Use of SLICE® to Reduce Infestations of *Salmincola californiensis* in Rainbow Trout *Oncorhynchus mykiss*

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Introduction

- SLICE® - aquaculture feed premix
  - 0.2% emamectin benzoate (EB)
  - Sponsor – Merck Animal Health
- EB – developed for the control of sea lice
  - Tested extensively – environmental safety, efficacy, and tolerance
    - Atlantic salmon, rainbow trout, and brown trout
  - Fed to fish, absorbed from gut and distributed to a variety of tissues
    - Binds to ion channels of nerve cells and disrupts transmission of nerve impulses – which results in paralysis and death of parasite
  - Excreted slowly – extended period of protection (up to 9 wks)
- Approved in
  - UK, Europe, Norway, Chile, and Canada
- Approved dosage
  - 50 µg EB/kg fish bw/d for 7 consecutive days

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Introduction

- Also shown to effectively reduce
  - Rainbow trout – *Argulus coregoni*
    - Hakalahti et al. (2004)
  - Brook trout – *Salmincola edwardsii*
    - Duston and Cusack (2002)
- Anticipate that EB will also be effective against
  - *S. californiensis*
  - Interest by many to gain approval in U.S.

- Requires data to support efficacy and safety
  - Conducted field efficacy trials on rainbow trout infested with *S. californiensis*
    - Study conducted under a FDA-accepted protocol

Study Objectives

- At the end of each study –
  - Demonstrate a significant difference in mean abundance of adult, female *S. californiensis* between treated and control groups ($P < 0.05$)

  AND,

  - Achieve percent reduction in mean abundance is ≥ 90% (treated tanks relative to control tanks)
    - Standard for terrestrial parasiticides
    - No standard established for fish parasiticides

Study Objectives

Additional criteria:

- Infestation level in control tanks ≥ 50% of baseline infestation established in the reference population (pretreatment)
- Dose administered must be ± 20% of target (50 µg EB/kg fish/d)

Studies Conducted

All female populations:

- SeaPac of Idaho
  - Magic Springs Hatchery, Hagerman, ID
  - June 2010
  - Fish size – 31.6 cm; 400 g
- Clear Springs Foods #1
  - Snake River Research Facility, Buhl, ID
  - June 2010
  - Fish size – 36.2 cm; 645 g
- Clear Springs Foods #2
  - Snake River Research Facility, Buhl, ID
  - October 2010
  - Fish size – 33.9 cm; 517 g

Mixed-sex population:

- Missouri Dept of Conservation
  - Maramec Spring Hatchery, St. James, MO
  - May 2011
  - Fish size – 48.4 cm; 1474 g

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Reference Population

- Suitable population determined by examining 30 fish
- Criteria:
  - Prevalence ≥ 70%
  - Mean abundance ≥ 3 parasites/fish

- Magic Springs
  - Prevalence – 93%
  - Mean abundance – 5.5 ± 4.4
- Clear Springs #1
  - Prevalence – 100%
  - Mean abundance – 7.9 ± 7.9
- Clear Springs #2
  - Prevalence – 100%
  - Mean abundance – 7.3 ± 6.0
- Maramec Spring
  - Prevalence – 77%
  - Mean abundance – 6.6 ± 12.6

Experimental Design

- Treatment conditions
  - Two (treated and control)
- Randomly assigned
  - Magic – 10 tanks (5 treated, 5 control)
  - Clear Springs #1 & #2 – 8 tanks (4 treated, 4 control)
  - Maramec Spring – 6 boxes (3 treated, 3 control)
- Randomly allocated
  - 20 fish/tank
- Treatment period
  - 7 d
- Post-treatment period
  - Magic – 30 d
  - Clear Springs #1 – 30 d
  - Clear Springs #2 – 42 d
  - Maramec Spring – 42 d
- Fed fish
  - 0.5 or 1.0 % bw (twice daily)

Data Collection

- Pre-study level of infestation
  - Counted parasites on 30 fish
  - Samples (n = 9) sent to LaCrosse FHC
    - Definitive identification
- Baseline fish health evaluations
  - 20 fish
- Mortality and behavior
  - Daily
- Feeding behavior
  - Daily
  - 5 point ordinal scale (0 = not eating; 4 = ~100% of the feed ration)
- Water quality
  - Water temp and DO
    - Daily
  - Hardness, alkalinity, pH
    - Two times during each study





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Data Collection

- Feed samples for dose verification
  - 3 samples from medicated feed bag
  - 1 sample from control feed bag
- End of study level of infestation
  - Counted parasites on all live fish
    - Fish sedated with MS-222
    - Number of fish/tank ranged from 12 to 20

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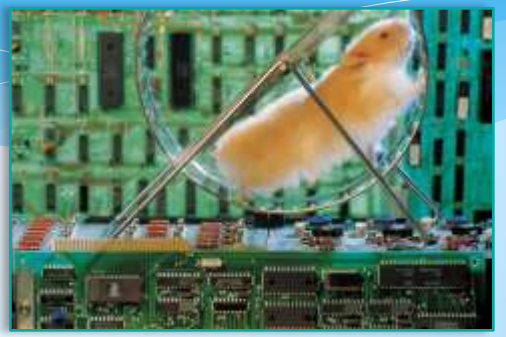
Data Analysis



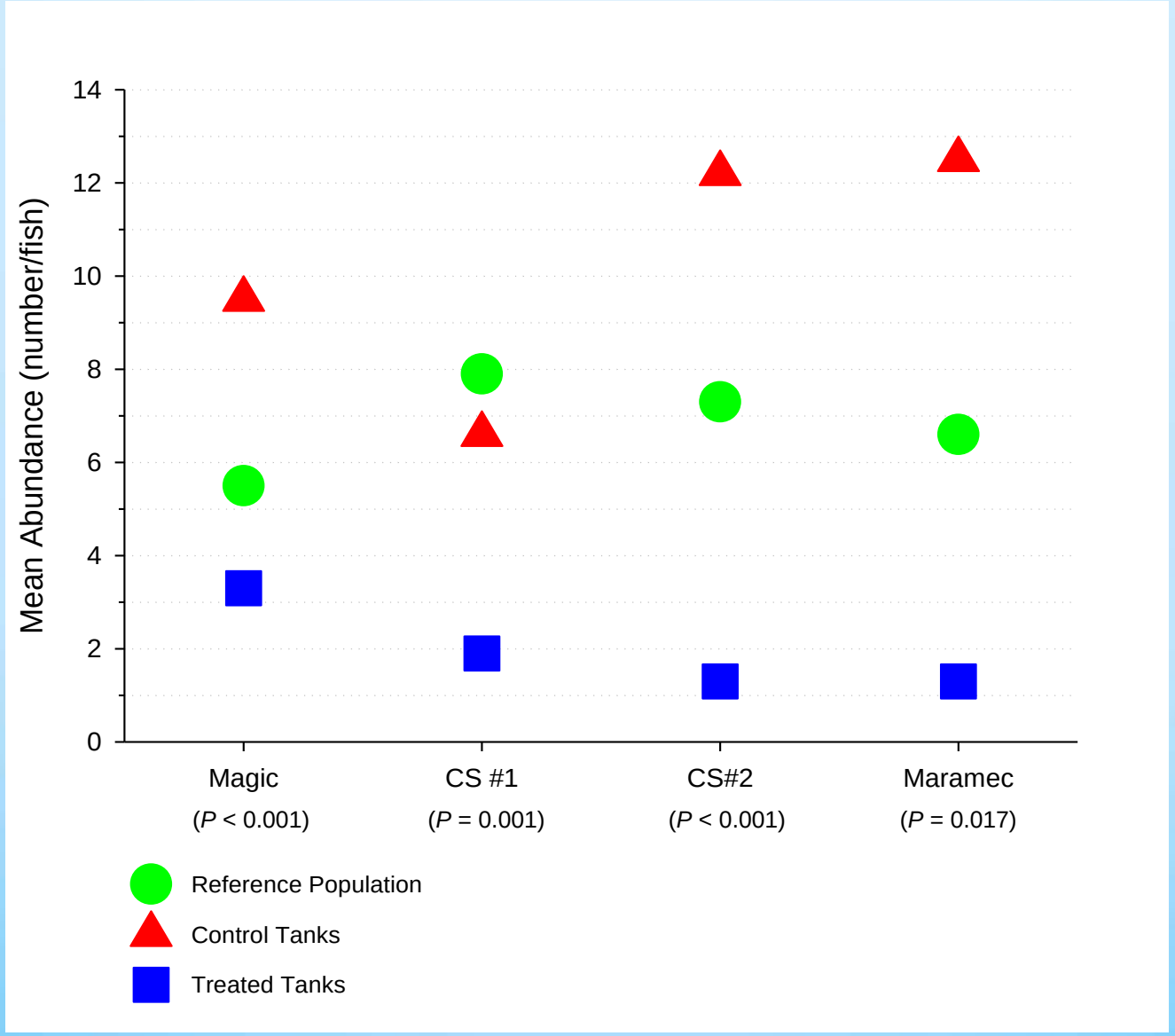
- Mean parasite abundance
  - Nested ANOVA ($P \leq 0.05$; two–sided)
- % reduction in abundance
  - Based on geometric means
  - Treated tanks relative to control tanks
- Mortality
  - Proc Glimmix ($P \leq 0.05$)

Results

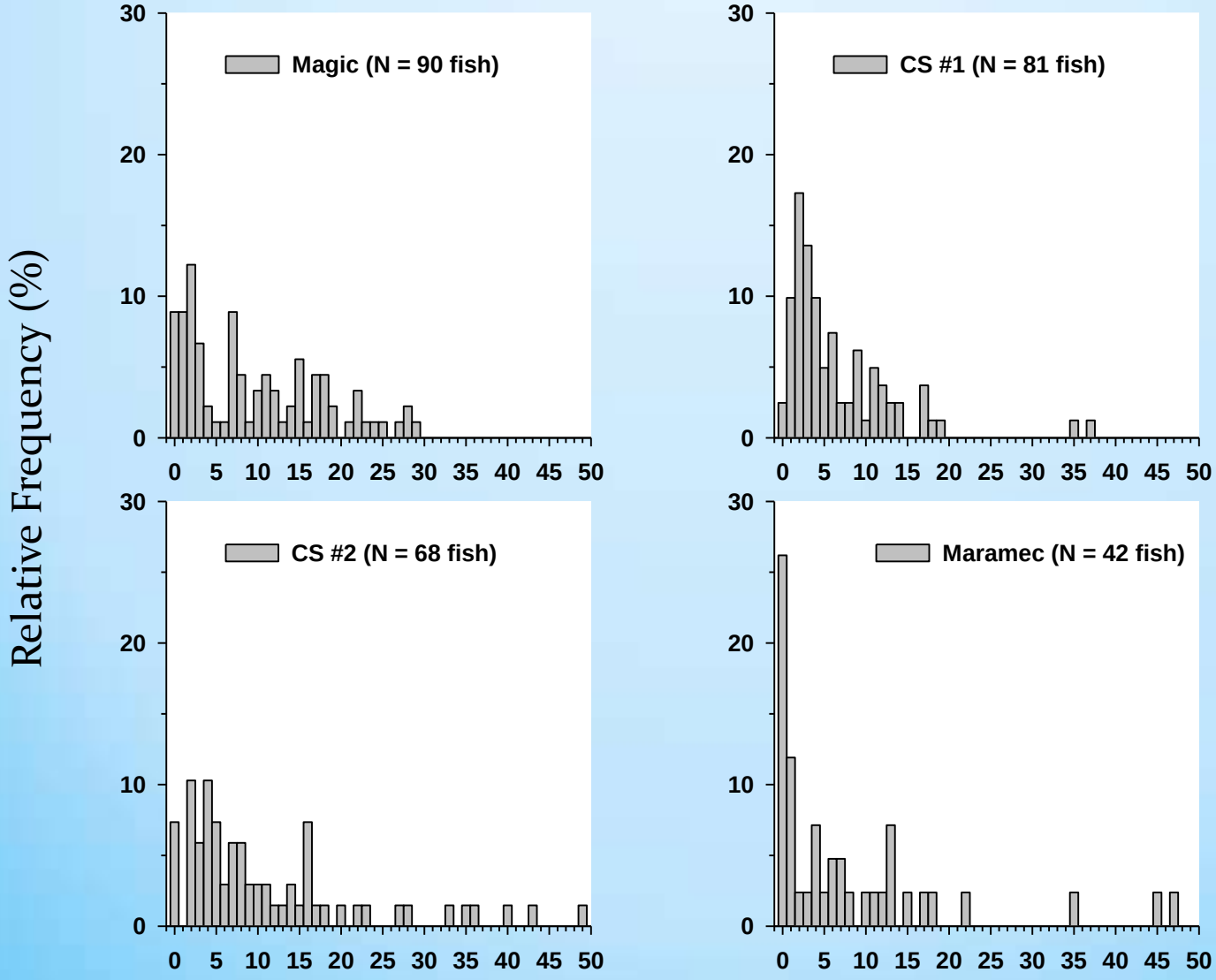
In all studies:

- Fish behavior was normal
- Feeding behavior was nearly identical in treated & control tanks
  - Treatment period – all tanks ate 50 -75% of feed
  - Posttreatment period – all tanks ate 75 – 100% of feed
- Water temperature, 13.4 – 15.3°C
- Dissolved oxygen, 6.3 – 8.7 mg/L
- Hardness, 160 – 236 mg/L $CaCO_3$
- Alkalinity, 125 – 158 mg/L $CaCO_3$
- Mean analytically verified dose in treated feed was 42 – 49 µg/kg fish/d (2 – 16% below target)
  - No EB detected in control feed

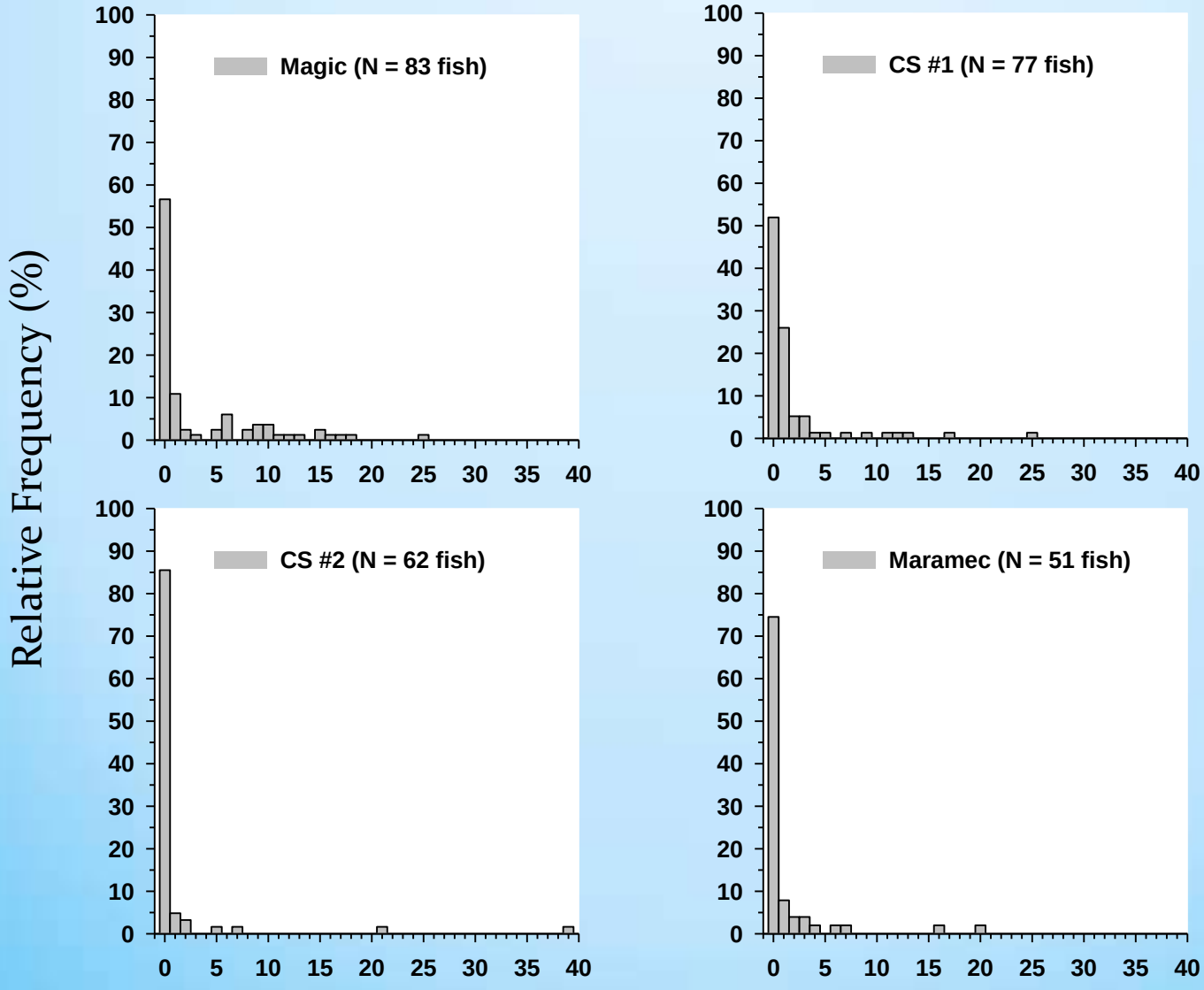
Mean Abundance



Parasite Frequency (controls)



Parasite Frequency (treated)



Percent Reduction

$$Percent\ reduction = 100 - \left[100 \times \frac{(geometric\ mean_{treated} - 1)}{(geometric\ mean_{control} - 1)} \right]$$

- Magic Springs – **79%**
  - 30 d post-treatment period
- Clear Springs #1 – **83%**
  - 30 d post-treatment period
- Clear Springs #2 – **96%**
  - 42 d post-treatment period
- Maramec – **90%**
  - 42 d post-treatment period

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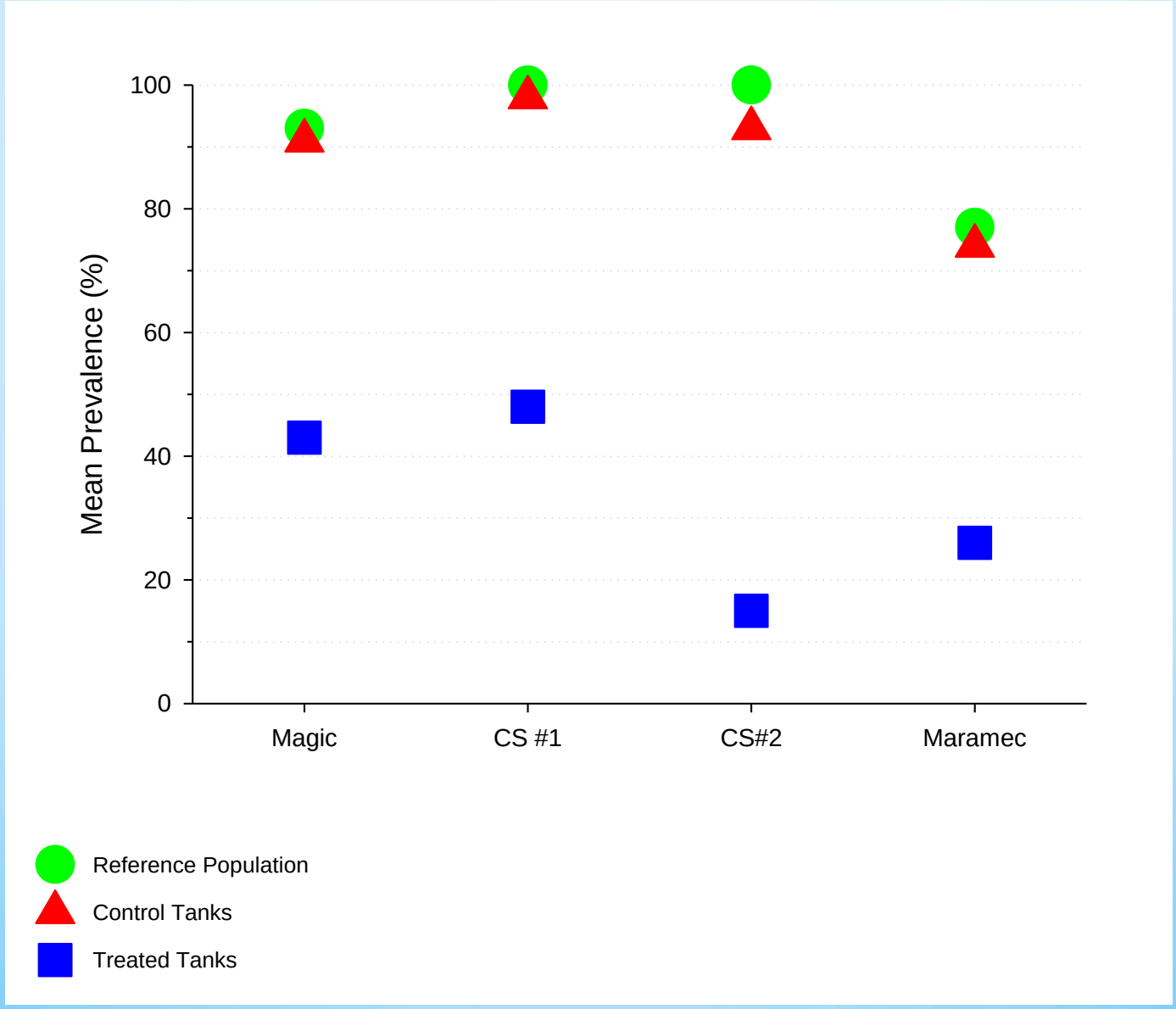
Prevalence



* No stats run

Mortality

Trial	Mean (range) cumulative mortality (%)		*P*-value
	Treated tanks	Control tanks	
Magic	16 (5 – 25)	10 (0 – 5)	0.2895
Clear Springs #1	2.5 (0 – 10)	1.3 (0 – 5)	0.6438
Clear Springs #2	25 (10 – 40)	15 (0 – 30)	0.3759
Maramec *	1.7 (0 – 5)	2.4 (0 – 7)	0.8952

* Fish escapement during post-treatment

Summary

- Significant difference in mean abundance between treated and control tanks in all 4 studies
- A 90% reduction was met in 2 of 4 studies
- Infestation prevalence decreased substantially in all treated tanks, but remained near pre-study levels in control tanks
- Fish did not eat all feed offered during treatment period
- EB dose administered was within ± 20% of target

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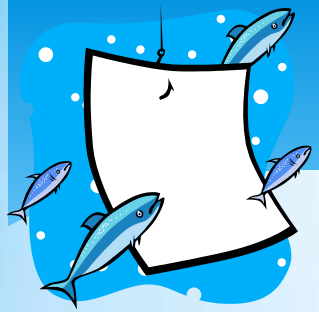
Discussion

- Numerous factors contributed to failure to meet 90% reduction:
  - FDA standard “brought over from terrestrial side”
  - Mean abundance “skewed” by 1 – 2 fish in each treated tank
    - Treating individuals vs. population
  - Continual exposure to parasites
  - Post-treatment period duration



Discussion

- Higher mortality in treated tanks in 2 of 4 studies
  - Differences were not statistically significant
  - Mortality occurred in both treatment groups
    - Not due to emamectin benzoate
    - Roy et al. 2000 – no mortality in ATS and RBT when fed 7-fold higher dose
    - Likely caused by infestation stress

Conclusions

- SLICE® was effective
  - Reducing *S. californiensis* infestations in male & female rainbow trout
- Greater level of reduction
  - likely after a 42 d compared with a 30 d post-treatment period
- Future studies conducted with > 42 d post-treatment period
  - Identify when maximum efficacy is achieved
  - Determine period of protection

Status

- Report for each study submitted to FDA for review
- Requested effectiveness technical section be considered complete
- Submitted manuscript to North American Journal of Aquaculture

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Questions?