problems that are presented during the training, and participating in activities (Robinson & Hullinger, 2008). Handelsman, Briggs, and Sullivan (2005) conducted two studies that explored the validity of a measure of participant engagement, the Student Course Engagement Questionnaire. The researchers utilized exploratory factor analysis to reveal four dimensions of a college student's engagement that were distinct and reliable. These dimensions were skills engagement, participation/interaction engagement, emotional engagement, and performance engagement. Handelsman et al. (2005) found a relationship between the four dimensions.

Mandernach (2009) later conducted a study that examined the impact of instructor-personalized multimedia supplements on student engagement. He compared student engagement between courses that featured instructor-personalized multimedia components with a modified version of the Handelsman et al. (2005) Student Course Engagement Questionnaire. Mandernach's questionnaire was modified to target an online learning environment that utilized four discrete factors. These factors were skill engagement such as note-taking or studying, emotional engagement such as personal involvement with class materials, participation/interaction such as asking questions or discussion, and performance engagement such as grades (Mandernach, 2009). A one-way ANOVA was conducted for each factor and also for the final exam. The qualitative feedback of the students in the study indicated enhanced engagement as a function of multimedia, while the quantitative data from the survey instrument yielded no significant differences. For the purposes of this study, Cru modified this questionnaire for participants in both an online training course and a face-to-face training course, specifically relating it to participants who would take the survey after each module of the training course.

Data Collection and Instrumentation

In the online and face-to-face training environments, participants were required to complete the same survey at the completion of each course module. In both environments the survey was administered using an online survey tool called Survey Monkey. The survey had 12 items and was designed to measure trainees' engagement with course materials. Each trainee indicated their level of agreement on a 4-point Likert scale (1=not characteristic of me; 4=very characteristic of me) to statements regarding course engagement. Engagement was scored according to three factors. These factors were skill engagement such as note-taking or studying, emotional engagement such as personal involvement with class materials, and participation/interaction such as asking questions or discussion (Mandernach, 2009). The Student Course Engagement Survey was modified to target trainees in both an online and face-to-face training environment (see Table 1.0). The survey data was collected at the end of each module from January 2012 until May 2012. Survey results were delivered in June 2012.

Table 1.0

Questions 1-9 Scale: Very characteristic of me, Characteristic of me, Not really characteristic of me, Not at all characteristic of me
I studied on a regular basis
I put forth effort into this module
I stayed up on all the readings
I found a way to make the module materials relevant to my life
I found a way to make the course materials interesting to me
I thought about this module even when I was not working actively participating with the course
I asked questions if I did not understand something
I had fun with this module
I am confident that I am doing well in this course

Questions 10-12 Scale: Agree, Somewhat Agree, Somewhat Disagree, Disagree
I really desire to learn more
Interaction with a coach helped me feel more engaged with the course
After completing this module, I feel equipped to pass these materials on to others

Data Analysis

For this study, the Rasch measurement model was used. The purpose for utilizing this model was to evaluate the effectiveness of the survey instrument in describing student engagement. Once the survey was evaluated, the first of five units were analyzed to obtain a person measure and an item measure for the face-to-face group and the online group. In order to obtain person measures and item measures for unit two through unit five, measures were anchored to unit one.
In order to understand whether survey successfully met the requirements for measuring engagement, a Rasch analysis of the survey was conducted. The Rasch analysis was conducted by utilizing the WINSTEPS software package. In order to provide an overall sense of how the survey instrument functioned, a rating scale analysis was implemented. Following the rating scale analysis, baseline statistics for modules 1 through 5 (both face-to-face and online groups) were reviewed in order to understand the dimensionality of the survey. Individual items and persons were also analyzed for misfit but were not removed due to a lack of significance in the results.

In order to understand whether there was a difference in the level of engagement of participants in the face-to-face course as opposed to the online course, a one-way analysis of variance (ANOVA) was conducted. Results from each group from modules 1 through 5 were compared and the means of each as well as the f statistic were reviewed.

Results

Rating Scale Analysis

Items 1 through 9 utilized a rating scale with categories related to characteristics. The scale had a step calibration greater than 1.4 and thus allowed participants to communicate through the measure (see Table 1.1). Items 10 through 12 utilized a rating scale with categories related to agreement. The scale also had a step calibration greater than 1.4. The calibration statistics indicated that the scale functioned properly.

Table 1.1
Rating Scale Threshold Structure

Observed Count	Observed Average	Infit MNSQ	Outfit MNSQ	Structure Calibration	Category Measure

Not at all characteristic of me	9	-1.25	.76	.75	NONE	(-4.23)
Not really characteristic of me	152	.16	.99	.98	-3.09	-1.64
Characteristic of me	356	1.19	.89	.89	-.18	1.56
Very characteristic of me	77	2.36	.95	.95	3.27	(4.39)
Somewhat disagree	8	.6	1.14	1.12	NONE	(-2.52)
Somewhat agree	71	1.49	1.19	1.33	-1.38	.00
Agree	119	2.41	1.26	1.21	1.38	(2.52)

Each category on the scale had a distinct curve, which indicated that each item had a distinct step from the other categories. Persons with a higher ability of 5 were more than likely to endorse step 3 (very characteristic of me), while a person who had an average ability such as a 0 (not at all characteristic of me) may have endorsed either 1 (not really characteristic of me) or 2 (characteristic of me).

Each category on the scale had a distinct curve, which indicated that each item had a distinct step from the other items. Persons with a higher ability of 5 were more than likely to endorse step 3 (agree), while a person who had an average ability such as a 0 (somewhat disagree) will more than likely endorse 2 (somewhat agree) and the person with a lower ability will more than likely endorse 1 (somewhat disagree). With a flatter curve of 2, this indicated that individuals were more than likely to endorse either 1 or 3.

Dimensionality Analysis

In order to define the separation and reliability of both persons and items, baseline statistics were reviewed for modules 1 through 5.

Person summary statistics of module 1 indicated that both separation and reliability were low with a separation of 1.54 and reliability of .70. Modules 2 through 5 showed similar results.

Person reliability and separation look to see whether the participants have variation across the latent variable (this being engagement). This indicated that there was low differentiation between participants who took each module and that participants were a part of two specific groups: participants who found each module very engaging and other participants who found the modules un-engaging.

Item summary statistics of module 1 indicated strong separation and reliability with a separation of 2.55 and a reliability of .87. Item reliability and separation look to see whether the items have variation across the latent variable (this being engagement) with some items being more difficult to agree with while others are less difficult to agree with. Separation and reliability within each module indicated two distinct groups of items, which is considered strong.

Principle Contrast Analysis

A principle contrast analysis was then conducted to address the dimensionality of engagement. The purpose of a principle contrast analysis is to determine whether the survey measures one construct or more than one. Results of the analysis revealed that the primary linear measure explained 34.7% of the variance in the data. The total unexplained variance was 65.3%. This indicated that residual contrasts other than engagement explained the variance. Therefore, 65.3% of unexplained variance would indicate that the test was not unidimensional (see Table 2.1). This could be due to one or more multiple factors.

Table 2.1
Explained and Unexplained Variance

	Measure	Model Error
Variance explained by the primary linear measure	6.4	34.7
Total unexplained variance	12.0	65.3
Unexplained variance in 1st contrast	2.2	11.7
Unexplained variance in 2st contrast	2.0	10.8
Unexplained variance in 3st contrast	1.6	8.5
Unexplained variance in 4st contrast	1.2	6.7
Unexplained variance in 5st contrast	1.2	6.4

One explanation for the survey not being unidimensional is that the questions were not created with one specific factor of engagement but multiple factors. Questions were produced from factors of engagement such as skill engagement, emotional engagement, and participation/interaction. Each group of questions related to one factor of engagement was analyzed for explained and unexplained variance but too few questions were in each factor to solicit a reliable explained variance. If one factor of engagement was used for producing questions, the likelihood of the survey being unidimensional would have been higher.

A more plausible explanation for the survey not being unidimensional is explained by the low person separation as determined by the examination of the baseline statistics. An analysis of the persons and the items will help to further determine whether a low person separation served as an explanation for the survey not being unidimensional.

Item Analysis

The survey that Cru utilized at the completion of each module was adapted from the Student Course Engagement Questionnaire (Handelsman et al., 2005). The survey had 12 items and was designed to measure trainees' engagement with course materials. Each trainee indicated their level of agreement on a 4-point Likert scale (1=not characteristic of me, 2=somewhat not characteristic of me, 3= somewhat characteristic of me, 4=very characteristic of me) to statements regarding course engagement for questions 1 through 9. Questions 10 through 12 were written on a different 4-point Likert scale (1=Agree, 2=Somewhat Agree, 3=Somewhat Disagree, 4=Disagree). A 4-point Likert scale was utilized in order to avoid future collapsing of categories, according to the Rasch model.

In order to measure engagement the questions were created out of three factors of engagement. These factors were skill engagement such as note-taking or studying, emotional engagement such as personal involvement with class materials, and participation/interaction such as asking questions or discussion (Mandernach, 2009) (see Table 3.1).

Table 3.1
Factors of Engagement

Skill engagement:	- I studied on a regular basis - I stayed up on all the readings - I put forth effort into this module
Emotional engagement:	- I found a way to make the module materials relevant to my life - I found a way to make the course materials interesting to me - I thought about this module even when I was not actively participating with the course - I had fun with this module - I am confident that I am doing well in this course - I really desire to learn more - After completing this module, I feel equipped to pass these materials on to others
Participation/interaction:	- I asked questions if I did not understand something - Interaction with a coach helped me feel more engaged with the course

Individual items were analyzed to determine if they fit the Rasch model's description of data that is suitable for measurement. This was determined by analyzing the fit statistics of each item. The infit and outfit mean-square value was analyzed to see whether items were productive for measurement. The infit mean-square value for each of the items revealed that all items were productive for measurement. The question 11 outfit mean-square value indicated that the item was less productive for measurement but not degrading. Point-measure correlation was reviewed to determine whether items were operating in the appropriate direction (whether positively or negatively). All items operated in the appropriate direction. Infit and outfit z-standard statistics were analyzed for each item. Misfitting items were those outside of the -2.0 and 2.0 logit window. Question 9 was considered misfitting due to it being too predictable for measurement. This was due to the question being very easy for participants to answer and thus contained no level of difficulty. This item was not removed from the data as it did not significantly affect the overall separation or reliability of the item data.

Person-item Map

To verify the means and difficulty levels of items and persons, person-item maps were reviewed to see how person ability matched with each item difficulty. If the survey was to successfully meet the requirements for measuring engagement according to the Rasch model, item difficulty and person ability needed to vary such that they represented a linear continuum from less to more on the logit scale. Items needed to have varying levels of difficulty with some being easier to answer positively to while others were more difficult to answer positively to. Persons needed to have varying levels of ability with some having a higher ability and others having a lower ability. After reviewing the item-person maps for each module, it was verified that with the exception of certain items, the difficulty of the survey was low and the ability of the participants was high. Based on the Rasch Analysis, it was determined that the survey was weak and did not truly measure the construct of engagement.

Analysis of Variance

In order to determine whether there was a significant difference in the level of engagement between the face-to-face group and the online group, a one-way analysis of variance was conducted for each module. Person measure statistics were compared between groups in each module. Module 1 was not significant: $F(1, 64) = .286$, $p < .001$. After analyzing modules 2 through 5, similar results were found with no significant difference between groups (see Table 4.1).

Table 4.1
One-Way Analysis of Variance for Module 1

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	367	1	.367	.286	.595
Within Groups	82.073	64	1.282		
Total	82.440	65			

Discussion

Research Question 1

The first research question asked "Did the survey successfully meet the requirements for measuring engagement according to the Rasch model?" The purpose of this question was to determine if the survey could be utilized to measure the construct of engagement. Rasch analysis was conducted to determine if the survey functioned as needed. Results from the Rasch analysis indicated that the survey did not perform successfully according to the Rasch model. The variance previously mentioned indicates that the survey needs questions that are of greater difficulty so that the likelihood of measuring engagement is greater.

The first test that was conducted was a rating scale analysis to understand whether a proper rating scale was created. It was determined that the rating scale on the survey functioned properly. This meant that participants could clearly differentiate categories on questions 1 through 9 between "Very characteristic of me" and "Somewhat characteristic of me." Participants could also differentiate categories on questions 10 through 12 between agree and somewhat disagree.

The second test that was conducted involved reviewing the summary statistics from the data for each module. The purpose of this was to review the separation and reliability for items and person data. Item reliability and separation look to see whether the questions have variation across the latent variable (this being engagement) from less difficult to agree with to more difficult to agree with. For items, separation and reliability were high at an average of 2.37 for separation and .846 for reliability across all five modules. This meant that some items on the survey were more difficult to agree with while others were less difficult to agree with.

An example of this was question 6 which was "I thought about this module even when I was not working actively participating with the course." This question was more difficult to agree with than question 10, which was "I really desire to learn more." Other questions related to studying, enjoyment of the course, and reading were more difficult to agree with while others relating to interaction and asking questions were less difficult to agree with.

Questions that related to studying, reading, and thinking about the module were very defined questions and thus were more difficult to agree with. Questions that were more general such as “put for the effort in this module” were much easier to agree with.

The separation and reliability of participants were also reviewed from the summary statistics. With all five modules, the person separation and reliability were low with an average separation of 1.738 and reliability of .75. This meant that there was not a continuum of people who took the survey with varying ability. After reviewing the item/person maps it was found that a large majority of participants, regardless of group, were above the mean and thus had a high person ability. Individuals could be part of one of two strata. One strata was those that were of higher ability while another strata (which was much smaller) was those of lower ability. This was true of modules 1 through 4 but not module 5, where more face-to-face participants fell below the mean and thus were considered to have low ability. A low person separation and reliability were due too low difficult of many of the questions or that participants chose to not take the survey in a serious manner.

As stated when reviewing the item separation and reliability, most of the items fell below the mean for each module meaning that the majority of the questions were easy for participants to indicate agreement. Regardless of the module, over half of the participants could answer every question with the exception of one or two with little to no effort, finding the questions to have no difficulty. The questions were too broad in nature such as “I put forth effort in this course” and much less defined.

Another possibility for low person separation and reliability was due to the fact that participants chose not to take the survey in a serious manner. It was originally thought that this could be due to the fact that the same survey was administered to the same participants for all five modules and thus participants became very familiar with the survey and thus did not process through the questions.

Multiple factors of engagement were utilized to form the questions. Factors that were utilized were skill, emotional, and participation/interaction. As stated earlier, engagement can have different interpretations.

With engagement having a very broad definition, Handelsman et. al. (2008) sought to utilize specific factors to help measure the construct. This may have contributed to the low dimensionality of the survey with only 34.7% explained by one construct. The dimensionality of the specific factors could have been analyzed but the factors of skill and participation had too few questions.

Research Question 2

To understand if there was a difference in the level of engagement between the face-to-face group and the online group, a one-way analysis of variance was conducted between groups for each module. While none of the statistical analysis revealed any significant differences, it was noted that the person mean scores did change from module to module.

The mean scores for both the face-to-face group and the online group increased from module 1 to module 2, from module 2 to module 3, and from module 3 to module 4. After module 4, the mean scores decreased for both groups for module 5. The mean score increase and decrease may have been due to the content of each module.

All five modules were reviewed for content. While there were similarities between modules that each group participated in, the differences between modules were that the online group watched videos, participated in online discussions, listened to audio podcasts, read articles, and participated in social activities, while the face-to-face group read articles and participated in social activities only. Each of the modules contained the same outline with three categories, which were education, exposure/experience, and evaluation.

Conclusion

After reviewing the survey with the utilization of Rasch measurement, it was determined that the survey contained questions that had a low level of difficulty and most participants had a high level of ability. Therefore, it was determined that the survey was unfit to provide a true measure of engagement between the face-to-face and the online group and as a result the survey data cannot provide a significant difference in the level of engagement between both groups. The weakness of

this study is the survey instrument that was used. In order to understand the differences in the level of engagement between two groups the survey must be able to provide a true measure of engagement of the participants in the course.

A one-way analysis of variance was conducted on each of the five modules to determine if there was a level of difference between the online group and the face-to-face group between modules. While there was a difference in the level of the mean between groups, the f statistic for each module revealed that it was not significant enough. The lack of difference supports the evidence that the survey was weak. Other reasons for no significance could be due too unclear expectations, a lack of media in the online course modules, or a lack of collaboration among participants.

Unclear Expectations

According to adult learning theory, individuals would be more motivated once rational learning objectives are clearly established and the objectives meet the needs of the learner (Knowles, Holton, & Swanson, 1998). While introductory paragraphs were present at the beginning of each module, it may have been more engaging had the expectations been presented either with less text to make them clearer or in a different format such as a video or audio presentation.

Lack of Media

Doolittle (2001) proposed the cognitive theory of multimedia learning, where individuals learn better by including words and pictures rather than from words alone. Modules may have lacked engaging content due to the poor quality or specific nature of the media that was used to present that content. If more media had been utilized such as pictures, video, and diagrams, rather than strictly PDF articles, the level of engagement in the online course might have been greater.

Lack of Collaboration

Richardson & Swan (2003) studied participants in an online course and found a correlation between social presence and students perceived learning. While each module in the Cru online course contained space for reflection and online

discussions, lack of mentor or student-to-student feedback or scaffolding during collaborative assignments may have led to students' lack of interest in using these forms of media or course engagement. All assignments within each module were individual assignments. To add more engagement, course designers might consider some form of explicit guidelines that students must follow in using the available media and interactive features of the course. Other types of interaction also could be considered such as synchronous text chat, audio chat or video chat. While these suggestions might lead to a greater sense of community, they could also undermine an advantage of online learning, which is asynchronous communication.

Future Research

To determine if Cru's new staff and intern training has a significant difference in the level of engagement between the online group and the face-to-face group, further research should be conducted. Adjustments to the training modules and the survey should be completed.

Items in Training

It was noted when reviewing the modules that the overall mean in the online group was greater than that of the face-to-face group. It was determined that this was due to the differences between the two groups. The online group participated in content that involved items such as videos, audio podcasts, and online discussions. The face-to-face group had the same content but utilized articles and had face-to-face discussions. While there were media-related items in the online group, it was noted that typically only one or two of these items were in each module. If Cru were to conduct another study, more items involving media should be utilized in the online group such as more videos and audio podcasts to show a greater difference in type of materials between groups. An analysis could then be conducted to determine if there was a difference in the level of engagement between groups at that point with a new survey.

Survey Adjustment

This study utilized survey questions from Handelsman et al. (2005). While some of the questions functioned with higher difficulty level, some questions were weak. In order for the survey to function properly, questions 2, 7, 10, and 11 should be replaced with questions of higher difficulty. Each of these questions fell below the mean in level of difficulty for all five modules. The questions should have a greater level of difficulty to answer positively. Other surveys could be reviewed for questions such as the National Survey of Student Engagement (NSSE 2012) or the Course Experience Questionaire (CEQ) (Brennan, Brighton, Moon, Richardson, Rindl, & Williams, 2002). A few sample questions that could be utilized in a revised survey are given (see Table 5.1). Questions that could be considered provide specific feedback on whether one form of media is more engaging than another. Other questions relate to the amount of time that participants were involved with each module.

Table 5.1 Question Suggestions for Survey

Questions	**Scale**
If the training were not required, I would still have wanted to participate.	Not characteristic of me, Somewhat Characteristic of me, Characteristic of me, Very characteristic of me
During this module, about how many hours of reading and writing have you done?	None, 1-4, 5-10, 11-20, More than 20
How many hours did you spend per week on this module?	None, 1-4, 5-10, 11-20, More than 20
How many conversations relating to the materials did you participate in?	None, 1-4, 5-10, 11-20, More than 20
I struggled to understand the materials in this module.	Not characteristic of me, Somewhat Characteristic of me, Characteristic of me, Very characteristic of me
Reading the articles was my favorite part of this module.	Not characteristic of me, Somewhat Characteristic of me, Characteristic of me, Very characteristic of me
The videos were my favorite part of this module.	Not characteristic of me, Somewhat Characteristic of me, Characteristic of me, Very characteristic of me
The audio podcasts were my favorite part of this module.	Not characteristic of me, Somewhat Characteristic of me, Characteristic of me, Very characteristic of me

The online interaction was my favorite part of this module.	Not characteristic of me, Somewhat Characteristic of me, Characteristic of me, Very characteristic of me

This study used a poorly constructed survey and thus the questions were too easy for students to say they agreed. Regardless, much can be learned at the completion of this study. A greater emphasis should be placed on the quality of questions that are asked in a survey instrument, specifically whether the question is difficult to agree with or not. Survey questions should cause the participants to pause and consider their response. Also, qualitative data should also be considered, as this was true of Mandernach's (2009) study. Lastly, obtaining secondary data from the participating organization, as was done in this study, can also lead to a lack of control of the survey instrument. Had the survey been created not by Cru, the survey may have been more challenging and thus yielded sufficient results for Rasch measurement. Because of the nature of the survey instrument, it cannot be stated with any certainty what differences existed between Cru's face-to-face and online training groups in the level of engagement. With a more valid and reliable instrument that measures the specific activities or media that tend to lead to higher levels of engagement, Cru may be more able to discover which training format is more successful.

Online training courses are being utilized more each year and sometimes without a plausible reason or purpose behind choosing one method or another. Often the purpose for online training that is considered is cost savings. While research is being conducted in the field of corporate and not-for-profit online training, more research in regards to the engagement of participants in online training, specifically in not-for-profit organizations must be considered. The purpose of doing so will help instructional designers, management and other stakeholders understand the best methods for delivering courses that create a sense of social presence, community, and provide constructivist learning that engages participants.

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An Analysis of the Indiana Reading List Primary Books for Scientific Misrepresentations and Connections to Teaching Science

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This study presents the results of analyzing a state sponsored K-2 reading list of fiction books (70 titles). Analysis was completed using the National Science Teachers Association's (NSTA) and Children's Book Council (CBC) criteria for selecting Outstanding Science Trade Books. Results indicate that 12 books (17%) possess credible presence of science content; 12 books (17%) contained scientific misrepresentations; and male characters are present in 38 books (54%), female characters are present in 14 books (20%), and gender-neutral characters are present in 18 books (26%). Strategies to accompany science and literature connections and more appropriate grade level curricular selections are offered.

Introduction

Educators know that books excite children and prompt them to imagine possibilities, ask questions, and enhance lessons. In general, children's books that are most commonly used in the elementary setting include nonfiction books and fiction storybooks (often called picture books). The nonfiction books normally chronicle a scientist's life, describe an ecosystem, or attempt to relate some scientific concept to a child's life. Since an apparent connection between science and literacy seems stronger between nonfiction books, teachers often incorporate these books into science reading. Porter's (2009) description of how elementary students can model the scientific process

reveals one way to effectively incorporate nonfiction books. She used *Science to the Rescue*, a book about how scientists help society, in order to help students solve a challenge they see in their own community.

Likely all primary teachers read fiction books in language arts lessons to promote interest in reading, vocabulary development, writing skills, and opportunities to integrate subject matter. Teachers may do so because the books typically cover fewer topics, contain fewer images, and better align to developmental and reading levels. Overall, they are simply easier and more enjoyable to read. The U.S. Department of Education reports that during a typical school week 1st through 4th grade teachers spend 21.9 hours teaching core subjects (language arts, math, social studies, and science). Language arts consume 11.7 hours (~36%), math consumes 5.6 hours (~17%), social studies consume 2.3 hours (~7%), and science consumes 2.3 hours (~7%) of these 21.9 hours. Therefore, it can be inferred that elementary teachers are mostly using fiction books in the elementary classroom since so much of the day is focused on language arts. Importantly, during such exercises science may not specifically be addressed and the threshold to scrutinize books for scientific misrepresentations may be lower because it isn't seen as "science time." This is important because these books can often misrepresent science concepts such as habitat, animal relationships, and the nature of animals. A misrepresentation is defined as "to serve badly or improperly as a representative." Marriott (2002) studied over 1000 pictures books and concluded that most presented misrepresentations of information about animals. Many other studies have come to the same conclusions. Trundle and Troland (2005) reported many children's books misrepresent concepts about the moon. Rice and Rainsford (Rice, 2002) report that many errors and exaggerations occurred throughout 50 commonly used trade, fantasy, and realistic fiction books. Ultimately, unaddressed and unchallenged misconceptions challenge learning later in school.

Indiana Reading List

The Indiana Reading List contains suggested choices for educators to use in their classrooms. The instructions on the website containing the list stated:

> These lists provide lots of good choices for reading across all grade levels. There is something here for everyone, but they are not all inclusive. Many of the authors on the list have written other interesting works. Many titles are the first in a series that may spur readers into reading the other books about the same characters. Parents and families will want to preview every title for appropriateness of content, interest, and reading level before selecting it for reading for their children. (https://learningconnection.doe.in.gov/Standards/PrintLibrary.aspx)

Additionally, when reviewing the Indiana Reading List for any grade level, one also finds another important statement.

> Designed as a companion piece to Indiana's Academic Standards in English/Language Arts, the following selections of the Indiana Reading List illustrate the quality and complexity of the suggested reading materials for students in Grades K – 2. The Indiana Reading List is not required reading nor is it meant to be all-inclusive. Teachers and parents are encouraged to review the selections to ensure suitability for the individual student. (https://learningconnection.doe.in.gov/Standards/PrintLibrary.aspx)

The disclaimers are noteworthy and transparent in that these selections need scrutiny by educators and that other selections might also be useful to educate children. What is missing is a checklist to help with that selection and strategies to address misrepresentations. This study will use a list developed by the National Science Teachers Association (NSTA) and Children's Book Council (CBC) to analyze commonly used books listed in the Indiana Reading List for grades K-2. The NSTA/CBC list is the one used for their selection of NSTA's annual Outstanding Science Trade Books list. The analysis will provide insight into any scientific misrepresentations and possible connections to science from the Indiana Reading List's suggestions.

It is important to note that even the most scientifically accurate, readable, and well-illustrated book won't teach

students on its own. A teacher lies at the heart of facilitating information exchange between the text and the reader. Their expertise is vital in making the connection between books and the learning of science concepts. To this end, several strategies to promote best practices in reading and learning science concepts with children's literature will be presented. Last, several quality fiction and nonfiction books aligned to the 2010 science standards will be presented that can help educators build connections between language arts and science.

Method & Results

This study selected the NSTA/CBC's criteria to evaluate the sections listed as Fiction: Picture Books and General Fiction; Folklore/Fairy Tales/Mythology; and Poetry on the Indiana Reading List for grades K-2. The list is found at https://learningconnection.doe.in.gov/Standards/PrintLibrary.aspx). The NSTA/CBC criterion list was chosen because of its national reputation and usage by the professional organizations under which it was developed. Its criteria to evaluate books are:

1. The book has substantial science content;
2. Information is clear, accurate, and up to date;
3. Theories and facts are clearly distinguished;
4. Facts are not oversimplified to the point where the information is misleading;
5. Generalizations are supported by facts and significant facts are not omitted; and
6. Books are free of gender, ethnic, and socioeconomic bias.

To begin the process the three reviewers selected 15 books from the list on which to perform an item analysis to establish inter-rater agreement. The reviewers discussed their initial reviews and results to agree about the interpretation of each criterion and how it should be implemented while reviewing the books. Criterion 1 was identified as appropriate for identifying a connection to science and criterion 2 was identified as appropriate to identify any inclusion of misrepresentations. Criteria 3, 4, and 5 were identified as more appropriate for nonfiction books and not used in this study. Criterion 6 was

included in the item analysis for its importance in meeting all students' backgrounds. After establishing inter-rater reliability, 70 of the 79 books from the identified sections of the Indiana Reading List were evaluated. Nine of the books were unavailable in any local public library or the university library. It was assumed that these books would not be widely used by classroom teachers given their limited availability.

Item analysis reinforced that few of the books on the list contained a significant connection to science or written in such a way as to allow a teacher to creatively use the book to promote interest in a science topic. Only 17% (12 books) met criterion 1 indicating substantial science content. Additionally, many of the selections were interpreted as outdated. The median initial publication date for the K-2 IRL was 1971. Only one book was listed as published after the year 2000.

Item analysis also revealed relatively few misrepresentations among all of the titles. Scientific misrepresentations were found in 17% (12 books) of the K-2 IRL. Since teaching with animal fantasy books included such basic strategies like ensuring students understand that organisms don't talk, the authors did not record this scenario, and similar aspects of teaching in the genre of fantasy, as a misrepresentation. Secondary science misrepresentations that might normally be overlooked when reading a book were those identified in this study. Examples included a person talking under water (misrepresenting sound waves), animals living outside their identified habitats (misrepresenting ecosystems), and animals coming back to life after being frozen (misrepresenting characteristics of living organisms). So, in answer to the research question about the abundance of misrepresentations the study concluded that there were few. However, this is not too surprising since so few of the books included topics connected to science.

Item analysis also revealed that too many books contained gender, ethnic, and socioeconomic bias. Fifty-four percent (38 books) of the books featured male main characters, 26% (18 books) were gender neutral, and 20% (14 books) featured female main characters. Significantly, most of the animal fantasy books referred to the main character as "he." Women were too often shown in aprons and doing domestic chores and diverse and ethnic populations were dramatically underrepresented in

the selected books. These finding supported those of a 2011 Sociologists for Women in Society study that also found similar results for about 6000 leading children's books that were published in the 20th century.

Discussion & Recommendations

Best practices in using children's literature call for more than just reading the book to students. While the various techniques are too numerous to detail here, there are several specifically aligned for science that can be effective. Fredericks's (2003) five-step guided reading strategy closely follows the Engage Explore, Explain, Elaborate, and Evaluate (5E) best practices teaching model in science lessons. Yopp & Yopp's (2006) three-step model follows the same general 5E sequence and to integrate reading activities utilizing science process skills (predicting what will happen in the story based on some of the pictures), incorporating fine arts (choose ten essential vocabulary words from the story to write a poem), and several other strategies like having students compare and contrast (as discussion and by graphing) their selection of key concepts in a story. Effective strategies such as these can lead to student achievement. Marzano (2001) reported that summarizing activities can lead to a 39% increase student achievement. When such activities ask students to compare and contrast similarities and differences student achievement increases by 45%.

All reading strategies can be summarized as having some sort of experience before reading, during reading, and after reading. Doing so helps students comprehend the text, build background knowledge and increase vocabulary. For example, many *before reading* activities require students to predict something about the story from the title or looking at the pictures in the book. *During reading* activities might include having students use sticky notes to record questions/thoughts/predictions while they read or pose problem based scenarios when you are conducting read alouds with students. *After reading* activities might include having students summarize what they have learned through writing paragraphs, drawing pictures, and verbal feedback or having students complete graphic organizers such as concept or semantic maps.

Generally, when using fiction books for a read aloud consider these effective strategies in selecting a book:

- *Setting a purpose*- Select a book that matches the purpose of the read aloud. For instance, is there an upcoming unit on weather? If so, selecting a book about seasons will ignite discussion and lead to questions about weather.
- *Knowing the book*- Preview the book to practice expression and to select places to stop for elaborating, questioning and monitoring comprehension. Also, if the book contains unfamiliar scientific vocabulary or text in another language it may be necessary to practice pronunciation before reading aloud. Many online dictionaries will play an audio pronunciation of the word.
- *Selecting new vocabulary*- The purpose of a read aloud is to expose students to rich vocabulary and text that they could not read on their own. Selecting three new words to highlight and discuss and add to a science word wall will enhance, not only vocabulary knowledge, but also comprehension.
- *Checking for inaccuracies*- If the book contains information about a scientific concept or species that is not depicted accurately it may not be the best choice for a real aloud. Because children are forming their thoughts and opinions about the world around them, it is important to select high quality picture books that are credible.
- *Enjoying the book*- Students will be more motivated to listen and become more involved in the story when they see the teacher enjoying the read aloud. Selecting books that interest not only the students, but also the teacher, is important.

Recently published picture books aligned to Indiana K-2 science standards

The following list of picture books was compiled by using the online catalog of the Indianapolis Marion County Public Library (http://sherloc.imcpl.org/). Keywords from the Indiana Science Standards (2010) were entered, and the reviews for each book were checked to determine the book's quality. The books

selected for the list are no more than two years old. While many of these books could be used with multiple grade levels, only one indicator for each book was identified and is located inside the parentheses.

Ashman, L. (2011). *Samantha on a roll.* Davenier, C. (Illus.). New York, NY: Farrar, Straus, Giroux. 40pp. ISBN 0374363994. Fiction. Samantha straps on her roller skates and goes on a wild ride through town (2.1.5 Observe, demonstrate, sketch, and compare how applied force (i.e., push or pull) changes the motion of objects).

Biggs, B. (2011). *Everything goes on land.* New York, NY: Harper Collins. 55pp. ISBN 0061958093. Fiction. A boy compares and contrasts a wide variety of vehicles as he rides in a car with his dad (K.1.1 Use all senses as appropriate to observe, sort, and describe objects according to their composition and physical properties, such as size, color, and shape).

Fleming, D. (2012). *Underground*. San Diego, CA: Beach Lane Books. 40pp. ISBN 1442458828. Fiction. Creatures that live in the soil are showcased through simple text, detailed illustrations, and endnotes (1.2.4 Observe over time the effect of organisms like earthworms in the formation of soil from dead plants. Discuss the importance of earthworms in soil).

Florian, D. (2012). *Unbeelievables.* San Diego, CA: Beach Lane Books. 32pp. ISBN 1442426527. Poetry. Poems reveal the life cycle of bees. Factual textboxes extend the content (2.3.1 Observe closely over a period of time and then record in pictures and words the changes in plants and animals throughout their life cycles-including details of their body plan, structure and timing of growth, reproduction and death).

French, V. (2011). *Yucky worms.* Ahlberg, J. (Illus.). Somerville, MA: Candlewick. 32pp. ISBN 0763644463. Fiction. A child learns about the vital role of earthworms while helping grandmother in the garden (1.2.4 Observe over time the effect of organisms like

earthworms in the formation of soil from dead plants. Discuss the importance of earthworms in soil).

Messner, K. (2011). *Over and under the snow.* Neal, C.S. (Illus.). San Francisco, CA: Chronicle Books. 44pp. ISBN 0811867846. Fiction. As a young girl glides over the snow on skis, her father points out clues to identify all of the creatures who make their homes beneath the surface (1.3.4 Describe how animals' habitats, including plants, meet their needs for food, water, shelter, and an environment in which they can live).

Fogliano, J. (2012). *And then its spring*. Erin E. Stead. (Illus.). New York, NY: Roaring Book. 32pp. ISBN 1596436247. Fiction. A young boy wonders if the brown of the winter will ever turn green enough to nurture the garden he has planted (2.2.7 Investigate how the sun appears to move through the sky during the day by observing and drawing the length and direction of shadows).

Munro, R. (2011). *Hatch!* New York, NY: Marshall Cavendish Children's Books. 44 pp. ISBN 054731583X. Nonfiction. A variety of habitats are showcased with the types of birds, nests, and eggs found within (2.3.1 Observe changes in plants and animals throughout their life cycles).

Seven, J. (2011). *The ocean story.* Christy, J. (Illus.). Mankato, MN: Picture Window Books. 32pp. ISBN 1404867856. Fiction. A child plays along the shore and learns about the various creatures that live in the ocean and how all living things depend upon one another (1.3.5 Observe and describe ways in which animals and plants depend on one another for survival).

Sidman, J. (2011). *Swirl by swirl: Spirals in nature.* Krommes, B. (Illus.). Boston, MA: Houghton Mifflin Harcourt. 32pp. ISBN 054731583X. Poetry. The beauty and functionality of spirals in plants and animals are explored through rhyme and illustration (K.3.1 Observe and draw physical features of common plants and animals).

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Application of rep-PCR to Screen for Enterotoxigenic *Bacillus* spp. in Artificially Contaminated Nonfat Dry Milk

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Bacillus spp. is normally a considered nonpathogenic soilborne saprophyte species of bacteria that may occasionally contaminate food. Subsequent ingestion of enterotoxigenic Bacillus spp. may cause emesis and/or gastroenteritis. Repetitive element palindromic polymerase chain reaction (rep-PCR) was applied in this study to differentiate Bacillus spp. in artificially contaminated reconstituted nonfat dry milk (NFDM). Thirty-three strains of Bacillus spp. obtained from environmental and food samples were grown in NFDM and subjected to rep-PCR. Agarose gel banding profiles of strains previously found to be enterotoxigenic (those harboring HBL and/or NHE operons) were compared to patterns generated from nonenterotoxigenic strains. Results reveal a distinct diagnostic band (1,230 bp) that appears in all enterotoxigenic strains tested, but not in strains lacking enterotoxin gene targets. Banding patterns among nonenterotoxigenic strains exhibited greater heterogeneity than the enterotoxigenic subgroup. Assay sensitivity in NFDM for B. cereus was found to be 10^3 CFU/ml. Biochemical profiling using the API 50 CHB/E medium and the API 50 CH system was shown to be less reliable in screening for potentially enterotoxigenic Bacillus spp. compared to rep-PCR. This rep-PCR assay holds promise as a useful means for quickly screening novel isolates of Bacillus spp. for potential enterotoxigenicity, even before the strains are identified using conventional methods.

Introduction

Because of their ubiquitous nature and spore-forming ability, *Bacillus* spp. routinely survive food processing treatments such as pasteurization. Surviving spores may then germinate if the food is temperature-abused, leaving vegetative cells to grow well in the absence of competing microflora (Becker *et al.* 1994, Christiansson *et al.* 1999, Cosentino *et al.* 1997, Crielly *et al.* 1994, Leuschner *et al.* 1998). *Bacillus cereus* is the representative food-associated pathogen within the family *Bacillaceae* that may cause illness through the production of an emetic toxin and/or diarrheal toxins (enterotoxins) (Ehling-Schulz *et al.* 2006, McKillip 2000, Schoeni and Wong 2005), occasionally at refrigeration temperatures. Within the last several years, a number of species other than *B. cereus* have been shown to possess enterotoxin genes, and to produce enterotoxin in model broth systems and in food (Beattie and Williams 1999, Damgaard *et al.* 1996, Gaviria *et al.* 2000, Perani *et al.* 1998, Phelps and McKillip 2002, Prüss *et al.* 1999, Salkinoja-Salonen *et al.* 1999, Stenfors *et al.* 2002, Yuan *et al.* 2002), further validating the need to develop reliable detection and screening tools for a microorganism considered by many to simply be a nuisance food spoiler and contaminant.

Foods frequently associated with *B. cereus* diarrheal syndrome include dairy products, although enterotoxigenic isolates have also been obtained from rice, cereals, bread, meat, pasta, spices, and seafood (Becker *et al.* 1994, Cosentino *et al.* 1997, Crielly *et al.* 1994, Damgaard *et al.* 1996, Griffiths 1990, Hsieh *et al.* 1999, in't Veld *et al.* 2001**,** Leuschner *et al.* 1998, Rusul and Yaacob 1995, Torkar and Mozina 2000). The diarrheal form of *B. cereus* food poisoning, arises from the production of at least two types of three-component enterotoxins during vegetative growth of *B. cereus* in the small intestine of the host (Granum and Lindback 2013, McKillip 2000, Schoeni and Wong 2005), making this scenario that of a toxicoinfection. Symptoms in susceptible individuals typically occur at least 12 hours following ingestion of enterotoxigenic *Bacillus* strains (viable cells and/or spores) and include abdominal pain, cramps, and diarrhea. Both hemolysin BL, (HBL) and a nonhemolytic enterotoxin (NHE) complex have been identified and characterized from contaminated foods (Granum *et al.* 1999),

and are considered the primary virulence factors of *B. cereus*. These multiple subunit toxin complexes are encoded by separate operons (Granum *et al.* 1999, Heinrichs *et al.* 1993, Ryan *et al.* 1997), the regulation of which involves the pleiotropic factor PlcR (Okstad *et al.* 1999, Salamitou *et al.* 2000), although the exact mechanism remains unclear.

A unique PCR-based approach to characterizing microbes, repetitive element palindromic-based PCR (rep-PCR), has been applied in clinical settings to differentiate the genetic diversity of bacterial pathogens from hospitals (Snelling *et al.* 1996). Rep-PCR is used to amplify repetitive, noncoding DNA sequences interspersed within bacterial genomes using primers specific to the repeated elements; differences in the resulting banding profile are used to categorize new isolates, or identify strains based on known DNA banding patterns (Versalovic *et al.* 1994). Such a strategy would prove useful if a DNA pattern or "fingerprint" could be established from known enterotoxigenic bacteria and then compared to profiles of new or unknown isolates. A match in the DNA profile would indicate that the new bacterial isolate was likely a pathogenic strain, without directly detecting the toxin gene(s). Herman *et al.* (1998) evaluated rep-PCR to differentiate *B. sporothermodurans* from other *Bacillus* spp., but the focus of the study was not to screen for enterotoxigenic strains specifically. Thus, we felt that the application of rep-PCR for testing artificially contaminated NFDM for enterotoxigenic *Bacillus* spp. would offer speed and cost-effectiveness with the ability for high throughput and sensitivity compared to conventional microbiological approaches. The practicality of a reproducible screening tool such as rep-PCR applied in a food system would therefore offer a number of advantages as a rapid method in food microbiology.

Materials and Methods

Bacterial isolation

The majority of *Bacillus* spp. used in this study are listed in Table 1, some of which were obtained as known pure cultures from other investigators, while others were isolated from food or environmental sources (Phelps and McKillip 2002). Unknown strains were identified at Mississippi State University,

Department of Food Science and Technology, using the MIDI™ system (Microbial ID, Inc., Newark, DE).

DNA extraction and rep-PCR.

In order to demonstrate the potential utility of this assay, a limited set of 33 strains of *Bacillus* spp. were separately grown in trypticase soy broth (TSB) (Difco, MD) and 2.5% (w/v) reconstituted NFDM powder, and incubated at 32°C, shaking (250 rpm) for 15 hours. When cell densities reached 10^6 CFU/ml (as monitored by standard plate count), DNA was isolated from each strain using the Ultraclean™ Microbial Genomic DNA Isolation Kit (MO BIO Laboratories, Inc., Solana Beach, CA) and quantitated using a BioRad SmartSpec™3000 (Bio-Rad, Hercules, CA).

Rep-PCR was performed using a commercial rep-PCR reagent kit (bioMèrieux, Durham, NC) and amplified using a Bio-Rad Gene Cycler (Bio-Rad, Hercules, CA). The rep-PCR reactions included 5X reaction buffer, 10 μg bovine serum albumin, 5 μl of dimethyl sulfoxide, 10 mM each dNTP, 600 ng rep-PCR primers, 2.5 U of *Taq* polymerase (Life Technologies, Grand Island, NY), and 200 ng template DNA in a total reaction volume of 50 μl. For the positive control reactions, 1 μl of template DNA (supplied in kit) was used, or 1 μl of HPLC-grade H_2O for negative control reactions. Amplification consisted of an initial denaturation at 95°C (120s), followed by 31 cycles at 94°C (3s), 92°C (30s), annealing at 40°C (60s), and extension at 65°C (480s). A final extension at 65°C (480s) followed. The rep-PCR products were analyzed on a 1.5% (w/v) agarose gel (Sigma, St. Louis, MO) stained with ethidium bromide, visualized under ultraviolet light, and the images digitally captured using an AlphaImager™1220 Documentation and Analysis System (Alpha Innotech Corporation, San Leandro, CA).

Assay sensitivity of rep-PCR

B. cereus (strain #56, Table 1) was used to determine assay sensitivity. This strain has been shown to harbor both HBL and NHE operons (Phelps and McKillip 2002). The strain was grown aerobically as described above in NFDM at 32°C, and standard plate counts completed to ascertain cell densities for the time points as follows: 0.5 h, 2.5 h, 4.5 h, and 6.5 h. DNA extractions and rep-PCR was performed as described above.

Biochemical profiling

The API 50 CHB/E Medium and the API 50 CH strips (bioMerieux, Inc., Hazelwood, MO) were used in biochemical typing of the isolates under study. *Bacillus* spp. were grown (in triplicate) in the API 50 CHB/E medium overnight at 37°C, shaking (250rpm), for 15 h, and the turbidity of each strain was diluted to a #2 McFarland standard before inoculation of the test strips, which were incubated at 37°C for 24-48 hours, and read thereafter according to the manufacturer's instructions.

Results and Discussion

TABLE 1. Majority of *Bacillus* strains screened in this study. Those in boldface denote enterotoxigenic isolates. The reference number and source for each is included.

Designation	Species/Strain	Source
23	***B. amyloliquefaciens***	**Air**
28	***B. cereus***	**P. Granum, Oslo, Norway**
56	***B. cereus* 1230-88**	**VWR Scientific**
19	*B. cereus* 0075-95	P. Granum, Oslo, Norway
22	***B. circulans***	**Whole milk**
3	*B. circulans*	Chocolate milk #1
48	*B. circulans*	Freshwater aquarium
5	*B. lentimorbis*	Tea
6	*B. lentimorbis*	Whey protein concentrate
8	*B. lentimorbis*	Black pepper
9	*B. lentimorbis*	MSG powder
11	***B. lentimorbis***	**Shrimp homogenate #1**
16	*B. lentimorbis*	Shrimp homogenate #2
17	*B. lentimorbis*	Vitamin supplement powder
27	*B. lentimorbis*	Salsa dip
29	*B. lentimorbis*	ATCC 14580
1	*B. licheniformis*	NFDM powder #1
2	*B. licheniformis*	NFDM powder #2

3	*B. licheniformis*	NFDM powder #3
13	*B. licheniformis*	Shrimp homogenate #1
15	*B. licheniformis*	Shrimp homogenate #2
24	*B. licheniformis*	Chocolate milk
38	*B. licheniformis* PLR-1	McKillip *et al.* 1997
40	*B. marinus*	Shrimp homogenate
21	*B. megaterium* DSM 319	F. Meinhardt, Univ. Munster, Germany
37	***B. pasteurii***	**Soil**
20	*B. subtilis*	Shrimp homogenate
26	*B. subtilis* PY79	L. Kroos, Michigan State Univ.
2	*B. subtilis* B4A	Washington State Univ.
34	*B. subtilis*	ATCC 23856
39	*B. subtilis*	Soy protein concentrate
4	***B. thuringiensis* subsp. *kurstaki***	**Soil**
7	*B. thuringiensis* subsp. *kurstaki*	Whey protein concentrate

TABLE 2. Biochemical comparison between enterotoxigenic and nonenterotoxigenic *Bacillus* spp. using the API 50 CH system. Parenthetical symbols denote slight positive or negative results.

	Enterotoxigenic strain number					**Nonenterotoxigenic strain number**				
	4	**11**	**22**	**23**	**28**	**1**	**3**	**20**	**21**	**38**
Glycerol	+	+	+	+	+	+	+	+	+	+
Erythritol	-	-	-	-	-	-	-	-	-	(-)
D-Arabinose	-	-	-	-	(-)	-	-	-	-	(-)
L-Arabinose	-	-	-	-	(-)	-	-	+	-	+
Ribose	+	+	+	+	+	+	+	+	+	+
D-Xylose	-	-	-	-	+	-	-	+	-	+
L-Xylose	-	-	-	-	+	-	-	+	-	+
Adonitol	-	-	-	-	-	-	-	-	-	-
β Methyl-xyloside	-	-	-	-	-	-	-	-	-	(-)
D-Glucose	+	+	+	+	+	+	+	+	+	+
Galactose	-	-	-	(-)	+	-	-	(+)	-	+
D-Fructose	+	+	+	+	+	+	+	+	+	+

L-Sorbose	-	-	-	-	-	-	-	-	-	(-)
D-Mannose	-	-	-	-	+	-	-	-	-	+
Rhamnose	-	-	-	-	-	-	-	-	-	+
Dulcitol	-	-	-	-	-	-	-	-	-	(-)
Inositol	(-)	-	-	-	-	-	(-)	+	-	+
Mannitol	-	-	-	-	+	-	-	+	-	+
Sorbitol	-	-	-	-	-	-	-	+	-	+
α Methyl-D-mannoside	-	-	-	-	-	-	-	(+)	-	(-)
α Methyl-D-glucoside	-	-	-	-	-	-	-	(+)	-	+
N Acetyl glucosamine	+	+	+	+	(-)	+	+	+	+	(-)
Amygdaline	(-)	(-)	-	(+)	-	(+)	+	+	(+)	+
Arbutine	(+)	+	+	(+)	(-)	+	+	+	(+)	+
Esculine	(+)	(+)	(+)	(+)	(+)	(+)	+	(+)	(+)	+
Salicine	(+)	+	+	+	(-)	+	+	+	+	+
Cellobiose	+	+	+	+	(-)	+	+	+	+	+
Maltose	+	+	+	+	(-)	+	+	+	+	+
Lactose	-	-	-	-	-	-	-	(+)	-	+
Melibiose	-	+	-	-	-	-	-	+	-	+
Saccharose	-	-	+	+	(-)	+	+	+	+	+
Trehalose	+	+	+	+	+	+	+	+	+	+
Inuline	-	-	-	-	-	-	-	+	-	-
Melezitose	-	-	-	-	-	-	-	+	-	-
D-Raffinose	-	-	-	-	-	-	-	+	-	+
Amidon	(+)	(+)	(+)	(+)	(-)	+	+	+	(+)	(-)
Glycogen	(+)	+	+	+	(-)	+	+	+	+	+
Xylitol	-	-	-	-	-	-	-	+	-	(-)
β–Gentiobiose	(-)	(+)	(+)	+	(-)	+	+	+	(+)	+
D-Turanose	-	-	(-)	-	-	-	+	+	-	+
D-Lyxose	-	-	-	-	-	-	-	-	-	-
D-Tagatose	-	-	-	-	-	-	-	-	-	+
D-Fucose	-	-	-	-	-	-	-	-	-	-
L-Fucose	-	-	-	-	+	-	-	-	-	-
D-Arabitol	-	-	-	-	(-)	-	-	-	-	-
L-Arabitol	-	-	-	-	-	-	-	-	-	-
Gluconate	-	-	-	-	-	(-)	(-)	(-)	(-)	(-)
2 ceto-gluconate	-	-	-	-	-	-	-	-	-	-
5 ceto-gluconate	-	(-)	-	(-)	(-)	(+)	(+)	(+)	(-)	(+)

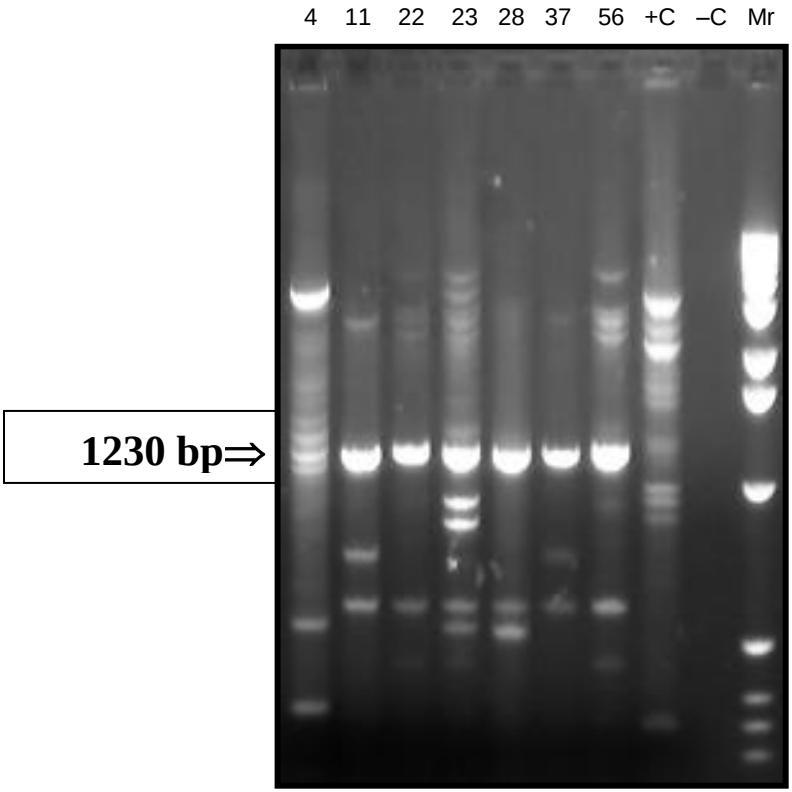

FIG. 1. Rep-PCR profiles of seven enterotoxigenic *Bacillus* strains earlier shown to harbor at least two enterotoxin genes separately grown in sterile, reconstituted NFDM for 15 h at 32^{o}C (as explained in Materials and Methods section). Strain numbers are indicated at the top of each lane and correspond to Table 1. +C denotes the positive control and –C, the negative control; Mr = 1kb mass ladder. Arrow indicates position of diagnostic band.

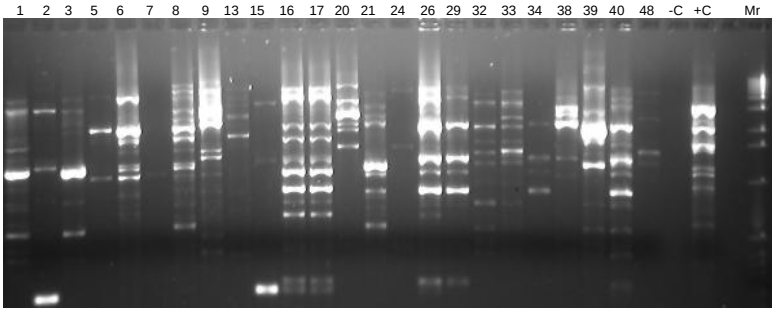

FIG. 2. Rep-PCR profiles of twenty-four non-enterotoxigenic *Bacillus* strains lacking enterotoxin genes separately grown in sterile, reconstituted NFDM for 15 h at 32^{o}C. Strain numbers for each are indicated at the top of each lane and correspond to Table 1. +C denotes the positive control and –C, the negative control; Mr = 1kb mass ladder.

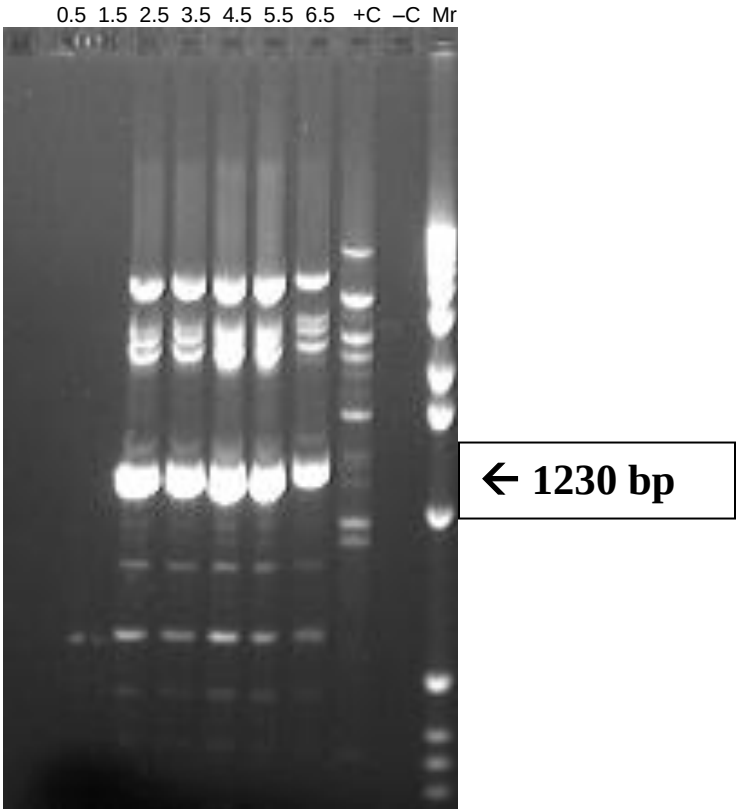

FIG. 3. Rep-PCR assay sensitivity profiles of strain #56 (*B. cereus*) at various time points (0.5 h, 2.5 h, 4.5 h, and 6.5 h growth in sterile, reconstituted NFDM at 32^{o}C). +C denotes the positive control and –C, the negative control; Mr = 1kb mass ladder**.**

Rep-PCR assay

Certain *Bacillus* spp. (shown as boldface in Table 1) had earlier been shown to harbor multiple enterotoxin genes using traditional PCR (Phelps and McKillip 2002). When grown in TSB (data not shown) and reconstituted NFDM (Fig. 1), all seven of these strains exhibited a common diagnostic band of 1,230 bp. This was not present in the DNA banding patterns of nonenterotoxigenic strains grown to the same density (2×10^8 CFU/ml) under identical conditions (Fig. 2). Digital gel images were analyzed using photodocumentation software to confirm the R_f and corresponding molecular weight values for each band in all samples tested compared to known migration distances for standard fragments. In no case did the distinct diagnostic band of enterotoxigenic strains appear in those isolates lacking enterotoxin operons. Moreover, the banding profiles of 24 *Bacillus* strains were heterogeneous with respect to each other.

The rep-PCR reaction conditions employed in this study consistently detected *Bacillus cereus* grown in reconstituted NFDM to densities as low as 10^3 CFU/ml (Fig. 3), as confirmed by standard plate count, which corresponded to 2.5h under the growth conditions utilized in this study.

Occasionally, enterotoxin production from non-*B. cereus* strains have been reported. Isolates of *B. circulans*, *B. lentus*, *B. licheniformis*, *B. mycoides*, *B. subtilis*, and *B. thuringiensis* demonstrated positive results using a commercial RPLA assay (Beattie *et al.* 1999). Several toxigenic strains of *Bacillus licheniformis* related to food poisoning have also been described (Salkinoja-Salonen *et al.* 1999), as have strains of *B. circulans* and *B. megaterium* isolated from clinical and food samples (Rowan *et al.* 2001). Phelps and McKillip (2002) reported several *Bacillus* spp. outside the *B. cereus* group as being enterotoxigenic when grown in milk. Toxin production by other *Bacillus* spp. has for the most part been sporadically associated with *B. thuringiensis*, a member of the *B. cereus* group. Damgaard *et al.* (1996) isolated several enterotoxin-producing strains of *B. thuringiensis* from pasta, bread, and milk. Perani *et al.* (1998) found that 29% of *B. thuringiensis* strains isolated from the environment produced *B. cereus* enterotoxin as measured by the Oxoid RPLA kit. Hansen and Hendriksen (2001) applied enterotoxin gene-specific PCR to a host of *B. cereus* and *B.*

thuringiensis strains with sporadic distribution of the target genes among the isolates.

A recent investigation concluded that within the *B. cereus* phylogenetic group (includes *B. cereus, B. thuringiensis, B. mycoides, B. anthracis, B. pseudomycoides,* and *B. weihenstephanensis*) enterotoxin production is quite variable and may result in immunological detection of toxins when the toxin genes are not detectable by PCR, and vice-versa (Hsieh *et al.* 1999). An additional report on a PCR-based assay for three HBL operon targets similarly provided negative results on RPLA assays (in't Veld *et al.* 2001). Work in our laboratory, in fact, has shown that in some cases only one or two of the three enterotoxin genes of each operon (HBL and NHE) may be detectable simultaneously, while other *Bacillus* spp. may yield signal from all three target genes in the operon (Phelps and McKillip 2002). These differences may be due to sample-to-sample variation in assay sensitivity, minor sequence differences in primer annealing regions among the isolates, or both. Therefore, one or more *hbl* and/or *nhe* genes may be detectable in enterotoxigenic strains by traditional PCR in virtually any combination. It must be assumed that the presence of one or more genes in either operon indicates enterotoxigenic potential of the *Bacillus* sp. being screened. Therefore, any PCR-based assay targeting only one of these genes is inadequate to properly screen dairy products for toxin-producing *Bacillus* spp. A need exists for a PCR assay that is not necessarily gene-specific, but still distinguishes potentially enterotoxigenic *Bacillus* spp. found in food from nonenterotoxigenic strains. Although this study is limited in scope, the data indicate the potential utility of rep-PCR as an approach with efficacy in these areas.

Biochemical profiling

The API biochemical system of differentiating the enterotoxigenic *Bacillus* spp. in this study from their nonenterotoxigenic counterparts took 48 hours to complete compared to 8 hours for the rep-PCR assay. The biochemical testing was unreliable in the ability to distinguish the subgroup of enterotoxigenic isolates from the nonenterotoxigenic strains, and frequently exhibited variable results in carbohydrate fermentation tests within identical replicates of the same strains (data not

shown). Table 2 summarizes results of the battery of API biochemical tests completed. Of the 49 available biochemical tests using the API system, only one carbohydrate and 5 ceto-gluconate yielded a possible means of differentiating the subset of enterotoxigenic *Bacillus* spp. from their nonenterotoxigenic counterparts. High sample throughput using the API system in screening for enterotoxigenic *Bacillus* spp. is not feasible, owing to the labor-intensive method of sample set-up and inoculation procedures, and variable results obtained from attempting to type phenotypic characteristics of these strains.

Traditionally, a biochemical approach has been used for the rapid identification of *Bacillus* isolates using a battery of results from substrate utilization tests in the API 20E and API 50 CHB strips (Logan and Berkeley 1984). The problem with this conventional technique is that it relies heavily on visual inspection, introducing a great deal of subjectivity when interpreting results. Moreover, certain strains exhibit inconsistent results on these biochemical tests, making reproducibility difficult. Since incubation of the test strips is necessary for the API system, this approach does not practically qualify as a 'rapid' means of identifying *Bacillus* spp. (or any bacteria). It should be noted here that our purpose in using the API system was to test these criteria – reliability, reproducibility, and speed – in the differentiation of *Bacillus* spp. from artificially contaminated food, compared to rep-PCR. The latter method exhibits greater potential utility as a screening tool for this purpose.

The prevalence of *Bacillus* spp. in the natural environment, and their spore-forming ability make members of this group inevitable contaminants of many foods. Moreover, the characterization of an expanding pool of psychrotrophic strains has brought increased attention to toxigenic *Bacillus* spp. The ability of enterotoxigenic *Bacillus cereus* to cause foodborne illness in humans via the consumption of contaminated foods such as cooked rice, meat, vegetables, sauces, milk, and a variety of other foods, has brought the focus in our laboratory to developing and implementing sensitive and versatile molecular screening tools that would differentiate enterotoxigenic *Bacillus* spp. from their non-enterotoxigenic counterparts. Some common methods employed to detect and differentiate *Bacillus* spp. include biochemical profiling (API system), random amplified

polymorphic DNA (RAPD)-PCR, arbitrarily primed (AP)-PCR, Real-Time PCR, and pulsed-field gel electrophoresis (PFGE) (Gracias and McKillip 2011, Logan and Berkeley 1984, Svensson *et al.* 1999, Swaminathan *et al.* 1999, Torkar and Mozina 2000), the latter of which is the basis for characterizing food pathogens using the PulseNet food safety database (Swaminathan *et al.* 1999). Rep-PCR, however, is not yet a high-profile tool for characterizing bacteria of relevance to the food industry.

The use of rep-PCR as a molecular screening tool to differentiate enterotoxigenic *Bacillus* spp. from nonenterotoxigenic strains in food is of importance to the consumer of products such as dry and fluid dairy products, spices, rice, and meats/sauces. This method has been recently employed in typing *Bacillus sporothermodurans* and other *Bacillus* spp. isolated from milk (Herman *et al.* 1998), to characterize *Bacillus anthracis* strains (Brumlik *et al.* 2001, Cherif *et al.* 2003), and to obtain enterotoxigenic *Bacillus* spp. DNA fingerprints from naturally contaminated NFDM (Cooper and McKillip 2006). Speed is essential when identifying *Bacillus anthracis* and other pathogenic strains via PCR (Lee *et al.* 1999). Rep-PCR shows broader species applicability and better discriminatory power than biochemical profiling, RAPD, and PFGE. This method allows consistent pattern formation and storage of the data in a database as a digitized image. This could enable the creation of large reference libraries of typed *Bacillus* species. Unknown strains can then be compared against the stored databases across laboratories for identification purposes and to monitor changes in microbial populations (Olive and Bean 1999).

Concluding Remarks

In our lab, rep-PCR has been shown to be a sensitive, reliable, and reproducible assay with respect to screening for potential enterotoxigenic *Bacillus* spp. in the model food system employed herein (reconstituted, artificially contaminated NFDM). Specific DNA fingerprints for each *Bacillus* spp. were obtained using this assay on a preliminary set of strains. The enterotoxigenic strains of *Bacillus* shared a common DNA diagnostic band of 1,230 bp. This fragment is currently being

cloned and sequenced for identification, although it is unlikely to be a toxin-encoding region, as the rep-PCR primers target extragenic DNA elements, not open reading frame sequences. It is important to note, however, that this band was common in all *Bacillus* strains harboring at least one of the two enterotoxin operons. The assay was sensitive in detecting *Bacillu*s cells at 10^3 CFU/ml of NFDM, reducing the need for time-consuming enrichment steps prior to setting up PCR reactions. Rep-PCR therefore holds promise as a useful means of screening for potentially enterotoxigenic *Bacillus* spp. in foods, and would serve as an effective quality assurance/control technology easily transferable for routine application.

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Molecular Forensic Analysis Using Ribonucleic Acid (RNA) - Based Approaches

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This review outlines the potential of ribonucleic acid (RNA) methods for biological evidence analysis. Nucleic acid sequence based amplification (NASBA) is a laboratory method for identification of body fluid type, as well as a more common RNA-based detection method, reverse transcriptase polymerase chain reaction (RT-PCR). NASBA and RT-PCR are directly indicative of gene expression levels in cells/tissues of interest. Either NASBA or RT-PCR could be used for extremely sensitive body fluid identification based upon amplification of tissue-specific mRNA transcripts present in a given forensic sample, commonly known as 'transcriptome' analysis, especially when combined with real-time detection chemistries.

Forensic Evidence Backlog

Forensic backlog occurs when the forensic evidence pertaining to specific criminal cases accumulates due to the inability to properly analyze such evidentiary samples and to produce results pertaining to a particular case. In 2000, the DNA Analysis Backlog Elimination Act was implemented. This act effectively dispersed grants to states to accommodate the collection and analysis of DNA samples from offenders for use in the FBI Combined DNA Index System (CODIS), among other purposes (Thomas, 2006). As of 2003, 200,000-300,000 convicted offender samples and over 540,000 evidentiary

samples (where no suspect has been identified) were still awaiting DNA analysis (Counterterrorism, 2004).

Since the Backlog Elimination Act was implemented, some $50,000,000 has been dispersed for DNA analysis of crime scene samples, as well as to increase public laboratories' capabilities to perform DNA analyses. The majority of these funds have been awarded to law enforcement or other government agencies. The average forensic laboratory possessed approximately 390 backlogged cases at the beginning 2002; each laboratory then received some 4,900 additional cases, and completed only 4,600 cases within the given year (Peterson & Hickman, 2005). Based upon this information, over 600 cases were backlogged and sample analyses were not performed for this evidence during that particular year per laboratory. In addition, the United States Government funded a census of publicly funded forensic crime laboratories in 2005 and discovered that number of cases requiring DNA analysis had increased by 38% since 2002 (DNA Initiative, 2010). Based upon this information, backlog of nucleic acid evidence has yet to be reduced by any substantial measure.

Nucleic Acid as a Tool in Forensic Analyses

Over the past several years, DNA typing has offered much to forensic science in terms of sensitivity and power to individualize biological evidence to match alleged perpetrator (Genge, 2002). Saliva, blood, and other bodily fluids, if properly collected and stored, provide invaluable material from which forensic investigators extract DNA for analysis.

Even though DNA is an excellent source for verifying the perpetrator of a crime, DNA analysis can be controversial. For example, several recent debates have circulated in the literature regarding how many variable regions must be examined to verify that no two individuals share the same DNA homology (Moore, 2004). Such debate is extremely important considering how easily a jury can be persuaded into believing that a suspect's genetic material was found on criminal evidence based upon the testimony of an "expert" forensic investigator.

Even though a DNA fingerprint is unique and can provide a vital link to the true criminal, it is important to remember that the DNA evidence retrieved from the scene of a crime is not

necessarily that of the perpetrator. For example, a hair may be found at the crime scene but the individual from whom it originated was not even at the scene when the crime occurred (Moore, 2004). It is common knowledge that the human body loses large amounts of genetic material in the form of skin, hair, etc., continually, and this genetic material can be dispersed anywhere from the workplace to multiple public areas where a crime could occur. Thus, an individual could be placed into custody and could face a trial for a crime that he or she did not commit simply because the DNA evidence was so quickly accepted as the primary source of evidence that links this person to the crime that was committed. For this reason, DNA evidence must be treated equally when compared to other evidence and requires corroboration with other sources such as witnesses or alibis.

RNA Analysis

Although DNA is typically extracted and analyzed from forensic case evidence, ribonucleic acid, or RNA, may also be isolated from an evidentiary sample for analysis. The need for RNA analysis using molecular-based technology to definitively identify a given body fluid and screen a sample for adequate DNA to perform traditional identification analyses has clearly been established in current forensic literature (Counsil & McKillip, 2010). Messenger RNA (mRNA) has great promise for forensic analysis because it is expressed in a tissue specific manner and thus can be used to differentiate multiple bodily fluids in a sample collected at a crime scene (Juusola & Ballantyne, 2005). Because each body fluid has a specific transcript profile (transcriptome), a particular fluid residue can be determined based upon its specific mRNA target sequence. For example, the housekeeping genes β-actin or GAPDH are constitutively expressed in saliva, semen, and blood samples, whereas the genes statherin or histatin 3 are present in great abundance solely within saliva (Juusola & Ballantyne, 2003). Thus, identification of tissue-specific genes could be utilized to more sensitively detect the presence of a given body fluid from a forensic sample via molecular methodology. Furthermore, by analyzing the precise abundance or lack of specific mRNAs that are present in an evidentiary sample along with other evidence

at the crime scene, investigators can gain insight into the type of crime (e.g. assault, murder) committed and whether DNA typing using STR analysis is necessary (Nussbaumer *et. al*., 2006). In a study by Nussbaumer *et al.* (2006), a real-time reverse transcriptase PCR (RT-PCR) application was employed which allowed for detection of multiple mRNA transcripts expressed in different body fluids ranging from saliva to semen and vaginal secretions. This procedure screened samples containing bodily fluids to quantify the levels of gene expression and to determine whether STR DNA analysis could be performed to produce successful results.

Messenger RNA is also an excellent source for forensic investigation due to its relative stability when properly collected and stored. Several reports have stated that RNA can possibly remain stable and therefore useful for analysis for weeks to months (Nussbaumer *et. al*., 2006). Another study found that mRNA used for forensic profiling was stable from stains up to ten weeks in age and could be successfully reverse transcribed in RT-PCR, amplified, and visualized using agarose gel electrophoresis (Juusola & Ballantyne, 2003). The field of forensic pathology has used mRNA transcripts retrieved from days-old cadavers to ascertain a more substantial link between the autopsy results and the physical evidence (Zhao *et. al*., 2009). Specifically focusing on saliva samples, mRNA has been analyzed from simulated forensic evidence for up to 547 days at room temperature, while saliva mRNA exposed to simulated environmental conditions (e.g. variable light, temperature, and rainfall) was extracted in nanogram quantities after seven days of continued exposure (Setzer *et. al*., 2008). Thus, one can determine that mRNA is a very persistent and reliable source of forensic evidence, even when exposed to harsh surroundings. Regardless of stability, use of RNA in forensic applications has drawbacks. If a forensic sample is not properly collected and stored, then the mRNA within the evidence can degrade. This could be rather problematic if biological material was collected as evidence. RNA degradation by ribonucleases (RNases) can quickly degrade RNA that is exposed to an unprotected environment laden with RNases (Int'l Society for Complexity, Information, and Design, 2005). For this reason, RNase contamination of a forensic sample is the greatest concern

investigators must face when working with RNA that is to be analyzed.

In addition, the methodology employed to retrieve and analyze RNA can cause problems with regards to quantity and stability. For example, an experiment by Kumar *et. al* (2006) indicated that there were no valid mRNA transcripts present in saliva evidentiary samples. Articles previously published by Ballantyne and additional forensic investigators have alluded otherwise, thereby calling into question the methods utilized for this particular experiment by Kumar *et. al*. (2006). Dr. Ballantyne responded to the journal from which the Kumar article was published, explicitly citing the fact that the researchers amplified genomic DNA pseudogene signals, which are a form of contaminant in their samples (Ballantyne, 2007). Thus, a researcher's experimental technique alone can ultimately impact the effect of mRNA evidence and its uses.

Saliva Evidence

The body fluid saliva possesses a wealth of genetic material that can be utilized to identify either a victim or perpetrator of a crime. Saliva samples excised directly from a crime scene or the surrounding area must be examined to determine sample origins and better clarify the events which occurred as the criminal act took place. Forensic serology involves the examination and identification of the effects and properties of saliva, sweat, semen, and blood (Crime Library, 2010). Forensic serology focusing on saliva has of late turned to novel fields of investigation. For example, saliva can be utilized in the field of Toxicology to detect the use of given drug such as marijuana based on the presence of tetrahydrocannabinol, or THC (Fatah & Cohn, 2003). Even though such applications of forensic serology are promising, a majority of cases in forensic analysis of saliva often specifically involve alleged sexual assaults or homicides (Bauer & Patzelt, 2002). Evidence collection from such cases, especially that originating from sexual attacks where the victim is still alive and traumatized, can be a grueling experience given the situation and the narrow time frame between the encounter and evidence collection.

With regards to salivary evidence, such material can be collected from a multitude of areas on the body. Areas often

directly associated with saliva evidence include bite marks, where the bodily fluid is directly deposited (Anzai-Kanto *et. al*., 2005), or from areas of forced interaction during the crime such as the oral cavity or genital areas; however, it should be noted that salivary evidence can be found on secondary items in need of investigation such as locations associated with the crime (e.g. fabric or carpet) or items used in an attempt to remove any incriminating evidence.

The former, more direct areas of contact during sexual assault often require a great deal of intrusive collection by a licensed medical practitioner or forensic nurse to properly collect saliva samples to be used in conjunction with forensic analysis. Such samples are collected via commercially-produced rape kits, which include several sterile cotton swabs for fluid collection and instructions with respect to locations in which samples should be taken. These areas of sample collection are varied, from the less-intrusive gathering of evidence from fingernail scrapings and articles of clothing to the in-depth swabbing of rectal/vaginal areas and the sampling of pubic hair (Petter & Whitehill, 1998). Such samples are to be collected from the victim within forty-eight to seventy-two hours to prevent any contamination or sample degradation.

Following collection, samples must be analyzed to determine whether saliva and additional bodily fluids are present. Investigators utilize colorimetric, presence/absence tests to verify whether saliva is in a forensic sample. For example, the SALIgAE saliva test provided via Abacus Diagnostics (West Hills, CA) utilizes the presence of the enzyme amylase in the bodily fluid for identification via a yellow color development in the presence of saliva (Miller & Hodges, 2005). Amylase is one of the initial enzymes utilized in the process of digestion, whereby it begins breaking down starch into sugar while in the saliva (Rolfes *et. al*. 2008). Several such presence/absence testing kits (e.g. Phadebas, Starch-Iodine mini-centrifuge kits) have become available and offer varying degrees of sensitivity with regards to saliva detection (Myers & Adkins, 2008). These kits also detect saliva based on the presence of amylase. Although such kits are relatively cheap and produce results quickly with little turnover time, the enzyme amylase can lead to many issues with regards to positive identification of saliva.

Foremost, it should be noted that the amylase enzyme is not exclusive to humans; rather, it can also be derived from primates and other mammals (Willott, 1974). Therefore, one can use the aforementioned saliva detection kits to verify the presence of saliva from the evidence collected, but it cannot definitively determine that the saliva present is human. Aside from the issue of human versus mammalian evidence, amylase is not found solely in saliva. In fact, it is well-documented that the enzyme is also found in bodily fluids such as semen (Auvdel, 1986). Although amylase is reported to be in much higher quantities in saliva compared to that of semen, such a fact is negated by colorimetric tests that lack a means of quantification.
Based upon the overlapping presence of the amylase enzyme in multiple bodily fluids, it is entirely possible that a sample analyzed by colorimetric testing might indicate the presence of saliva, when in fact a mixed forensic sample consisting of various bodily fluids might actually contain semen or a mixture of both fluid types. It is due to these conflicting possibilities in the outcome of colorimetric results that bodily fluids such as saliva are now undergoing analysis utilizing modern approaches entrenched in molecular applications. Moreover, mRNA as of recent has been viewed as a promising candidate for specific identification of saliva.

The use of mRNA transcripts present in saliva has numerous applications in a wide variety of medical fields. For example, one can utilize mRNA transcripts associated with the spermidine/spermine N1-acetyltransferase (*SAT*) gene as an oral cancer biomarker (Park *et. al*., 2006). In terms of high specificity and sensitivity with molecular-based mRNA applications, the aforementioned transcript is but one of several that have been shown to detect the presence of oral cancer in 91% of samples examined (Zimmerman *et. al.,* 2007). In terms of forensic implications, several previously mentioned studies by Juusola and Ballantyne (2003, 2005) have alluded to a need for mRNA analysis of bodily fluid samples for definitive identification. Simulated exercises involving time-degradation of mRNA have even led investigators to the discovery up to fourteen stable mRNA markers for use in identifying both blood and saliva in forensic samples (Zubakov *et. al*., 2008). Small Proline Rich Protein (SPRR) markers such as *SPRR3* and *SPRR1A* are

present only in saliva and are therefore not detected in semen, thereby eliminating the dilemma discussed with colorimetric detection assays. Aside from the use of mRNA taken directly from saliva, bacterial isolates and the mRNA therein can also be utilized to determine whether the bodily fluid is present. Such investigation falls under the scientific branch of microbial forensics.

Microbial Forensics

Predating back to 1995, microbial forensics was suggested as a tool to combat acts of bioterrorism (Jain *et. al*., 2005). Due to the terrorist attacks on September 11, 2001 and the resulting bioterrorist attacks *via* the use of the pathogenic bacterium *B. anthracis*, microbial forensics has propelled itself to a mainstay discipline in forensic investigation. In its modern infancy, microbial forensics was implemented to identify strains of potential pathogenic bacteria, verify those laboratories and individuals possessing such strains for distribution, and then link the subculture of bacteria used for a potential attack to the original culture. The U.S. government actually defined microbial forensics as "a scientific discipline dedicated to analyzing evidence from a bioterrorism act, biocrime, or inadvertent microorganism/toxin release for attribution purposes" (Budowle *et. al*., 2003). Since the time following the development of microbial forensics, this discipline has branched out beyond its government-derived definition and broadened its investigative horizons.

Current microbial forensics has developed into a large field with many applications. One such function involves the use of microbial forensics and diagnostic microbiology (Atlas, 2004). This collaboration combines the best in analysis and determination of the causative agent (diagnostic microbiology) with the sample collection, evidence handling, and investigation of the pathogenic source (microbial forensics). Microbial forensics has recently delved into aspects of animal health monitoring, as well. For example, it is possible to determine whether a pathogen has been discretely released into the population by monitoring animal morbidity and mortality rates, areas of increased disease states or death, etc (McEwen *et. al*., 2006). Aside from investigation into bacterial forms of

bioterrorism, microbial forensics also examines additional forms of pathogens which might be utilized in an attack on a person or group of people. A case involving the use of HIV as a weapon of harm against another person is just one example of microbial forensics extending its reach beyond the realm of bacteria and widespread bioterrorism applications (Budowle & Harmon, 2005). These advances in microbial forensics have even led to its establishment in current forensic investigation pertaining to the identification of bodily fluids.

A study by Nakanishi *et. al.* (2009) successfully utilized bacteria present in saliva to verify the presence of said body fluid. The investigators utilized molecular real-time PCR techniques to amplify mRNA transcripts from the Chaperonin gene associated with the oral streptococci bacteria *S. salivarius* and *S. mutans* present in saliva. Previously, such bacteria were only utilized to confirm bite mark evidence; however, a PCR-based application focusing on bacteria within the body fluid could provide yet another unique method by which saliva identification could be performed. Thus, the use of microbial forensics and advanced PCR techniques could provide a wealth of information to be used in multiple facets of investigative research.

Although the use of microbial forensics to identify saliva via PCR-based methodology is stimulating, it is important to note that there are multiple real-time chemistries available to perform such an analysis, and that each of these chemistries possesses unique properties in regards to sensitivity, yield, etc. Numerous forensic experiments discussed previously herein focus on mRNA analysis through the use of Reverse-Transcriptase PCR, known more commonly as RT-PCR. Although widely used and accepted, it is important to compare this method to a lesser-known yet equally efficient technology known as nucleic acid sequence based amplification, or NASBA. Only through the most extensive, analytical comparisons of these similar technologies will the most proficient and responsive chemistry for forensic saliva analysis prevail.

The Use of Polymerase Chain Reaction for Forensic Evidence Analysis: NASBA and RT-PCR

In a molecular study (Juusola & Ballantyne, 2003), mRNA was utilized for identification of body fluid samples. The study

used the PCR application known as RT-PCR to verify whether mRNA was present in total RNA extracts retrieved from evidentiary samples containing bodily fluids.

Traditional RT-PCR is rather simple in its methodology, consisting of four basic phases (Bustin, 2002). The primary target molecule in the reaction is mRNA, with an intermediate target consisting of cDNA, and the end-product (known in PCR as an amplicon) is DNA. The first phase of RT-PCR is known as reverse transcription, whereby the single-stranded mRNA is used as a template to create a complimentary DNA (cDNA) strand. The enzyme responsible for the production of the cDNA strand is known as reverse transcriptase. The mRNA and cDNA strands are bound together in what is referred to as a heteroduplex, termed as such due to the differing types of nucleic acids bound together within the complex. The second phase of RT-PCR is known as the denaturation phase. During denaturation, the mRNA:cDNA heteroduplex is separated via high temperature, leaving the cDNA ready for the third phase: annealment.

During annealment, sequence-specific primers designed by the researcher via computational program or simply included in the commercial RT-PCR kit will bind to the cDNA strand. At this point, a DNA polymerase enzyme will proceed to recognize the primer sites and begin synthesis of a DNA strand complimentary to that of the intermediate reaction target. Thus, the final amplicon produced for detection during RT-PCR is DNA.
The final phase of RT-PCR is quite variable, as it focuses on detection of the DNA end-product. Figure 1 displays the overall RT-PCR procedure utilizing scorpion primers employed for real-time detection of the amplicon (Bustin, 2002). Currently, there are many real-time detection chemistries available to aid in both the detection and quantification of the RT-PCR amplicon. Although a complete explanation of real-time PCR detection chemistries is outside the scope of this review, the reader is encouraged to refer to two recent sources that proved a comprehensive perspective on the theory and practice of these approaches (Reddy *et. al*., 2007; McKillip & Drake, 2004). Having examined the process behind RT-PCR, it is imperative that one examine its applications in forensics.

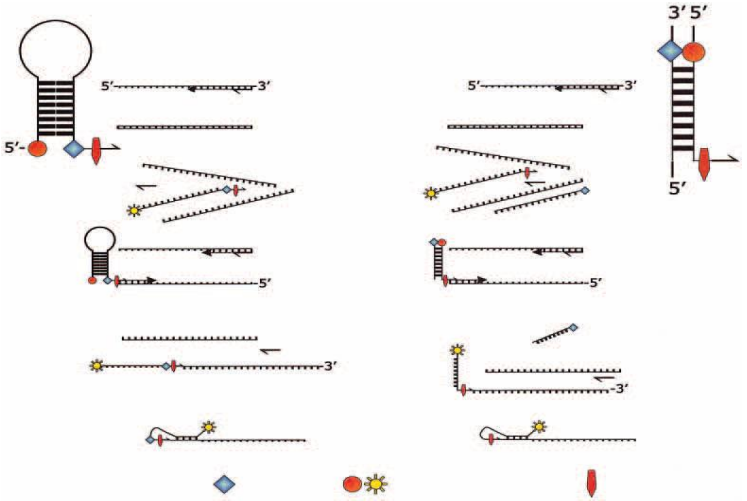

Figure 1. RT-PCR procedure as directed according to the text. Scorpion primers utilized therein act as the real-time detection chemistry.

If the precise type and quantity of mRNA from a forensic evidence sample can be determined, then it is possible to identify the precise body fluid or tissue from which the specific mRNA molecule originated. Thus, one could use RT-PCR to screen a forensic sample to determine whether there is an adequate amount of genetic material within for forensic analysis to be performed. The study by Bauer and Patzelt (2002) utilized RT-PCR and mRNA to identify menstrual blood stains. This study showed that RT-PCR could successfully identify body fluids from dated forensic cases by using mRNA from the case evidence. By verifying mRNA levels substantial enough to yield RT-PCR results, the likelihood of similarly being able to purify adequate DNA amounts for fingerprint (STR) analyses is also high.

Although using RT-PCR seems to be an excellent idea, this application is not without its drawbacks. Because traditional RT-PCR has been determined to be only semi-quantitative due to the application's insensitivity to ethidium bromide (the stain used to observe separated DNA molecules in an electorphoretic gel). However, microcapillary electrophoresis has been used to

assess RT-PCR results. Though this assay is similar to traditional gel electrophoresis, it uses narrow capillary tubes and an electrical current to effectively separate molecules such as DNA or RNA based upon molecular weight (Beckman Coulter 2007). Additionally, through the advent of real time detection chemistries in PCR procedures (Hunt, 2010), unparalleled sensitivity is now possible for forensic DNA analyses. Real-time PCR is capable of detecting extremely small amounts of mRNA found within a minuscule tissue sample, primary cells, or in the case of forensics, traces amounts of bodily fluids found at the scene of a crime. Aside from RT-PCR (traditional or real-time) aforementioned mRNA applications, there is yet another process by which the mRNA amounts present in a sample can be successfully measured using a PCR reaction. This PCR procedure is known as NASBA.

NASBA is a highly sensitive *in vitro* RNA transcription based amplification system (Dieman *et. al*., 2002). The NASBA reaction (Figure 2) is an isothermic procedure (Compton, 1991) used to detect expression of a gene of interest present in a particular sample (Rodriguez-Lazaro *et. al.,* 2004). The process begins when an oligonucleotide antisense primer (P1) anneals to the RNA target present in the nucleic acid extract obtained from a particular sample. The 3' end of the P1 primer is complementary to the beginning of the target sequence; the 5' P1 end contains the T7 RNA polymerase promoter (Innovative Biotechnologies Int'l., 2006). After annealing, the reverse transcriptase activity of AMV-RT generates a cDNA complement to the RNA target. The RNA portion of the resulting DNA:RNA hybrid molecule is removed through the action of RNase H. This permits the sense (P2) primer (which is complementary to an upstream portion of the RNA target) to anneal to the cDNA strand. Subsequently, the DNA-dependent DNA polymerase activity of AMV-RT creates a double stranded DNA copy of the original RNA target with a fully functional T7 RNA polymerase promoter at one end.

The promoter is then identified by the T7 RNA polymerase, producing a large amount of antisense, single stranded mRNA corresponding to the original RNA target. These antisense RNA transcripts can then function as templates for repeated rounds of NASBA, all performed isothermically (Gracias & McKillip, 2007).

The typical level of mRNA amplification is at least a factor of 10^9 (Innovative Biotechnologies Int'l., 2006). An intermediate product of this reaction is the RNA:DNA heteroduplex formed during the initial amplification cycle (Uyttendaele *et. al.,* 1994). The major NASBA amplicon produced during amplification is mRNA, and this amplicon can be detected in a real time format as it is being amplified. For instance, fluorescent dyes or molecular beacon probes (Gionata *et. al*., 1998) can be used to detect the RNA product produced by NASBA using real time detection methods. Because NASBA is a closed tube, relatively inexpensive, isothermic procedure that does not require time consuming thermocycling steps that are necessary for most PCR applications, this system could be used to effectively process forensic evidence samples and thereby reduce case evidence backlog.

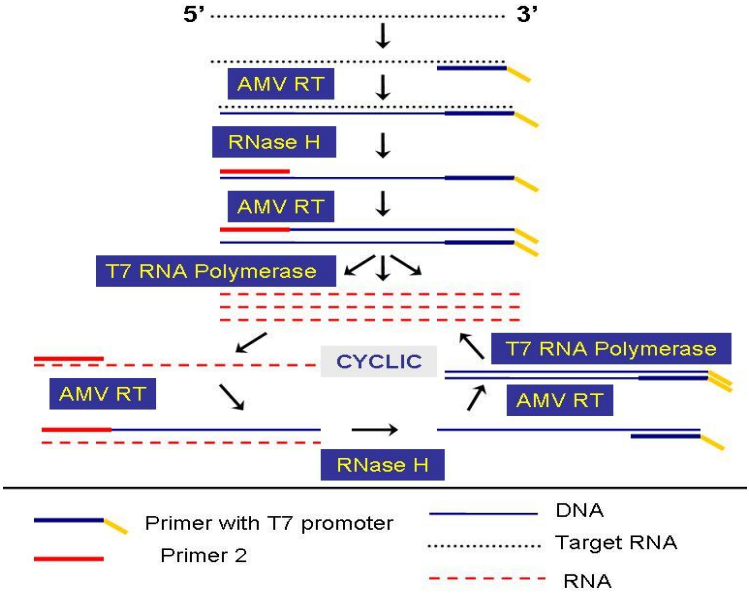

Figure 2. NASBA procedure as directed according to the text (Fig. adapted from Counsil & McKillip, 2010).

NASBA has many practical applications in terms of RNA-based research and even provides overlap into multiple fields of

scientific inquiry, thereby making this technology most beneficial. NASBA can be collectively utilized for both genotypic analysis and virology, as determined by its ability to ascertain the *CCR5* chemokine receptor genotype in humans and better understand the interaction between the receptor and the HIV virus, which uses the receptor as a portal of entry (Romano *et. al*., 1999). NASBA may be used within a clinical setting, as well. For example, NASBA could be used in hospitals and health clinics to detect the presence of a myriad of sexually-transmitted infections (STIs) including but not limited to Chlamydia, herpes, hepatitis, and human papillomavirus (Dieman *et. al*., 2007). Moreover, clinical diagnosis of infectious disease could involve the use of NASBA, which has been able to detect the presence of pathogens such as *Mycobacterium tuberculosis*, West Nile virus, and influenza. Food safety and health department laboratories could benefit from NASBA when inspecting food and water products for the presence of a myriad of pathogenic bacteria such as *Salmonella enterica* or *Legionella pneumophilia*. In addition, the use of NASBA as a means to both determine nucleic acid quantity and reduce forensic case backlog via timely evidence analysis has been well documented (Counsil & McKillip, 2010). Thus, NASBA is a versatile tool capable of use in a broad range of scientific research.

Now that both mRNA-based applications have been addressed, a few observations of comparison between RT-PCR and NASBA must be examined, specifically in terms of processing time, cost, and sensitivity. One study by Van der Meide *et. al* (2007) determined that RT-PCR seems to possess an advantage in terms of sample process time. In the study, both NASBA and RT-PCR commercial kits were utilized for *Leishmania* parasite analysis. According to the researchers, the RT-PCR kit required a mere two handling steps to process a sample, with assay completion occurring at approximately two hours and twenty minutes. However, the NASBA kit utilized required eight steps to process a sample and utilized an assay run time of four hours and thirty minutes. Although it seems that RT-PCR dominates the field of mRNA analysis in terms of sample throughput, it should be noted that this study used NASBA with electrochemi-luminescence (ECL) detection. Such real-time detection chemistry is both expensive and time-

consuming, especially when compared to the short time duration necessary to detect mRNA amplification via intercalating dyes such as SYBR Green II or in-situ detection of a given amplicon *via* scorpion primers, molecular beacons, etc. Thus, the advantage with regards to turnaround and processing time appears coincide with RT-PCR, but only because the kit-based procedure requires less preparation and handling before utilizing the assay.

In addition to its victory in the realm of processing time, RT-PCR also appears to have the advantage over NASBA in terms of cost effectiveness. Excluding the cost of primers and real-time detection chemistries, a single Nuclisens NASBA kit (Biomereiux, Durham, NC) capable of analyzing 100 samples costs approximately $1,300. An Epicentre MasterAmp RT-PCR kit (Epicentre, Madison, WI) also capable of analyzing 100 samples costs nearly three-fold less, at $400. Thus, a simple cost analysis determines that NASBA is quite expensive, especially given the current economic climate and the general sentiment favoring cost cutting measures in the workplace.

Although NASBA would appear to be avoided as a useful tool based upon processing time and cost, it is an advantageous technology based upon its heightened sensitivity with respect to amplification versus its RT-PCR counterpart. In a study comparing PCR-based methods and traditional culture techniques for detecting Rhinoviruses, NASBA out-performed RT-PCR in terms of sensitivity. The study determined that NASBA sensitivity peaked at 80.9%, while RT-PCR detection of Rhinovirus serotypes achieved 61.9% sensitivity (Ieven *et. al*., 2000). A comparative study between the two technologies was performed in conjunction with SARS virus detection, as well Keightley *et. al*. (2005). The study revealed that NASBA SARS *pol* 1 target sequence was more sensitive in terms of detection when compared to that of the RT-PCR TaqMAN SARS *pol* 1 target, surpassing the RT-PCR by a two-fold magnitude. Another SARS-based study performed by Chantratita *et. al* (2004) strengthens the support for NASBA, claiming a ten-fold increase in sensitivity when using NASBA with molecular beacon detection versus RT-PCR utilizing a hybridization probe (Chantratita *et. al*., 2004). Thus, it appears that NASBA could prove itself useful in terms of increased detection due to its

heightened sensitivity, especially when challenged with a sample containing a low copy-number of nucleic acid template. Such a scenario is completely plausible when analyzing forensic evidence, especially given the fact that most evidence is exposed to various contaminants, temperature variations, etc. which all lead to degradation of nucleic acids used for analysis. NASBA would therefore be more cost-effective in the long term scope of forensic body fluid identification if it could successfully amplify all mRNA extracted from a sample of varying quality. Costs would build exponentially through the use of RT-PCR kits that fail to identify the bodily fluid due to diminished sensitivity.

The aforementioned experiments pertaining to NASBA sensitivity are medically-related and focus upon virology. With applications in a forensic setting, it is imperative that an in-depth comparative analysis of both RT-PCR and NASBA be performed to truly determine the most appropriate and useful technology. Such an experiment must focus upon several key elements, namely sensitivity, processing time, and low-template quantity. Only after such validation studies have been completed will forensic investigators truly be able to determine which technology is the most appropriate tool for mRNA analysis leading to body fluid identification.

Based upon current literature regarding the identification of saliva using genetic markers from oral streptococci found therein, this research seeks to determine the best methodology by which to identify said transcripts. To this end, NASBA and RT-PCR have been utilized in conjunction with varying quantities of mRNA template from the Chaperonin gene of the saliva associated bacterium *S. mutans* to determine which application is most suited for continued forensic analysis and research of body fluids based on sensitivity and reaction efficiency.

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Measuring Elementary Teachers' Perceptions as an Initial and Partial Assessment of the Impact of the Indiana Science Initiative

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This study provides insights into elementary teachers' perceptions of classroom environments spanning the launch of a major state science initiative. The initiative promotes science literacy through inquiry-based science kits. Results indicate that, after the launch, inquiry-based kits are more prominent in regional classrooms, there is an absence of science safety plans, and nearly 60% of students participate in science fairs. Reported challenges and rewards of teaching elementary science were examined. Teachers report a diminishing challenge for needed equipment and technology and a significantly rising challenge for balancing kit dynamics. Rewards of student interest and using hands-on learning science were reported.

Introduction

The survey was born out of a goal to investigate, at a regional level, the state of science education in the elementary school setting. In doing so, educational stakeholders are provided a view into current practices and environments. The need for such a process originates from comprehensive efforts to promote science education at all levels of education. A concern for the state of education in Indiana is evident from data reported in the Nation's Report Card, a report developed by the National Council for Education Statistics (NCES) as part of the National Assessment of Education Progress (NAEP). Among other things, the report card provides a comparison of the performance of

students in fourth, eighth, and twelfth grades across several demographics. Indiana students score only marginally higher than the national average. Using the national comparison data from 2009, Indiana's fourth graders' average score of 153 ranked above the national average of 149 (U.S Department of Education, 2009a). Fourth grade males scored 153 while the females scored 152.

In 2009, Indiana's eighth grade students' average score of 152 ranked above the national average of 149, with males, once again, scoring higher (151 versus 147) than females (U.S Department of Education, 2009b). Researchers have attributed several factors that might contribute to this disparity of males scoring higher than females. Teachers and systems may hold perceptions that males have more innate abilities related to math and science and may subconsciously preference, motivate and track males toward math and science as appropriate interests (Osborne, Simon, & Collins, 2003). Also, tests may be designed in such a way as to preference males versus females (Penner, 2003).

Indiana NAEP scores, as do national scores, begin to show even greater discrepancies when comparing science achievement between races. At the fourth grade level, white students in Indiana score 158 while black students score significantly lower at 129. Hispanic students average 136 and score only marginally higher than black students and rank significantly below white students. The gap widens as all populations retest in eighth grade. Much has been written about this achievement gap and efforts to combat it (Lee & Luykx, 2007).

Irrespective of race, students who qualify for free and reduced lunch face continually score lower on test of academic achievement such as the NAEP. Indiana scores reflect that. Indiana fourth grade students qualifying for free and reduced lunch averaged a score of 141 points compared to 162 points for those that did not. Similarly to racial gap increases, the gap for students qualifying for free or reduced lunch widens from the fourth grade (21 points) to eighth grade (26 points). Similar findings across the nation also exist and help propel much of the existing reform movements at the national and state levels.

The Indiana Science Initiative

As highlighted above, Indiana scores are only slightly better in science than the national average. Because of the desire and need for Indiana to improve, the Indiana Department of Education unveiled a reform effort called The Indiana Science Initiative (ISI) in 2010 to promote inquiry-based science in grades K-8. Research shows that inquiry-based science greatly improves student interest and success in the classroom (Anderson, 2007; National Research Council, 2011). The ISI focuses on several key aspects of invigorated efforts to help students learn. They include incorporating commercially based science kits and utilizing student notebooking that emphasizes data collection, student writing, and student drawing of science phenomena as they study it. Working with several educational stakeholders and following training and pilot programs, the ISI began distribution of the kits to schools in fall 2011 (Indiana Science Initiative, 2010). The ISI reform movement focuses in inquiry-based kits and notebooking because of their demonstrated success in foster scientific thinking and student learning. And, importantly, it creates an agenda to move science into the primary grades for many schools that overlook it while focusing on language arts and mathematics. As science becomes more commonplace in these primary classrooms, educators will need to adapt practices and environments to handle the realignment. Questions such as, “Are safety protocols in place” and “Does the initiative foster interest in science” need to be addressed.

Key Strategies of Indiana Science Initiative

Commercially available kits are paramount to the ISI, and science education reform overall, because of they are intended to deliver a more focused and better designed curricula than textbooks. Textbooks, and their accompanying teacher’s manuals, may be filled with various activities that present information in a stepwise fashion and struggle to connect concepts in a temporal and integrated fashion (Fulton & Campbell, 2004). Because of this presentation, elementary students mostly experience lessons that are disjointed, do not build upon one another, and are forgettable due to the textbook’s lack of inquiry-based, problem-solving approach. Inquiry-based

kits on the other hand, are designed to present a sequence of lessons that build upon one another conceptually and developmentally. Therefore, prominent reform movements call for utilizing inquiry-based science kits in order to promote best practices in science teaching and assessment (National Sciences Resource Center, 2011).

Many of these assessment protocols require students to write out (often in notebooks) project designs, data collections and analyses, and explanations and justifications in student work. Such practices address the National Science Education Standards (NSES) Standard A (Science as Inquiry) and the NSES Teaching Standards A (Teachers of science guide and facilitate learning) and C (Teachers of science engage in on-going assessment of their teaching and of student learning) (National Research Council, 1996). The outcome is that notebooking allows students to form cognitive structure for the information they learned. Rowell's (1997) meta-analysis on writing in science concluded that students could improve their conceptual understanding of science through notebooking. Herman (2005) reported that fifth grade students, and in particular those with defined low socioeconomic status, that participated in a notebooking experience outperformed those that did not as measured by the state's high stakes assessment test. Other researchers have also consistently proven the promises of using student writing in notebooks (Baxter, Bass, & Glasser, 2001; Fellows, 1994; Glynn & Muth, 1994; Hand, Prain, & Wallace, 2002).

In addition to helping students through writing, notebooks also enhance learning through student drawings and graphic organizers (Glynn & Muth, 2008). Edens and Potter (2003) Investigated fourth and fifth grade drawings and concluded that they can help students achieve. These results paralleled others by Gobert and Clement (1999) and Van Meter (2001). The later two studies concluded that when intermediate elementary students generated diagrams and drawings in their learning they outperformed students utilizing text only learning on instruments that measured conceptual understanding and problem solving. Other researchers support similar conclusions about the use of visual literacy in science notebooks such as the impact on observational skills and nature portrayal (Bricker, 2007) and

conceptual understanding beyond the human scale (Minogue, Wiebe, Madden, Bedward, & Carter, 2010).

Purpose of the study

Striving to answer the research goal to investigate the environments of elementary classrooms and opinions of teachers as schools transitioned to the ISI, the authors created a survey that was used to address the following five research questions to meet the goal for investigating the initial, regional impact of the ISI upon elementary classrooms.

1. What percent of teachers report that they are using commercially based inquiry kits in their teaching at their school?
2. What percent of teachers report that their school has a science safety plan?
3. What percent of teachers report that their school conducts a science fair?
4. What do teachers report as their three greatest challenges about teaching science in their elementary setting?
5. What do teachers report as their three greatest rewards about teaching science in their elementary setting?

The safety plan question is an important question since many primary level classrooms are now conducting science, whereas they may not have been doing so before. Additionally, the type of science in elementary classrooms may be shifting with the incorporation of inquiry-based kits. Examples include raising organisms, incorporating engineering and constructing projects, and testing of natural materials like rocks, minerals, and plants. These processes mirror the types of science activities that warrant safety plans that are not as evident in cookie cutter, disjointed activities in traditional textbooks. And, as students start to participate in authentic based science experiments, research question three becomes relevant as a way to initially and partially assess if students, teachers, and schools are extending their culture of science learning.

Method

To answer the research questions, an open-ended survey was created and face and construct validated. Called the Survey of Elementary Science Teaching (SEST), the instrument was distributed to regional K-5 elementary teachers. The sample of convenience was derived because of the researchers' connections to teachers through a university-based field practicum program. The sample includes responses representing 31 different elementary schools which are all are located within four southern Indiana counties. School demographics included urban (52%), suburban (33%), and rural (15%) schools. The teaching population mirrors the U.S. Census population for the region; American Indian (0.3%), Asian (1.6%), Latino/a/Hispanic (6.0%), Black (12.6%), White/not Hispanic (81.5%), Native Hawaiian (0%), and two or more (2.9%) (United States Census Bureau, 2010).

Multiples surveys from the same school were deemed acceptable since prior conversations with regional teachers resulted in differing answers to the first three research questions and would have differing answers to the final two research questions.

The surveys questions were straightforward and well aligned to the research questions.

1. Are you using FOSS, STC, SEPUP, or other inquiry-based science kits in your teaching at your school?
2. Does your school have an official science safety plan and what is it?
3. Does your school conduct a science fair, why or why not?
4. What are the three greatest challenges about teaching science in your current elementary setting?
5. What are the three greatest rewards about teaching science in your current elementary setting?

Research questions one and two required standard frequency counts to scrutinize. Research question three required a frequency count and sorting and collapsing of the responses into similar categories. Research questions four and five were the most challenging to answer since answers varied. Similar to

research question three, the researchers sorted and collapsed responses to these research questions into representative categories. To complete this process, the researchers selected 15 surveys on which to perform an item analysis to establish inter-rater agreement. The researchers discussed their initial reviews and results to agree about the interpretation of each response and into which representative category it should be summarized. Item analysis was then performed on the remaining surveys. As the surveys were analysed, researchers came to agreement, if discrepancies existed, to create a final entry for tabulation. A small number of surveys were returned with no answers on individual items and resulted in a lower response for those questions. Similarly, if a response indicated the teacher did not know the answer then the question was not included in the tabulation. Other questions on the survey, if answered appropriately, were maintained.

Results

Eighty-five surveys were returned and yielded the following data to answer the research questions. Thirty-eight surveys were collected during spring 2011 (before ISI implementation), 20 surveys were collected during fall 2011 (Initial semester of ISI implementation), 27 surveys were collected during spring 2012 (second semester of ISI implementation).

Research question one asks, “What percent of teachers report that they are using commercially based inquiry kits in their teaching at their school?” The results indicated that after the first year of the Indiana Science Initiative the percent of teachers reporting that their school used inquiry-based kits rose from 52 percent to 96 percent (see figure 3).

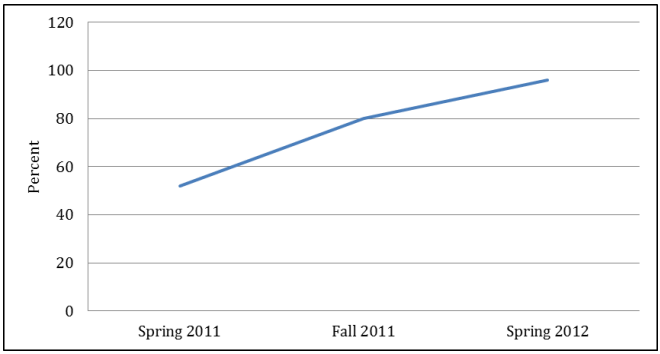

Figure 1. Percentages of teachers reporting that their school uses commercially based inquiry kits (n=79).

Research question two asked, "What percent of teachers report that their school has a science safety plan?" The results shown in figure 2 indicated the percentages of teachers that reported they have a safety plan was lower following the first year of the ISI implementation (21% compared to 7.5%).

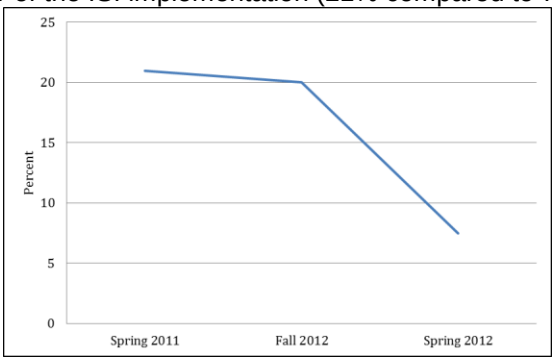

Figure 2. Percentages of teachers that reported that their school has an official science safety plan (n=85).

Only fourteen teachers over the entire survey period reported they had an official science safety plan (spring 2011 = 8, fall 2012 = 4, and spring 2012 = 2). The common elements for the safety plans are categorized and shown in figure 3. The most common element reported included the use of safety equipment,

such as an eye wash station. The second most common response included appropriate attire such as gloves and goggles.

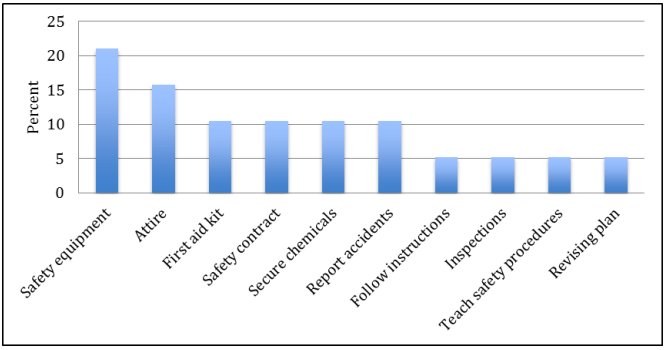

Figure 3. Percentage of teachers reporting common elements of an official school science safety plan (n=14; 8 in spring 2011, 4 in fall 2011, and 2 in spring 2012).

Research question three asked, “What percent of teachers report that their school conducts a science fair?” The results shown in figure 4 indicated a one-year trend upward from 50% to 58% of schools that incorporate a science fair into students’ experiences.

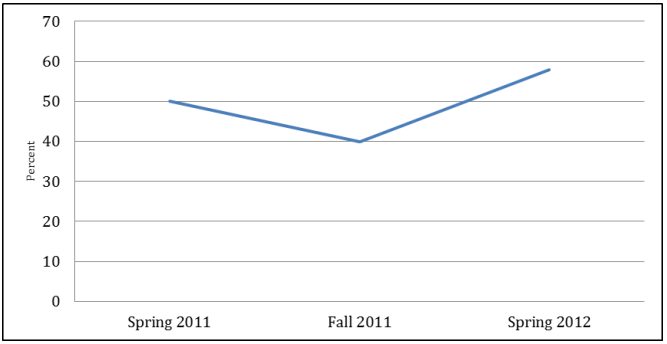

Figure 4. Percentages of teachers reporting that their school conducts a science fair (n = 84).

Research question four asked, "What do teachers report as their three greatest challenges about teaching science in their elementary setting?" Tables 1 and 2 list the greatest challenges prior to and after ISI implementation, respectively.

Research question five asked, "What do teachers report as their three greatest rewards about teaching science in their elementary setting?" Tables 3 and 4 list the greatest rewards prior to and after ISI implementation, respectively.

Table 1. *Challenges expressed by teachers on the SEST prior to the ISI implementation (spring 2011).*

Challenges	Frequency	Percent of responses
Attention of students	4	5
Cost/Budget	7	8
Kit dynamics	0	0
Lack of tech/equipment	19	22
Meeting standards	8	9
New to science education	1	1
Other	0	0
Physical dynamics	9	10
Safety	1	1
Student deficiencies	8	9
Support administration	6	7
Switching classes	2	2
Time management	23	26
Totals	88	100

Table 2. *Challenges expressed by teachers on the SEST after the ISI implementation (fall 2011 and spring 2012).*

Challenges	Frequency	Percent of responses
Attention of students	13	12
Cost/Budget	2	2
Kit dynamics	20	18
Lack of tech/equipment	9	8
Meeting standards	6	5
New to science	2	2

education		
Other	3	3
Physical dynamics	5	4
Safety	2	2
Student deficiencies	10	9
Support administration	1	1
Switching classes	10	9
Time management	30	27
Totals	113	100

Table 3. *Rewards expressed by teachers on the SEST prior to the ISI implementation (2011).*

Rewards	Frequency	Percent of responses
Hands on learning	7	9
Influencing academic and social growth	17	21
Integrating learning	10	12
NOS	4	5
Other	5	6
Parent support	0	0
Resources	2	2
School organization	7	9
Student creativity	6	7
Student interest	10	12
Teachable moment	13	16
Totals	81	100

Table 4. *Rewards expressed by teachers on the SEST after the ISI implementation (fall 2011 and spring 2012).*

Rewards	Frequency	Percent of responses
Hands on learning	23	19
Influencing academic and social growth	14	11
Integrating learning	8	7
NOS	8	7

Other	1	1
Parent support	1	1
Resources	7	6
School organization	3	2
Student creativity	12	10
Student interest	29	24
Teachable moment	16	13
Totals	122	100

Discussion

Research question one indicated that the ISI has successfully integrated kits into regional schools. With such an immediate infusion of the kits into classrooms, professional development and school team level planning that correlates to the teaching and assessment strategies accompanying these kits is important. Notably, teachers were, and will be, more fully implementing the strategies of notebooking and narrative writing in science as part of the ISI curriculum. Knowing these kits are in classrooms will help teachers, school administrators, and professional development partners begin efforts to assist teachers in the strategies.

Research question two revealed that about only 10% of teachers reported their school had an official science safety plan. Although many elementary teachers understand the importance and promote safety in the classroom, it is imperative that teachers, school administrations, students, and parents know a plan is in place. Such protocol helps with classroom management, promoting best practices in science, and legal necessities. This is especially important to primary classrooms where students are learning to follow proper science protocols emphasizing safety. This counterintuitive finding is interesting. Why is science safety decreasing if the amount of science is increasing? One explanation may be that with an increase in science teachers are realizing a general safety plan for the classroom/school is a different than an “official” safety plan for science. Upon communicating these findings with regional schools and administration, plans and timelines are in place to develop and establish safe practices in the elementary science classroom.

Research question three indicated an upward trend in the number of schools that were conducting or participating in science fairs. This finding is encouraging as an indicator for the ISI's impact on promoting inquiry in science through capstone events like science fairs. Perhaps, the day-to-day inquiry nature of the inquiry-based kits impacted teachers' perceptions of the reward for open-ended investigations promoted in science fairs. Additional data collection will help establish a more reliable measure and insight into this finding.

Research question four revealed some intriguing results and concerns. Before and after implementing the ISI, the greatest challenge for teachers was time management in the classroom. This isn't surprising and is an often-cited concern by classroom teachers. As expected, kit dynamics rocketed to the top of teachers' challenges (0% to 18%). Comments often included statements such "kits not arriving on time" or "kits/materials not delivered in proportion to students". Though concerns about kits were apparent, it may have helped lead to a decrease in lack of technology and equipment (22% to 8%) since the kits normally contain all of the materials needed to teach their lessons. Regional efforts to increase technology in the classroom were also underway and may have also influenced a decrease in this concern. However, a more likely and very important consideration is how an increase in teaching with kits may be leading to a decrease in the use of technology since many of the kits' lessons don't overtly incorporate technology. Teachers and researchers should explore the question, "Are inquiry-based kits impacting the time and resources teachers might use to incorporate technology into science." Additionally, a drop in the challenge of meeting standards was noticed (9% to 5%) and may be attributable to the alignment of kits to the state standards.

Research question five revealed some interesting and key dynamics about life in the elementary science classroom. Student interest and hands-on learning ranked forth and fifth before ISI implementation and ranked first and second after implementation. Expressions for student interest doubled (12% to 24%), as did hands-on learning (9% to 19%). With a more scripted curriculum it wasn't surprising to see a drop in the reward for integrating learning (12% to 7%). Perhaps the scripted

curriculum also attributed to a decrease in teachers' expression for influence on academic and social growth (21% to 11%).

Conclusions

The ISI has successfully infused science kits into elementary classrooms in the southern region of the state, but teachers are experiencing concerns about teaching them. As science is becoming more commonplace in the elementary grades, many teachers need to develop and follow appropriate safety protocols to help students practice safe science. Intermediate grade teachers may need to support other teachers with developing safety protocols since they have more routinely previously taught science. National models of safety could be adopted or other community resources could be leveraged to develop safety policies in school buildings or system wide levels. Increased participation in science fairs, a form of inquiry-based science activity, was reported and may be attributed to the increased focus on inquiry-based science.

More research is needed to deeply and broadly measure teachers' perceptions and student learning in elementary classrooms through the sustained efforts of the ISI. For now, several interesting inferences can be drawn from this collection of data using the SEST into elementary teachers' perceptions of the state of science education in their regional classrooms. An emerging glimpse into these rooms revealed initial success for infusing kits into the classrooms to promote inquiry that increased teachers' perceptions for student interest and engagement.

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Tales from the Front Curve: Experiences of a Female STEM Educator taking an Undergraduate Physics Class

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This article contains the experiences of a female mathematics educator taking an inquiry-based undergraduate physics course as a student. The struggles of female students in science classes are told from the viewpoint of a credentialed, extroverted insider.

Introduction

What follows in this essay are tales from my experiences as a female mathematics educator being on the front line as a student in an undergraduate physics course at Penn State Erie: The Behrend College. I decided to take Physics 211, a freshman-level Mechanics course, because a team of faculty members at Penn State Erie were researching the effectiveness of the course, which had been redesigned to be "lectureless" and use active learning. I am very much in favor of inquiry-based learning and have facilitated graduate-level education courses on inquiry, so I signed up with the research team to help give them an inside look at what was going on in their class. The professors saw some gender inequities in the class when it was offered in previous semesters and they were working to fix these issues. We informed all the students on the first day of class that I was a researcher participating in the class - a fact which was forgotten rather quickly, as I was mostly treated the same as other females in the class (as you'll see, neither my mathematics expertise nor my faculty status got me any preferential treatment).

About Me

I have a bachelor's in mathematics, a master's in applied mathematics, and a doctorate in mathematics and science education and work as an Assistant Professor of Mathematics Education. When I started taking physics in the Spring 2008 semester, it was the first time I had seen physics since high school (14 years ago). While I did not take physics as an undergraduate or graduate student, I took several biology and chemistry courses during my college career. I never took physics in college because, unlike my other high school science classes, my high school physics class wasn't very good (there were no labs and it was all rote learning). In high school, I signed up for as many science courses as my schedule would allow, sometimes two or more science courses at a time, and I won the mathematics and science department awards as a graduating senior (the first time both departments had ever given their awards to the same person). So, I have a good working knowledge of both mathematics and science.

About the Class

The physics class met three times per week, for two hours on Mondays and Wednesdays and one hour on Fridays. My section of the class was full, with a total of 72 students. Roughly 15% of the class was female, and roughly 10% were minority students. Roughly 80% of the students were engineering majors, with the other 20% being science majors. Eight large round tables were filled by three groups of three-student teams. Those three-student teams had three possible seating choices: left, middle, or right. The middle seat was where most of the action was: in front of every middle seat was a computer (three computers per table, one computer per group), and when the equipment for the labs was set up it often went primarily in front of the computer and, hence, the middle person.

Each student was assigned to a team of three. The teams were switched after each exam, so over the course of the semester a student had four different teams. The teams were created based on the research findings of Beichner (2000), Beichner, et al. (2006), and Handelsman (2004), the pioneers of this inquiry-based physics course, which has now taken hold at more than 50 universities nationwide. The first teams of the course were

split by gender (all of the females were put into female-only groups). The research about this physics course (Beichner, 2000; Beichner, et al., 2006; Handelsman, 2004) indicates that this guarantees that female leaders will emerge in the class. The second, third, and fourth teams of the course were created by taking one person with an exam score from the top third of the class, one with a score in the middle third of the class, and one with a score in the bottom third of the class. Research by Beichner (2000), Beichner, et al. (2006), and Handelsman (2004) indicates that mixed-ability grouping is the best way to proceed with the rest of the physics course groupings.

My First Group

According to the grouping protocol, my first group was composed of myself and two other white females. I sat in the left seat (I did not get to use the computer or put any of the lab equipment set-ups together at all in this group). We were at a table with two other all male groups. One day we were working on a task that involved slopes and other concepts straight from calculus. I felt right at home with my mathematical knowledge and we finished our sheet and I was 100% positive that our answers were correct (I've been teaching calculus for 10 years and all of the female students knew this and knew that the questions on the sheet were mathematical questions). We had worked through each question and carefully justified the answer using ideas from calculus. The professor then asked us to check our answers with the other groups at our table. This is when chaos ensued. The two male groups at our table had the wrong answers (they had gone with their preconceived notions of how things should be physically rather than applying mathematics). One male student got up and came around the table to get me to explain my answers. I had to turn away from my group to explain the answers to him over my left shoulder. He wanted me to justify every one of my answers and yet did not actually change his answers when presented with reasoned explanations from a mathematics faculty member.

When I turned back around to my group, both of the other girls in my group were changing their answers to be incorrect to follow what the male groups had. They had not asked for any justification for changing their answers; rather the boys in the

other group had simply said, "These are the right answers," and the females changed our correct answers on our team report. They were about to turn the one team paper in with all wrong answers on it, when the professor decided to put the worksheet up on the overhead and go through the answers. As the professor went through the answers, the girls just changed their answers back again. The males railed against the answers that the professor was putting up, but they begrudgingly changed their answers. As the teams turned in their three papers with all correct answers, I wondered if the males had even listened to any of my mathematical explanations or whether they couldn't see past my pink notebook to hear my reasoning.

My Second Group

As per the mixed-ability grouping protocol, my second group was composed of one white female from the top third of the Exam 1 scores (me - I received a 100% score on Exam 1), one white male student from the middle third, and one white female student from the bottom third. In most of the all-male groups, the person from the top third emerged as the group leader, but that was not the case in my group. Our group's male student dominated the group for the entire month. Neither myself nor the other female got to use the computer in that group as the male student usurped the middle seat the first day and pretty much never let us touch anything after that, including using the computer or setting up the labs. Sometimes I was allowed to turn in my paper as the one team paper (if I had the neatest handwriting that day).

For example, one day during this group, the professor put on the directions sheet that we should switch roles for the day (whoever had put the set-ups together the least should do it today, take turns doing the computer tasks for the day, take turns being the scribe on the one team paper). Having heard in the physics research group faculty meeting that I had never put a set-up together or used the computer, the professor made a special effort to visit our table at the beginning of class and say "Who has put the set-ups together least in this group?" Now, the other girl had yet to put any set-ups together either, but after a long pause, I realized she wasn't going to pipe up, so I said "me." The professor said "Okay then, Danielle, you're putting the

set-up together today." The male student was standing there listening, holding the track (a fairly large device that's used with a cart to simulate frictionless movement) in his hands. He didn't move to give me the track. The professor actually then told the male student to put the track down so I could do the set-up. After the professor walked away, the student still didn't put the track down, murmuring, "Let me just help you adjust this," while he was setting the feet of the track in place. When he finally put the track down, I got to work setting up the lab: a cart with a force meter was supposed to be set up on the left end of the track and a motion detector and some other things were to be set up on the right end of the track. As I installed one bolt to connect the force meter to the cart on the left side of the track, the male outfitted the entire right side of the track. Then, he took the left side equipment back out of my hands and finished setting up that side as well. Apparently, installing one bolt somehow counted for me setting up the lab.

When we were getting ready to move on to the second activity about an hour later that day, the male student remarked, "My friend from the earlier class said this worksheet is impossible to get done in time because there's too much computer work on it." In a snarky tone, I said back, "We'll get it done, you're really fast at the computer," and he replied, "You know that's illegal, we have to take turns using the computer today." So, even though he had just acknowledged what was *supposed* to happen, he then proceeded to do all six parts of the computer work for the activity. Despite the fact that I had asked to participate in setting up the lab and using the computer, and the professor verbalized that he wanted our group specifically to share in the responsibility for the labs, the other female student and I were powerless to do anything in the group.

My Third Group

Having never used the computer, I decided that the first day of my third group that I would get to class as early as possible to secure the middle seat. I did so, and as it drew near to class time, one of my group members, an Asian-American student, showed up and sat on my left. As I was to find out later, I was in the top third of the Exam 2 scores, the male Asian-American student was in the middle third, and our other team member, a

white male, was in the lower third. The white male showed up about 15 minutes late to class and did not sit on my right, rather he sat on the left of the Asian-American student and gradually started pushing us over until I was no longer in front of the computer. Now, the professor knew of my plan to get there early to secure the computer seat, so when he looked over and saw me not at the computer, he came over and asked "Danielle, I thought you were going to run the computer in this group?" I replied that I was and asked the Asian-American student if he would move to my right. As I was helping him move his things, the white male, reaching over from the left, logged into the computer and started the computer work. A few times that period I got to touch the computer mouse, but often, the low-achieving white male reached bodily across me from the left to take the mouse out of my right hand to take over. That particular white male student had one of the lowest Exam 2 scores of any student in all of the sections of the course, so he dropped the course shortly after that day.

The first day that the Asian-American student and I were alone in our group, we quickly realized that neither of us had ever done a full lab set-up nor run the computer for an entire class. It was two months into the course and we were doing these things for the first time. That day we had to put a set-up together with a lot of tricky parts and, as we struggled to put it together, the strangest thing happened. The professor came over, took the clamp out of my hands, and clamped the set-up to the desk for us. I had never seen him actually help a group put a set-up together before, so I was a bit in shock that he was squatting down and fiddling with the clamp. The physics research team and I now call this scenario "Damsel in Distress." We began to wonder if I was sending off unconscious signals for the teacher and teaching assistant to help me. Even though I resented myself for it later, somewhere deep inside, I was relieved that he took that clamp out of my hand. It saved me from having to take a risk. It saved me from having to defeat the stereotype threats I was facing in the course.

At one point that month, we actually had to ask the teaching assistant to stop hovering near our table that group (he was perched a few feet away, ready to swoop in at the slightest sign of trouble). One objective of the course was for the students to

struggle with science on their own and, because of unconscious gender issues, the teacher, the teaching assistant, and I had all inadvertently undermined my learning. Luckily, we had quite a few more class periods to go in that group and eventually, because of my efforts to share responsibilities equally between myself and the Asian-American student, both he and I became fairly fluent with the computer and putting together the set-ups.

Moving Forward

As the course is coming to a close, we have switched to our fourth groups. I am the member of the group from the top third of the Exam 3 scores, another white female is the middle third representative, and a white male is the bottom third member of our group. So far, the other girl and I have shared the leadership role of the group. We take turns running the computer, doing the set-ups and turning in our paper. The male member of our group is sort of a "third wheel" who is struggling to keep up with us, even when we take the time to be sure to "leave no child behind." In the group next to us, there is another group with a girl who is a strong, intelligent co-leader of her group that we often share ideas with in productive ways.

As the course has gone on, I think the gender gap in terms of leadership and achievement may be closing (the female students started the course with more females proportionally in the lowest third than is statistically probable and by Exam 2 had worked their way up to a statistically even split across the thirds). After Exam 3, the course averages for the females were no longer statistically different from the male students. Through the hard work of all involved to be more transparent about gender issues in the classroom, we have definitely made some headway. Because of this transparency, the professors and teaching assistants worked together to find strategies to alleviate gender issues in the class.

For future semesters, the physics professors at Penn State Erie will definitely have my experiences on their radar. The research group and I presented at a gender conference at the university and many female students expressed the idea that my stories were giving more of a voice to their experiences (both in science classes and in classes in other disciplines). We are now working to find the proper vehicle to use to bring potential gender

issues to light in future semesters of the physics course. I am very thankful to the physics department members for letting me see this course from the inside (since becoming consciously aware of gender issues as part of my doctoral thesis research, I hadn't been back in the classroom as a student) and for working so hard to implement change. When I stand on the front line with my big female voice, it is definitely a "front curve." It's important for people to see that even someone who is as credentialed and extroverted as I am can face these issues as a female member of an undergraduate science class, so we must keep working hard to promote gender equity in schools.

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