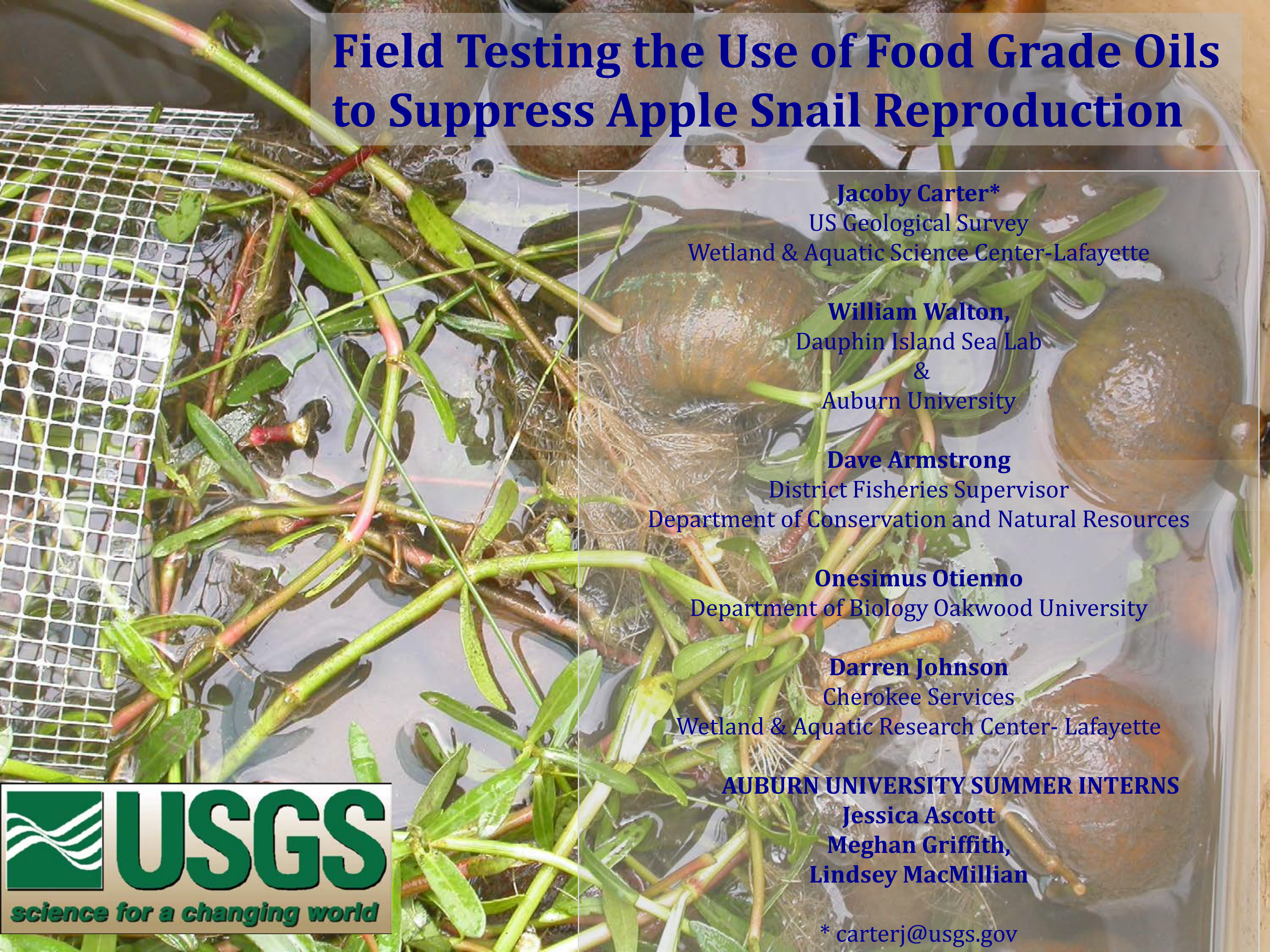

Field Testing the Use of Food Grade Oils to Suppress Apple Snail Reproduction

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Apple snails can devastate the community of submerged and floating leaved aquatic vegetation.

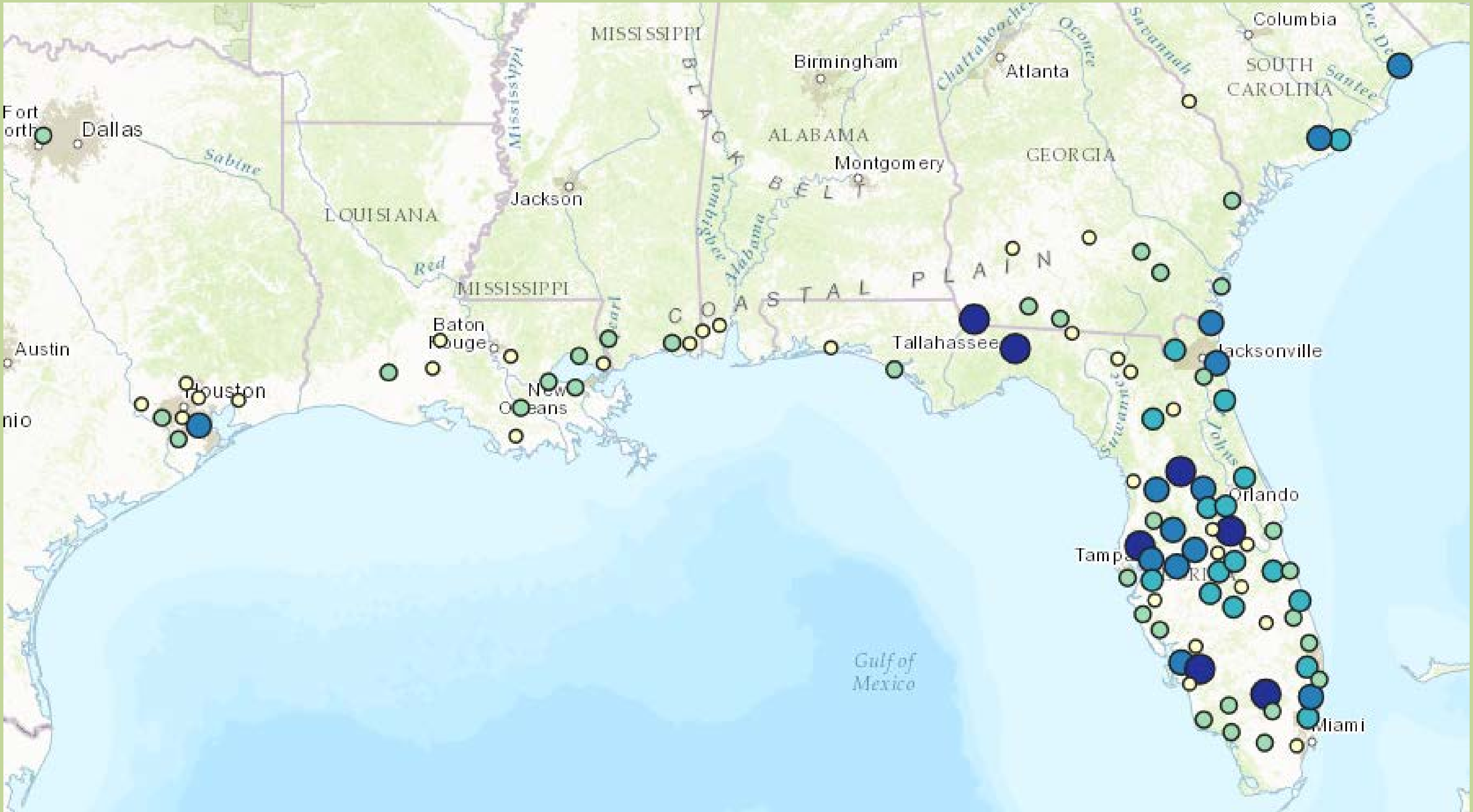
In south Louisiana we are already hearing reports by owners of duck leases of habitat quality loss due to overgrazing of submerged aquatic vegetation by apple snails.

In various regions around the world rice and taro production have been negatively impacted by apple snails.

They may impact amphibian reproduction by eating their eggs.

Since egg masses are the most prominent aspect of their natural history we wanted to develop a method that effectively reduced their reproduction.

Distribution of maculata apple snails (*Pomacea maculata*) in our region



Source:
http://nas.er.usgs.gov/
October 3, 2016

Why use food grade oils?

Barriers for approval should be low.

-Food grade oils may be exempt from EPA oversight since they area already approved as inert ingredients.

-They are already considered safe for human consumption.

Mode of action is physical not chemical.
The oil suffocates the egg mass.

Inexpensive and easily obtained.

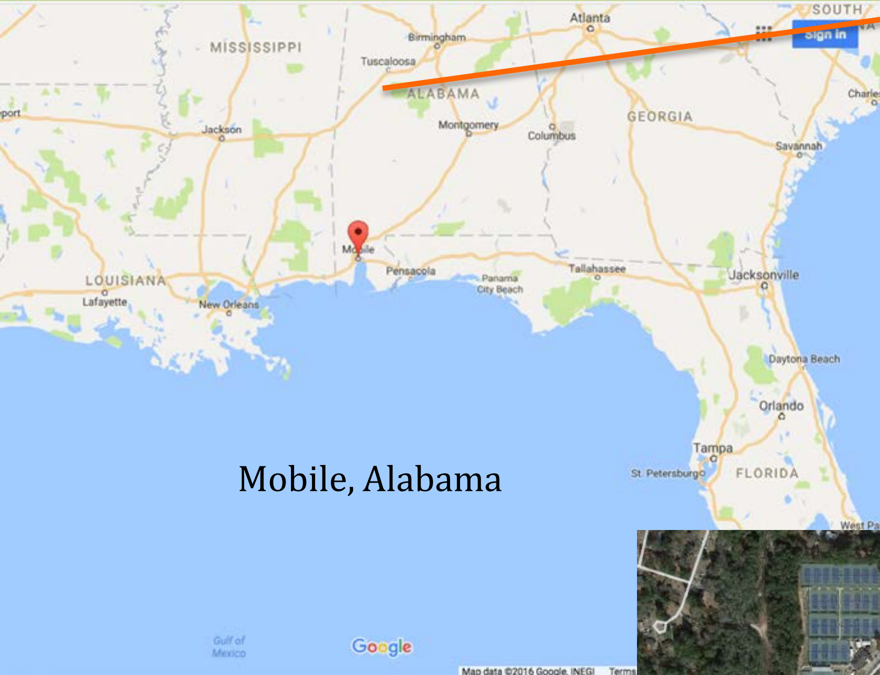
The use of a spray may have advantages over physical destruction of the egg masses.

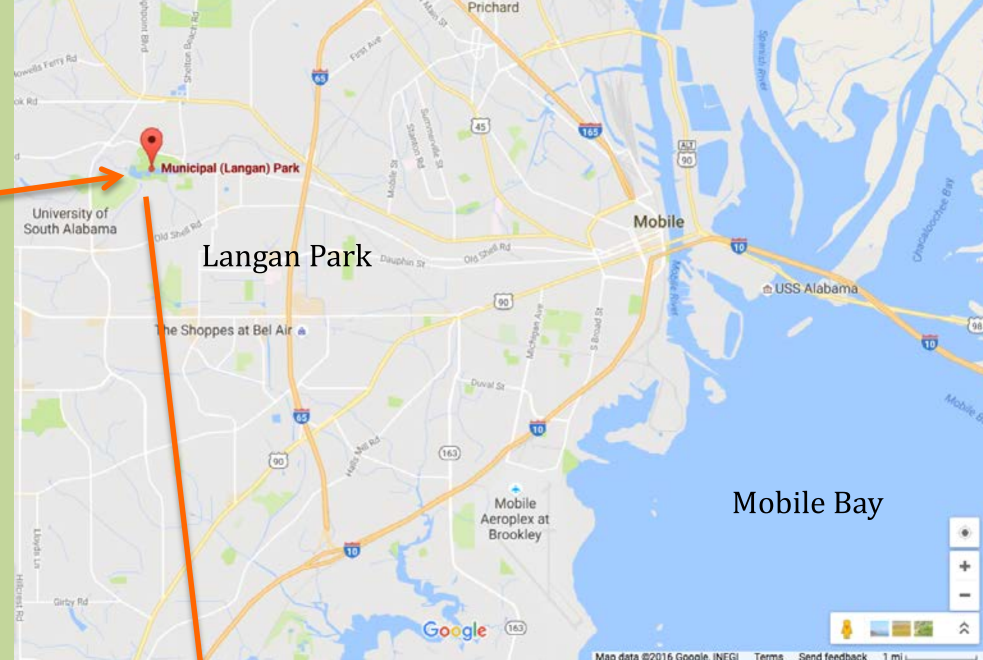
Treatments

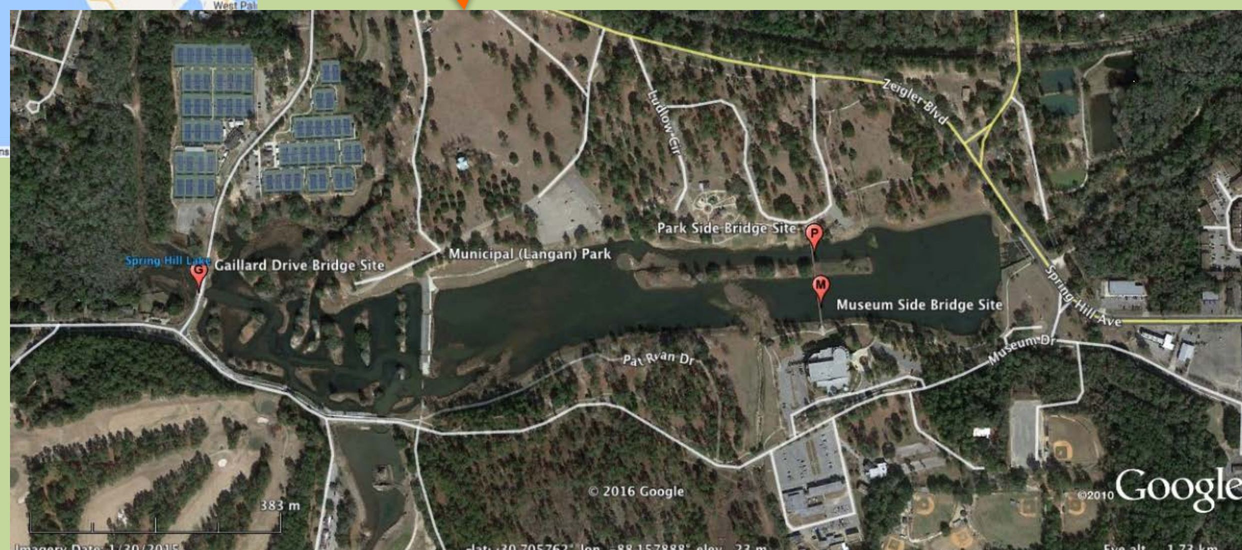
- Pam® - which is mostly canola oil (a.k.a. rapeseed oil) and emulsifiers.
- Undiluted coconut oil applied with a hand pumped agricultural sprayer.
- Control- Not sprayed.

After treatment, egg masses were observed for a minimum of three weeks, after which % hatching was evaluated.

Study Site







Location of Three Study Sites in Langan Municipal Park, Mobile AL, USA.
The sites were all on bridge piers and abutments. G: Gaillard Drive Bridge (30.705665° -88.16504); M- Foot bridge across the lake, Art Museum side (30.705439°, -88.154868); and P- foot bridge across the lake , park side (30.70617°, -99.154976°).

MG 7/1

Egg Mass Control Experiment Data Sheet (version: June 20, 2016) #3 of: 3 for day

Today's Date: 6/30/16 Date Site Last Checked: 6/27/16 Number of Days Since Last Checked: 3

Highest Envelope Number for Site Before Today (new envelops start at this number + 1): 75

Team (names, minimum 2): Lindsay, Megan, Jessica

General Location: Langan Site Name (& Code): B Camera Used: ... 850

Envelope ID: Z = Site; ### = sequential number at site; T = treatment; SME (Structure, Material, and Exposure)

Egg Mass Color Key

Average the color over the entire egg mass (from 1 freshest, to 6 oldest). If any hatching is apparent, score as '7'.

See back for other keys.

#	Envelope ID (Z-###-T-SME)	Treat	Color	Date	Length	Width	% Hatch	H_2O	Env
70	B	P		6/30			NH		
71	B	C		6/30			NH		
72	B	CO		6/30			NH		
73	B	P		6/30			NH		
74	B	C		6/30			NH		
75	B-75-CO-BMO	CO	1	6/30	10.6 cm				
76	B-76-P-BCU	P	1	6/30	7.1				
77	B-77-C -	C	1	6/30	8.1				
78	B-78-CO-	Co	2	6/30	6.1				

Notes on Back (Yes/No): **Mission Leader's Initials:**

Envelopes to catch hatchlings & label the treatment

235 egg masses were entered into the field study from May 25th through July 5th, 2016

Egg masses less than 3 weeks old that were lost before they hatched out were removed from our analyses.
If they hatched out before 3 weeks (most hatched out in 2), they were retained for analysis.
Egg masses 3 weeks or older had their final status recorded and were then removed.

A modified Braun-Blanquet Index was used to record percent hatching at the end of three weeks.

Index	Visual Estimate of Hatching
0	0% (no hatching)
1	trace - 10%
2	10 – 25%
3	25-50%
4	50-75%
5	75-100%

40% of potential observations were lost do to flooding or other unknown causes before the full three weeks of observations could be completed.

Analyses were done using the PROC LOGISTIC in SAS, version 9.4

Overview of Analysis

(1) Model Selection

A stepwise analysis was used to determine the best cumulative logit model for the data. We used a cumulative logit model because the response categories (hatching index) were ordinal.

(2) Hatching Probabilities

The resultant maximum likelihood model was then used to derive hatching probabilities given a treatment.

(3) Evaluating the Effectiveness of Treatment

Since hatching probabilities of a given treatment were spread across multiple indices and were affected by egg mass length (see next) we estimated the number of hatchlings and compared overall effectiveness of the three treatments.

Model Selection:

We analyzed the effects of treatment on apple snail egg mass while controlling for other independent variables.

(1) The **independent variables** were

Site (B-Gaillard Drive Bridge, M- Museum bridge, and P- Park bridge),
Treatment (Pam, Coconut Oil, or control = no spray)
Color of the egg mass (as an index of age)
Length of the egg mass
Maximum Age (time since last site visit when the egg mass wasn't present)
Interactions of **Site &Treatment**
Interaction of **Color &Treatment**
and
Interaction of **Length &Treatment**.

(2) The **response variable was hatching index** with the **ordinal levels 0-5**.

(3) The **stepwise procedure** was run on all variable using **forward**, **backwards**, and stepwise on the **cumulative logit** of the **hatching probabilities**.

(4) These variable reduction procedures were run with an **alpha = 0.05**.

We had a total of 140 observations.
Since the total number of observations was insufficient in hatching categories 2-5 for analysis (rule of thumb minimum ≥ 25), we had to combine several hatching categories.

Hatching Index, Definition, Observations & Analysis Categories.

Modified Braun-Blanquet Hatching Index	% hatching observed	n	Analysis Category	n
0	no hatching	55	1 (0)	55
1	<10%	41		
2	10-25%	12	2 (1-2)	53
3	25-50%	8		
4	50-75%	5		
5	75-100%	19	3 (3-5)	32

(1) All three stepwise variable reduction procedures (forward, backward, and cumulative logit) were in agreement and kept the following two variables: **treatment** and **length**.
(2) Interactions were not significant.
(3) For the overall model, the p-value was < 0.0001, with the individual variable significant (Treatment p-value <0.0001, Length p-value = 0.0220).

Model Design

Class	Value	Design Variables	
Treat	C	1	0
	CO	0	1
	P	-1	-1

Model Convergence Status

Convergence criterion (GCONV=1E-8) satisfied.

Score Test for the Proportional Odds Assumption

Chi-Square	DF	Pr > ChiSq
17.3912	3	0.0006

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	91.8459	3	<.0001
Score	63.6977	3	<.0001
Wald	56.1109	3	<.0001

Type 3 Analysis of Effects

Effect	DF	Wald Chi-Square	Pr > ChiSq
Treat	2	54.5833	<.0001
Length	1	5.2441	0.0220

Analysis of Maximum Likelihood Estimates

Parameter		DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	3	1	-3.0431	0.7891	14.8724	0.0001
Intercept	2	1	-0.00734	0.7027	0.0001	0.9917
Treat	C	1	3.1853	0.4348	53.6776	<.0001
Treat	CO	1	-1.2662	0.2786	20.6626	<.0001
Length		1	0.2028	0.0885	5.2441	0.0220

Was spraying with cooking oil effective in preventing hatching?

Contrast Test Results

Contrast	DF	Wald Chi-Square	Pr > ChiSq
Control – Coconut Oil	1	45.1394	<.0001
Control-Pam	1	53.2910	<.0001
Coconut Oil-Pam	1	2.7560	0.0969

Summary

(1) Both oil applications significantly reduced hatching as compared to control.

(2) The longer the egg mass, the greater the likelihood of hatching, but the effect was small

Hatching Probabilities

To assess effectiveness of the oil treatment (how much they reduced hatching) we need to calculate the probability of each hatching category (1-3) occurring across all size classes for each treatment.

Running the probabilities across all lengths of egg masses and summing we get the following.

The Sum of the Probability of Hatching of a Given Treatment

Hatching Index/Treatment	Probability
25-100%/Control	0.869
Trace-25%/Control	0.123
No Hatching/Control	0.008
25-100%/Coconut Oil	0.100
Trace-25%/Coconut Oil	0.540
No Hatching /Coconut Oil	0.360
25-100%/Pam	0.056
Trace-25%/Pam	0.442
No Hatching /Pam	0.502

How Effective Were the Treatments?

For each combination, to calculate the expected number of hatchlings
(# eggs per egg mass * % hatching * # egg mass) = number of expected hatchlings.

Assuming one female produces 1.5 egg masses per week for a 30 week season = 45 egg masses.
Further assume each egg mass has an average of 1,000 eggs
The number of expected hatchlings is:

Treatment	Max Possible	Category 3 100% hatch	Category 2 25% hatch	Category 1 0% hatch	Hatchlings
Control:	45*1000*[	(1.0*0.869) +	(0.25*0.123) +	(0.0*0.008)]=	40,490 (baseline)
Coconut Oil:	45*1000*[	(1.0*0.100) +	(0.25*0.540) +	(0.0*0.360)] =	10,575 (-73.9% reduction)
Pam®:	45*1000*[	(1.0*0.056) +	(0.25*0.442) +	(0.0*0.502)] =	7,536 (-81.2% reduction)

This is across all lengths, and is conservative in that is uses the maximum percentage hatching of the combined indexes in a category.

Therefore the actual number of hatchlings will probably be fewer than the above estimates.

The percent reduction (e.g., the effectiveness of the treatment) remains unchanged even if we make different assumptions about the number of egg masses laid in a season or the average number of eggs per egg mass

Conclusions

- Spraying with food oils can significantly reduce overall hatching (between 70-80%). This fraction might improved upon with further research into the technique.
- Pam® was cost effective. One can sprayed over 80 egg masses. However, its effective range is short, whereas a conventional sprayer can spray at a distance.
- While the reduction in percent hatching was significant, that still leaves a lot of hatchlings given the number of eggs per egg mass.
- Oil sprays may be useful in those situations where physical destruction of the egg mass is not practical.
- Reduction of reproducing females still needs to be part of any mitigation strategy.