Quantification of Fecal Bacteria Grazing by Micro-Zooplankton in Stormwater Control BMPs

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Stormwater Runoff

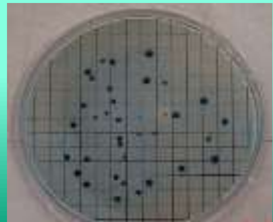
- Major source of coastal water pollution
- Problem enhanced by increasing urbanization within the watershed
- Not only health concerns, economic also (i.e shellfish harvesting areas closing)

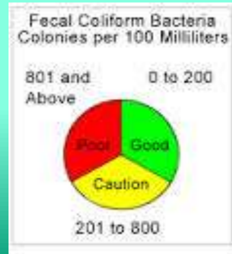
Fecal Bacteria

- most directly impacts human health and economy
- commonly sourced from concentrated animal feeding operations (CAFOs), sewage effluents, and widely sourced from **urban and suburban stormwater**

Fecal Bacteria Regulations

- 14 coliforms per 100 ml to close a shellfish bed
- 200 coliforms per 100 ml is considered unsafe to for humans to swim





Basic BMP Guidelines

Plan for stormwater management
- Sustainable and eco-friendly
- Improve water quality
- Low impact development (LID)

Mimic natural hydrology
- Promote infiltration, retention and ET

Treat stormwater runoff
- Wet detention ponds
- Wetlands

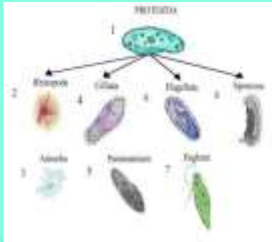
Micro-zooplankton

- Heterotrophic and mixotrophic planktonic organisms
- Normally between 10 and 200 µm
- 2 main categories, metazoan & protozoan

Nauplius

Rotifer

Protozoans



Study Objectives

1. Verify that micro-zooplankton grazing is a significant factor in fecal bacterial removal from storm water.
2. Compare removal rates in a constructed wet detention pond and a constructed wetland.
3. Examine the effects of seasonality on micro-zooplankton removal of fecal coliforms.

Study Objectives (continued)

4. Conclude if there's a correlation between chlorophyll *a* levels and micro-zooplankton removal of fecal coliforms.
5. Calculate DOC concentrations and look for correlations with grazing rates.
6. Determine the importance of rainfall on concentration of fecal coliforms.

Hypotheses

1. The wetland will have higher fecal coliform removal rates than the wet detention pond.
2. Grazing rate is inversely related to Growth rate.
3. Seasonality is a factor in the efficacy of fecal coliform removal.
4. Rainfall significantly impacts the fecal concentrations, and BMP effectiveness.

Hypotheses (continued)

5. Dissolved Organic Carbon (DOC) concentrations will be positively correlated with micro-zooplankton Grazing rates at both sites.
6. Chlorophyll *a* will be positively related with growth rates.
7. Grazing rates will be higher in the summer (increased temperature, vegetation, etc…)

Constructed Wetland
JEL Wade

- Constructed to treat stormwater runoff
- Drains a 2,393 ha watershed
- 22% impervious surface coverage
- Dense, diverse vegetation
- Average fecal load reduction of 99%

Constructed Wet Detention Pond
Kings Highway Pond

- Located behind a retail parking lot
- Significant drainage and run-off from impervious surfaces.
- Little vegetation
- Small resident population of geese

Field Analysis

- Field analysis using YSI
- Water collection in 25-L carboys
- Amber 150ml bottles for chla analysis

3-Day Grazing Tests

2 Treatments: whole water and filtered*

***Initially filtered with 20 µm mesh, later began 10 µm 06/2015**

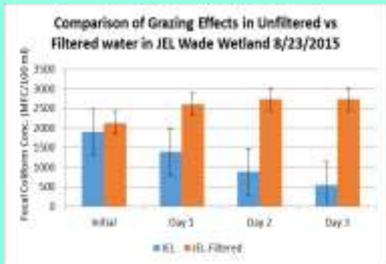
Sub-samples taken initially, and every 24 hours for 3 days.

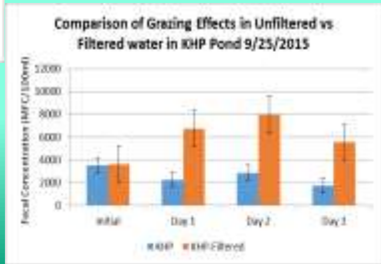
Fecal bacteria was cultivated using Rosolic Acid & Difco mFC broth base, incubated in a bath at 44.5 C for 18-20 hrs

Samples counted microscopically and calculated per 100 ml

3-Day Grazing Experiments

Results from 10μm filtered experiments.

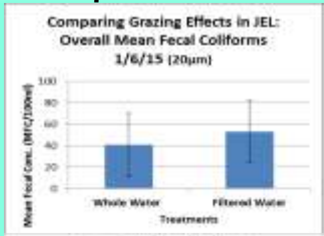


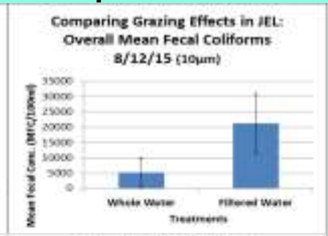


3-Day Grazing Experiments

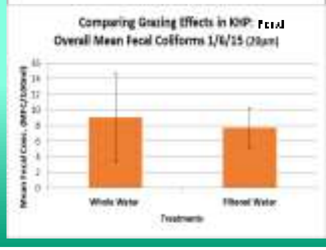
20μm filtration **10μm filtration**

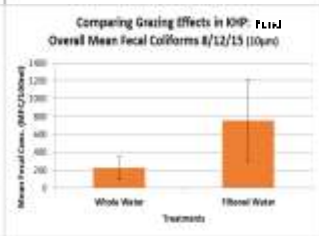
JEL



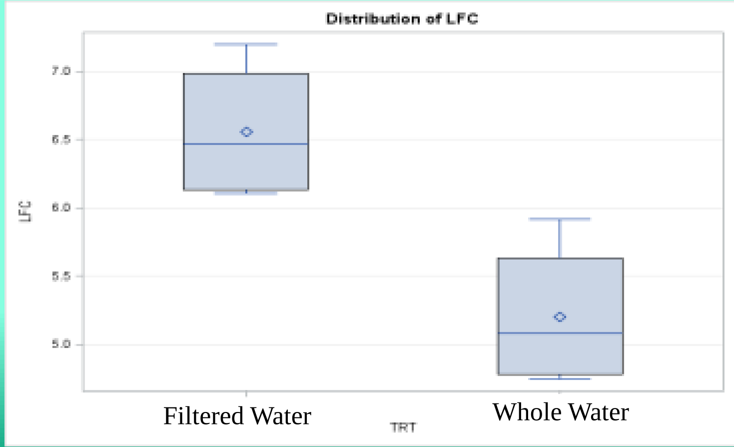


KHP





3-Day Grazing Experiments Demonstrating Fecal Coliform Removal by Micro-zooplankton



Least Squares Difference (LSD) statistical results from experiments in JEL Wade constructed wetland (JEL) and King's Highway wet detention pond (KHP) using 20µm mesh for filtration

Site	Date	Whole Mean	Filtered Mean	Grazing Sig? ($p<0.05$)
JEL	7/15/2014	172	150	No
JEL	7/29/2014	611	456	No
JEL	9/1/2014	125	155	No
JEL	1/6/2015	41	53	No
JEL	2/11/2015	58	70	No
KHP	7/23/2014	2622	2622	No
KHP	7/29/2014	123	105	No
KHP	9/1/2014	20	17	No
KHP	1/6/2015	9	8	No
KHP	2/11/2015	18	16	No

Least Squares Difference (LSD) statistical results from experiments in JEL Wade constructed wetland (JEL) and King's Highway wet detention pond (KHP) using 10µm mesh for filtration.

Site	Date	Whole Mean	Filtered Mean	Grazing Sig? ($p<0.05$)
JEL	**8/12/2015**	**5250**	**21275**	**Yes**
JEL	**8/23/2015**	**1187**	**2552**	**Yes**
JEL	8/28/2015	878	1246	No
JEL	9/25/2015	5350	9383	No
JEL	**10/6/2015**	**3375**	**3050**	**Yes**
KHP	**8/12/2015**	**225**	**755**	**Yes**
KHP	8/19/2015	44	54	No
KHP	**8/23/2015**	**888**	**3517**	**Yes**
KHP	**8/28/2015**	**81**	**335**	**Yes**
KHP	**9/25/2015**	**2583**	**5975**	**Yes**
KHP	10/6/2015	1971	2008	No

Results of 3-Day Grazing Experiments

- Statistics run with SAS.
- Data were tested for normality using the Shapiro-Wilk test and log-transformed.
- 20µm filtration results showed 0 significant results in the five experiments ran at both sites.
- 10µm filtration results showed 3 out of 5 significant results in the wetland, and 4 out of 6 at the detention pond

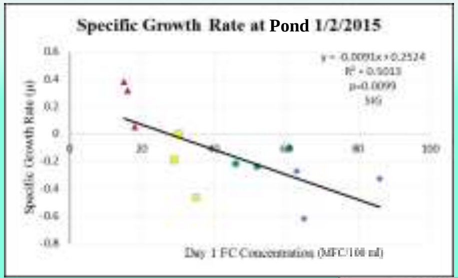
24 Hour Dilutions

4 Treatments: 100%, 75%, 50%, & 25% whole water
***filtered using 0.45µm millipore filters**

Sub-samples taken initially, then 24hrs later.

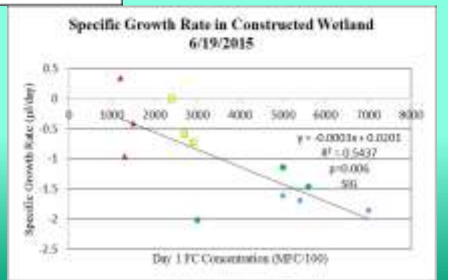
Fecal bacteria was cultivated using Rosolic Acid & Difco mFC broth base, incubated in a bath at 44.5 C for 18-20 hrs

Samples counted microscopically and calculated per 100 ml



24-Hr Dilution Assays

Blue diamonds
100% whole water
Green circles
75% whole water, 25% filtered
Yellow squares
50% whole water, 50% filtered
Red triangle s
25% whole water, 75% filtered



24-Hour Dilution Assays JEL Wade Wetland

Site	Dilution	Intercept	Slope	P	Sig. (-) slope	Grazing sig.?
JEL	**8/12/2014**	**0.9115**	**-0.001**	**0.003**	**Yes**	**Yes**
JEL	**8/26/2014**	**0.3543**	**-4E-04**	**0.022**	**Yes**	**Yes**
JEL	9/2/2014	0.2203	-0.001	0.168	No	No
JEL	**12/11/2014**	**0.6958**	**-0.002**	**0.0004**	**Yes**	**Yes**
JEL	1/25/2015	0.2161	-5E-04	0.526	No	No
JEL	**6/8/2015**	**0.2007**	**-0.003**	**0.0002**	**Yes**	**Yes**
JEL	**6/19/2015**	**0.0201**	**-3E-04**	**0.006**	**Yes**	**Yes**
JEL	**12/8/2015**	**0.8268**	**-0.019**	**9.03E-05**	**Yes**	**Yes**
JEL	**2/10/2016**	**0.902**	**-0.015**	**1.16E-06**	**Yes**	**Yes**
JEL	**2/15/2016**	**0.2558**	**-0.002**	**0.018**	**Yes**	**Yes**
JEL* forebay	**2/25/2016**	**0.6006**	**-0.021**	**1.81E-05**	**Yes**	**Yes**
JEL* outfall	**2/25/2016**	**0.5739**	**-0.019**	**0.015**	**Yes**	**Yes**

24-Hour Dilution Assays Kings Highway Detention Pond

Site	Dilution	Intercept	Slope	P	Sig. (-) slope	Grazing sig.?
KHP	8/6/2014	0.6221	-0	0.249	No	No
KHP	**8/11/2014**	**0.7889**	**-0**	**0.0006**	**Yes**	**Yes**
KHP	8/19/2014	-1.58	0.01	0.357	No	No
KHP	9/18/2014	0.0166	-0	0.069	No	No
KHP	12/16/2014	0.3614	0.004	0.779	No	No
KHP	**1/2/2015**	**0.2524**	**-0.01**	**0.009**	**Yes**	**Yes**
KHP	1/19/2015	0.1976	-0.02	0.072	No	No
KHP	**12/9/2015**	**0.8268**	**-0.02**	**9.03E-05**	**Yes**	**Yes**

Environmental Factors Influencing Micro-zooplankton Grazing in the Wet Detention Pond

- Initial Fecal Coliform concentrations positively correlated with Water Temperature (R=0.74, p=0.04)
- Grazing Rate has a near-significant positive relation with Initial Fecal Coliform concentrations* (R=0.63, p=0.09)
- Water Temperature negatively correlated with Turbidity (R= -0.73, p=0.04)

Environmental Factors Influencing Micro-zooplankton Grazing in the Constructed Wetland

- Initial Fecal Coliform concentrations strongly correlated with Water Temperature* (R=0.75, p=0.005)
- Grazing Rate strongly correlated with Initial Fecal Coliform concentrations* (R=0.83, p=0.0009)
- Grazing Rate positively correlated with Water Temperature (R=0.57, p=0.051)
- Bacterial Growth Rate negatively correlated with microzooplankton Grazing Rate (R= -0.64, p=0.023)
- Chlorophyll *a* strongly correlated with Rainfall amount (R=0.94, p=0.006)

Results of 24-Hour Dilution Assays

- Regressions were run on all 24 hour assays
- 10 out of 12 experiments in the wetland yielded significant removal by grazing ($p<0.05$)
- 3 (almost 5) out of 8 trials in the detention pond experiments showed significant removal by grazing ($p<0.05$)

Additional Laboratory Analyses

Dissolved Organic Carbon (DOC)

- 20 ml samples filtered
- 2 duplicates per site
- Initial concentration observed
- Used EPA 415.3 method for analysis

Chlorophyll *a*

- 50 ml samples filtered
- 2 duplicates per site
- Initial concentration observed
- Used EPA/600/R-97/072 method for analysis

Overview of Results

- Seasonal changes showed no obvious difference on grazing effects
- The constructed wet detention pond had significant removal of the bacteria, but less often than the wetland
- Overall, results show grazing is a significant factor removing fecal coliform bacteria at both the constructed wetland, and wet detention pond

Future Research

- Qualitative analysis and comparison of micro-zooplankton found at both locations.
- Interpret DOC and chlorophyll *a* samples from both sites and look for correlations.
- Collect and analyze macrophyte vegetation species role in stimulating micro-zooplankton grazing.*

Thank you for your time, QUESTIONS?

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