Assessing critical habitat and threats to endangered Stickleback Species pairs on the forested land base

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Jordan Rosenfeld

ABSTRACT

Stickleback species pairs are nationally and globally red listed and contribute uniquely to provincial and global biodiversity. The purpose of this project is to carry out research on federally endangered Stickleback Species pairs in British Columbia to better understand the threats to their persistence on the forested land base. More specifically, the goals of the project are 1) to collect information that will inform the identification of critical habitat attributes for stickleback species pairs, and 2) to identify threats to their persistence, including landuse impacts, but also focusing on identifying the cause(s) of hybridization in Enos lake and the potential role of exotic species, particularly crayfish. Identifying the cause of hybridization of the species pair in Enos Lake is essential if the remaining populations on the forested land base on Texada Island are to be managed to avoid the same fate.

The management implications of this research will be to provide a more informed basis for determining management priorities for stickleback species pairs, based on a more robust understanding of factors that are putting them at risk, including i) exotic species, particularly crayfish, ii) water management (potential influence of abstraction on water level fluctuations), and iii) watershed development. Results of this research should provide guidance to managers for protecting stickleback species pairs, for prioritizing recovery activities, and for reducing the uncertainty associated with different management actions.

Project delivery was primarily through 2 graduate student theses, which are ongoing. Thesis one focused on sampling limnological and biological attributes of species pair and single species stickleback lakes in 2008, and performing a multivariate analysis to identify whether unique attributes exist for species pair lakes. Thesis two focused on cattle tank and aquaria experiments to determine the potential role of invasive crayfish impacts on hybridization of stickleback in Enos Lake. 2008 cattle tank experiments were unsuccessful, and are being repeated in 2009 along with crayfish-nesting stickleback behavioural challenge experiments. Both theses should be complete by spring 2010.

INTRODUCTION

One of the key biodiversity goals and responsibilities of the province of B.C., private landowners, and the forest industry is to ensure the persistence of globally endangered species on the forested landbase. B.C. is legally committed to recovery planning for endangered species under the Species At Risk Act, which requires identifying critical habitat for species at risk, as well as non-habitat related threats to species persistence and how to manage them. Recovery planning is a critical step in resource planning if B.C. is to fulfill its commitment to maintain endangered species. Stickleback species pairs are nationally and globally red listed and contribute uniquely to provincial and global biodiversity. In addition to their intrinsic biodiversity value they have supported some of the most advanced research in evolution and genetics

since Darwins' finches (e.g. Rundle et al. 2000; Peichel et al. 2001; Colosimo et al. 2005; Keneddy 2005).

Stickleback species pairs are 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 and the limnetic species feeding on zooplankton in the pelagic zone. 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). 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 watershed development or crayfish introduction; Boughman 2001; Gow et al. 2006; Taylor et al. 2006). Only two of four original species pairs are extant in the four remaining lakes on Texada Island, three of which are on public or private forested land subject to industrial logging, and one additional species pair has recently been discovered in Little Quarry lake on Nelson Island. The importance of proper habitat and watershed management in these forested watersheds is heightened because they represent 75% of the remaining global distribution of extant species pairs. 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. Establishing critical habitat attributes and identifying and minimizing threats to species persistence is a priority commitment for provincial agencies responsible for management of endangered species and their habitats on provincial forested lands (i.e. Ministries of Environment and Forests).

Although stickleback species pairs have been subject to enormous research focused on their evolutionary ecology and genetics, little is known about their ecological requirements or habitat associations. This project involved targeted research to collect data to aid the identification of critical habitat attributes (Rosenfeld and Hatfield 2006) and the priority habitat and non-habitat related threats to species persistence (e.g. forestry impacts, exotic species) on the forested land base, in support of prioritizing management actions to minimize these threats. Specifically, we collected information through a combination of 1) habitat identification and mapping in species pairs lakes, 2) assessment of the habitat attributes that are necessary for species persistence based on the distribution and productivity of habitats in species pairs lakes relative to single-species stickleback lakes, 3) assessment of seasonal changes in habitat availability and conditions (e.g. littoral macrophyte beds vs. pelagic habitat), and 4) habitat-explicit Population Viability Analysis (PVA; through matching funding from the federal Interdepartmental Recovery Fund).

Key threats that are being assessed are the roles of development, exotic species, land-use impacts, and water quality in extirpation and hybridization of species pairs (Seehausen 2006; Taylor et al. 2006), and their potential threat to remaining species pairs. In particular, identifying with certainty the cause of hybridization for the Enos Lake species pair is essential if we are to ensure the remaining species on the forested

land base do not suffer the same fate. Observational and manipulative experiments have been or are being performed to determine the potential roles of 1) watershed development, 2) changes in water quality, and 3) introduction of crayfish as causative factors in hybridization in Enos Lake. Once the cause of hybridization has been identified with confidence, appropriate management actions to prevent hybridization from occurring in remaining lakes on the forested land base will be identified, providing planners and resource managers with the necessary information to ensure species persistence.

Research is being delivered over a 2-3 year period by faculty in UBC Zoology and graduate students and research associates working in collaboration with the BC Ministry of Environment, the stickleback species pair Recovery Team, Recovery Implementation Groups, and local stakeholders. One graduate thesis is focusing on identifying whether there are unique attributes of stickleback species pairs lakes by comparing biological (e.g. zooplankton, benthic invertebrate, and macrophyte abundance), physical habitat (e.g. bathymetry and extent of littoral zone), and water chemistry (e.g. nutrient) conditions in species pair and non-species pair lakes to define the range of lake conditions required for species persistence. This information will help inform the location, area, and specific identity of critical habitat attributes within a lake (i.e. extent of littoral zone, spawning and rearing habitat) that are required for species persistence. The second thesis is focusing on assessing and clarifying threats to species pairs, and how these threats can be mitigated by management. The majority of this work has focused on determining the cause of hybridization in Enos Lake so that this can be avoided in the remaining populations on Texada Island.

This project has been delivered with participation from member of the recovery team, and matching funding from the federal Interdepartmental Recovery Fund and NSERC.

METHODS

Overview

This was funded as a two-year project involved two different parts corresponding to two M.Sc. theses. The first was a limnological comparison of attributes of stickleback species pair lakes and single species lakes. This involved field collection of physical (bathymetry), chemical (nutrients), and biological (aquatic plant distribution and abundance, zooplankton biomass, benthic invertebrate biomass on sediment and rocks) information on lake characteristics, which took place primarily in July and August 2008. The second part of this project was an experimental assessment of the impacts of crayfish on stickleback nesting behaviour and hybridization, which involves cattletank experiments on nesting success and hybridization in the presence and absence of aquatic plants (which are consumed by crayfish), and direct observation of crayfish impacts on stickleback nesting behaviour in aquaria. These are described in more detail below.

Because we were unable to recruit graduate students to begin their theses in April 2007 at the project start date, we hired research technicians to carry out a preliminary experiment on documenting crayfish impacts on aquatic plants before graduate work began in Sept. 2007; this experiment is described below. Since it is hypothesized that crayfish may deplete the abundance of the benthic invertebrate resource favoured by the benthic stickleback species, 2007 field work also included collection of invertebrates on benthic and rock substrate from Enos and Paxton lakes to compare benthic invertebrate abundance in the presence and absence of crayfish.

2007 Methods –

Enclosure experimental design

The objective of this experiment was to determine whether crayfish decrease abundance of aquatic plants or benthic invertebrates. Aquatic plants are a key habitat feature in stickleback species pair lakes that provide important habitat for nesting, shelter, and foraging, and benthic invertebrates are a key food source for the benthic stickleback species.

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.

Enos and Paxton Lake benthic sampling

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.

2008 Methods –

Limnological surveys

Study Sites

Fourteen lakes in southwestern British Columbia containing threespine stickleback were sampled for this study (Table 1). These lakes can be divided into two main groups: "Species pair lakes" and "Solitary" or "non-species pair lakes". Seven of the sampled lakes contain, or have historically contained, threespine stickleback species pairs and the other seven lakes contain only a

solitary population of threespine stickleback (Table 1). All known species pair lakes were sampled whereas solitary lakes were chosen based on elevation, distance to sea, and fish assemblage. Lakes included were between 45-100 m in elevation and were <= 5000 m distant from the sea to allow the solitary lakes the same probability of being colonized by a marine ancestor as the species pair

Lake	Location	Elevation (m)	Distance to Sea (m)	Fish Assemblage
Species Pair Lakes				
Balkwill	Texada Is.	78	5000	S+C
Emily	Texada Is.	31	3150	S+C
Paxton	Texada Is.	97	3840	S+C
Priest	Texada Is.	78	4720	S+C
Little Quarry	Nelson Is.	53	285	S+C
Enos	Vancouver Is.	53	1500	S (Hybridizing) + C
Hadley	Lasqueti Is.	61	1330	C+Ca (S Extinct)
Solitary Lakes				
Cranby	Texada Is.	72	2530	S+C
Ambrose	Sechelt Pen.	59	940	S+C+Sc
Brown	Sechelt Pen.	49	496	S+C+Sc
North	Sechelt Pen.	45	945	S+C+Sc
Stowell	Salt Spring Is.	77	1400	S+C+R
Weston	Salt Spring Is.	69	1890	S+C+R
Chemainus	Vancouver Is.	83	2650	S+C+R+B

Table 1: Species pair and Solitary lakes sampled in summer 2008. B smallmouth bass (*Micropterus dolomieu*), Ca brown bullhead catfish (*Ameiurus nebulosus*), C coastal cutthroat trout (*Oncorhynchus clarkii clarki*), Sc prickly sculpin (*Cottus asper*), S threespine stickleback (Gasterosteus aculeatus), R rainbow trout (*Oncorhynchus mykiss*)

lakes. Elevation and distance to sea were chosen following Vamosi (2003) and McPhail (1993) with an estimated maximum 10% error in measuring these parameters. It has been found that all species pair lakes contain only one other species of fish, coastal cutthroat trout (*Oncorhynchus clarkii clarkii*). Thus, it would have been advantageous to sample solitary lakes that contain only stickleback and coastal cutthroat trout, however, this was not possible because of the limited number of lakes with this specific simplified fish community. Therefore, the group of solitary lakes can be further broken down into 4 subgroups based on fish assemblage: 1) stickleback + coastal cutthroat trout, 2) stickleback + coastal cutthroat trout + prickly sculpin (*Cottus asper*), 3) stickleback + coastal cutthroat trout + rainbow trout (*Oncorhynchus mykiss*) and 4) stickleback + coastal cutthroat + rainbow + (introduced, non-native) smallmouth bass (*Micropterus dolomieu*).

By sampling lakes of differing fish assemblage to the species pair lakes I hope to see a contrast in available resources. For example, the prickly sculpin is a benthic fish and feeds mainly on benthic invertebrates (Brown et al. 1995) and is therefore likely a better competitor than stickleback for benthic resources, as well as a potential predator. As such, I would expect lakes containing prickly sculpin to have fewer benthic invertebrates available for sticklebacks, which could prevent the sticklebacks from becoming fully benthic. Rainbow trout are known to feed on benthic invertebrates as well as small fish (Nilsson and Northcote, 1981). Therefore, their presence could add increased competition and/or predation on sticklebacks, possibly limiting stickleback habitat.

The seven species pair lakes sampled includes Balkwill, Emily, Little Quarry, Paxton, Priest, Enos, and Hadley Lakes (Table 1). The first five of these lakes contain relatively unperturbed species pairs while Enos Lake contains a hybridizing species pair (Kraak et al. 2001; Taylor et al. 2006) and the pair of Hadley Lake has become extinct (Hatfield, 2001). Therefore, species pair lakes sampled can be broken into three subgroups based on species pair condition: 1) unperturbed or intact, 2) hybrid, and 3) extinct. For this report, data collected will be presented in two groups: Species pair lakes and Solitary lakes.

Water Chemistry

A total of 16 water chemistry variables were measured at each lake between July 6 and August 28, 2008 and again at Paxton and Cranby Lakes between November 29 and December 1, 2008. These variables include: pH, conductivity, turbidity, dissolved oxygen, temperature, water transparency, chlorophyll a, dissolved organic carbon (DOC), total organic carbon (TOC), coloured dissolved organic matter (CDOM), dissolved inorganic carbon (DIC), total nitrogen (TN), total phosphorous (TP), alkalinity (total as CaCO3), total suspended solids (TSS), and total dissolved solids (TDS).

Turbidity samples were taken at a depth of 0.5m and measured using a Lamotte meter. Surface readings of pH and conductivity were measured using a WTW340i meter. Dissolved oxygen (DO) and temperature were measured using an YSI model 58 oxygen meter every 0.5m of depth to create DO and temperature profiles of each lake. Water transparency was measured using a Li-Cor Model LI-250 light meter at 0.12m, 0.5m, 1.5m, 2.5m, and 3.5m and k-values were calculated. The more negative the k-value the less clear the water. For all other variables, water samples were taken within 100m of the geographic centre of each lake at a depth of 0.5m and analysed by Maxxam Analytics (Burnaby, BC).

Zooplankton and Benthic Invertebrates

At each lake, three vertical zooplankton tows were collected using a width Wisconsin plankton net of mesh size. Samples were preserved in 5% formalin. Volume of water filtered for each tow will be calculated using the formula $V=\pi r^2 h$, where r is the radius of the plankton net mouth and h is the depth of the vertical tow. Sampling methodology followed Tonolli (1971).

Benthic invertebrates were collected from both sediment and rock substrates. Sediment samples were collected by scooping sediment from a known area into a 250µm mesh net attached to the end of a pole and held against the lake bottom, filtering the sediment through a 250µm mesh, and preserving the sediment in 5% formalin. Rocks were collected and scrubbed with a brush to wash off all invertebrates. Invertebrates were preserved in 5% formalin. The length and width of each rock were measured and the surface area of each rock was calculated. From this, number and biomass of invertebrates per surface area will be determined.

In the lab, zooplankton and benthic invertebrates will be identified to genus or family. All individuals will be counted and 30 individuals from each taxon will be measured for length using a digitizer. Dry weight of individuals will be calculated using published length-weight regressions and from this total biomass of each taxon can be determined.

Macrophytes

Total macrophyte coverage was surveyed and calculated at each lake. Using a canoe, I travelled along the perimeter of each lake and the area of macrophyte beds were either estimated by eye or measured using a range finder. Macrophytes were divided into two groups: emergent and submergent. Macrophytes provide habitat for fish, including benthic stickleback, especially during the breeding season when benthics build nests around and using macrophytes (McPhail 1994). Consequently, I predicted that species pair lakes will have a generous amount of macrophytes while still having a large amount of open water, the latter of which is presumably necessary for the persistence of limnetic sticklebacks. In this way, habitat is available for both benthic and limnetic stickleback.

Bathymetry

Bathymetric data was collected using a Lowrance LMS-525C DF GPS Fishfinder. Data collected will be used to create bathymetric maps using a computer program called Surfer. Littoral area and maximum depth will be extracted from these maps. The Littoral area can be defined as either the portion of a lake that is less than 2m deep or the area that stretches from the shoreline out to where sufficient light for plant growth reaches the lake bottom. For this study, I will use the former definition to calculate littoral area. I expect species pair

lakes to have a large amount of littoral area but still have areas that are too deep for plant growth. Again, this will provide habitat for both benthic and limnetic stickleback. If the entire lake was so shallow that plants could grow everywhere, limnetic stickleback would probably not exist.

Proposed Analysis

A multivariate analysis (such as a principal components and perhaps subsequently discriminant function analysis) will be performed to determine if any of the species pair and solitary lakes differ overall and if so which of the environmental variables measured contribute most to this segregation between lake groups. These analyses will group lakes together according to like characteristics. If the final analysis groups species pair lakes separate from solitary lakes, this will mean there are environmental variables that are different between lake groups and by inference are important in the persistence of the species pairs. It will also give support to the idea that conditions in sympatry are important to the persistence if not the origin of each species pair. If the analysis cannot differentiate between the two lake groups, then based on the variables measured, there would be no evidence for conditions in sympatry as being important or that discrete environmental variables are needed for species pair evolution persistence relative to solitary lakes.

2008 Cattletank experiments

Cattletank experiments were used to test the effect of habitat homogenization (from crayfish consumption of aquatic plants) on hybridization between limnetic and benthic stickleback. Cattle tanks are 1100 L plastic containers with an open top, and dimensions approximately 0.6m deep, 1.2m wide, and 1.8m long. For the first season of experimentation during the spring and summer of 2008, the tanks were set up as follows, using materials commonly found in the stickleback habitats of Paxton Lake.

1. The bottom was covered with coarse sand, roughly 5 cm deep, to allow rooting of aquatic plants and digging of nests by male sticklebacks. Sand was rinsed approximately 8 times to improve clarity.

2. All cattle tanks were filled with approximately 850L of Vancouver municipal water on April 23, 2008, and allowed to stand for seven days to allow the chlorine to dissipate.

3. Planktonic invertebrates were collected from UBC research pond #8 via plankton tows and were subsequently concentrated and added in equal amounts to each cattle tank on April 29, 2008. A voucher sample of this inoculation of

planktonic invertebrates was collected for initial quantification. The aim was for these invertebrates to multiply and form a natural food source for limnetic sticklebacks during the experiments.

4. Approximately 1 L of peat moss was added to each tank to provide a nutrient base.

5. Leaf litter and substrate from the littoral region of UBC research pond #13 was added to all tanks on May 7-8, 2008 in order to stock the substrate with benthic macroinvertebrates. Litter and substrate was collected via kick-netting, and was filtered through a 300µm sieve. Approximately 3.5 L of this material was added to each tank. As with the planktonic invertebrates, these macro invertebrates were intended as a food source for the benthic sticklebacks.

6. Aquatic plants (*Potamogeton* sp.) obtained from UBC research pond #13, was randomly applied to half of the cattle tanks on May 9, 2008 as the control treatment. In each of the control tanks, aquatic plants were planted in four dense clumps, halfway between each corner of the tank and the centre. Open and covered sections of the tank were roughly equal in area. The other half of the tanks were left bare as the clear-cut treatment.

7. One month was allowed for the invertebrate and plant communities to establish themselves in all of the tanks before any fish were added to the tanks. Chlorophyll a (Chl a) was measured in each tank periodically in June of 2008 to monitor the availability of phytoplankton as food for the stocked planktonic invertebrates such as cladocerans and copepods. Because all but one tank had very low initial levels of Chl a, all tanks were supplemented with 10 µg/L of Phosphorus and 160 µg/L of Nitrogen.

Three tanks with aquatic plants ("Plants" treatment) and three without ("Open" tretment) were used in the first season of experiments (2008). Due to the late start of experiments, this was considered a pilot. Two of each of limnetic males, limnetic females, benthic males, and benthic females were added to each tank for a total of eight fish per tank. Tanks were monitored daily to observe any nesting behaviour. Some isolated observations were made in the tanks, but no mating behaviours or nests were observed.

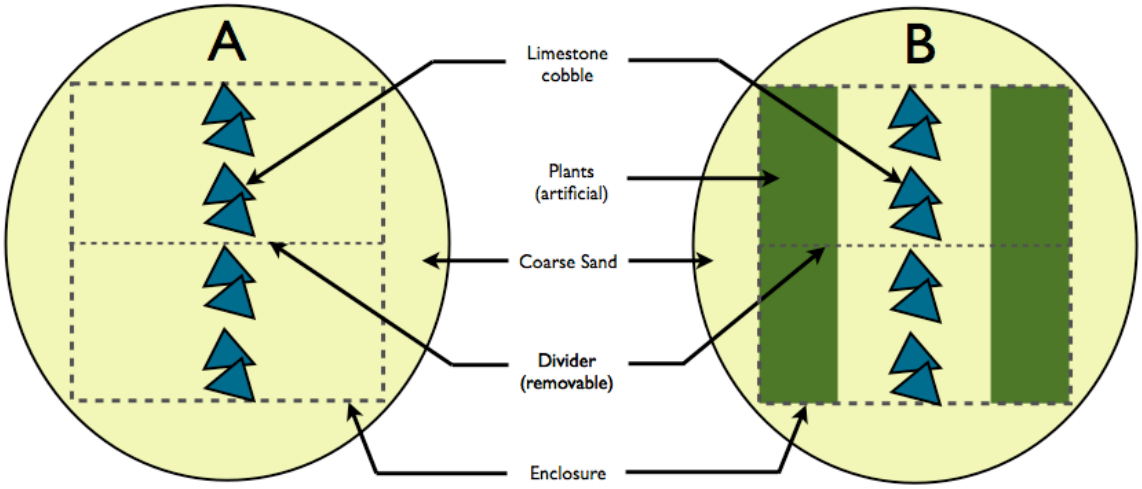


Figure 1. Set up of cattle tanks for phase 1 including modifications made for the second season. The experiment will consist of multiple replicates of a control treatment (B) with equal amounts of "cover" and "open" habitat and a clear cut treatment (A) designed to simulate the effects of plant removal by crayfish in Enos Lake.

2009 Methods –

Cattletank experiments

These 2009 experiments are being carried out between the months of March and June of 2009, as these months coincide with the appropriate temperature and photoperiod required for maintaining suitable breeding conditions in cattle tanks. A pilot of this phase of research was run during the spring and summer of 2008, but no results were obtained due to in-tank mortality likely caused by poor timing and inhospitable (excessively warm) conditions. Various modifications have been made in setup and methodology in 2009 cattle tank trials (described below).

Threespine sticklebacks were obtained from Priest and Paxton Lakes on Texada Island. These individuals have never been exposed to crayfish and therefore will be a good model for determining how the naïve Enos Lake species pair may have reacted when first exposed to this invader. Stickleback were

captured using unbaited minnow traps and dip nets, sorted by visual means as either benthic or limnetic, and held separately in large tanks overnight to reduce transportation stress. Once transported back to the Vancouver campus of UBC, fish collected in the spring of 2009 are being held in holding tanks either adjacent to the cattle tanks, or in an array of aquaria set up in an environmentally controlled chamber in the Biological Sciences building, where the 2008 fish are still being held. Benthic and limnetic sticklebacks are held in separate aquaria. Juvenile sticklebacks are fed to satiation with a mixture of frozen *Daphnia*, live brine shrimp and live *C. elegans*. Adult sticklebacks in holding tanks are fed to satiation with frozen chironomid larvae.

Crayfish have been, and will continue to be obtained from Enos Lake using minnow traps and prawn traps baited with cat food. Those crayfish that will be used for aquaria experiments will be transported live to the Vancouver campus of UBC, where they will be held in secure quarantine tanks in the environment chamber described above and fed algae wafers to satiation. Crayfish used in phase 3 will be immediately frozen in dry ice (at approximately -40^{o}C) to preserve gut contents.

Below are the 2009 modifications to the 2008 cattle tank methods described above:

8. After the first field season in late July of 2008, each cattle tank was topped off with additional water from one of the new (2009) UBC research ponds. This water contained planktonic invertebrates, thus re-charging the stock of plankton in each tank. Dead material was pruned from the aquatic plants and the tanks were allowed to sit undisturbed for 7 months before preparations for second season of trials will be performed.

9. Lime (Calcium Carbonate) will be added to each tank to bring the pH up to approximately 8, which is the pH experienced by the fish in their home habitat of Priest Lake, as well as the pHmaintained in the environmental chamber holding facility.

10. During the pilot study in the summer of 2008, it was evident that the irregular shape of the inside of the cattle tanks was problematic for standardizing the breeding area available to sticklebacks and for tracking purposes. To solve this problem, rectangular enclosures composed of fibreglass screen secured to a wooden frame, were constructed and placed inside each cattle tank. Enclosures measure 1m x 1.5m, and reach from above the water surface to below the surface of the substrate. (See Figure 1). These enclosures also provide a frame on which the apparatus described in the following point will be mounted.

11. It is possible that some of the fish mortality observed during the 2008 field season was due to territorial aggression of male sticklebacks. To avoid this problem, a removable divider, composed of the same materials as the enclosure, will also be constructed and fit across the width of the enclosure (see Figure 1). At the start of each trial, benthic and limnetic males will initially be separated by this barrier to discourage any territorial aggression. Once nests are

constructed, mesh barriers will be removed. Since this divider will bisect the enclosure across its width, identical nesting opportunities – in terms of habitat options – will be available to the fish placed in each side of the divider.
12. Based on low survivability of plants during the first season, artificial plants, made from weighted strips of black plastic bags, will be used in addition to real plants (providing the real plants survive the winter) in the control treatments. For the control, the arrangement of aquatic plants will also be changed for the second season to better provide equivalent areas of the two habitats; they will be arranged in 25cm-wide lengthwise strips along the two sides of the enclosure to simulate the "cover" breeding habitat preferred by benthics (area = 2 [25cm x 150cm] = 7500 cm^2) thus leaving a 50cm strip in the middle of the enclosure open to simulate the "open" nesting habitat of limnetics (area = 50cm x 150 cm = 7500 cm^2, see Figure 1).
13. Limestone cobble (approximately diameter of 10-20cm) will be added in a single lengthwise strip along the centre line of each enclosure to provide more realistic cover such as that found in the open areas of Priest Lake. This habitat structure should correct for the obvious confounding effects of an overly sterile open habitat.
14. During the first field season, no nest construction was observed in the cattle tanks. It is possible that males were capable of building nests, but did not have sufficient building supplies. In the second season, a fresh stock of pine needles and java moss will be added to each tank for use as nest-building materials.
15. During the June, 2008 experiments, Chlorophyll A measurements ranged from 0.5 µg/L to 9.2 µg/L, with only 3 tanks ever exceeding a measurement of 5 µg/L, my minimum Chl a target considered to be adequate to sustain a population of plankton. A supplement of chironomid larvae will be added to each tank in the second season of these experiments to ensure sufficient nutrition for the experimental fish. In the first season of experimentation, the first fish were captured in the field on May 11, 2008 and the first fish were added to the cattle tanks on June 10, 2008. By June 30, all but 2 of the 48 fish added to the cattle tanks had survived. In late June/early July, water temperature and dissolved Oxygen in the cattle tanks ranged from 18^{o}C and 7.5 mg/L, respectively at sunrise to 25^{o}C and 10 mg/L at sunset. Starting these experiments so late in the summer probably contributed significantly to the high levels of fish mortality due to a variety of factors: poor physical condition following two to three months of mating behaviour, additional stress from handling and relocation, high temperatures, low oxygen due to high temperatures, etc. To ameliorate these various effects, and avoid high levels of mortality, the second season of sampling and experimentation is taking place from March – June of 2009.

Two cattle tanks are being used for each replicate, one with aquatic plants and one without. For each replicate, two benthic and two limnetic stickleback males will be added to each tank. Gravid females of both morphs will be placed

in jars and floated in each tank for 1-2 hours each, to encourage nest building behaviour in the males. Once males have constructed their nests, either two limnetic or two benthic females will be added to each tank, determined by a coin toss. Nests will be monitored on a regular basis and behaviour will be observed as thoroughly as possible without disturbing the fish. When eggs are laid, the owner of the nest will be recorded and the eggs will be removed and preserved in 95% ethanol. After each round, the females will be removed from the cattle tank, weighed and measured. A fin clip will also be taken and preserved in 95% ethanol. This process will be repeated with the same males for a second round, this time using two females of the opposite morph from those used in the first round. Once the males in a given tank have been exposed to both limnetic and benthic females, they will be replaced with a fresh set of males. When males are removed from the cattle tanks, they will be weighed, measured and fin clips will be taken and preserved in 95% ethanol. Retired males will be used in later aquaria experiments.

As it is possible that breeding may not occur in some of the tanks, supplemental behavioural observations will be carried out throughout the trial period to record any other differences between the treatment and control tanks. These observations may include, but will not be limited to the following:

a) approximate measures of distance between benthic and limnetic sticklebacks to test the segregating qualities of aquatic plant cover.
b) frequency of nest approaches by females of both members of the species pair as a proxy for actual mating.
c) nest location to determine the preferred nesting habitat of benthic and limnetic males. Preserved eggs and fin clips from each pairing of males and females will be genotyped to determine the parents of the eggs, thus validating observations made during the experiment.

Aquaria experiments

Glass aquaria will be used in a temperature-controlled environmental chamber to assess crayfish effects on stickleback nesting behaviour and success. Interactions between crayfish and stickleback will be observed to test whether or not *P. leniusculus* disrupts the nesting behaviours of benthic and limnetic males in a controlled setting.

In the UBC BioSciences building, an environmental chamber was used to house several 180L glass aquaria measuring 90 cm x 45 cm x 50 cm (see Figure 2). These aquaria are much smaller than the cattle tanks, but large enough to reasonably assume no forced conflict between crayfish and sticklebacks due to crowding. Temperature and photoperiod in the environmental chamber is controlled to simulate seasonal changes in Enos Lake, allowing direct and long term - albeit less ecologically relevant – observations of the behavioural

interactions between sticklebacks and crayfish. Experimental aquaria were set up in a similar way to the cattle tanks, with some key differences:

Aquaria setup:

1. Experimental tanks were filled with dechlorinated tap water from the UBC Biological Science building and fitted with a single air stone to oxygenate the water.

2. One or two pieces of limestone will be added to each experimental aquarium to simulate the relatively high pH found in Priest Lake (pH = 8). The limestone will be placed in one corner of the aquarium (see Figure 2) to allow for some shelter for nesting male sticklebacks.

3. Artificial plants will be placed in each of the experimental aquaria to provide a more natural environment for crayfish and stickleback.

4. A nesting box, containing filter sand for nesting substrate, will be placed in one corner of the aquarium. The floor of the remainder of the aquarium will be kept clear of substrate, thus forcing males to nest in the space provided (see Figure 2). This will allow observers to more easily score proximity measurements.

5. A jar will be fitted with a sliding barrier of mesh in its lid and attached to a string to allow remote removal. In the case of treatment replicates (as we will hereafter see) this mesh will keep the crayfish confined in the jar while still allowing visual and chemosensory contact between stickleback and crayfish.

6. A layer of duck weed (*Lemna*) will be added to the surface of the water in each test aquarium to provide an additional buffer from outside disturbance while still allowing diffuse light to enter the tank.

7. A cloth will be draped over the sides of the experimental tank to reduce disruption from outside of the tank. A viewing hole will be cut in the cloth to allow observations to be made.

8. One 5cm section of PVC pipe will be placed in one corner of each aquaria to provide crayfish with refuge in treatment replicates (see Figure 2).

As male stickleback begin to exhibit nuptial colouration, they will be moved from holding tanks to an experimental aquarium (as seen in Figure 2). Separate aquaria will be used for each treatment and control replicate, randomly assigned each time. In both the treatments and controls, gravid females will be presented to the male in a floating glass jar to induce nest building behaviour, and promptly removed once nest construction begins. If live gravid females are not available, an artificial gravid female will be used in a similar manner.

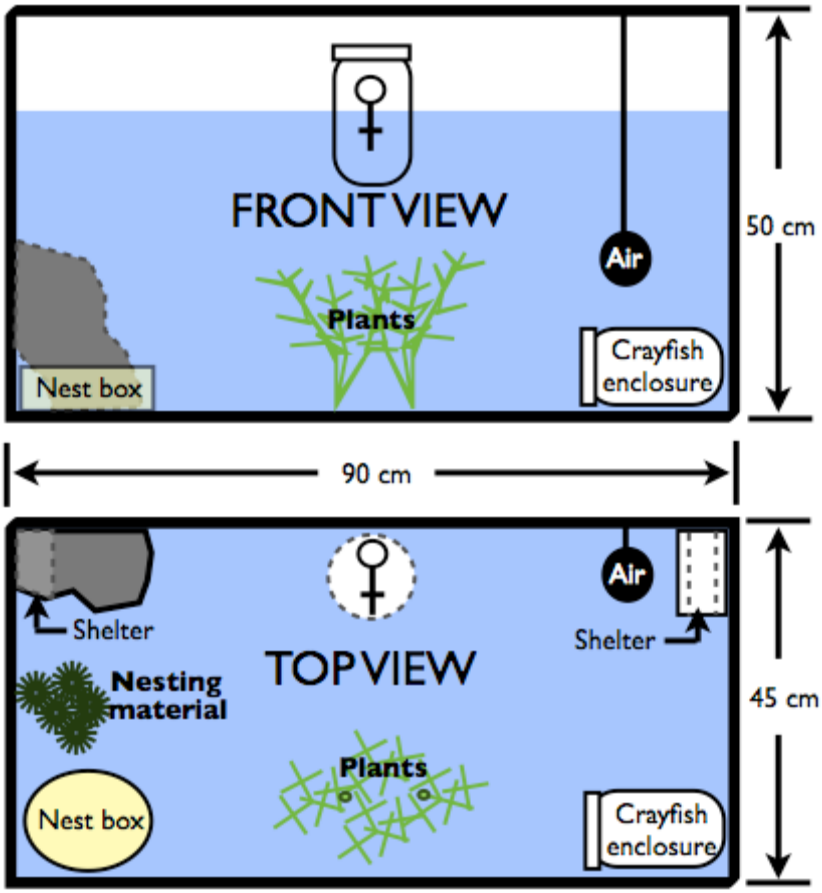


Figure 2. Experimental aquarium set up for studying crayfish effects on nesting behaviour and success. Front and top views illustrate the various components of the apparatus. In treatment replicates, the crayfish enclosure jar will contain a crayfish; in control replicates, it will not. Observations will be made through the front of the aquarium (Front View).

Once the nest is constructed, a glass jar will be added to the opposite end of the aquarium from the location of the nest. In the treatment aquarium, the jar will contain one crayfish (see Figure 2); in the control aquaria, the jar will contain nothing. The test crayfish will be fasted for at least 48 hours prior to the experiment to ensure willingness to eat plant material once released from its jar enclosure. At the same instant as the crayfish jar is added, a gravid conspecific female will again be floated in the aquarium in a glass jar (see Figure 2).
The behaviours of both the stickleback and the crayfish will be scored for an initial 10 minute period for each tank. In each 10 minute period, one scoring will be made for each 5 second interval. After this period of observation, the mesh door on the crayfish jar in the aquaria, regardless of treatment, will be removed by pulling on a string. Behaviours will again be scored for a ten minute period to

measure any differences in nesting disruption based on physical contact as opposed to visual and chemosensory contact alone. For each replicate, the treatment and control will be run one after the other to allow direct observations. Order will be determined by coin toss.

Behavioural scoring will include various male stickleback reproductive behaviours described by Wootton (1976) and summarized by Ridgway (1982), such as: *a)* Direct or Zig-zag Approach – Male approaching female (in jar) by swimming in either a straight or weaving line. Positive approach behaviours would indicate a priority for courtship, thus suggesting a lack of external disruption. *b)* Direct or Meandering Lead. A behaviour in which the male swims from the female's position back to his nest with the female in tow, in a straight or weaving path, respectively. As the female will be confined to a floating jar, leads will be scored if the male swims at least one body length towards the nest when in proximity to the female. This behaviour would indicate a commitment to mating by the male, thus further suggesting a lack of external disruption. *c)* Nest Maintenance – Various male stickleback behaviours including the fanning of the nest entrance with pectoral fins, poking at the nest with his snout, or gluing the nest by pressing his cloaca against the nest while swimming over it. These behaviours could indicate either readiness to mate, or defensiveness, depending on whether he is oriented towards the female or the crayfish jars. *d)* Null – swimming in a non-directional way, or remaining motionless in the water column. This behaviour (or lack thereof) may be difficult to attribute to the presence or lack of disturbance. Further modifications to the scoring of these null behaviours may be necessary as the first results are recorded. *e)* Refuge – Taking refuge in the shelter provided in the aquarium. This behaviour, if not attributable to disruption by the experimenter, would indicate extreme disruption by the crayfish and would result in complete nesting failure if prolonged.

In addition to these previously described behaviours, more generalized measurements will also be made, including the relative proximity of the male stickleback to the gravid female (in jar), his own nest, and the crayfish. This will indicate the nesting male's priorities with respect to reproduction. Greater proximity to the crayfish would indicate a confident and aggressive display of territoriality, whereas a lower proximity to the crayfish, combined with a lowered proximity to the female and nest, would indicate a more severe impact on male nesting behaviour, as the male is not simply wasting nesting time confronting an intruder, but fleeing
altogether.

Behavioural scoring of crayfish during the second 10 minute period will also be included in a similar fashion, and will include:

a) Proximity to the stickleback nests and adults, *b)* Direct nest approach - approaching the stickleback nest in a direct line, *c)* Nest contact – Touching any part of the male stickleback's nest. This would be considered a disruptive

behaviour, *d)* Nest consumption – Consuming any part of the male stickleback's nest. This would be considered a disruptive behaviour, *e)* Materials consumption – Consuming any left over materials of the type that the males have used for the construction of their nests. This would be considered a disruptive behaviour, *f)* Direct attack – Posturing aggressively and approaching the stickleback male (i.e. with chelipeds elevated and open). This would be considered a disruptive behaviour, *g)* Null – Not moving or cruising in a direction not oriented directly towards the male stickleback or his nest. This behaviour would be considered neutral. h) Refuge – Taking refuge from defensive behaviours exhibited by the stickleback male. This behaviour would be highly significant in demonstrating the ability of a stickleback male to ward off disruptive advances by a crayfish. Tallies of all behaviours will be made while watching through the glass from under a darkened hood while the inside of the aquarium is illuminated. This practice should reduce the possibility of disruption by the experimenter.

RESULTS

Overview of progress to date

2007

We had difficulty finding qualified graduate students to start this project in April 2007, and consequently both students started in Sept. 2007. This unfortunately limited the scope of the work that could be performed in 2007, and delayed much of the work by one year. Nevertheless, summer undergraduate research technicians were hired and did an enclosure experiment studying crayfish impacts on stickleback habitat that directly addressed the goals of the research, and provided a sound basis for the graduate students to build on for their 2008 and 2009 field work.

2008

Cattletank experiments to determine crayfish impacts on aquatic macrophytes and stickleback hybridization were completed in 2008 but were unfortunately unsuccessful due to high mortality of collected stickleback, partly due to inadequate holding facilities, as well as high water temperatures in the cattletanks in late May-July, among other factors. The limnological survey of stickleback lakes was completed in July and August 2008.

2009

Cattletank experiments were restarted in April of 2009 and are ongoing, and should have a higher probability of success because of an earlier start date with lower water temperatures than in 2008. Aquaria experiments to determine crayfish impacts on

breeding stickleback behaviour have begun in April and are also ongoing. Several stickleback lakes were re-sampled in April 2009 to measure seasonal variation in limnological characteristics, and this completes the limnological sampling.

Chad Ormond has entered all of the data from the limnological survey, and is presently completing sorting of benthic invertebrate samples. This should be complete by May, and identification and measurement of invertebrate and zooplankton samples should be completed by the end of August. Both graduate students are anticipated to have completed their data analysis and thesis writing by the end of May 2010 at the latest, and hopefully by Dec. 2009.

2007 Results -

1) Enos lake enclosure experiment

Crayfish significantly reduced abundance of aquatic plants that were stocked in enclosures (Figure 3), typically by more than 50% over the 5 week experiment. Exceptions were *Utricularia* (bladderwort), which were apparently not consumed by crayfish.

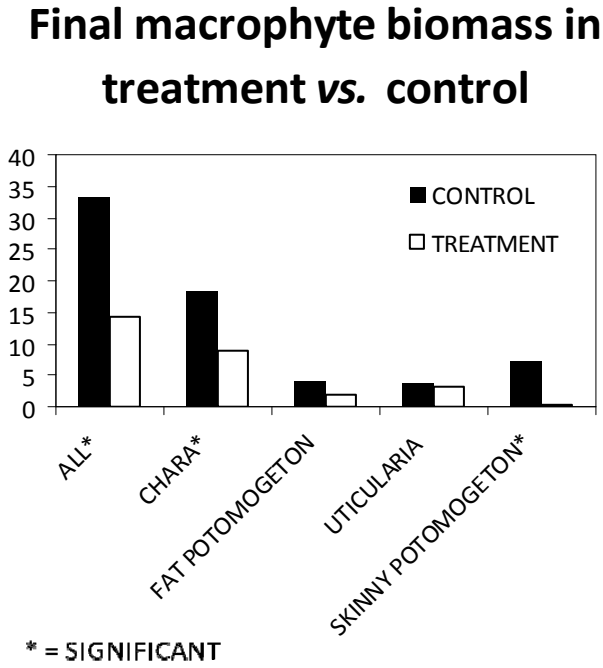


Figure 3. Biomass (g wet weight) of different plant species remaining at the end of the experiment in treatment (crayfish present) and control enclosures.

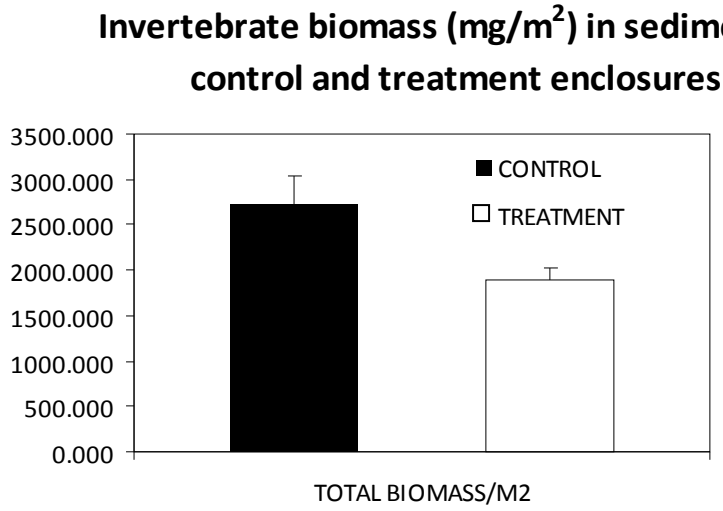


Figure 4. Biomass of benthic invertebrates on sediment in control and treatment (crayfish present) enclosures.

Crayfish also reduced the average biomass of benthic invertebrates in sediment in treatment enclosures relative to controls (Figure 4), although the difference was not statistically significant ($p = 0.09$).

Stickleback stocked in control and treatment enclosures did not survive in sufficient quantity to estimate crayfish effects on stickleback growth. Mortality of stickleback was likely attributed to handling and temperature-induced stress, since lake temperatures were above 20°C when fish were stocked in early August.

2) Comparison of benthic biomass between Enos and Paxton lake

Benthic invertebrate biomass was significantly higher on rock substrate (Figure 5) in Paxton lake (crayfish absent) than in Enos lakes (alien invasive crayfish present), and non-significantly higher in Paxton lake on sediment substrate (Figure 6).

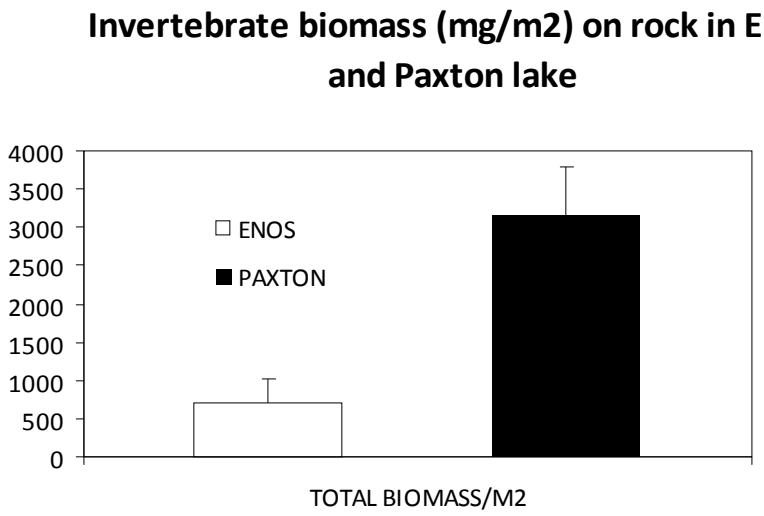


Figure 5. Invertebrate biomass on rock substrate in Enos and Paxton lakes.

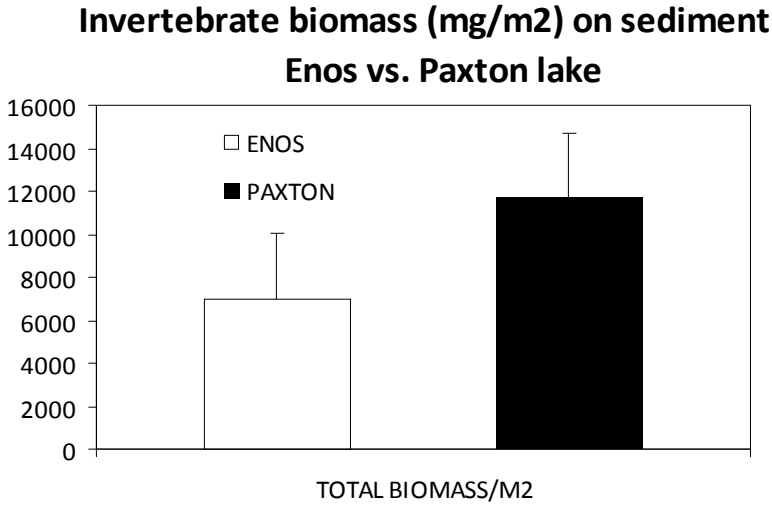


Figure 6. Invertebrate biomass on sediment substrate in Enos and Paxton lakes.

The results of both the enclosure experiment and the comparison of benthic invertebrate biomass between Enos and Paxton lakes are consistent with strong crayfish effects on macrophytes (aquatic plants) and benthic invertebrate abundance. Crayfish reduced macrophyte abundance in enclosures, and this is consistent with the potential for crayfish to have reduced macrophyte abundance in Enos lake itself. Reduced benthic invertebrate biomass in Enos lake (relative to Paxton where crayfish are absent) is also consistent with crayfish reduction of benthic invertebrates in Enos lake enclosures. It would appear that crayfish are likely responsible for both reduction in plant and invertebrate abundance in Enos lake.

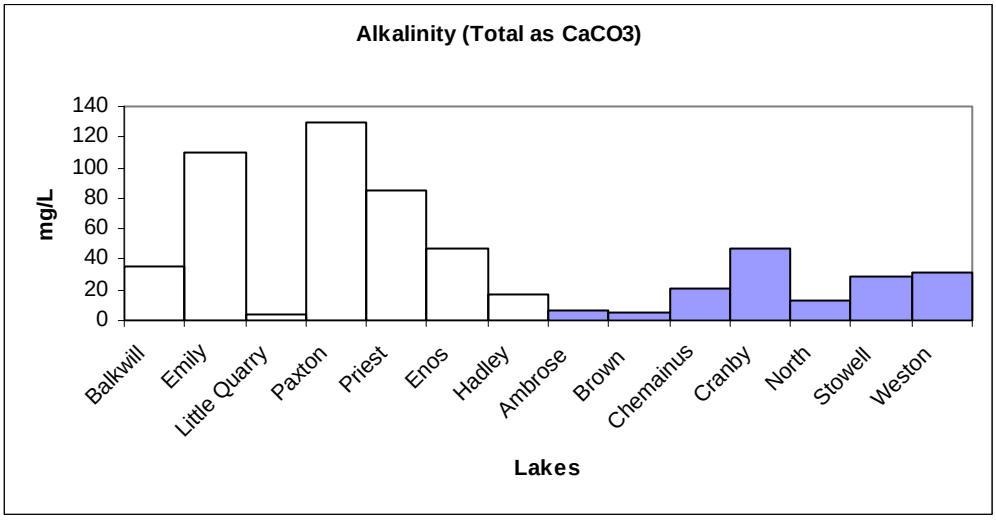
The exact mechanism whereby crayfish may have initiated hybridization remains unclear, but is likely related to removal of macrophytes and reduction of benthic invertebrate abundance. Macrophytes are important spawning and rearing habitat for stickleback species pairs, and may be an important cue in spatial segregation of breeding pairs, which is the focus of Gerrit Velema cattletank experiments. Benthic invertebrates are the primary food source of the benthic stickleback species, and reduction in prey abundance could influence final adult body size, which is a primary cue in mate selection.

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 organisms) out of the remaining stickleback species pair lakes remains the highest management priority.

2008 Results –

The majority of physical and chemical data collected between July 6 and August 28, 2008 has been collated but not yet analyzed. Benthic invertebrate and zooplankton data are currently being processed in the laboratory (samples sorted and invertebrates counted and measured), and bathymetry data also remains to be analyzed.

Simple analysis (Figure 7, see Ormond 2009 Thesis Proposal for details) indicates that species pair lakes may have higher alkalinity (total as CaCO3), total dissolved solids, and dissolved inorganic carbon, although a proper multivariate analysis is required to verify these patterns.



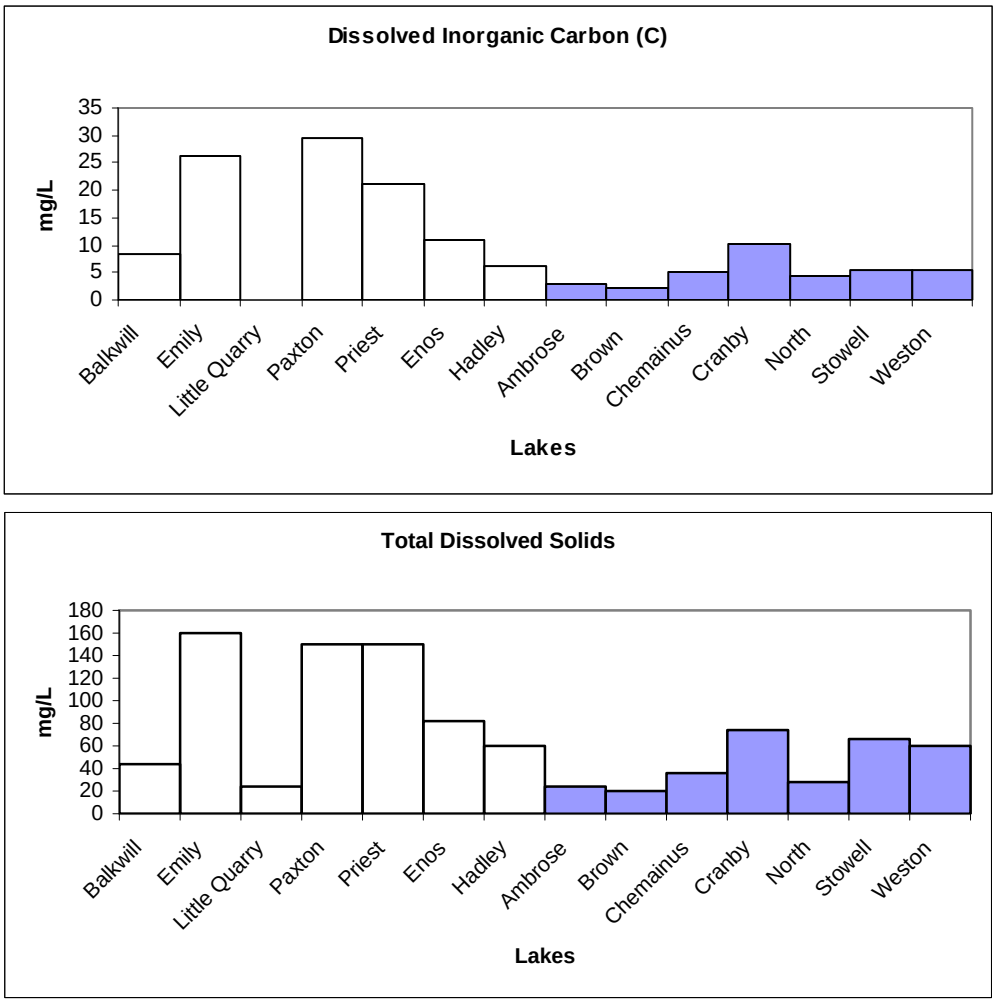


Figure 7. Differences in alkalinity, dissolved organic carbon, and total dissolved solids between species pair (open bars) and solitary stickleback lakes (filled bars).

2009 Results –

Cattletank and aquaria experiments to determine crayfish effects on stickleback nesting behaviour and success are in progress. Data from these experiments will become available in fall 2009.

DISCUSSION

Limnological uniqueness of stickleback species pair lakes

Management of lakes with endangered species like stickleback species pairs requires knowledge of the range of conditions that need to be maintained to allow species to

persist. The fact that species pairs occur in a very limited number of lakes suggests two possibilities. One is that the lakes have a very unique set of limnological conditions that are required for species evolution and persistence; the other is that there is nothing intrinsically unique about the limnology of the lakes (other than a depauperate fish fauna, which appears to be a pre-requisite; Vamosi 2003), and that it was random vagaries of colonization during the second invasion leading to speciation that determined which lakes evolved species pairs. In the first case management of the lakes is simplified to some extent – if there is nothing limologically unique about species pair lakes, they may require only the management necessary to maintain water quality within the range that prevents hybridization. In the second case, there may be additional attributes of species pair lakes that define a potentially narrower range of limnological conditions for species evolution, and by inference species persistence.

Preliminary results from the lake survey (Figure 7) suggest that stickleback species pair lakes may have somewhat higher productivity than non-species pair lakes, but this remains an anecdotal observation that requires a full multivariate analysis to allow proper assessment. This should emerge as part of the completion of Chad Ormonds M.Sc. thesis.

Role of crayfish in hybridization

The 2007 enclosure experiments in Enos lake demonstrated 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 invertebrates (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 and 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 either removal of macrophytes or reduction of benthic invertebrate abundance or both. Macrophytes are important nesting 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 also a primary cue in mate selection.

Nesting behaviour and success experiments currently being conducted by Gerrit Velema for his M.Sc. thesis should directly test these hypotheses and inform the mechanisms whereby crayfish impact stickleback species pair, and provide the basis for accepting or rejecting pre- or post-reproductive impacts leading to hybridization.

Management Implications

Results of the limnological survey, once analyzed, should help clarify the habitat conditions required for stickleback species pair persistence, as well as the conditions required for speciation to initially take place. This information should help define habitat requirements in a concrete way and supplement more general definitions of critical habitat (e.g. Hatfield 2008).

Although the mechanisms whereby crayfish may have initiated hybridization remain 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. Immediate 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.

Project Extension Activities to Date

The information collected during the course of this project to date has been considered during joint DFO - BC MOE recovery planning meetings in 2008, as well as during Stickleback Species Pair Recovery Team Meetings, and has been instrumental in decisions concerning the management of these species. Information from this project has also been incorporated into the Recovery Strategy for stickleback species pairs and documents defining critical habitat. The latest Recovery Team Meeting concerning stickleback species pairs and their habitat took place on March 25, 2009 3:00-5:00 PM. This recovery team meeting followed the BC Non-Game Species at Risk Symposium at UBC where both graduate students working on this project presented their thesis work to date.

Project Completion

This project is unfortunately well behind schedule. The original reason for this was that we were unable to line up suitable graduate students to begin their first field season

in 2007 at the original project start date in April, which effectively delayed field work associated with each thesis by 1 year. Although the 2008 field work for the limnology survey contrasting stickleback and non-stickleback lakes was successful, the 2008 cattle tank experiments were not, which further delayed the experiments related to crayfish effects on hybridization.

These delays are unfortunate, but are part of the normal vagaries of field ecology. This project will continue and will be completed using non-FSP funding sources (NSERC, Dept. of Zoology funding). We anticipate that both graduate theses and associated science and management/conservation results will be completed between Dec. 2009 and May 2010.

LITERATURE CITED

Blumenshine, S. C., Lodge, D. M., and Hodgson, J.R. 2000. Gradient of fish predation alters body size distributions of lake benthos. Ecology 81:374-386.

Boughman, J. W. 2001. Divergent sexual selection enhances reproductive isolation in sticklebacks. Nature 411: 944-947.

Brown, L.R., S.A. Mattern, and P.B. Moyle. 1995. Comparative ecology of prickly sculpin, *Cottus asper*, and coastrange sculpin, *C. aleuticus*, in the Eel River, California. Environmental Biology of Fishes 42: 329-343.

Colosimo, P.F., Kim E. Hosemann, Sarita Balabhadra, Guadalupe Villarreal, Jr., Mark Dickson, Jane Grimwood, Jeremy Schmutz, Richard M. Myers, Dolph Schluter,[4] David M. Kingsley. Widespread parallel evolution in sticklebacks by repeated fixation of ectodysplasin alleles. 2005. Science 307: 1928-1933.

Foster, S. A., J. A. Baker, and M. A. Bell. 2003. The case for conserving threespine stickleback populations: protecting an adaptive radiation. Fisheries 28(5):10-18

Gherardi, F. and Acquistapace, P. 2007. Invasive crayfish in Europe: the impact of *Procanbarus clarkia* on the littoral community of a Mediterranean lake. Freshwater Biology 52: 1249-1259.

Gow, J.L., Peichel, C.L., and Taylor, E.B. 2006. Contrasting hybridization rates between sympatric three-spined sticklebacks highlight the fragility of reproductive barriers between evolutionarily young species. Mol. Ecol. 15, 739–752.

Gow, J. L., S. M. Rogers, M. Jackson, and D. Schluter. 2008. Ecological predictions lead to the discovery of a benthic-limnetic sympatric species pair of threespine stickleback in Little Quarry Lake, British Columbia. Canadian Journal of Zoology. *In press*

Hatfield, T. 2001. Status of the stickleback species pair, *Gasterosteus* spp., in Hadley Lake, Lasqueti Island, British Columbia. Canadian Field-Naturalist 115:579-583.

Hatfield, T. 2008. Identification of critical habitat for sympatric stickleback species pairs and the Misty lake parapatric stickleback species pair**.** Pacific Scientific Advice Review Committee (PSARC) Report.

Kennedy, D. 2005. Breakthrough of the year (Editorial). Science 310: 1869.

Kraak, S. B. M., B. Mundwiler, and P. J. B. Hart. 2001. Increased number of hybrids between benthic and limnetic three-spined sticklebacks in Enos Lake, Canada; the collapse of a species pair? Journal of Fish Biology 58:1458-1464.

Nilsson, N.A. and T.G. Northcote. 1981. Rainbow trout (*Salmo gairdneri*) and cutthroat trout (*S. clarki*) interactions in coastal British Columbia lakes. Canadian Journal of Fisheries and Aquatic Sciences 38: 1228-1246.

Ormond, C. 2009. M.Sc. Thesis Proposal. The effect of environmental factors on threespine stickleback (*Gasterosteus aculeatus*) evolution and continued persistence.

McPhail J.D. 1993. Ecology and evolution of sympatric sticklebacks (Gasterosteus): origin of the species pairs. Canadian Journal of Zoology 71: 515-523.

McPhail, J.D. 1994. Speciation and the evolution of reproductive isolation in the sticklebacks (*Gasterosteus*) of south-western British Columbia. pp. 188-206. *In* Bell, M.A., and S.A. Foster (eds.), The evolutionary biology of the threespine stickleback. Oxford Univ. Press, Oxford.

Peichel, C. L., Nereng KS, Ohgi KA, Cole BL, Colosimo PF, Buerkle CA, Schluter D, Kingsley DM. 2001. The genetic architecture of divergence between threespine stickleback species. 414: 901-905.

Ridgway, M.S. 1982. Reproductive isolation between two coexisting populations of stickleback (*Gasterosteus*) in Enos Lake, Vancouver Island, M.Sc. thesis, University of British Columbia, Vancouver.

Rosenthal, S. K., Stevens, S.S. and Lodge D. M. 2006. Whole-lake effects of invasive crayfish (*Orconectes* spp.) and the potential for restoration. Canadian Journal of Fisheries and Aquatic Sciences 63: 1276–1285

Rundle, H. D., Nagel, L., Boughman, J. W., and Schluter, D. 2000. Natural selection and parallel speciation in sticklebacks. Science 287: 306-308.

Taylor, E.B., Boughman, J.W., Groenenboom, M., Sniatynski, M., Schluter, D., and Gow, J.L. 2006. Speciation in reverse: morphological and genetic evidence of the collapse of a three-spined stickleback (Gasterosteus aculeatus) species pair. Mol. Ecol. 15, 343–355.

Tonolli, V. 1971. Methods of collection, zooplankton. pp. 1-16. In Edmonson, W.T. and G.G. Winberg (eds.), A manual on methods for the assessment of secondary productivity in fresh waters. Blackwell Scientific Publications Oxford and Edinburgh.

Vamosi, S.M. 2003. The presence of other fish species affects speciation in threespine sticklebacks. Evolutionary Ecology Research 5: 717-730.

Wood, P.M. 2003. Will Canadian policies protect British Columbia's endangered pairs of sympatric sticklebacks? Fisheries 28: 19-26.

Wootton, R.J. 1976. The biology of the sticklebacks, Academic Press, London.