Premise plumbing factors leading to mycobacterial growth in homes in the greater Philadelphia region

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Background

- Nontuberculous mycobacteria (NTM) are naturally occurring in water sources and are native to drinking water systems [1-3]
- NTM can cause pulmonary infections in healthy individuals and are difficult to treat [4]
- *Mycobacterium avium* complex (MAC) accounts for most NTM related diseases in the US [1-3]
- NTM disease incidence had risen six fold (1.5 – 9 per 100,000) from 1997 – 2003 [5]
- Recent estimates put NTM disease cases at 15 per 100,000 [1]

NTM Characteristics

- Resistant to chlorine, chloramine and ozone [6]
- Resistant to temperature - over 90% survive exposure to 50 ^{o}C (125 ^{o}F) for 60 mins or more [9]
- Tolerate long periods of water stagnation [10]
- Grow in low levels of oxygen [12]
- Form biofilm on the insides of pipes [7,8]
- Ideally adapted to growth in drinking water systems and premise plumbing [7,8]

Previous work

- Montgomery County NW of Philadelphia, PA has high incidence of NTM disease
- Lande et al. 2015 [13] findings:
    - NTM present in source water and distribution system – 1-5 CFU/mL
    - NTM replicate in water heaters - >1,000 CFU/mL
    - Water heaters set at higher temperature were less likely to grow NTM
    - Elevating water heater temperature controlled growth of *M. avium* but at the risk of growth of *M. chelonae*

Objective

Determine premise plumbing factors leading to NTM growth in homes in greater Philadelphia region

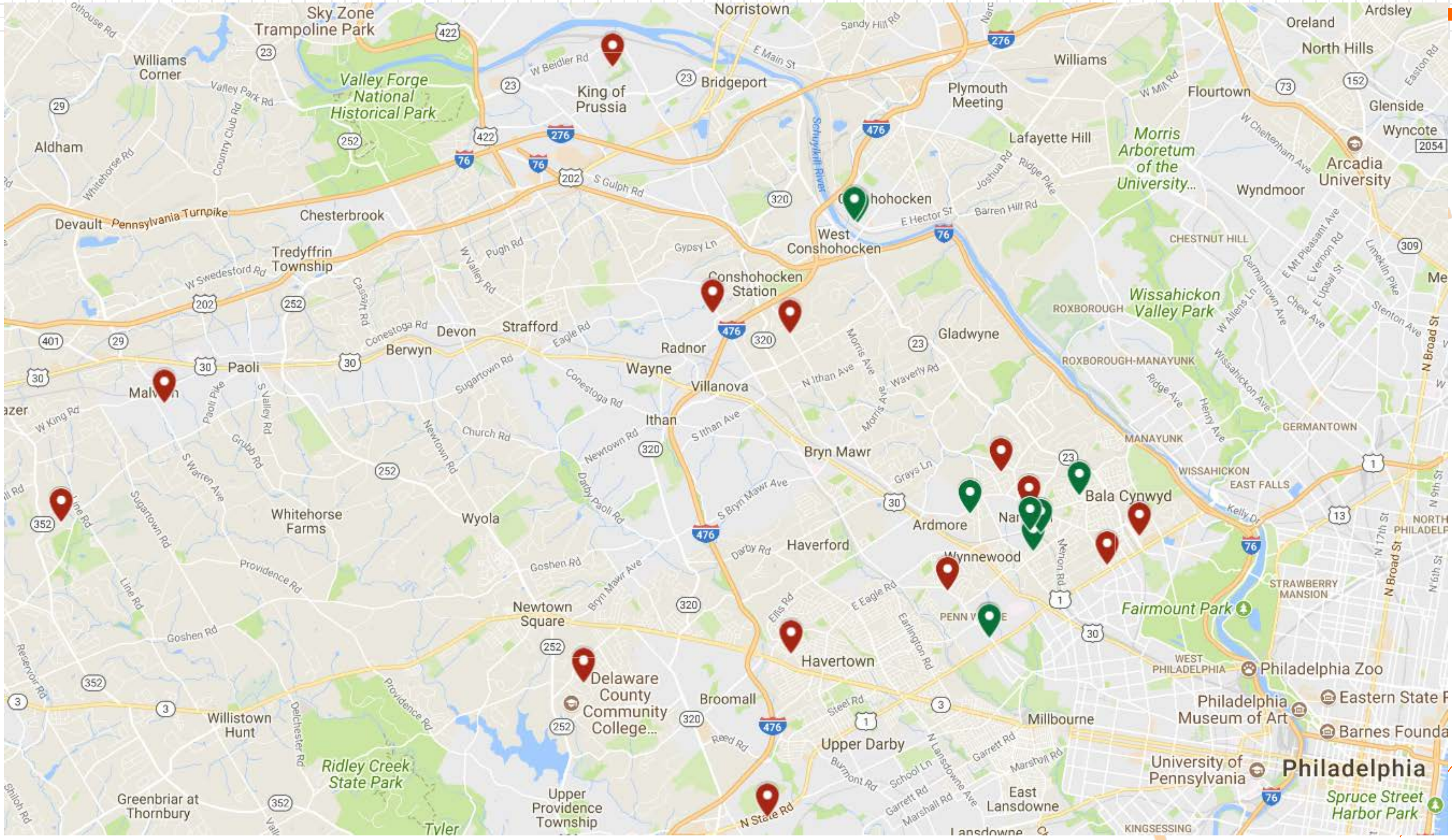
Hypotheses

- Patient homes are more conducive to NTM growth
- Least used bathrooms are more conducive to NTM growth
- *Methylobacterium spp.* can control growth of *Mycobacterium avium* in the biofilm

Sampling Sites

13 Patient
- 11 Large
- 2 Small

9 Control
- 8 Large
- 1 Small



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Data Collection per home

- Up to 10 Biofilm samples
- Water for qPCR
- ICP-MS for dissolved metals
- pH

- Total Chlorine and Temperature
  - Cold Stagnant
  - Hot Stagnant
  - Cold Flushed
  - Hot Flushed
- Ammonia and Nitrite
  - Cold Stagnant
  - Hot Stagnant
  - Cold Flushed

Data Collection per home

- Number of residents
- Plumbing materials
- Pipe and water heater sizing
- Fuel source
- Water heater model and make
- Insulation
- Time to hot water at fixture
- Age of the house
- Size of the house

Sampling Order

Small homes:

- Cold water first draw
- Hot water first draw
- Flushed hot
- Flushed cold
- Biofilm samples

Large homes:

- Cold water first draw (least)
- Hot water first draw (least)
- Flushed hot (least)
- Flushed cold (least)
- Cold water first draw (most)
- Hot water first draw (most)
- Flushed hot (most)
- Flushed cold (most)
- Biofilm samples

Key Takeaways

- In patient vs control homes, there were no differences in:
  - *Mycobacterium spp.* in biofilm
  - *Mycobacterium spp.* in water
  - *M. avium* in water

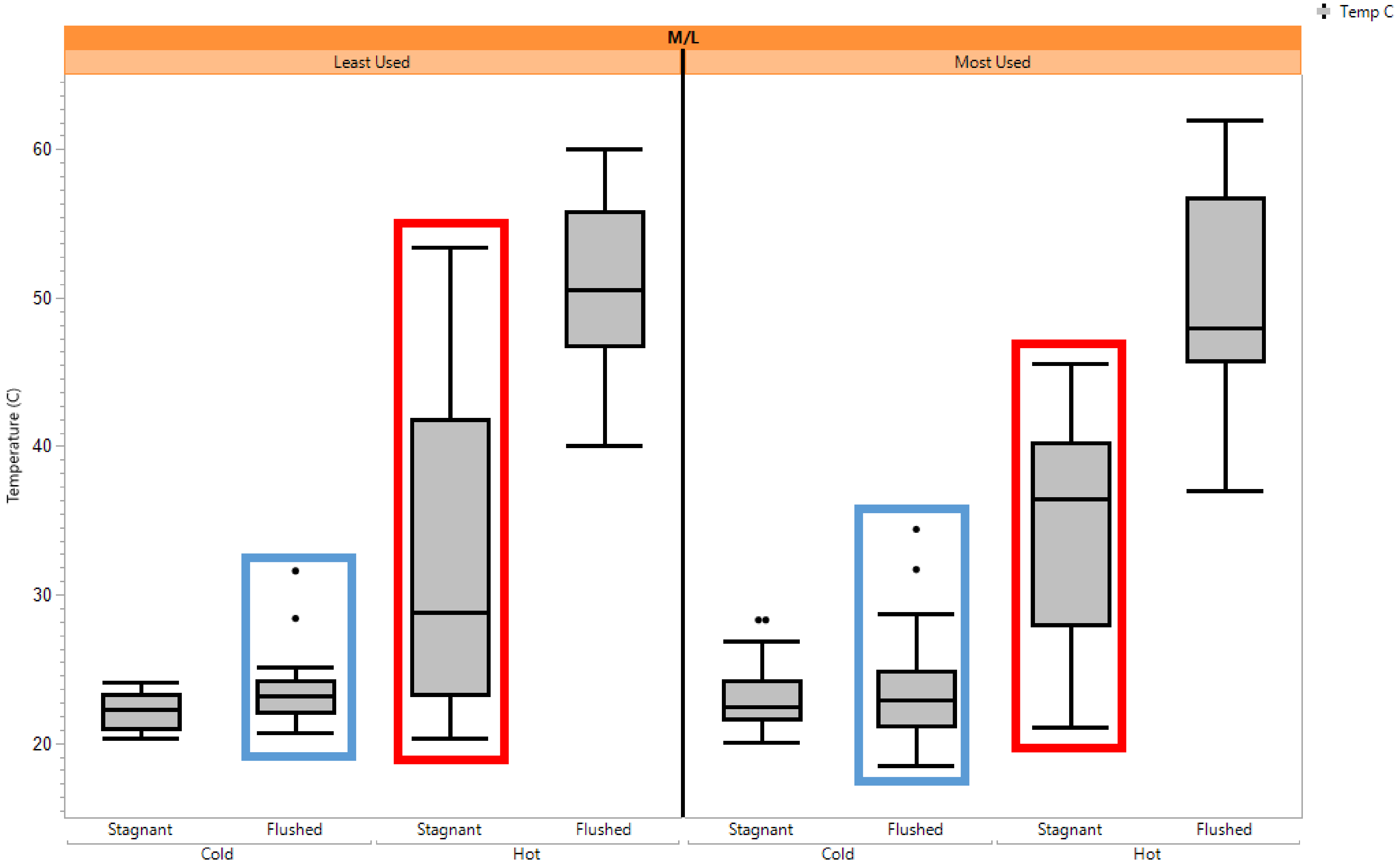
- Significantly more mycobacteria in biofilm cultures and water samples collected from least used showers compared to most used showers
  - Confirmed by other factors that co-vary with stagnation
    - Metals
    - Ammonia
    - Chlorine
    - Nitrite

Key Takeaways

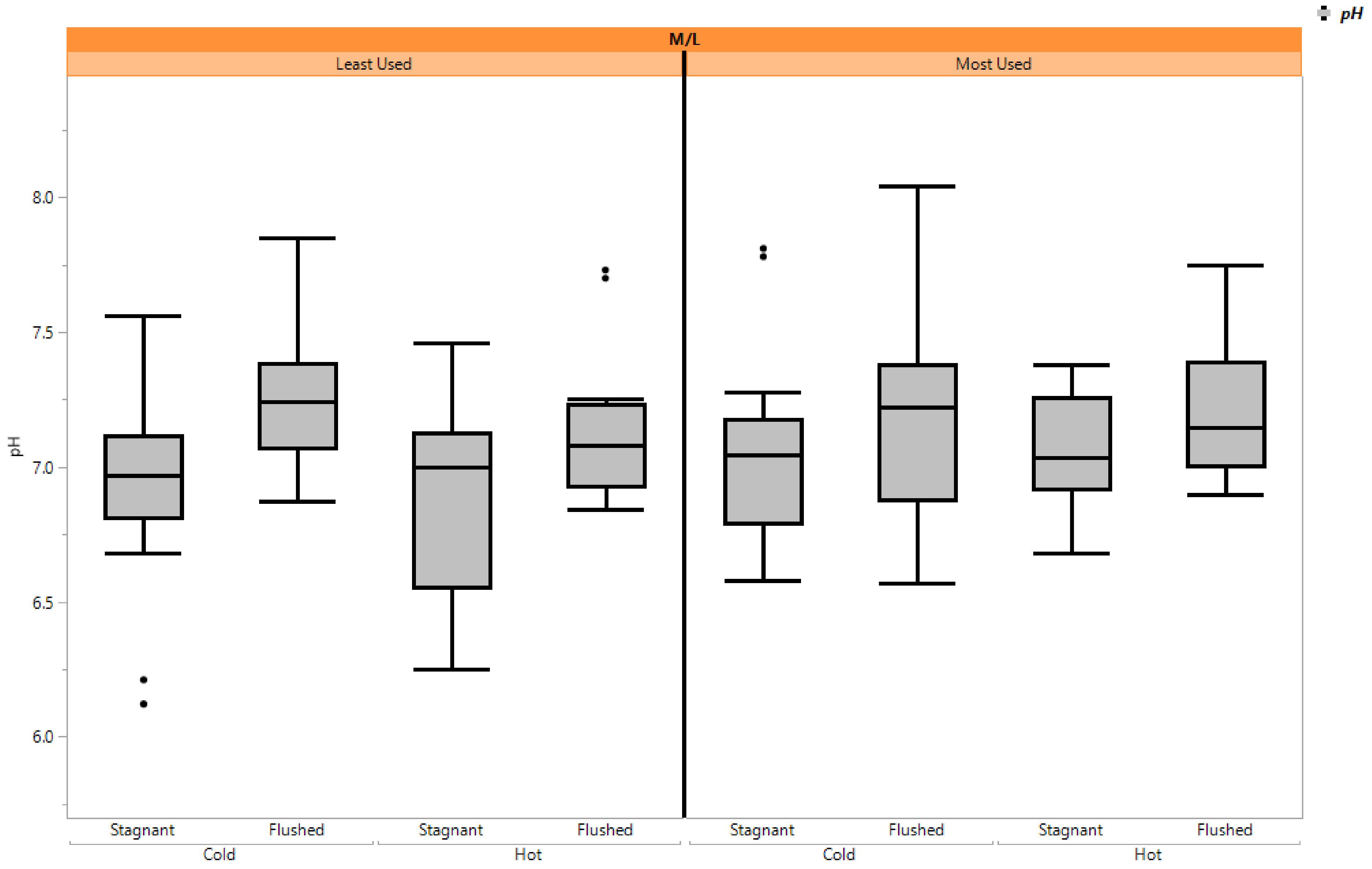
- High counts (>100 CFU/swab) of *Methylobacterium* spp. was a significant indicator for lower *Mycobacterium* spp. counts in biofilms, and vice-versa

- Factors affecting *M. avium* DNA (gc/mL) in water:
  - 16S rRNA, M. spp. (collinear)
  - Sampling characteristics – M/L, S/F (stagnation)
  - Size of the house (stagnation)
  - Age of the house
  - Recirculation systems

Temperature



pH



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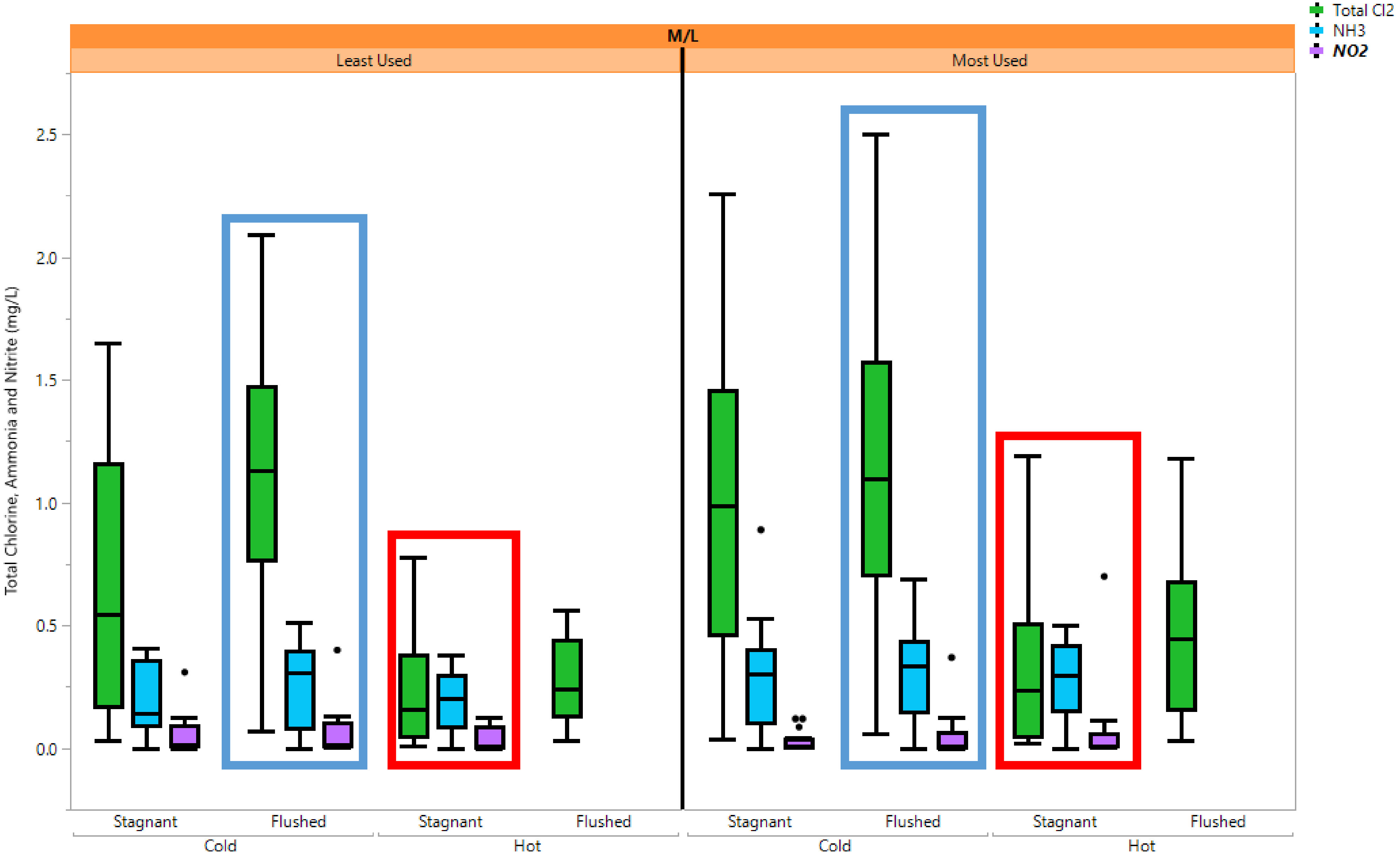
Total Chlorine, Ammonia and Nitrite



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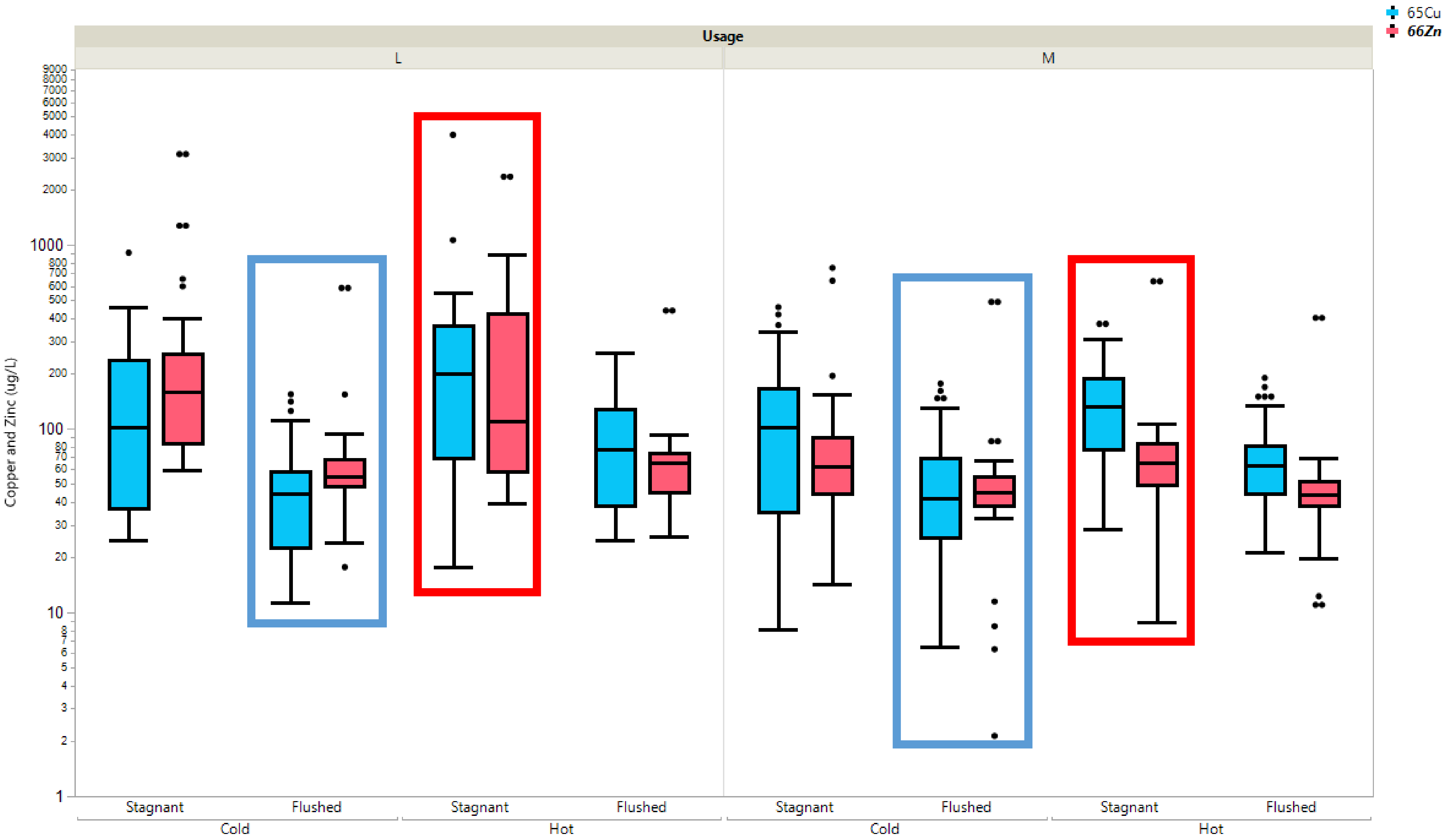
Copper and Zinc



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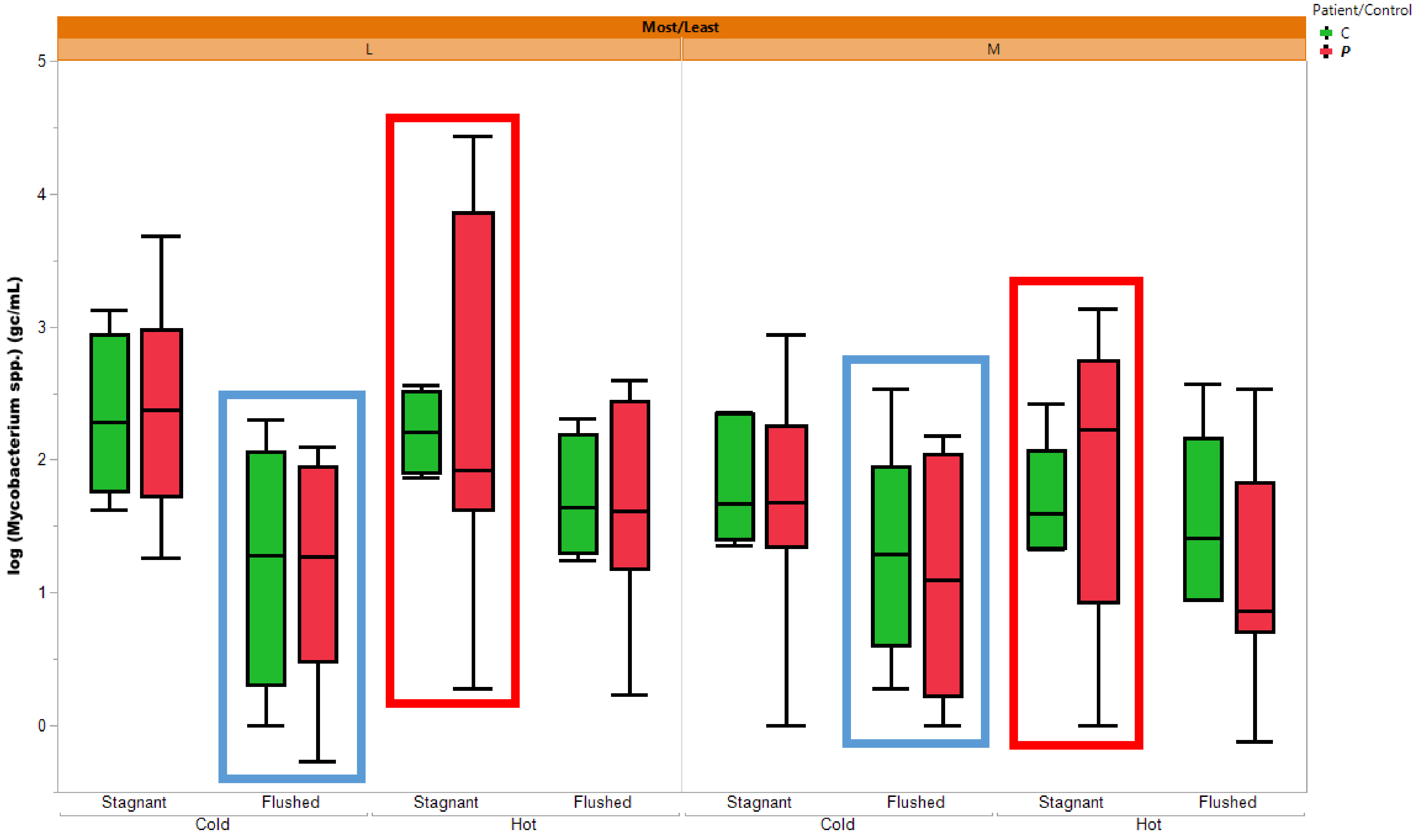
Location		Biofilm (n)	Biofilm	Sample		qPCR (n)	M. spp.	M. avium
Patient	Most	11	4 (36%)	Cold	Flush	10	9 (90%)	4 (40%)
							46 ± 62	1 ± 1
					Stag	11	10 (91%)	5 (45%)
							172 ± 279	1 ± 2
			24 ± 59	Hot	Flush	11	10 (91%)	5 (45 %)
							72 ± 122	17 ± 45
					Stag	11	10 (91%)	8 (73%)
							335 ± 421	76 ± 158
	Least	9	4 (44%)	Cold	Flush	9	9 (100%)	4 (44%)
							42 ± 50	9 ± 27
					Stag	9	9 (100%)	6 (67%)
							859 ± 1539	**23 ± 67**
			5009 ± 11514	Hot	Flush	9	9 (100%)	6 (67%)
							124 ± 155	39 ± 76
					Stag	9	9 (100%)	7 (78%)
							5024 ± 9511	**823 ± 1621**
Control	Most	5	0 (0%)	Cold	Flush	5	5 (100%)	2 (40%)
							80 ± 148	6 ± 13
					Stag	5	5 (100%)	2 (40%)
							109 ± 105	8 ± 17
			0 ± 0	Hot	Flush	5	5 (100%)	2 (40%)
							94 ± 156	2 ± 5
					Stag	5	5 (100%)	3 (60%)
							80 ± 103	21 ± 35
	Least	4	2 (50%)	Cold	Flush	4	4 (100%)	1 (25%)
							59 ± 93	0 ± 0
					Stag	4	4 (100%)	3 (75%)
							441 ± 597	0 ± 1
			5 ± 6	Hot	Flush	4	4 (100%)	3 (75%)
							80 ± 87	1 ± 1
					Stag	4	4 (100%)	4 (100%)
							196 ± 133	11 ± 15

Mycobacterium spp. (Patient vs Control)



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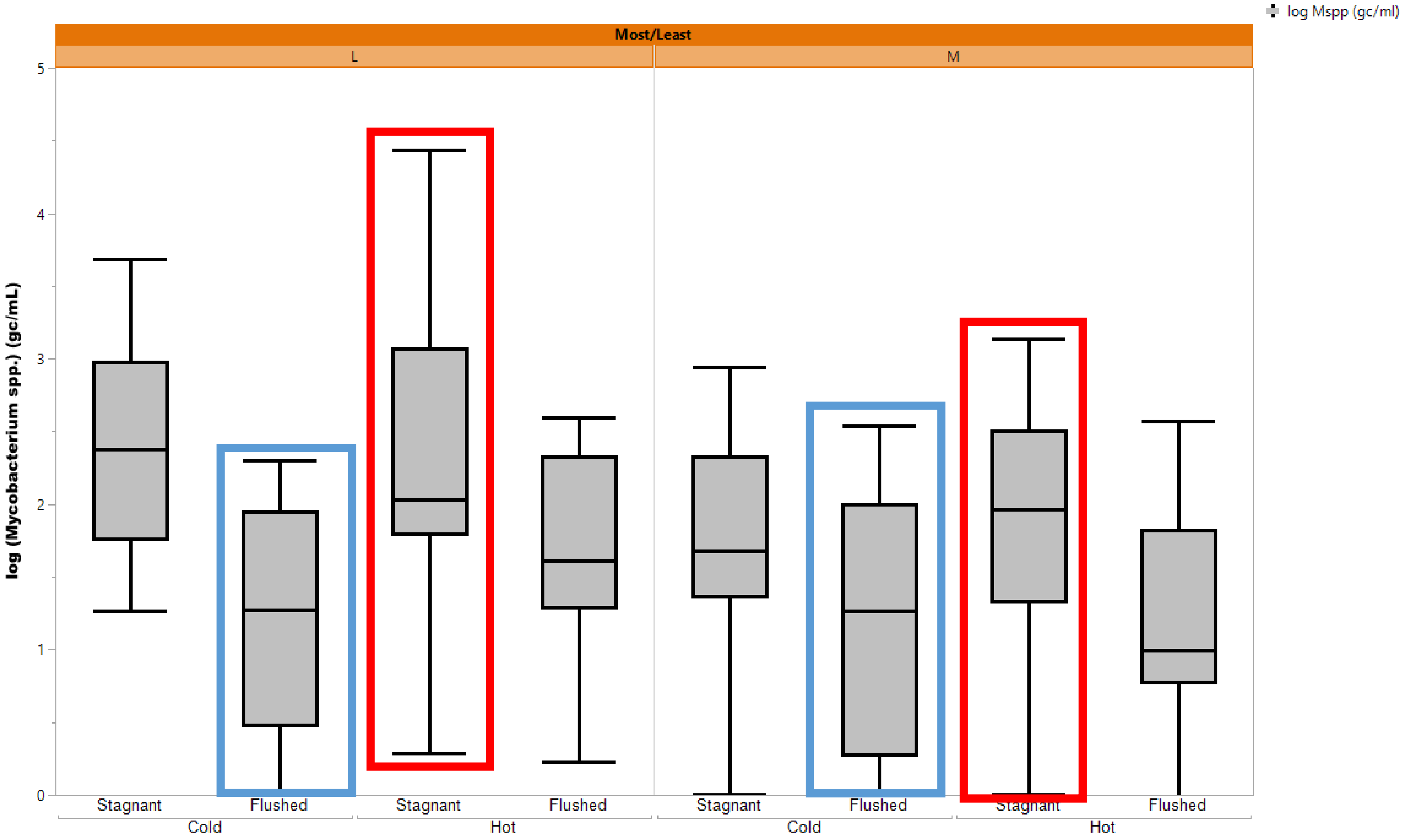
Mycobacterium spp.



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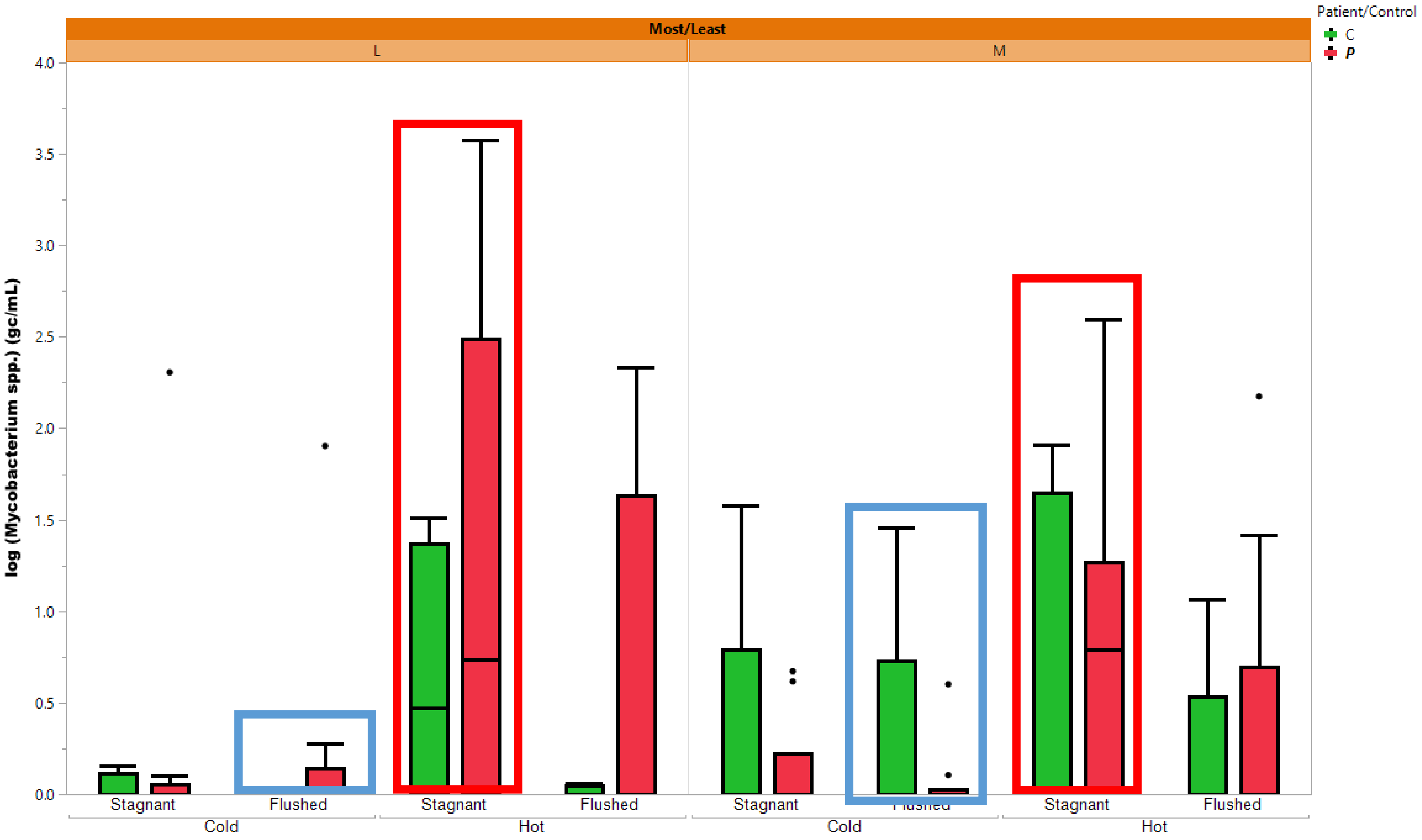
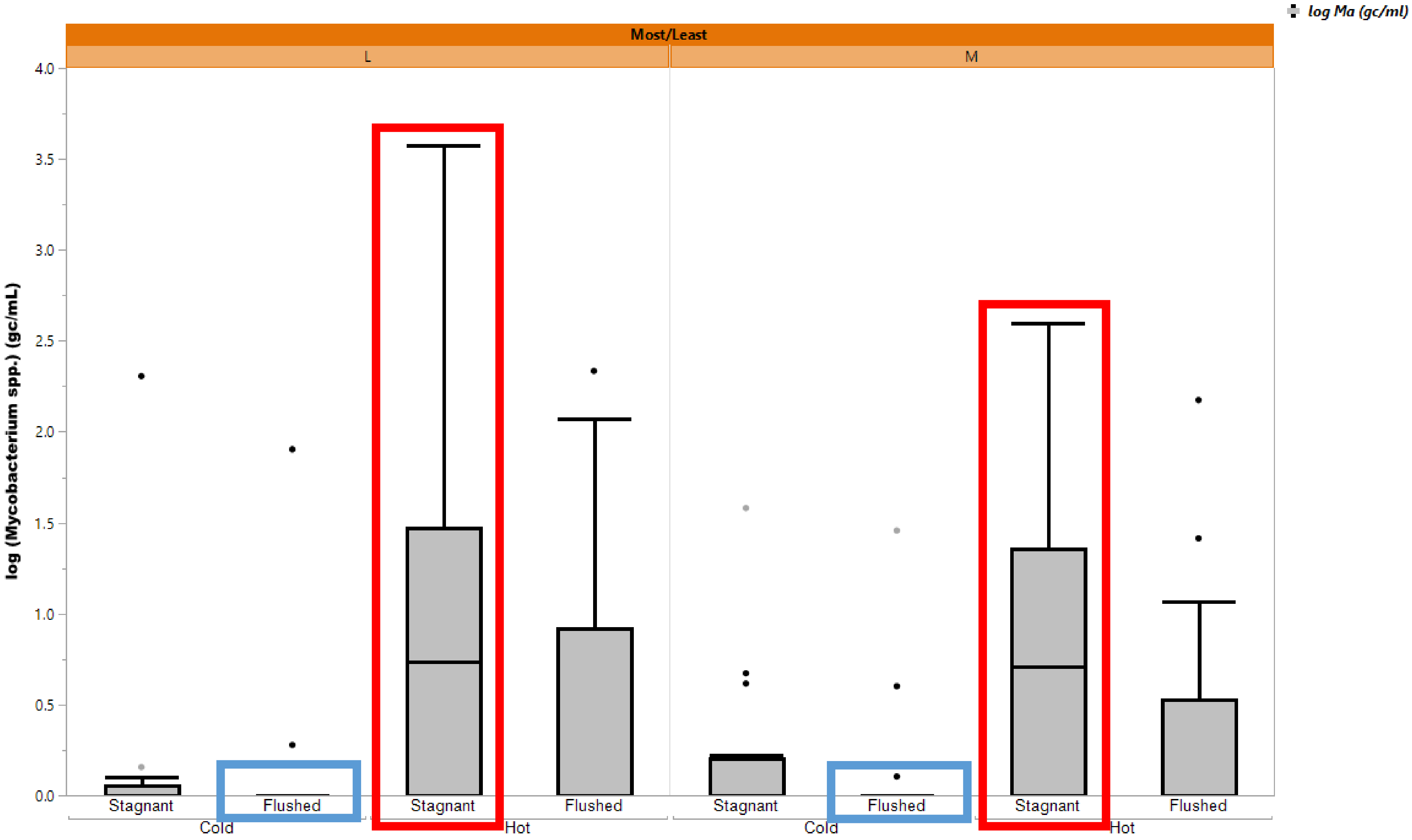
Mycobacterium avium (Patient vs Control)



Mycobacterium avium



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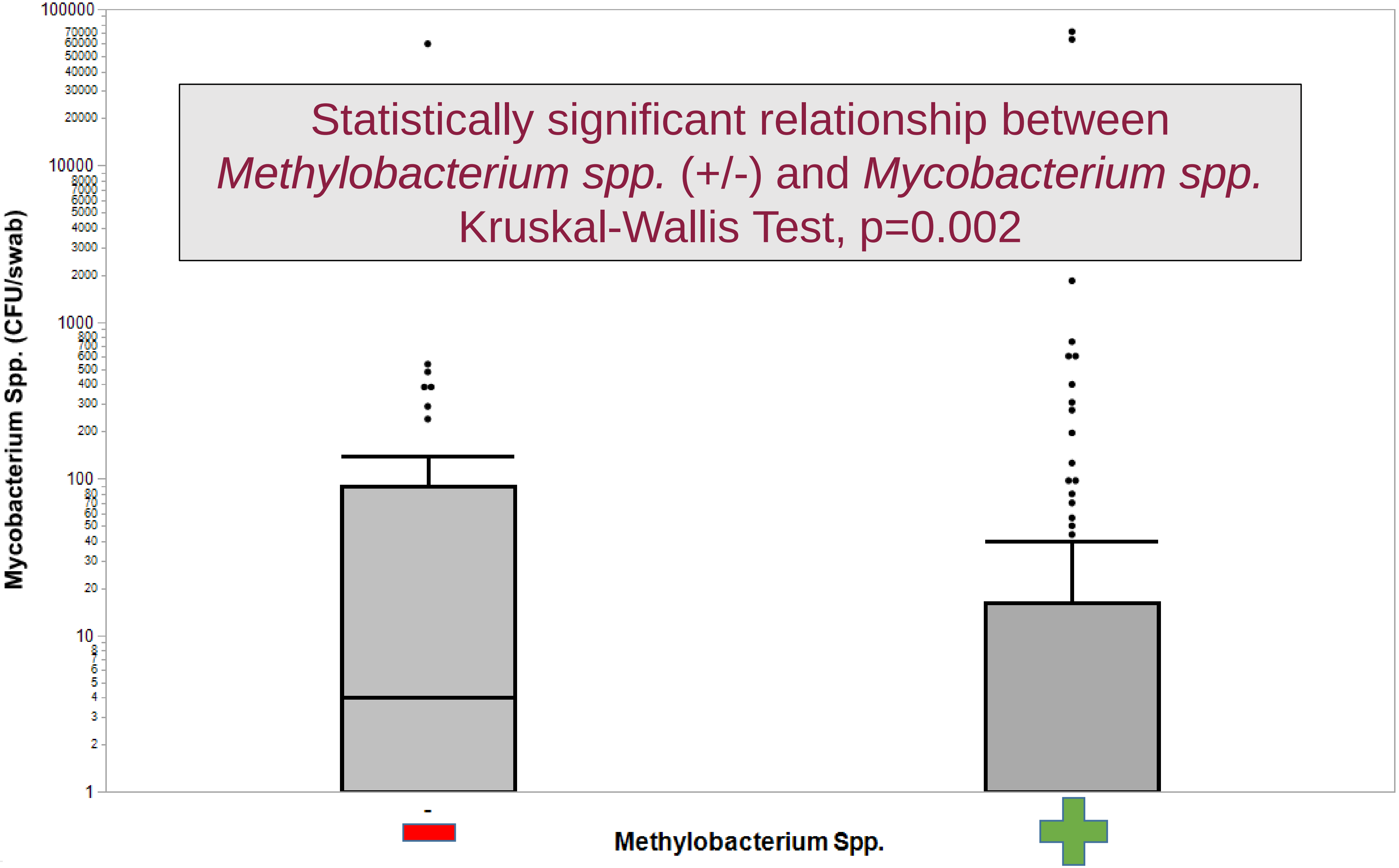
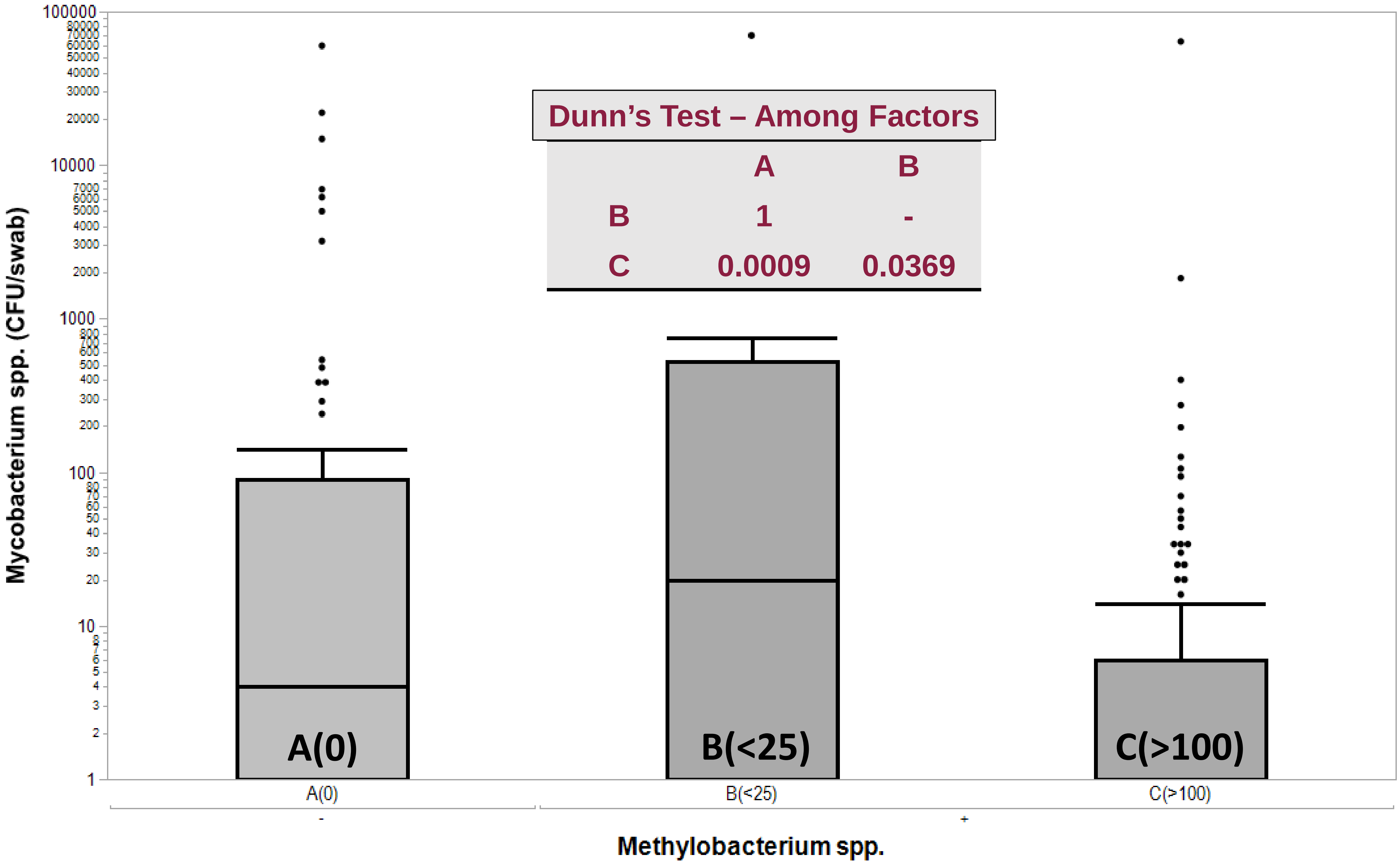
Mycobacterium spp. Vs Methylobacterium spp.





Dunn’s Test – Among Factors

	A	B
B	1	-
C	0.0009	0.0369

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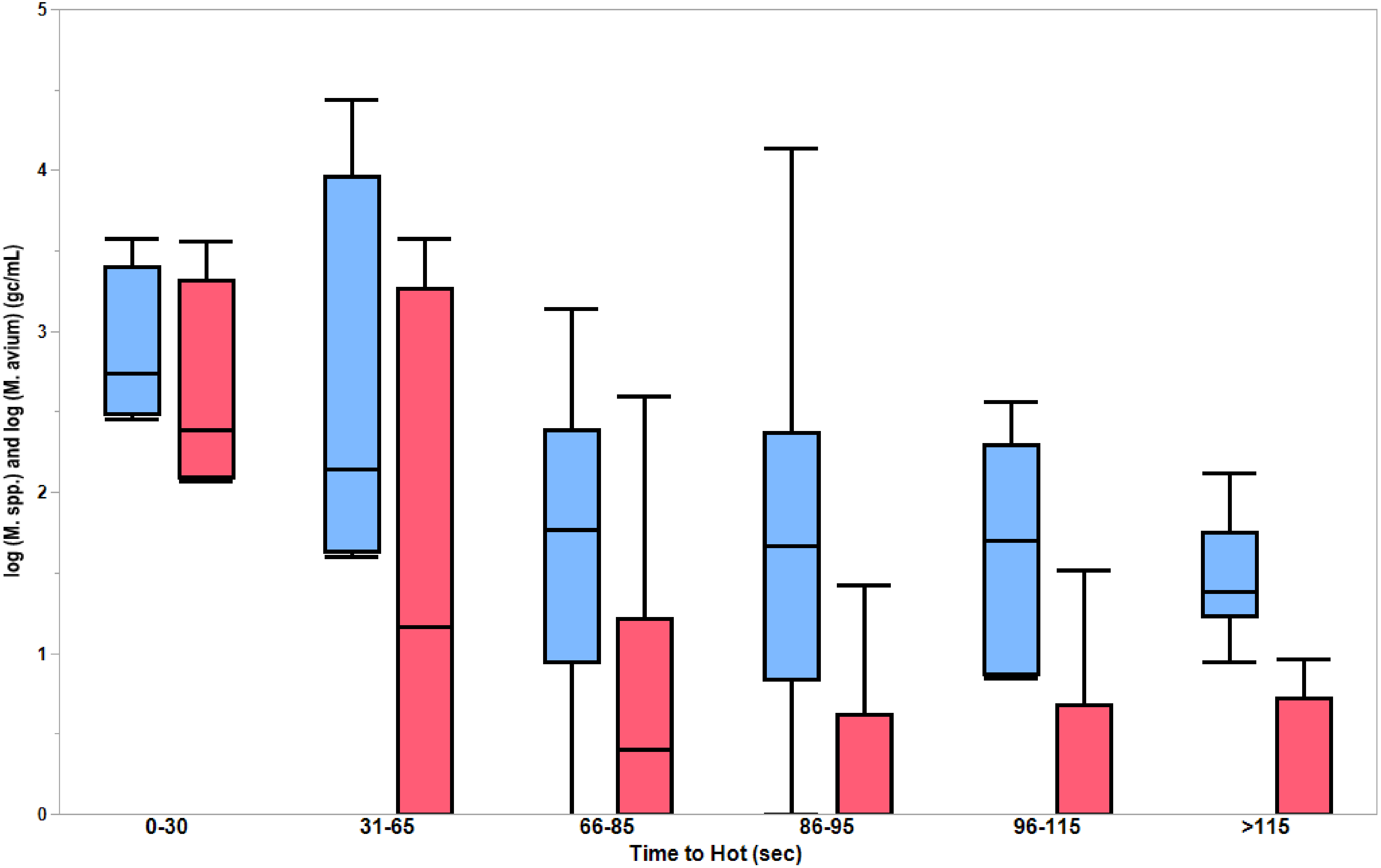
Mycobacterium spp. and Mycobacterium avium



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Factors affecting M. avium at the tap

- Most/Least - stagnation
- Stagnant/Flush – stagnation
- 16S rRNA – collinear
- *Mycobacterium spp.* – collinear
- Area of house - stagnation
- Floors - stagnation
- Year built in
- Recirculation

Future Work

- 16S amplicon sequencing on samples to determine microbial community makeup
- Follow-up sampling to study impact of raising water heater temperature as a control strategy

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