An Astro-Bug Summer

NASA Internship 2013
Tiffany M. Swarmer
University of North Dakota
Mark C. Ott Ph.D.
Microbiology Laboratory

SPACE LIFE SCIENCES
SUMMER INSTITUTE

Background

- Known to have a gypsy foot
  - 7 countries
  - 28 states
- Bachelors of Science in Biology with a focus on Microbiology
- Lead lab tech in the Sonoma State University DNA sequencing Lab
- 2-3 years working in a hospital as:
  - An Emergency First Responder
  - Risk Management
  - Administrative Clinical Researcher
- Currently working on my Masters of Science in Space Studies at UND with a focus on human factors for long duration spaceflight.


UND THE UNIVERSITY OF NORTH DAKOTA
NASA
LUNAR/MARS HABITATION MODULEPROTOTYPE
SPACE

Summer Goals

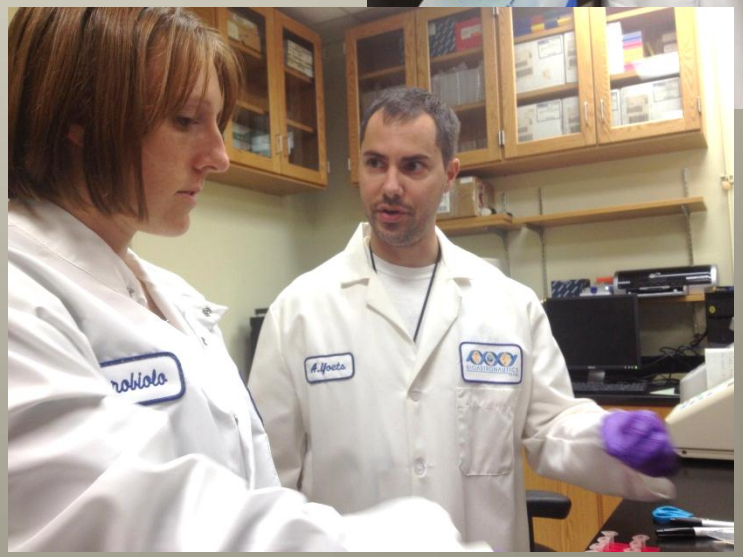
- Gain an understanding of how NASA functions and operates on a day to day basis.
- Gain an understanding of the future direction of human spaceflight.
- Gain an understanding of the Microbiology Departments role and operational function within NASA.
- Perform a summer project utilizing the resources available in the Microbiology project.

Microbiology Lab

- Microbial monitoring for the ISS.
  - Onboard monitoring
  - Identification of returned samples
  - Pre/Post Flight
  - Food
  - Cargo
  - Mitigation
    - Unexpected contamination
- Development and testing of new technologies for microbial monitoring during spaceflight.
- Providing local and on site microbial monitoring.
- Research
  - Antibiotic/MRSA study
  - Viral Shedding

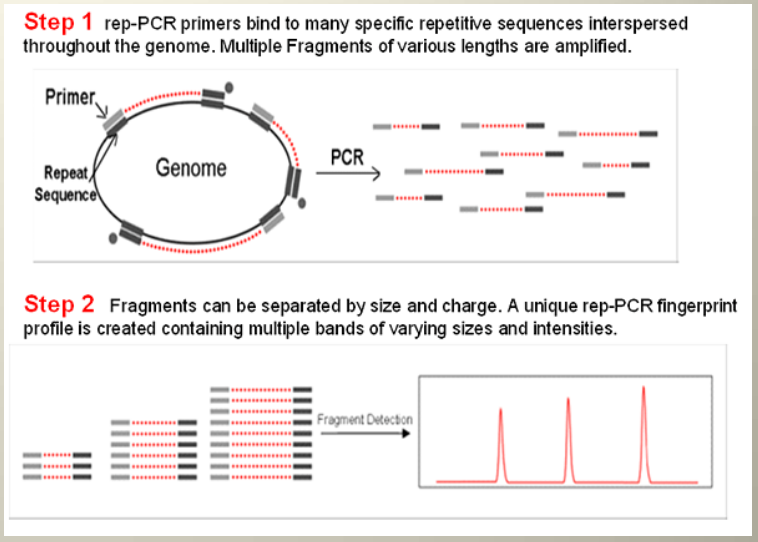
In Lab Training

- Aseptic technique
- Bioreactors (theory and use)
- Plate streaking
- Colony counting
- DNA extraction
- PCR
- Electrophoresis
- DNA sequencing
- DNA sequence analysis



My Monster…. I Mean Project

- Troubleshoot and use the Diversilab system
  - Genetic fingerprint of isolates from the PWD system on the ISS
  - Focus on genetic drift of isolates
- Sadly, this project had to be abandoned due to system IT concerns.

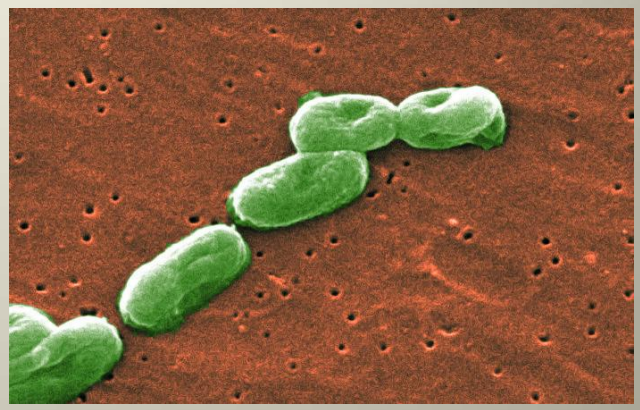


Microbial Identification Project

- Re-identified *Burkholderia cepacia* samples isolated from the early Space Shuttle program.
- Original method for identification was through the Vitek system.
- The goal was to use 16s rDNA sequencing to confirm or refute the original identification.

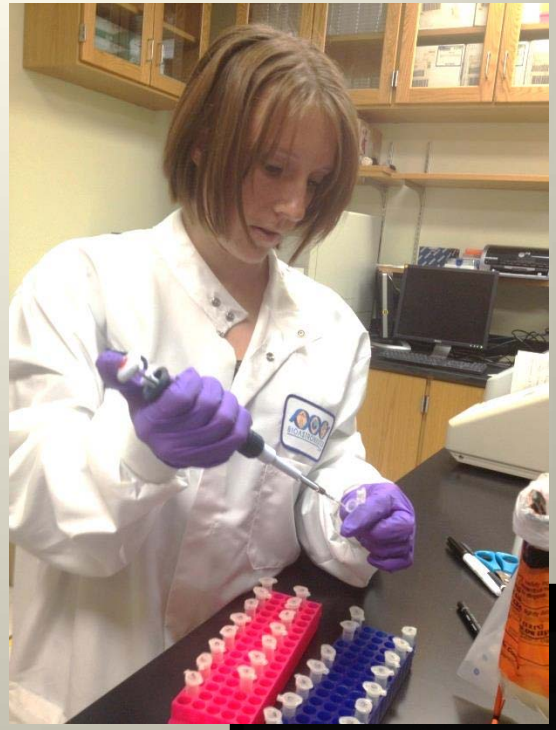
Burkholderia cepacia complex (Bcc)

- Members within the *Bcc* have a 16s rDNA similarity higher (97.7%) than other *Burkholderia* species (97.0%).
- Identification of a specific species within the *Bcc* and other closely related gram negative bacteria difficult.

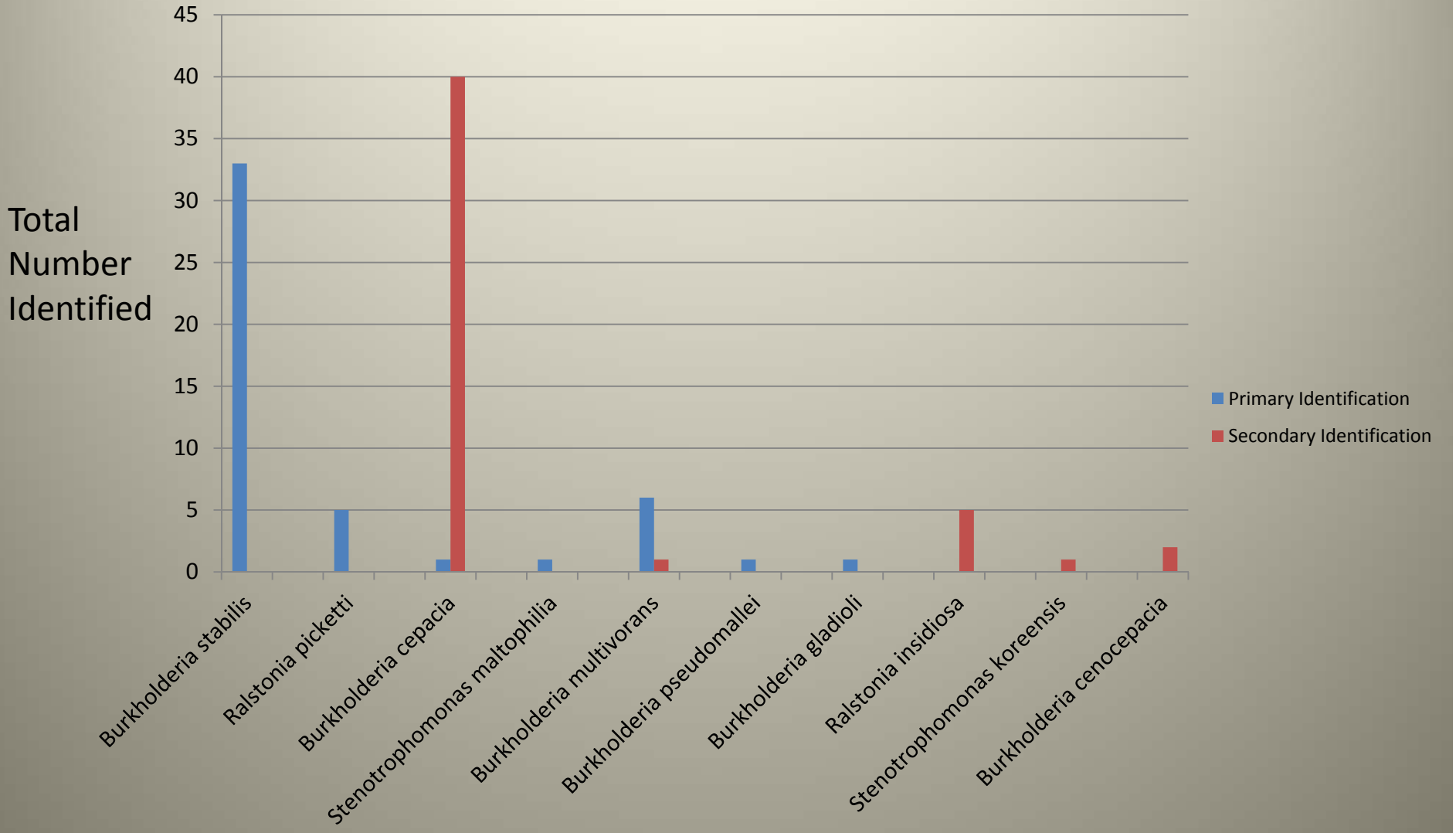


Process

- Location…Location….Location
- DNA extraction
  - Ultraman Prep extraction kit
- PCR amplification
  - Kit
- Gel electrophoresis
- Exo-sap
- Sequence cycle Reaction
- Filters
- Sequencing
- Analysis of Sequence data



Results


Total Number Identified
45
40
35
30
25
20
15
10
5
0
Burkholderia stabilis
Ralstonia picketti
Burkholderia cepacia
Stenotrophomonas maltophilia
Burkholderia multivorans
Burkholderia pseudomallei
Burkholderia gladioli
Ralstonia insidiosa
Stenotrophomonas koreensis
Burkholderia cenocepacia
Primary Identification
Secondary Identification

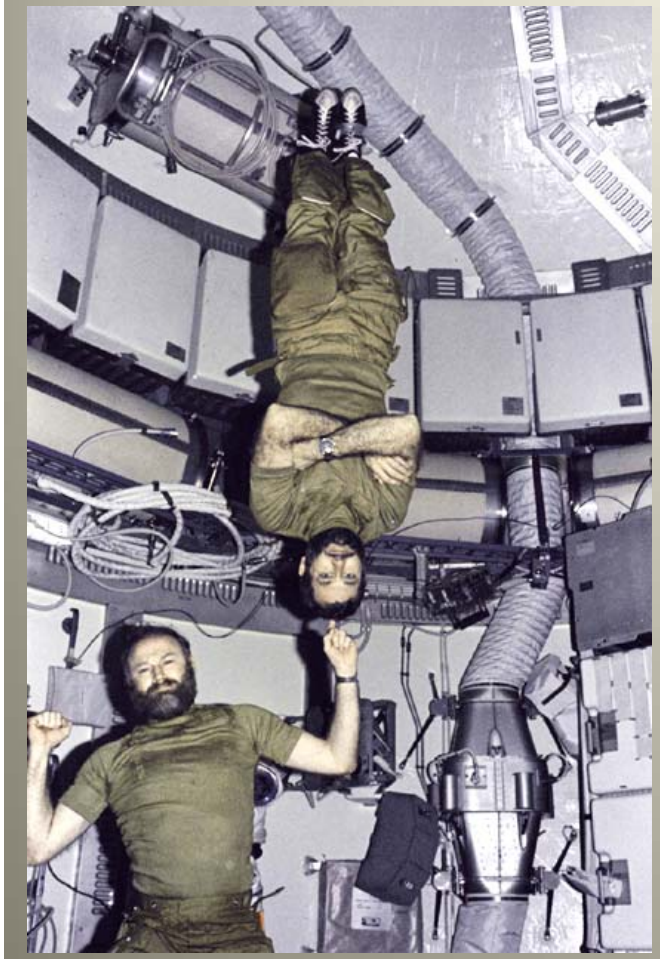
Non-*Burkholderia cepacia* complex isolates:

Organism	Sample Number	Origin
Ralstonia Picketti	6 and 7 42,43,and 44	CWC-20 STS-91 Tank B#1
Stenotrophomonas maltophilia	16	STS-87 L-15
Burkholderia pseudomallei	37	STS-87
Burkholderia gladioli	38	STS-90 Ambient H2O

Conclusions

- Out of 50 samples
  - 8 (16%) appear to be bacteria not contained in the *Burkholderia cepecia complex.*
  - 2 (4%) of these 8 are within the genus *Burkholderia*
  - 2 (4%) isolates are unknown
  - 40 (80%) are bacteria within the closely related *Burkholderia cepacia* complex.
- *Ralstonia Picketti* is the most commonly misidentified bacteria and shares genotypic/phenotypic similarities.
- *Stenotrophomonas maltophilia* was isolated from one of the samples and is also know to be similar to Bcc bacteria.

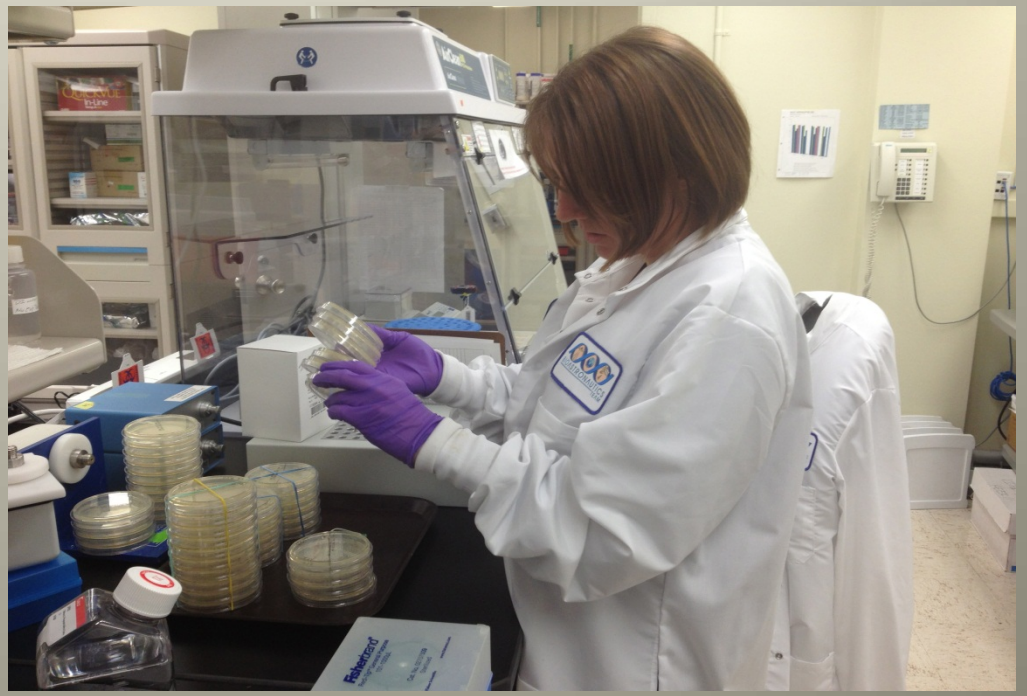
Future Considerations



- In future identifications of *Burkholderia* isolates the use of Bcc selective media such as BCSA may eliminate misidentifications of non-*Burkholderia* bacteria.
- Methods utilizing selective amplified genomic fragments, genetic fingerprinting, may also be beneficial in discriminating between the species within the Bcc.

Antibiotic Study

- Sarah Castro, PhD
- Testing the bioreactors as models for microgravity.
- Examined how *methicillin resistant Staph aureus* (MRSA) responds to antibiotics after exposure to a low-fluid-shear (LSMMG) environment created by the bioreactors

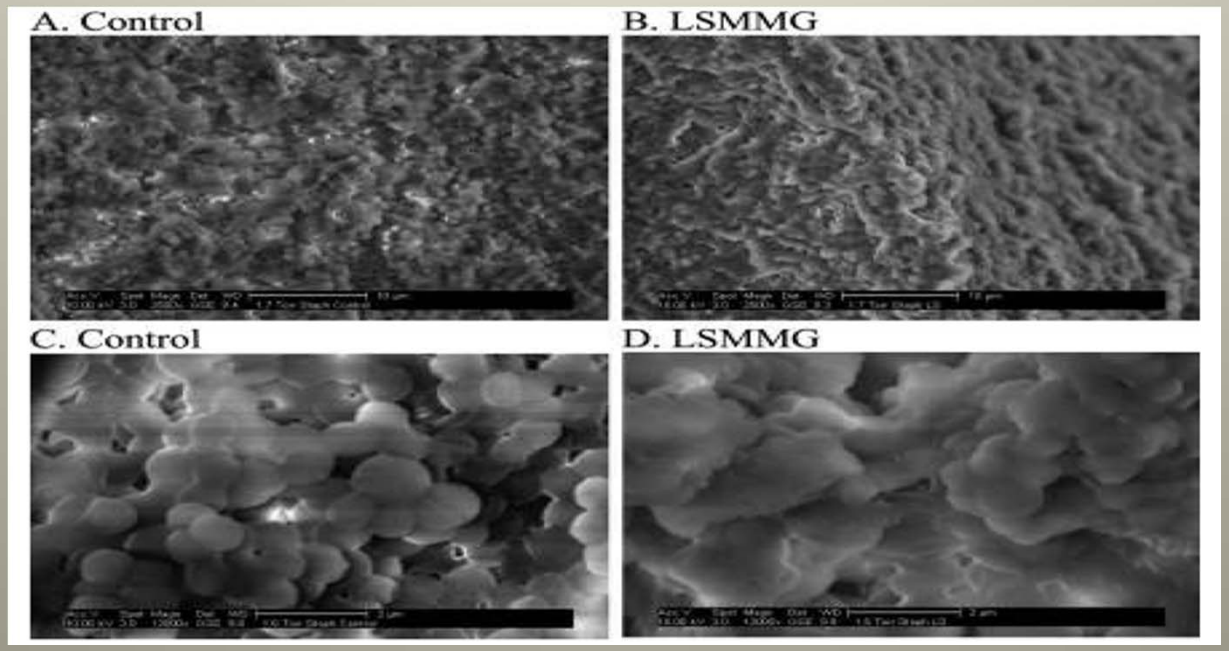


Process

- Grow the MRSA in normal Earth conditions and also in the LSMMG conditions
- Inoculate culture with varying amounts of clindamycin (antibiotic)
- Incubate and observe the growth of the MRSA overtime to determine how the LSMMG environment affected the MRSA

Physical Changes in MRSA Exposured to LSMMG

- The *S. aureus exposed to the LSMMG* has a decrease in carotenoid production causing a loss of the golden yellow color noted in the Control.
- Biofilms (extracellular polymeric substance) appear to be more prevalent and completely encase the MRSA exposed to the LSMMG.



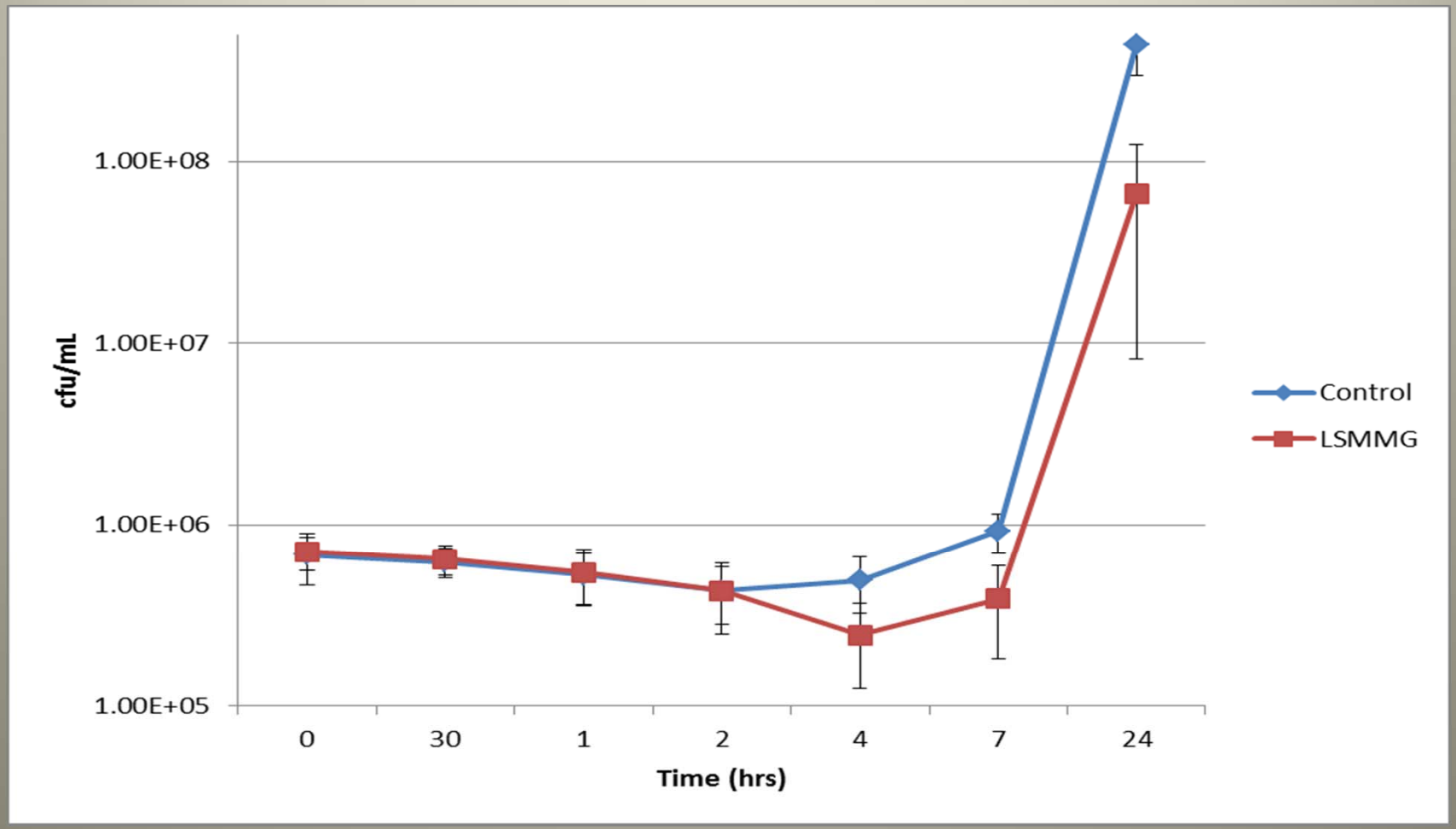
Environmental scanning electron microscopy images of control and LSMMG-cultured *S. aureus* N315. Control-cultured *S. aureus* at 2,5003 (A) and 10,0003 (C) magnification revealed individual cells that were clearly visible. For low-fluid-shear-cultured *S. aureus*, at 2,5003 (B) and 10,0003 (D), the cells were much less visible and completely embedded in an EPS matrix.

Physical Changes in MRSA after Clindamycin/LSMMG exposure

- *S. aureus* exposed to LSMMG has a decrease in carotenoid pigmentation.
- Exposure to 750mg clindamycin appeared to decrease biofilm formation as noted by the decrease in aggregates within the culture at 24 hours.

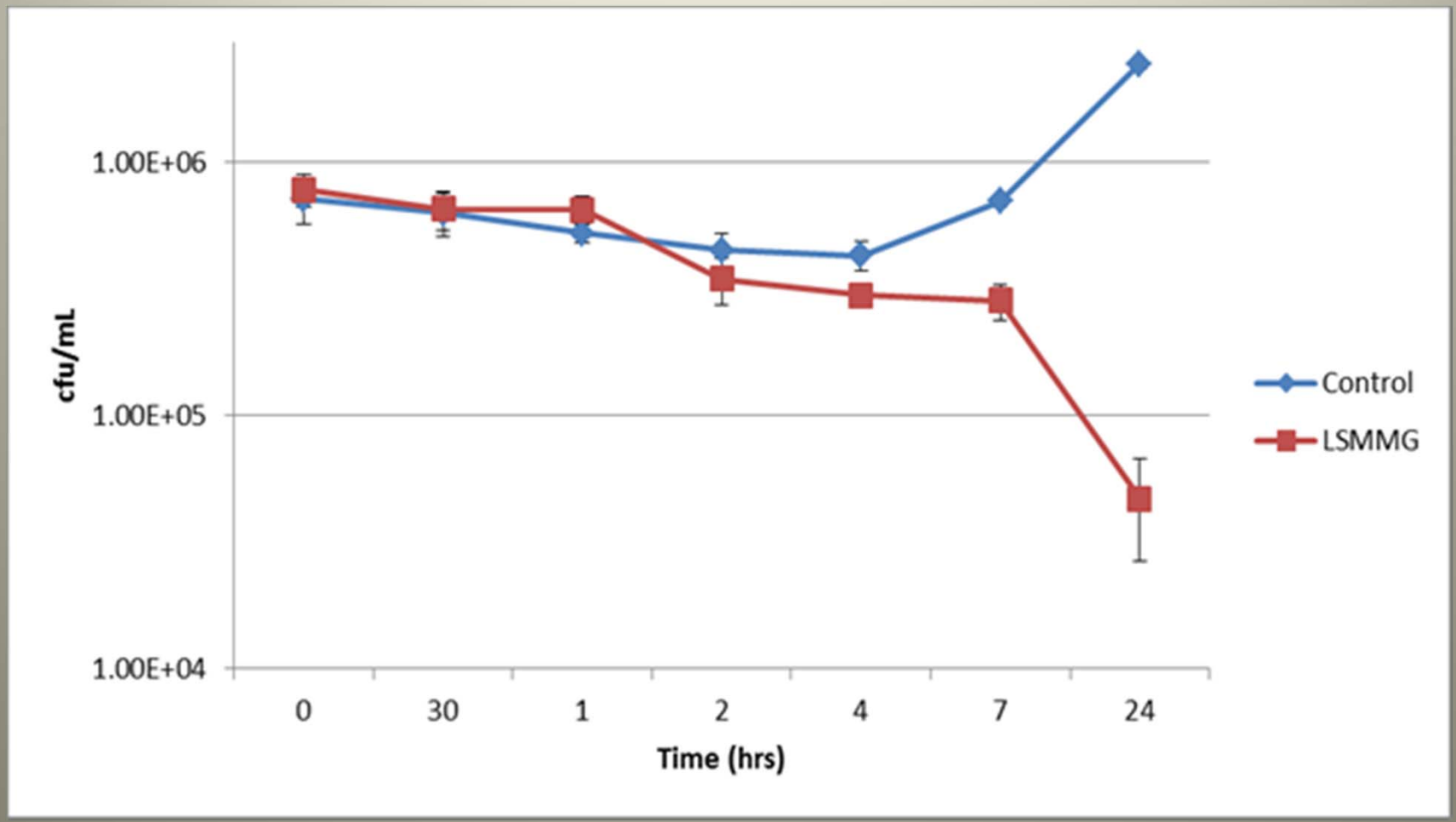
Results

Preliminary results for 750mg clindamycin.



Results

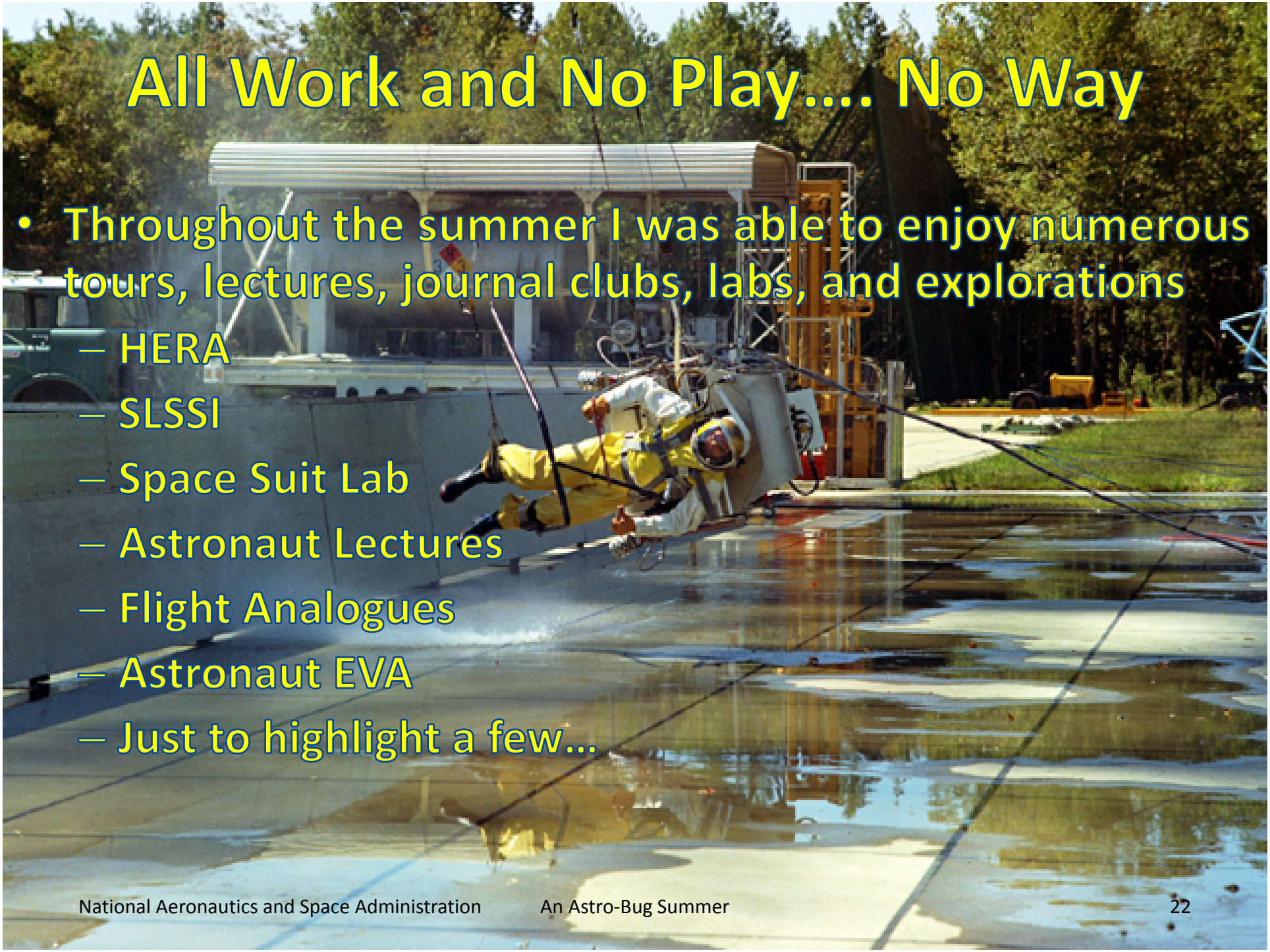
Preliminary results for
1000 mg clindamycin



Conclusions

- Clindamycin appears to cause a delayed growth cycle at 750 mg and decreased overall MRSA growth
  - Seen predominantly at the 4 and 7-hour time point.
  - With the 24-hour time point demonstrating rapid growth in both the control and LSMMG, with the LSMMG total cfu less than the control
- 1000 mg demonstrates a loss of growth in the LSMMG sample with no rebound at 24 hours

All Work and No Play…. No Way

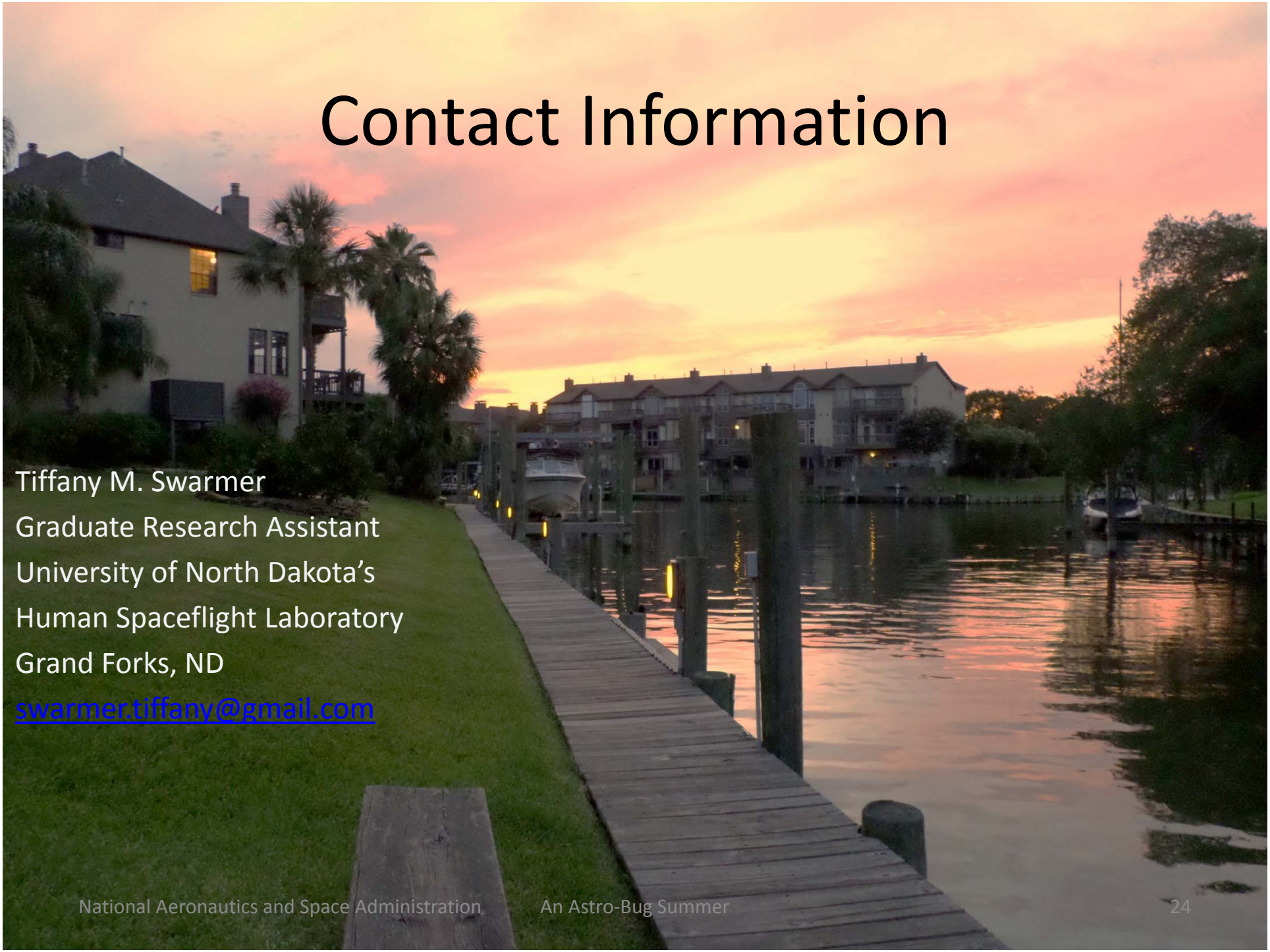


- Throughout the summer I was able to enjoy numerous tours, lectures, journal clubs, labs, and explorations
  - HERA
  - SLSSI
  - Space Suit Lab
  - Astronaut Lectures
  - Flight Analogues
  - Astronaut EVA
  - Just to highlight a few…

Thank you!!!!

- **The list of individuals who have helped me over the Summer is lengthy and I apologize for anyone I missed:**
  - **Mark C. Ott PhD**
  - **Lauren Merkle PhD**
  - **Judy Hayes**
  - **Victoria Castro**
  - **Airan Yoets**
  - **Sarah Castro PhD**
  - **Doug Botkin PhD**
  - **Melanie Smith**
  - **Jackie Reeves**
  - **JSC Microbiology**
  - **UND**
  - **North Dakota Space Grant**
  - **And many, many more**

Contact Information



Tiffany M. Swarmer
Graduate Research Assistant
University of North Dakota's
Human Spaceflight Laboratory
Grand Forks, ND
swarmer.tiffany@gmail.com


QUESTIONS???

Refrences

- Castro SL, Nelman-Gonzalez M, Nickerson CA, Ott CM. Induction of attachment-independent biofilm formation and repression of hfq expression by low-fluid-shear culture of staphylococcus aureus. Appl Environ Microbiol, 2011;77(18), 6368-6378.
- Coenye T, Vandamme P, Govan Jr. W, Lipuma JJ. Taxonomy and identification of the burkholderia cepacia complex. J Clin Microbiol, 2001;39(10), 3427-3436.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