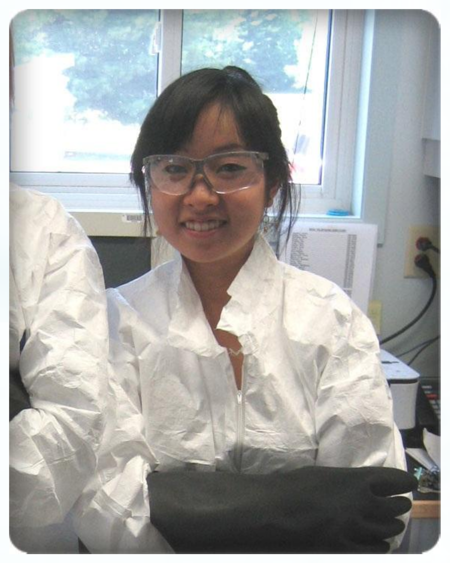
Minimal effects of low calcium levels on *Bythotrephes* life history: Implications for establishment in Canadian Shield lakes



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ABSTRACT

To examine the possible effects of low calcium (Ca) on *Bythotrephes*, we conducted a controlled study in which neonates born in the lab were reared over 23 days at 0.1, 0.5, 1, 1.5, 1.9 or 2.4 mg Ca^{++} L^{-1}. Parameters considered included: intrinsic rates of natural increase (*r*), survival, development time, growth, clutch size, offspring length, and offspring gender. We found that *r* declined only at 0.1 mg Ca^{++} L^{-1} as a result of decreased survival but still remained above 0, indicating population increase. There were some significant differences in clutch sizes among 1st and 2nd broods and offspring tailspine lengths among Ca treatments, but little effect of Ca deficiency on all other parameters examined. Field data indicate that while *Bythotrephes* has not been found in Canadian Shield lakes with <1.5 mg $Ca^{++}L^{-1}$, in Norway *Bythotrephes* also occurs at <0.5 mg Ca^{++} L^{-1}. Therefore, in the absence of other stressors, future *Bythotrephes* establishment in novel habitats will likely not be hindered by low water Ca levels.

INTRODUCTION

Calcium is a crucial component of crustacean exoskeletons. Ambient Ca levels are currently falling in Canadian Shield lakes (Jeziorski et al. 2008), a region that is also being affected by the rapid spread of the invasive spiny water flea *Bythotrephes*. Past studies have identified a threshold of <1.5 mg Ca^{++} L^{-1} as detrimental to *Daphnia* (e.g. Ashforth & Yan 2008), a favoured prey of *Bythotrephes*. Effects of Ca deficiency on *Bythotrephes* is unknown, however. Though comparatively soft-bodied, *Bythotrephes* grows quickly and females undergo 2-3 moults over their life cycle, with an additional moult upon release of each successive brood. If found to be sensitive to low Ca, future establishment success of *Bythotrephes* in Shield lakes may be compromised. Thus, the aims of this study are to:

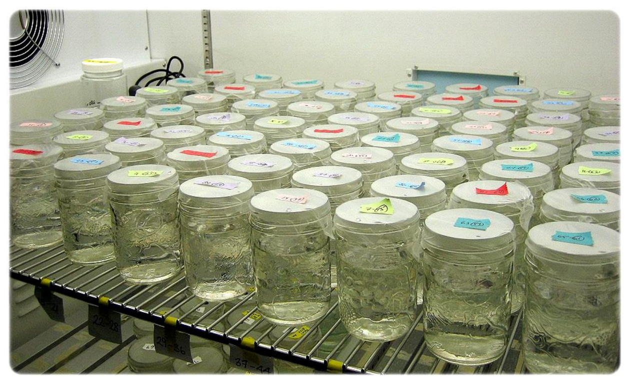
1) **Determine possible effects of low Ca on *Bythotrephes* life history**
2) **Compare lake Ca levels where *Bythotrephes* occurs in Norway (populations have long been established) versus Canada (new populations are becoming established)**

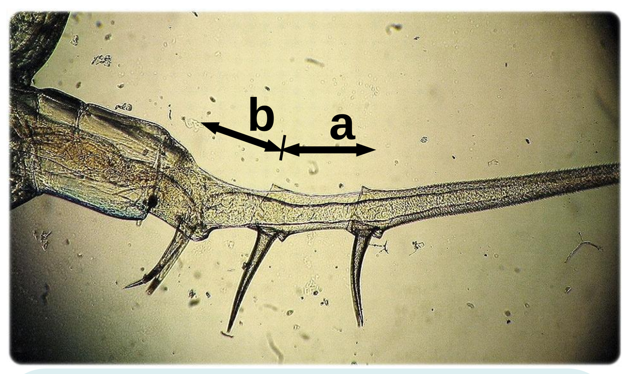
Implications of our findings for future population establishment in Shield lakes are discussed.

METHODS

Lab bioassay

We reared lab-born F_1 *Bythotrephes* neonates in an artificial softwater culture medium with ambient [Ca^{++}] adjusted to six treatment levels: 0.1, 0.5, 1, 1.5, 1.9, 2.4 mg Ca^{++} L^{-1} (*n*=11 individuals treatment^{-1} initially). Temperature was maintained at 21°C. Complete field collection and culture conditions are described in Kim and Yan (submitted). Once daily, each *Bythotrephes* was transferred to her own container of 175 ml fresh media, and prey were added to total approximately 50 *Artemia* nauplii, 10 *Bosmina freyi*, 7 *Daphnia ambigua* and 5 *D. pulex*. Test animals were scored daily for survival and reproduction.





TOP: Glass jars containing individual *Bythotrephes* being reared at different Ca levels within the growth chamber

BOTTOM: A) Growth at Instar 1, and B) growth at Instar 2 indicated by lengths between barb pairs

Data analysis

Bioassay results were analyzed by calculating intrinsic rates of natural increase (*r*) of the treatment "populations", as this metric simultaneously incorporates "complex interactions among life history traits and provides a more relevant measure of ecological impact" (Forbes & Calow 1999). We assessed factors contributing to *r* (survival, fecundity), development time, growth at instars 1 and 2, clutch sizes, lengths of *Bythotrephes* offspring, and offspring sex ratios via t-tests and one-way ANOVAs with post-hoc Tukey tests. Where applicable, we square root-transformed heteroscedastic data prior to analysis.

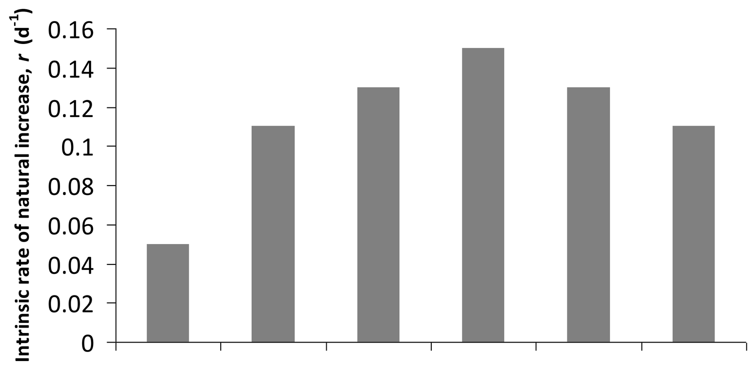
To examine the frequency of *Bythotrephes* presence in Norwegian versus Canadian Shield lakes (Watershed 2EB survey data), we excluded all non-invaded lakes and lakes with pH <6 or >7 from both datasets (lab bioassay pH was ~6.5). We then categorized the lakes into intervals of approximately 0.5 mg Ca^{++} L^{-1} to reflect our Ca range of interest (0 – 2.5 mg $Ca^{++}L^{-1}$).

RESULTS

Summary of lab bioassay findings:

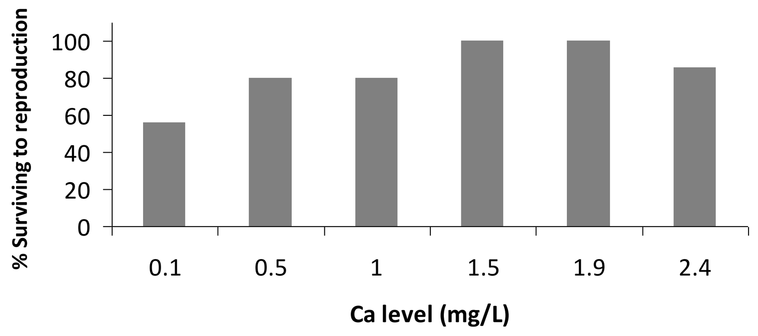
1) Intrinsic rate of natural increase

- Substantial decrease at 0.1 mg Ca^{++} L^{-1}
- Peak at 1.5 mg Ca^{++} L^{-1}
- But *r* remains positive, indicating population increase



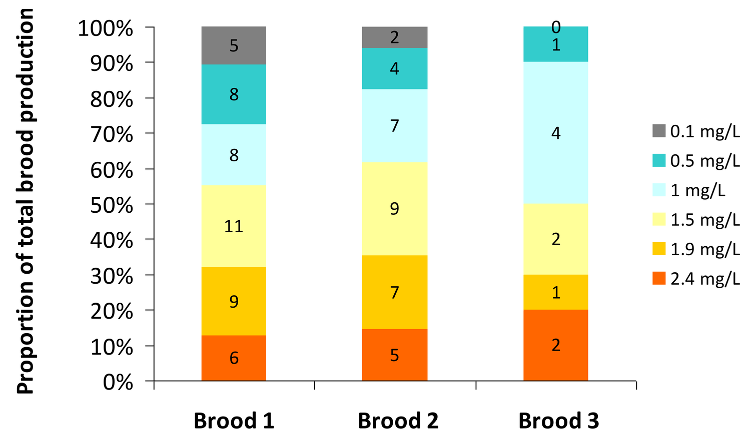
2) Survival to reproduction

- Decrease at 0.1 mg Ca^{++} L^{-1}
- All other treatment levels had ≥80% survival



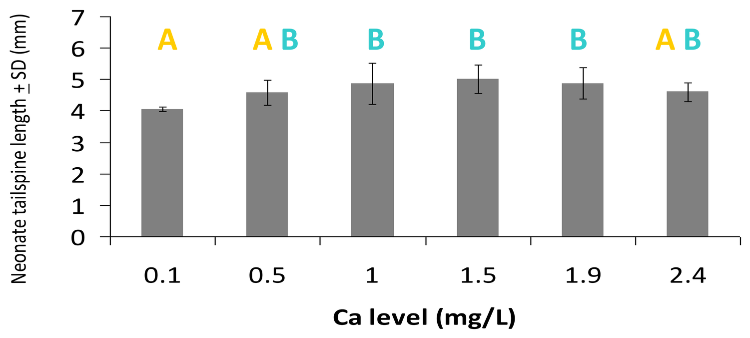
3) Multiple brood comparisons

- At all Ca levels (except 0.1 mg Ca^{++} L^{-1}), 2nd & 3rd broods produced
- At all Ca levels (except 0.1 and 0.5 mg Ca^{++} L^{-1}), clutch sizes increased significantly from 1st to 2nd brood (*p*<0.05)
- At all Ca levels (except 1 mg Ca^{++} L^{-1}), body + tailspine lengths of offspring increased significantly from 1st to 2nd brood (*p*<0.05)
- At 0.1 mg $Ca^{++}L^{-1}$, 43% of 1st broods aborted/non-viable



4) Tailspine length of brood 1 offspring

- Significantly shorter at 0.1 mg Ca^{++} L^{-1}, but not significantly different than 1 and 2.4 mg Ca^{++} L^{-1}

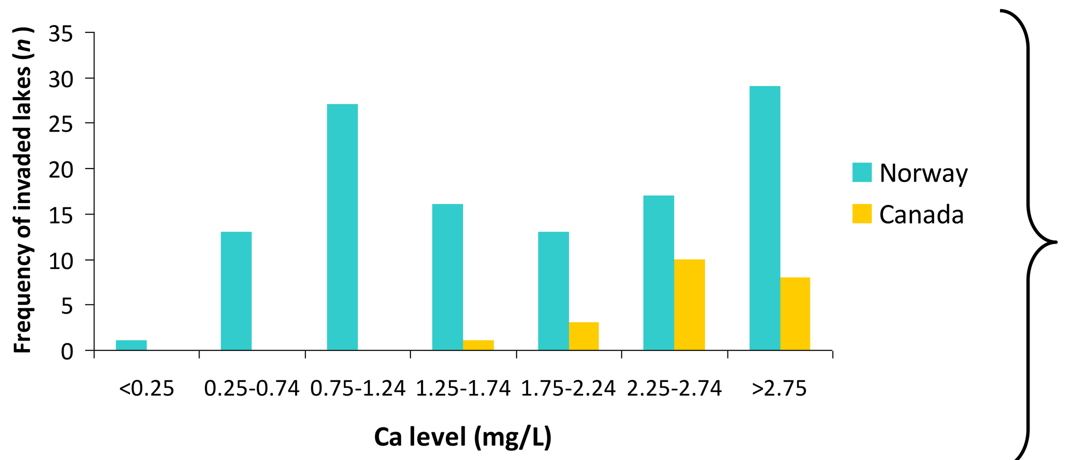


(Treatments not connected by the same letter grouping were significantly different, *p*<0.05)

5) BUT NO SIGNIFICANT DIFFERENCES AMONG CA TREATMENTS IN:

- **Development time to maturity (Instar 3)**
- **Time to reproduction**
- **Growth at Instar 1**
- **Growth at Instar 2**
- **1st brood clutch sizes**
- **2nd brood clutch sizes**
- **Offspring sex ratios (no male production)**

Bythotrephes presence in Norwegian vs. Canadian Shield lakes:



Frequencies of *Bythotrephes* presence in lakes of differing Ca levels in Norwegian (*n*=116) and Canadian Shield (*n*=22) lakes.

Norwegian data from B. Walseng. Canadian data from Cairns et al. 2007.

1) In Norway, *Bythotrephes* occurs in lakes with Ca as low as 0.2 mg Ca^{++} L^{-1}

2) In surveyed 2EB lakes of Canadian Shield, *Bythotrephes* has only been found in lakes with >1.5 mg Ca^{++} L^{-1}

CONCLUSIONS

Our lab results indicate that there is a minimal effect of low Ca on *Bythotrephes*. Despite the decrease in survival at the lowest Ca level (0.1 mg Ca^{++} L^{-1}), the ability to produce a 2nd brood and the lack of male production contribute to a positive intrinsic rate of natural increase. Nearly half of the 1st brood neonates in this treatment were aborted or non-viable but 2nd brood offspring appeared healthy and body lengths increased substantially.

A novel finding of this study is that *Bythotrephes* frequently produce multiple broods. This should be considered when constructing population viability models.

The peak in *r* at 1.5 mg Ca^{++} L^{-1} is noteworthy. Although differences were not statistically significant, survival, adult growth, clutch sizes (both 1st and 2nd broods), and offspring tailspine lengths are also highest at 1.5 mg Ca^{++} L^{-1}. This may be indicative of a hormetic response by *Bythotrephes* as a result of low Ca, but more research is required to confirm this.

Upon comparing the ranges of Ca levels in which *Bythotrephes* occurs in Norwegian lakes (where it is relatively well established) vs. Canadian Shield lakes (where it is a relatively new invader), it appears as though *Bythotrephes* should not be limited by low Ca. Explanations for why it has not been found in Shield lakes with <1.5 mg Ca^{++} L^{-1} may include a lack of propagule pressure, a lack of detection effort, or other unknown stressors acting in concert with low Ca to hinder establishment. Higher-Ca lakes also tend to be larger and more prone to human activity, which has been implicated as a major predictor of *Bythotrephes* presence (Weisz & Yan 2010). Lower-Ca lakes may be smaller and more difficult to access. Given that their *Daphnia* prey are overall more susceptible to low Ca than *Bythotrephes*, the negative impacts of this invader on native cladoceran communities could be enhanced by falling Ca.

In conclusion, it appears that low Ca should not inhibit future establishment success in novel environments, in the absence of other stressors. Areas for further research include the determination *Bythotrephes* body Ca content and studies on the possible effects of low Ca in conjunction with additional stressors (e.g., limited food availability, thermal stress, pH, predation).

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