Using the Fluoromarker Calcein to Assess Growth Rates of Quagga Mussels *in situ*

Kerrin Mabrey[1,*], Ashley Elgin[2], Paul Glyshaw[1]
[1]University of Michigan CILER
[2]NOAA Great Lakes Environmental Research Laboratory
[*]Contact: kmabrey@umich.edu

OBJECTIVE

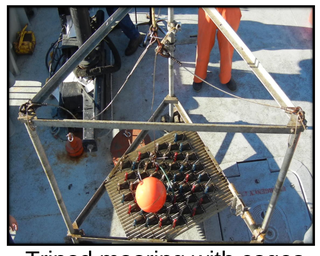
To assess the effect and efficacy of using calcein to mark dreissenid mussels for *in situ* growth experiments.

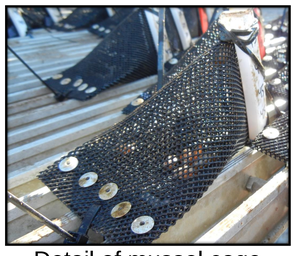
INTRODUCTION

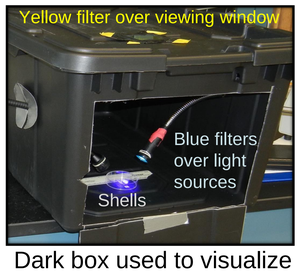
- A better understanding of growth rates for the invasive quagga mussel, *Dreissena r. bugensis,* in their natural setting would help us predict their population expansion patterns and environmental impacts.
- Calcein is a fluorescent marker that has been successfully used to study growth in marine shelled organisms such as snails and mussels[1,2,3], but fewer studies have been done in freshwater environments.
- Calcein could be a useful tool to help measure individual growth of dreissenid mussels *in situ,* but we need to first evaluate if: (1) calcein has an effect on mussel growth; and (2) if we can accurately evaluate shell growth using calcein markings. We conducted a field test during 2015-2016 to address these questions.

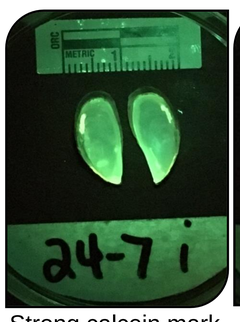
METHODS

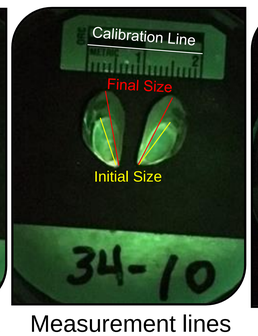
- We established three experimental factors: calcein exposure (yes or no), mussel size class (small=8-10mm or large=14-16mm), and deployment duration (5 or 11 mo).
- We collected mussels from 45m in L. Michigan in April 2015
- Half of the individuals were given a fluorescent mark by exposure to 100 ppm calcein[2] for 18 hours.
- We measured initial shell length and assigned the mussels to replicate cages. The cages were deployed on a tripod mooring in L. Michigan at the same site of collection.
- At each time point we re-measured the mussels and photographed both the inner and outer surfaces of each valve using a 12 MP iSight iPhone camera. Fluorescence was viewed using a blue excitation filter (λ=460nm) over the light sources and a yellow filter (λ=495nm) over the camera lens.
- We used ImagePro software to measure and evaluate the images.

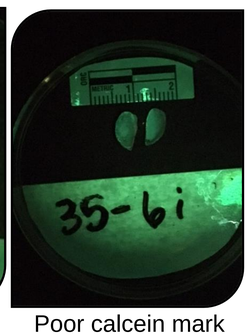












RESULTS

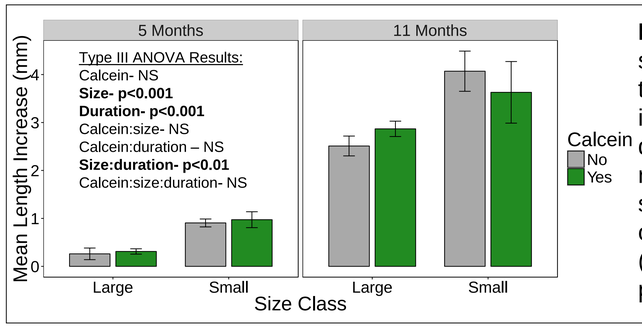


Fig 1. Overall, calcein did not significantly affect growth, but there is a significant interaction between size and duration. Further analysis revealed that calcein significantly enhances growth of large mussels at 11 months (1-way ANOVA: $F_{1,10}$= 11.55, p <0.01)

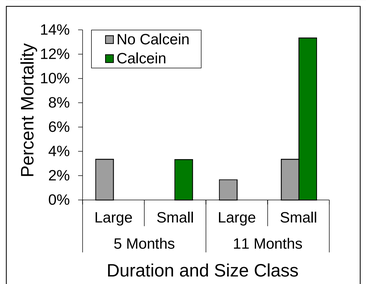


Fig 2. Small, calcein-exposed mussels, at 11 months duration, experienced the highest mortality rate among all groups.

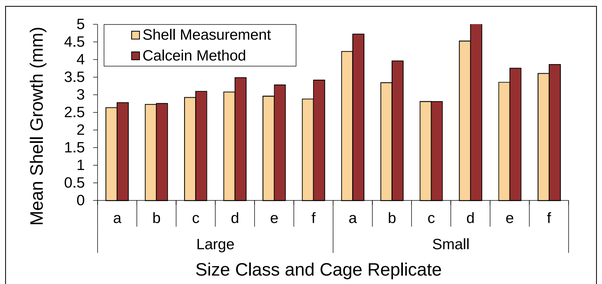


Fig 3. Mean shell growth estimated by calcein marks was significantly higher than growth determined from direct initial and final shell measurements (paired t-test: t_{11}= -5.51, p <0.001).

DISCUSSION

Given the size-specific responses in mussel growth and mortality during longer deployments, we recommended caution in using calcein to mark dreissenid mussels for growth studies.

- Calcein had no effect on mussel growth at 5 months, but large mussels exposed to calcein showed a growth enhancement after 11 months (Fig 1). Growth among small mussels was higher and more variable than for large mussels, regardless of calcein exposure. These findings contradict what was found for marine bivalves[4] and juvenile green sea urchins[5], where no calcein effect was detected.
- Small mussels may be more sensitive to calcein, as demonstrated by higher mortality rates, particularly among those deployed for 11 months (Fig 2). Large mussels exposed to calcein exhibited no mortality and mortality among no-calcein treatments was low.
- Overall, shell length was overestimated by a mean difference of 10% when using image analysis on calcein markings, as compared to using the difference between the initial and final shell measurements by calipers (Fig 3). We found that new shell growth sometimes overlaps the area marked by calcein, potentially obscuring the original terminus of the shell.

ACKNOWLEDGEMENTS

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