Effects of alien crayfish on macrophytes and benthic invertebrates in Enos Lake: implications for hybridization of limnetic and benthic stickleback species pairs

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This project was funded by the BC Forest Science Program, the federal Interdepartmental Recovery Fund, and the BC Conservation Corps

Introduction

Stickleback species pairs have evolved independently in a limited number of lakes in British Columbia. They are listed as endangered in Canada, and globally unique in that a benthic and limnetic species have recently evolved and differentiated in the same lakes, with the benthic species feeding on benthos in the littoral zone while the limnetic feeds on zooplankton in the pelagic zone. In addition to their intrinsic biodiversity value they have supported some of the most advanced research in evolution and genetics since Darwin's finches (e.g. Rundle et al. 2000; Peichel et al. 2001; Colosimo et al. 2005, Keneddy 2005). Current status of stickleback species pairs in B.C. is not encouraging (Foster 2003; Wood 2003). Four pairs have been identified in six different lakes (Foster et al. 2003; a new species pair has also recently been discovered in Little Quarry Lake, Nelson Island; Gow et al. 2008). One of the pairs (Hadley Lake) has been extirpated due to introduction of alien fish (*Ictalurus* catfish; Hatfield 2001), and another species pair (Enos Lake) has collapsed into a hybrid swarm for unknown reasons (circumstantial evidence implicates habitat change associated with crayfish introduction or watershed development; Boughman 2001; Gow et al. 2006; Taylor et al. 2006). Given that half of the original species pairs have become extinct over a relatively short period, the remaining species are likely to suffer the same fate unless threats to their persistence are properly identified and managed. Although it appears that introduction of alien invasive species represent the greatest threat to persistence of the stickleback species pairs, the mechanism whereby the Enos lake species pair has collapsed into a hybrid swarm, and the potential role of crayfish, remain unclear.

Although stickleback species pairs have been subject to enormous research focused on their evolutionary ecology and genetics, less is known about their ecological requirements, habitat associations, or the mechanisms whereby they are impacted by invasive species. In this report we describe the results of a field study designed to determine the potential impact of crayfish on stickleback species pairs and their habitat, in particular the abundance of aquatic macrophytes and benthic invertebrates.

Hypothesized mechanisms whereby crayfish could have led to hybridization of stickleback specie pairs include:

1) Increased water turbidity as a consequence of macrophyte removal by crayfish, thereby interfering with colour transmission and impairing the ability of limnetics and benthics to discriminate mating colouration between species.
2) Hybridization through closer proximity of limnetic and benthic nests in the absence of aquatic macrophytes (benthics nest among plants), or removal of habitat cues (i.e. macrophytes) that stickleback use for nest discrimination.
3) Differential susceptibility of nests of one species to crayfish predation, so that i) gravid females either resort to laying their eggs in the nests of the other species, or ii) a declining population of one of the species leads to

difficulty in finding an appropriate mate and therefore increased mating with the other species.

4) Reduction in benthic invertebrate abundance by crayfish decreases prey availability for the benthic species, leading to a smaller maximum adult body size, thereby increasing hybridization rates because body size is a primary cue in mate discrimination between species.

There is no evidence for a contemporary change in turbidity in Enos lake, and current turbidity levels do not differ much from Paxton lake on Texada Island, although Enos lake does have a distinctive brown colouration. Changes in dissolved organic carbon following the 1m increase in lake level in the early 90s, and seasonal draw downs in water levels for irrigating a golf course, could also have trigger hybridization. In this study, however, we focused on whether crayfish could trigger the necessary changes in macrophyte and benthic invertebrate abundance required for hypotheses 2-4 above. We did this by i) constructing enclosures in Enos lake, and stocking them, macrophytes, artificial substrates, and juvenile stickleback, and then documenting the response to crayfish introduction; and ii) comparing benthic invertebrate abundance on rock and sediment substrate in Enos lake (crayfish invaded) and Paxton lake (crayfish free). Our expectations were i) that enclosures with crayfish would have reduced macrophyte abundance, benthic invertebrate abundance, and stickleback growth relative to controls, and ii) that benthic invertebrate abundance would be lower in Enos than in unimpacted Paxton lake. Below we describe the methods and results of our experiment.

Methods

Enclosure experimental design

We installed 8 enclosures in the littoral zone of Enos lake during August 2007. Enclosures were 120 cm by 120 cm square, and were constructed of 6mm mesh hardware cloth (galvanized steel screen) secured to the lake bottom with re-bar. Enclosures were placed in 70-110 cm of water over fine sediment substrate. Enclosures were closed on the bottom with a sheet of 6mm hardware cloth that was sunk into the sediment to a depth of approximately 5 cm, and the sides of enclosures extended above the water surface by 10-25 cm. A hardware cloth lid with a sampling hatch was fixed to the top of each enclosure to allow limited sampling while preventing escape of stocked crayfish.

We added 4 species of macrophytes to each enclosure from August 20-23 2007. Macrophytes were collected from a small pond upstream of Enos lake, and included both a broad and narrow leaved species of *Potomogeton, Utricularia vulgaris* (bladderwort), and *Chara*. Macrophytes stocked in enclosures were spun for 10 revolutions in a salad spinner, and weighed wet to the nearest 0.1 g. An average 78g of *Chara*, 47g of wide leaf *Potomogeton*, 85g of narrow-leaved *Potomogeton*, and 27g of *Chara* was added to each enclosure by threading roots through one of four 15 cm x 15 cm square pieces of hardware cloth per enclosure, which were then sunk into the bottom sediment of each enclosure.

Two immature hybrid stickleback (one each of a benthic-type and limnetic-type morphology) were stocked in each enclosure on August 24 2007. Average stickleback total length was 41mm. Four enclosures were designated as controls and four as treatments. Three crayfish (6 – 10g each) were added to each enclosure. Crayfish were also inadvertently added to controls at the start of the experiment, and subsequently removed from controls 12 later when this error discovered.

The experiment was terminated on Oct. 1-2, 38 days after fish and crayfish were stocked initially in enclosures. Two replicate benthic sediment samples (for assessing benthic invertebrate abundance) were collected from each enclosure using a benthic sampler with a 250um mesh net. Sediment samples were rinsed through a 250um sieve to remove fine organic detritus, and preserved in 5% formalin for future processing of invertebrates in the laboratory. All remaining aquatic macrophytes were removed, rinsed of sediment, and placed in ziplock bags for transport back to the laboratory. No crayfish were recovered from control enclosures, and an average of 1.3 crayfish were recovered from treatment enclosures.

Aquatic plants were dried to a constant temperature and weighed in the laboratory to a constant weight at 55 C. A conversion from wet weight of plant to dry weight was derived for each species using collected samples of known wet weight. Benthic invertebrates were sorted from detritus in the laboratory under a binocular microscope at 10X magnification. Invertebrates were then identified to family, and length was estimated to the nearest 0.05 mm using a digitizing system and binocular microscope equipped with a drawing tube. Biomass of invertebrates was estimated using taxa-specific length-weight regressions from the literature.

<u>Enos and Paxton Lake benthic sampling</u>

Four rocks were collected from the shoreline of each of Enos and Paxton lakes on July 12 and 5, 2007, respectively. Each rock was scrubbed in a bucket to remove invertebrates, and the contents of the bucket was then filtered onto a 250um mesh sieve and preserved in 5% formalin for processing in the laboratory as described above. The dimensions of each rock were also measured so as to estimate invertebrate abundance per unit area. Three samples of sediment were collected from each of Paxton and Enos lakes on the same dates using a 0.5mm mesh net. Contents of the sediment samples were rinsed in a 250um sieve and preserved in 5% formalin, and total biomass of invertebrates in each sample was estimated by digitizing as described above.

Results

<u>Enclosure experiment</u>

The presence of crayfish in enclosures reduced abundance of all macrophytes other than *Utricularia* (Figure 1) by approximately half. Macrophyte biomass also decreased in the control enclosures over the course of the experiment, possibly because of the presence of crayfish in all enclosures during the first 12 days of the experiment.

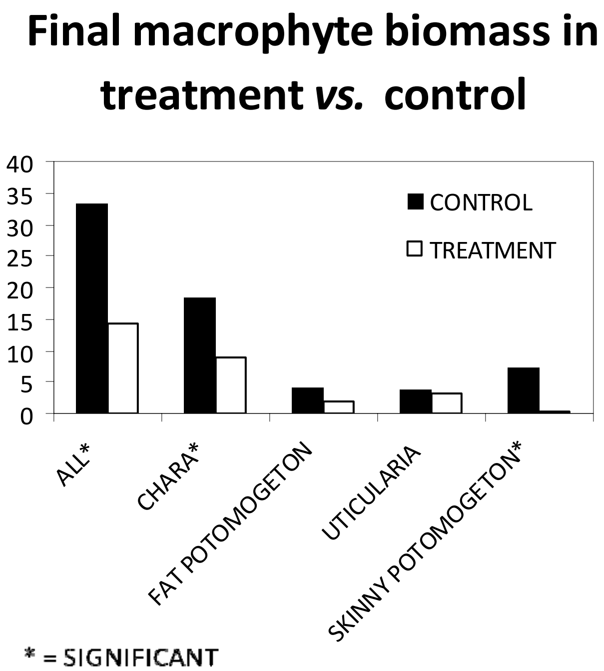


Figure 1. Wet mass of macrophyte present in control and treatment (crayfish present) enclosures at the end of the experiment.

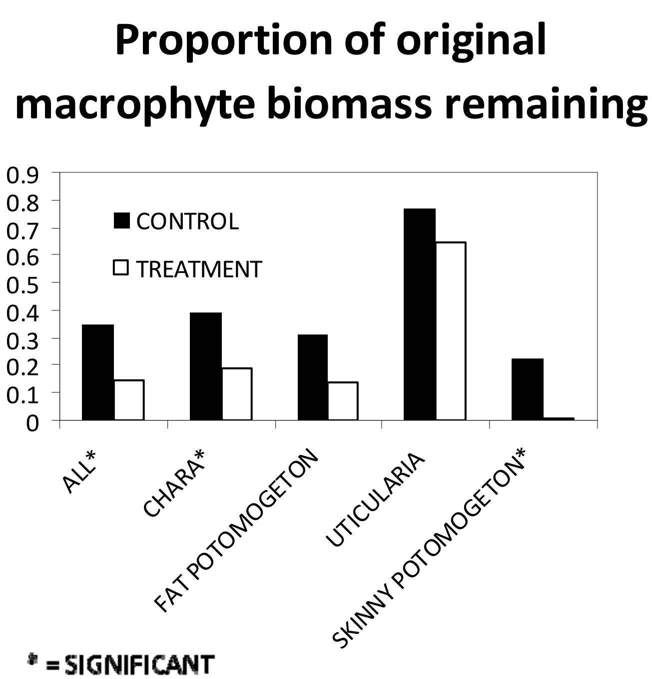


Figure 2. Proportion of original macrophyte biomass remaining in control and treatment (crayfish present) enclosures at the end of the experiment.

Anecdotal observations of macrophytes placed outside of enclosures in quiescent littoral habitats confirmed their rapid removal by crayfish. All taxa other than *Utricularlia* would generally disappear overnight (although crayfish were visibly active during the day in Enos lake, they were even more active at night in shallow water).

Benthic invertebrate biomass and abundance on sediment in enclosures (Fig. 3) was lower in the presence of crayfish as predicted, but the difference was not statistically significant (p = 0.097 for a one-sided Wilcoxon test on biomass).

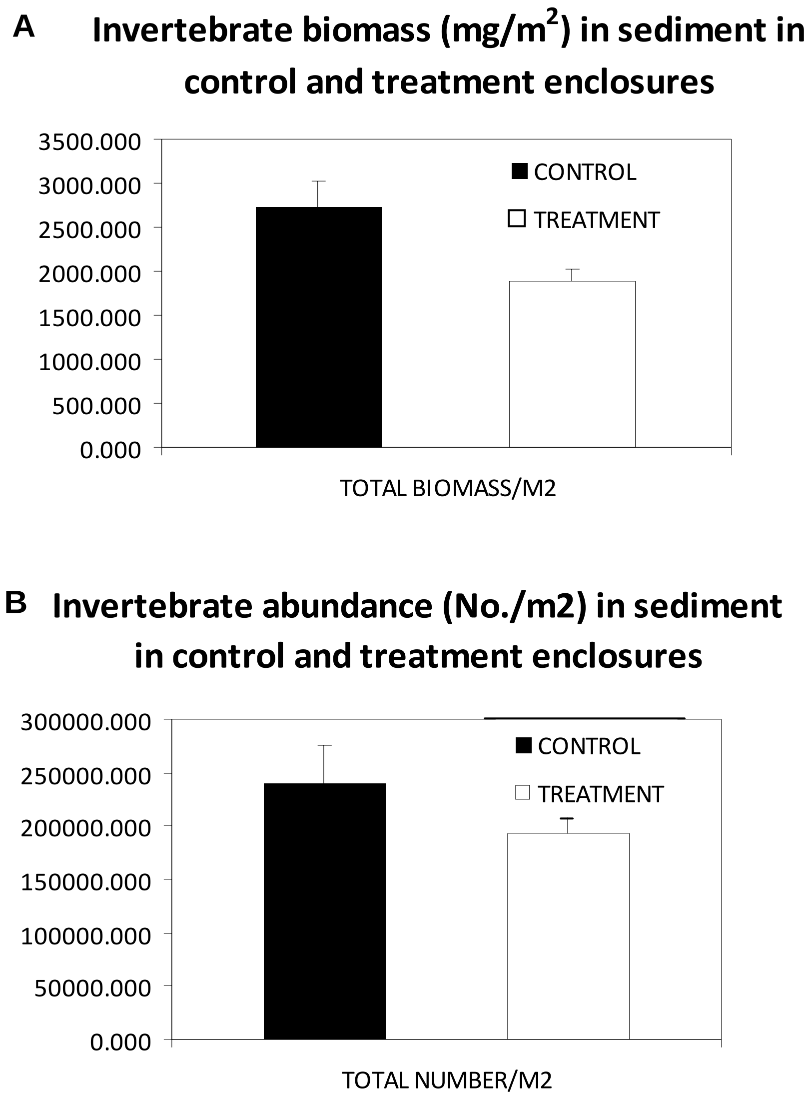


Figure 3. Benthic invertebrate biomass (A) and abundance (B) per m2 of sediment substrate in control and treatment (crayfish present) enclosures.

Stickleback appeared stressed at the time of stocking, and mortality in enclosures was high, probably because of the warm lake temperatures (approximately 20 C) and handling stress when enclosures were stocked.

Insufficient stocked fish were recovered (3 of 16) to make inferences about crayfish effects on growth, and differential colonization of enclosures by smaller stickleback was an added source of variation in growth rate within enclosures.

Comaprison of benthic invertebrate abundance on rock and sediment substrate in Enos and Paxton lake

Both invertebrate biomass and abundance were higher on rock substrate in Paxton lake (crayfish absent) than in Enos lake (Figure 4; $P < 0.06$ for biomass, $P < 0.005$ for number using a one-tailed t-test), consistent with our hypothesis of lower invertebrate biomass in the presence of crayfish. Similarly, invertebrate biomass on sediment was also higher in Paxton than in Enos lake (Figure 5), although the difference was not statistically significant ($p < 0.28$). However, abundance of benthic invertebrates on sediment was non-significantly higher in Enos lake ($P < 0.54$) than in Paxton, indicating a smaller average size of invertebrates on sediment in Enos lake (not significant, $P < 0.09$ for a one-tailed t-test). A reduction in average invertebrate prey size is also consistent with crayfish impacts, since predators like fish and crayfish typically differentially impact larger prey items (e.g. Blumenshine et al. 2000, Gherardi and Acquistapace 2007).

Discussion and Conclusion

Our enclosure experiment in Enos lake demonstrates that crayfish can substantially reduce abundance of aquatic plants in a relatively short time. This effect of crayfish on macrophtes has been commonly observed in other waterbodies (e.g. Rosenthal et al. 2006, Gherardi and Acquistapace 2007). Given that crayfish were not historically present in Enos lake (Paul Bentzen, pers com.), it would seem reasonable to conclude that the qualitatively observed reduction in abundance of aquatic plants in Enos lake over the last 10-15 years is likely a consequence of the introduction and subsequent increase in population size of crayfish in Enos lake.

Crayfish also reduced abundance of benthic invertebrates on sediment substrate inside treatment enclosures (Figure 3). Although this difference was not statistically significant, it is consistent with the commonly observed effects of crayfish on benthic invertebrate elsewhere (e.g. Gherardi and Acquistapace 2007). Average benthic invertebrate size was also non-significantly smaller in Enos lake sediment relative to Paxton (crayfish absent), suggesting that crayfish may differentially reduce abundance of larger benthic invertebrates that are likely important prey items for benthic stickleback.

Collectively, these results indicate that crayfish likely caused a substantial reduction in macrophyte abundance in Enos lake, and may also reduce abundance of benthic invertebrates (over an above the reduction in epiphytic invertebrates associated with consumption of aquatic plants). This strongly implicates alien invasive crayfish in Enos lake as the causative agent that has led to hybridization of the limnetic and benthic species pair. The exact mechanism whereby crayfish may have initiated hybridization remains unclear, but is likely related to removal of macrophytes and reduction of benthic invertebrate abundance, although it is impossible to differentiate the relative likelihood of

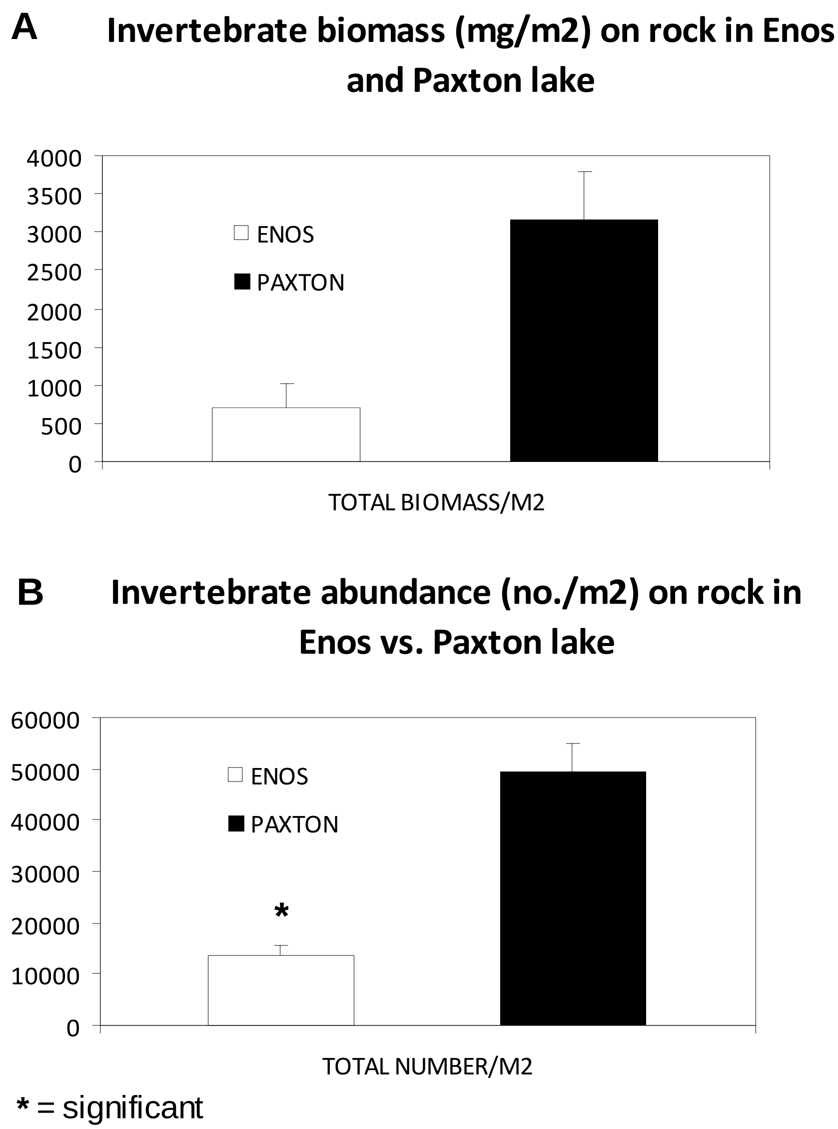
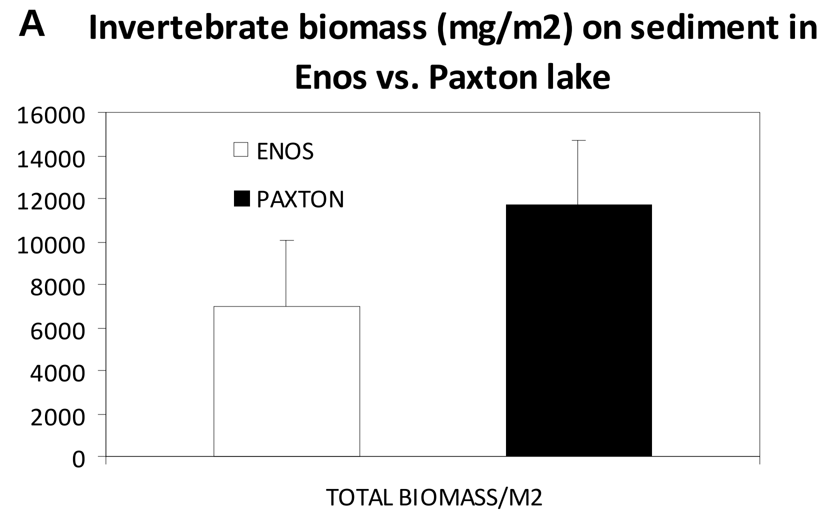


Figure 4. Differences in invertebrate biomass (A) and abundance (B) on rock substrate between Enos and Paxton lake.

hypotheses 3-4 outlined in the introduction based on our data. Macrophytes are important spawning and rearing habitat for stickleback species pairs, and may be an important cue in spatial segregation by breeding pairs, or limnetic and benthic breeding success may be differentially impacted by crayfish. Benthic invertebrates are the primary food source of the benthic stickleback species, and reduction in prey abundance by crayfish could reduce benthic adult body size, which is a also primary cue in mate selection.

Although the mechanism whereby crayfish may have initiated hybridization remains unclear, our results suggest that crayfish impacts are sufficiently large to provide the necessary preconditions for any of the potential pathways of hybridization described above, and targeted research is needed to determine the most plausible pathway. Management implications are that crayfish are likely the causative agent of stickleback hybridization in Enos Lake, and that keeping alien invasive crayfish (or other aquatic invasives) out of the remaining stickleback species pair lakes remains the highest management priority.

A



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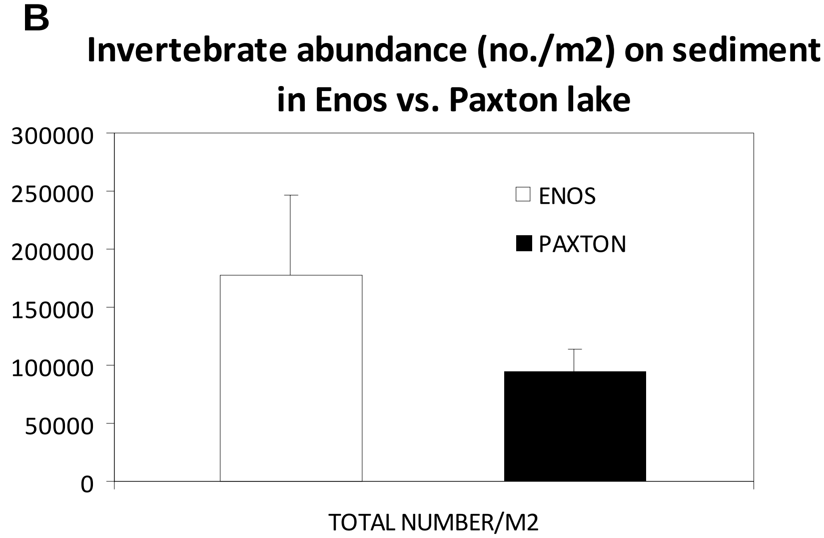

Figure 5. Differences in invertebrate biomass (A) and abundance (B) on sediment substrate between Enos and Paxton lake.

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