

## Monmouth County Department of Health

### Environmental Laboratory

#### ANNUAL ACTIVITY REPORT 2006

#### About the Laboratory

The Monmouth County Department of Health Environmental Laboratory is a NJDEP certified laboratory(#13417) and operates in compliance with the requirements of N.J.A.C. 7:18 Regulations Governing the Certification of Laboratories and Environmental Measurements. Samples are analyzed for the purpose of establishing compliance with NJDEP regulatory programs and therefore the laboratory is required to maintain certification in accordance with the chapter. In recent years the laboratory

Total Suspended Solid	pH
TR Chlorine	Enterococcus
Fecal Coliform	Total Coliform
E. coli	Hetero Plate Count
Specific Conductance	Salinity
Temperature	Ammonia
Nitrate-N	Phosphorus (total)
Turbidity	Dissolved Oxygen

Figure 1. NJDEP Laboratory Certified Parameters 2006

has shifted away from maintaining an extended list of certified parameters and currently holds certifications in tests that are integral to our Ambient and Coastal Monitoring Programs and related Water Pollution Control activities(Figure 1). Some drinking water certifications are maintained which are used for evaluating well water. A testing services contract is held with a commercial laboratory for other parameters. In addition, macro-invertebrate bio-assessment and phytoplankton identification/cell counts are performed.

Proficiency testing is administered by the NJDEP Office of Quality Assurance. The laboratory was audited by the NJDEP Office of Quality Assurance in September of 2006.

## Highlights

### Educational Outreach and Collaborative Efforts

In March, Becky spent a morning at Ranney School with the 7<sup>th</sup> grade science classes. The students were entertained by the aquatic macroinvertebrate collection. The creatures were represented by over 21 Orders and families of bottom-dwelling, swimming, case-making, crawling, skating and diving (to mention a few) all predator and prey alike, aquatic macroinvertebrates. In the classroom, the students got an idea to have teams measure the length of the super-hellgrammite, and, as it turned out, it actually exceeded the size range stated in our aquatic entomology book. The hellgrammite, which is a dobsonfly, is an incredible 94 mm. The event was informal, educational and inspirational for all. Students used petri dishes and magnifying lenses in order to closely examine characteristics of aquatic organisms and the adaptations for the niche that they occupy.

### The Halloween Bug-off Event

The laboratory organized an Entomological program for the Health Department (October 31<sup>st</sup>), which featured a powerpoint presentation on Forensic Entomology in the



Figure 3. Hematophagous bed bugs *Cimex lectularius*

conference room. The answer to the mystery of *what ate the hole in the dead guys arm and then lived in it* was answered. A laboratory kiosk was set up to loop our powerpoint presentation on Chagas Disease, a protozoan illness spread by Triatome bugs (Figure 2) that is endemic in Central America. A selection of the lab's Entomology collection was made a museum for



Figure 2. Hematophagous Triatomine bug

microscopic viewing of various parasitic and filth insects. The hematophagous bed-bugs (Figure 3) were probably the most viewed specimens. A "Bugoff" identification challenge took place using 17 insects, also from the lab collection.

### Speaking Engagements

In October, Becky presented at The Microbial Source Tracking Workshop. The presentation was on how our lab has applied Polymerase Chain Reaction (PCR) on coastal bacterial isolates to identify species such as *Enterococcus faecalis*, *E. faecium*, and *Staphylococcus pasteurii*. The recreational bathing regulatory community will at some point be using the revolutionary technology of qPCR to provide rapid bacterial enumeration of waters. The MCHD laboratory effort using qPCR technology is to explore the field while we develop our ideas on how it can be used as an investigatory tool.

In April, a 2-day Technical Water Monitoring Workshop sponsored by the NJDEP was held in Bordentown, NJ. The workshop included a series of speakers presenting innovative research on water monitoring issues. Becky spoke in the recreational bathing

breakout session on the topic of *Enterococcus*.

Becky presented an overview of MCHD water monitoring activities to the NJ Water Monitoring Coordination Council. The Health Department is a NJWMCC member. The mission of the council is to promote collaboration, communication, and coordination between groups that collect water monitoring data.

### **Training**

On March 10<sup>th</sup>, Dr. Barry Kreiswirth and Dr. Natalia Kurepina of The Public Health Research Institute in Newark visited the laboratory to learn how the enterococcus test was performed for the recreational bathing program. The laboratory staff visited the PHRI lab on March 29<sup>th</sup> to use the Cepheid SmartCycler for DNA amplification and fluorometric detection, using probes specific for *E. faecalis* and *E. faecium*.

Becky attended a 2-day Polymerase Chain Reaction(PCR) Course at the Waksman Institute of Microbiology at Cook College. She learned various techniques including cloning DNA to RNA followed by amplification of RNA using polymerase and primers. PCR involves repeated copying of a small target of DNA using 2 DNA primers that flank the target. Gel Electrophoresis was used to visualize the migration of nucleic acids to compare banding patterns. Also included sample purification, and a RAPD quick technique to compare 2 samples only to see if they are a match. All of these techniques are widely used in various disciplines.

Becky and Dyna attended a free midge(Diptera, Chironomidae) identification course which was offered by the NJDEP as part of the Technical Water Monitoring Workshop. The identification of midges to species is added value to bioassessment surveys. Not speciating midges is a loss of information, especially here in the coastal plain, where sometimes 50% of a sample is midges. Chironomid midge species have a range of tolerance values within their Order. Macroinvertebrate data submitted for inclusion on the NJ Integrated Site List will, most likely, need to be identified to the species level in the near future.

Tick identification, educational material, and counseling was provided to residents who brought in their ticks. Residents were also made aware that the MCMosquito Commission would be able to test a black-legged tick for the *Borrelia burgdorferi* spirochete. Many residents seemed to be interested in this service.

Dyna was invited to speak at a MC Health Officer Meeting. Many of the water quality complaints that the environmental staff and laboratory investigates are those caused by nuisance algae blooms. Dyna described problems that are commonly encountered in freshwater lakes and ponds, such as the pea-soup green cyanophyta, as well as those algae encountered at our bathing beaches, such as “red-tides or mahogany-tides”. In 2006, species implicated in bloom events were captured by digital imaging.

Many dinoflagellates are listed by UNESCO as HABs, that is, Harmful Algae Bloom forming species. Identification of the species are made. Educational posters are created using the digital images and sometimes the reference material, along with additional water quality data/physical characteristics of the event. These posters preserve the events from year to year and increase awareness. Having real images from particular incidences available in the laboratory is a great aid for the laboratory technician that may be unfamiliar with an organism too. Posters are displayed at educational events.

### Microscopy

The laboratory uses a Leica compound microscope and stereoscope with a Windows based imaging system (PAX-it) for a closer look at everything from bacterial colonies and gram stains to pantry pests. Digital images have been most useful for reports, research, presentations, posters, training and other outreach. In addition, the laboratory is often called on to use our equipment and investigative skills to identify unknown substances or to rule out the presence of a harmful substance.

In 2006, a Windows upgrade was made to the “microscope computer” in order to network it. The Windows upgrade then dictated that the current version of PAX-it software, that drives image capturing, be installed. In addition, the frame grabber card, required an upgrade(FlashBus Spectrim Pro). This work has been completed and the lab can now take advantage of new software features and build our reports in our network file folders.

### Performance Evaluations

In January, and again in June, the NJDEP administered Proficiency Testing(PT) Study for NJ Laboratory Certification were completed acceptably for both Microbiological and Chemical Parameters.

### Recreational Bathing

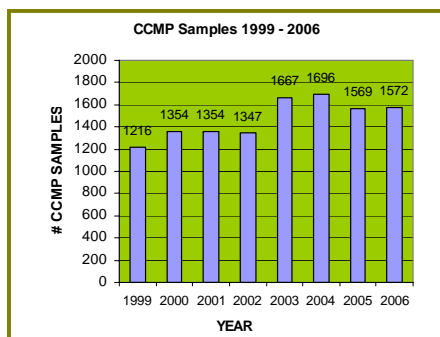


Figure 4. CCMP samples for years 1999 – 2006

Monday morning bacterial monitoring of 61 recreational bathing beaches and environmental sites in the Cooperative Coastal Monitoring Program(CCMP) totaled 1413 samples. In addition, 159 resamples with brackets for enterococcus were collected after exceedances of the 104 cfu/100 ml bathing standard. The number of resamples required in 2006 was a 58% increase over of those required in 2005. In addition, coastal investigation samples(CINV) added 48 enterococcus samples for a grand total of 1572.

The amount of samples received in 2006 is essentially the same as the 2005 amount (Figure 4). The laboratory prepares mEI agar plates, confirmation media and reagents for use with the USEPA Method 1600 for enterococcus.

USEPA Method 1600 for enterococcus was republished with a July 2006 revision date. 40CFR will be changed to allow the use of the newer version soon. The definition of a typical enterococcus colony in the July 2006 method is any blue haloed colony that is > 0.5 mm in diameter. Plates having a mixture of <0.5 mm and > 0.5 mm colonies require extra measuring and attention (Figure 5). It is unclear which of the QC procedures in the newest method will be required by the NJDEP.

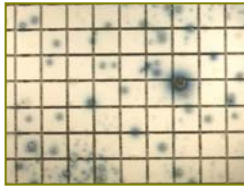


Figure 5. Enterococcus plate having a mixture of < 0.5 mm and > 0.5 mm sized colonies.

### Correlations between Enterococcus and Fecal coliform

Two different microbial indicators are recommended for freshwater and salt water. This is due to differential survival rates. Enterococci are more salt tolerant so are the preferred indicator for saline waters. Many of our sampling areas are the interface, that is, estuaries, so the relationship between enterococcus bacteria and fecal coliform recoveries in samples are useful.

For the third study year, enterococcus is not a good predictor of fecal coliform levels based on results of *overall* 2006 data ( $r = 0.45$ ). Nevertheless, and perhaps more logical, a great improvement is realized by breaking down results by “types” or project code. The correlations between Enterococcus and fecal coliform counts were calculated for “types” of samples or project codes. The Pearson Correlation Coefficient was applied to the paired sets of the 2 parameters where data exists.

Higher correlations are found between fecal coliform and enterococcus when “like” sample types are compared as opposed to *overall* (all) sample types (Table 1). This

2004 PROJECT CODE	r value	n (# samples)	2005 PROJECT CODE	r value	n (# samples)	2006 PROJECT CODE	r value	n (# samples)
ALL SAMPLE TYPES	0.46	356	ALL SAMPLE TYPES	0.55	234	ALL SAMPLE TYPES	0.45	313
			ALL COMP	0.55	67	ALL COMP	0.56	84
MANR	0.56	33	MANR	0.49	114	MANR	0.87	92
MONP	0.998	25	MONP	0.80	18	MONP	0.91	69
CINV	0.73	51	CINV	0.87	19			

Table 1. Correlations between the indicators enterococcus and fecal coliform by “overall” and by project code groups for the years 2004, 2005, and 2006.

pattern of stronger correlation may reflect samples that are taken closer to the source of contamination before additional

time and mixing occur. Monmouth Park samples have yielded strong correlations for 3 years of data. Correlations calculated for “all sample types” together, have remained weaker. Fewer data pieces were generated in 2005 and 2006 because the CCMP brackets were no longer sampled for both parameters. Some projects collect data for both tests. In the near future the laboratory will use *E. coli* as the surface water quality indicator for freshwater. *E. coli* is a sub-group of fecal coliform. Relationships between *E. coli* and fecal coliform will be tracked for one year as the data transitions from the one fresh water indicator bacteria to the other.

## Staphylococcus Update

	GLU	FRU	MNE	MAL	LAC	TRE	MAN	XLT	MEL	NIT	PAL	VP	RAF	XYL	SAC	MDG	NAG	ADH	URE
1	POS	POS	POS	POS	POS	POS	NEG	NEG	NEG	POS	NEG	POS	NEG	NEG	POS	NEG	POS	NEG	POS
2	POS	POS	NEG	POS	NEG	POS	NEG	NEG	NEG	NEG	POS	POS	NEG	NEG	POS	NEG	NEG	POS	POS
3	POS	POS	NEG	POS	NEG	POS	NEG	NEG	NEG	NEG	POS	POS	NEG	NEG	POS		POS	POS	POS
4	POS	POS	NEG	POS	POS	POS	NEG	NEG	NEG	NEG	POS	POS	NEG	NEG	POS	NEG	NEG	POS	POS
5	POS	POS	NEG	POS	NEG	POS	NEG	NEG	NEG	NEG	POS	POS	NEG	NEG	POS	NEG		NEG	NEG
6	POS	POS	NEG	POS	NEG	POS	NEG	NEG	NEG	NEG	POS	POS	NEG	NEG	POS	NEG		NEG	NEG
7	POS	POS	NEG	POS	NEG	POS	NEG	NEG	NEG	NEG	POS	POS	NEG	NEG	POS	NEG	NEG	POS	POS
8	POS	POS	NEG	POS	NEG	POS	NEG	NEG	NEG	NEG	POS	POS	NEG	NEG	POS	NEG	NEG	POS	POS
9	POS	POS	NEG	POS	NEG	POS	NEG	NEG	NEG	NEG	POS	POS	NEG	NEG	POS	NEG	NEG	POS	POS
10	POS	POS	NEG	POS	POS	POS	NEG	NEG	NEG	NEG	POS	POS	NEG	NEG	POS	NEG	NEG	NEG	POS
11	POS	POS	NEG	POS	POS	POS	NEG	NEG	NEG	NEG	POS	POS	NEG	NEG	POS	NEG	NEG	NEG	POS
12	POS	POS	NEG	POS	NEG	POS	NEG	NEG	NEG	POS	POS	POS	NEG	NEG	POS	NEG	NEG	POS	POS
13	POS	POS	NEG	POS	NEG	POS	NEG	NEG	NEG	NEG	POS	POS	NEG	NEG	POS	NEG	NEG	POS	POS
14	POS	POS	NEG	POS	NEG	POS	NEG	NEG	NEG	POS	POS	POS	NEG	NEG	POS	NEG	NEG	POS	POS
15	POS	POS	NEG	POS	POS	POS	NEG	NEG	NEG	POS	NEG	POS	NEG	NEG	POS	NEG	NEG	POS	POS

Table 2. Results for 15 BioMerieux API runs for Staphylococcus species identification.

*Staphylococcus* is suspected to be associated with algae or detritus in that area. Heavy to confluent growth of this bacteria prompted resampling of sites due to interfering confluent growth.. The yellow growth was isolated and the species tentatively identified in our lab by BioMerieux API STAPH kits as *near to Staphylococcus warneri* (Table 2). Coagulase Negative Staph(CNS) is known to be difficult to determine because many CNS isolates show indeterminate traits. Although a pattern does emerge in the biochemical characteristics, this is most likely the reason for the varying results for the 15 analysis by API. We also suspected that the species was not one the kit codes for, which, in the end of the story, proves to be correct.

Three of the above CNS isolates were sent to EMSL for PCR and DNA sequence analysis (Table 3). The decision that the bacteria warranted genotyping was based on the significant Enterococcus method interference. EMSL ran the amplification, sequenced the nucleotide and searched the genome bank to conclude with 99.9% certainty that the organism was *Staph. pasteurii*. After the result was received from EMSL we learned that *Staph. pasteurii* can be phenotypically distinguished from all other novobiocin susceptible *Staph. spp* EXCEPT *Staph. warneri*, from which it can only be differentiated by genotyping! (which was accomplished in 1993). So, our in house methods for determining the unknown bacteria were as accurate as could be without the aid of PCR. Nucleotide sequencing is required to differentiate *S. pasteurii* from *S. warneri*.

Location	Date Coll	Species by PCR and DNA sequence analysis
Horseshoe Cove	10/03/05	<i>Staphylococcus pasteurii</i>
Sandy Hook Light	06/06/06	<i>Staphylococcus pasteurii</i>
Spermacetti Cove	07/17/06	<i>Staphylococcus pasteurii</i>

Table 3. Locations and dates for isolates sent to EMSL for PCR and DNA sequencing. All three were identified as *S. pasteurii*.

Looking forward to the 2007 bathing season, the sites that are affected by the *S. pasteurii* will be run in dilution to quantify Enterococcus. The occurrence of the *S. pasteurii* growth will be noted as it relates to patterns and trends.

During the summer of 2006, a Coagulase Negative Staphylococcus(CNS) continued to interfere with Enterococcus analysis as a competitor for space and nutrients on mEI plates for Enterococcus. Staphylococcus is also a gram + bacteria that will tolerate salt. Problems were mostly in the Raritan Bay/Sandy Hook area and the

Success in our attempts to determine species for *Enterococcus* isolates have been limited. Our goal is to “characterize” sources of bathing beach contamination by identifying species composition. The percentage of enterococci that are *E. faecalis* may differ by site/contamination source, as this species occurs at high frequency in human faeces and sewage. Some contamination events or closures may be caused by re-suspended sediments, birds, runoff from Monmouth Park horse stables, the feeding of birds on mussels or human source. The species of *Enterococcus* present may have correlations with the source of contamination. The hypothesis would be something such as, *the profile of enterococcal bacteria is different at Wreck Pond outfall than it is from other contaminated ocean sites, therefore the source is different.* However, further biochemical tests will be required to differentiate the various species. Below are some preliminary findings using the API 20 Strep gram positive identification system (BioMerieux).

### Results of API 20 Strep gram positive identification system (bioMerieux)



Figure 6 and 7 API 20 Strep gram positive ID system is a profile of enzymatic activity.

The API 20 Strep gram positive identification system (bioMerieux) is a suite of biochemical tests that condense to a numerical index to profile bacteria. API has been used in our lab to identify species of gram positive, catalase negative growth (Figures 6 and 7); our

goal is to determine *Enterococcus* species. Bacterial isolates from bathing beach samples profiled with the API in our lab have included *Enterococcus faecium*, *E. faecalis*, *E. durans*, *E. avium*, *E. gallinarium*, *Streptococcus uberis*, and *Aerococcus viridans*. Our overall success with these kits has been variable. Often a profile is not obtained or the profile is nonsensical. In one example, 15 isolates from Deal Casino beach did confirm for *Enterococcus* by traditional biochemical tests but the API profile provided LAP negative results, ruling out the possibility that the isolates were *Enterococcus*. These mixed results are bothersome and analysis by qPCR would provide resolution. Some of the reasons why the API kits are problematic are that (1) kits code for only 5 species of enterococcus, (2) accuracy of API kits varies by species, and (3) they are designed for clinical isolates. The literature reports variable success for environmental isolates. In conclusion, to collect meaningful *Enterococcus* species data would require performing an array of individual biochemical tests on a huge number of bacterial isolates. This brings us to the reason our laboratory has investigated the use of qPCR as an investigational tool. The value added feature being the eventual implementation of this technology as the method for bathing beach water quality analysis.

### Using qPCR on *Enterococcus* isolates

The laboratory staff were invited to visit the laboratory of Barry Kreiswirth and Natalia

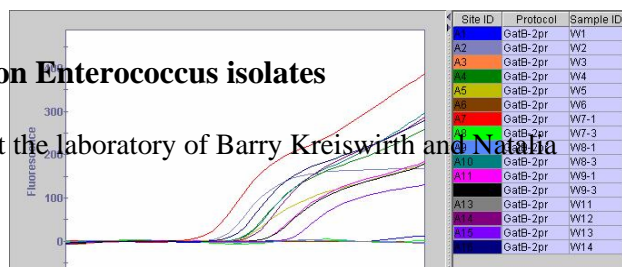


Figure 6. Results of fluorometric detection using Cepheid Smart Cycler II. The detection of the fluorescence increases with amplification of the nucleotide sequence of interest.

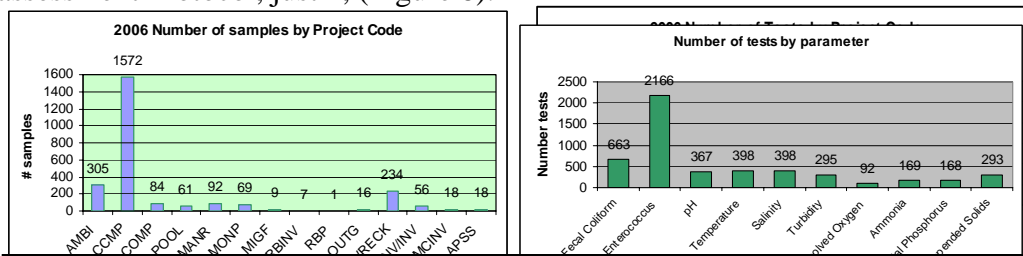
Kurepina of The Public Health Research Institute in Newark. The PHRI lab trained us in the use of Cepheid Smart Cycler II for DNA amplification and fluorometric detection, using probes specific for *Enterococcus faecalis* and *E. faecium*. The Smart Cycler II System is a rapid, real-time thermal cycler used for identifying DNA/RNA from prepared biological samples. The Cepheid Smart Cycler System II has automated much of the testing process, and making each reaction site individually programmable. It is the fastest, easiest to use, and most flexible system now on the market. A set of samples collected from Manasquan River and from two septic overflow events were used to collect some preliminary data. The *Gat B gene area* was targeted to determine if the isolates were *E. faecalis* and *E. faecium*. Ten of the sixteen isolates were positive. Isolates that are not *E. faecalis* or *E. faecium* would need further analysis with other probes or biochemical tests.

Enterococcus isolates have been stored from summer bathing samples (including those from York Avenue, The Terrace, Philadelphia Avenue) impacted by Wreck Pond outfall. In the near future, we expect to have results by PCR and DNA sequencing to give us some preliminary data on Enterococcus species. A further collection of this data in the summer of 2007 may or may not show correlations with the source of contamination. It will also provide confirmation that the colonies being counted toward the bathing standard are indeed enterococcus and not a false positive.

The EPA is currently researching using qPCR technology on fresh whole water samples. The next study is to validate for salt water testing. A presentation, Water Quality Monitoring by PCR by Richard Haugland(USEPA ORD-NERL) was attended in May. Another talk in May was given by Diane Calesso (USEPA Region 2) on her research in the same area. This technology could provide 2 hour bathing beach enterococcus results. The product expected to be used for testing is the Cepheid Smart Cycler II. The Smart Cycler II for amplification and fluorometric detection comes complete with laptop or desktop, monitor, centrifuge, vortex and 1 year service contract.

### Sample Types/Project Codes/Testing

A variety of sampling projects and investigations, are tracked by project codes as they are entered into Sample Tracking and Information System(STIS) laboratory sample database. The Manasquan River Project totaled 92 samples and Monmouth Park totaled 69. Samples named COMP are just miscellaneous problems that required investigation. The lab received 234 samples for the Wreck Pond Rain Event Study, Rapid Bioassessment Protocol, just 1, (Figure 8).



Figures 8 and 9. Number of samples received and number of tests performed on samples, by projectcode, by the laboratory in 2006.

Each sample often has a number of tests that are associated with it (Figure 9). For example, Ambient water monitoring stations(68) were sampled 292 times (4 times a year). At each sample site, there are between 5 and 10 tests are performed. This brings the total number of tests for Ambients to 2386. Some tests are meter measurements and others are chemical or microbial. CCMP samples usually just have one test performed. County Park pools, of which there are 3 plus a “sprayground”, totaled 61. The total number of individual tests that were performed for all sample types are shown below (Figure 10).

Figure 10. Number of tests performed sorted by test type, in 2006.

During 2006, the laboratory received 32 complaint/investigation samples for Phytoplankton Identification and Enumeration. The majority of these samples were collected in July and August. Sample types ranged from bathing to non-bathing beaches as well as lakes and outfalls. There were six samples from fresh water sites that contained blooms of the blue-green algae *Microcystis*. In August, *Microcystis* was the cause of a heavy bloom (>21,000 units/ml) at Deal Lake. This bloom seems to be an annual occurrence for this particular site. In the Raritan and ocean, the dinoflagellate *Prorocentrum spp.* was present during June, July and August of 2006. This genus is listed as a Harmful Algal Bloom causative agent by UNESCO. There were six samples containing low blooms of two different species of *Prorocentrum*. Posters were created using our 1000X magnification digital images for Raritan Bay Flagellates. (Figures 12, 13, 14).

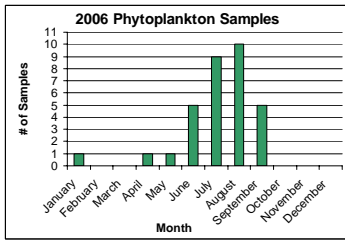


Figure 11. Number of phytoplankton samples and month collected, in 2006.