

**INVESTIGATING THE ROLE OF INVASIVE AMERICAN SIGNAL
CRAYFISH (*PACIFASTACUS LENIUSCULUS*) IN THE COLLAPSE OF
THE BENTHIC-LIMNETIC THREESPINE STICKLEBACK
SPECIES PAIR (*GASTEROSTEUS ACULEATUS*)
IN ENOS LAKE, BRITISH COLUMBIA.**

by

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Abstract

Biodiversity is of critical importance to the quality of life on Earth. In light of rapidly increasing extinction rates in recent years, understanding threats to biodiversity such as habitat destruction, over-exploitation, and the introduction of invasive species is of utmost importance to conservation efforts. Invasive species, in particular, are a threat of great concern in aquatic environments, due, in part, to their potential role in facilitating introgressive hybridization between closely related species. In recent decades, such a case of hybridization has led to genomic extinction in a benthic-limnetic species pair of threespine stickleback (*Gasterosteus aculeatus*) in Enos Lake, British Columbia, following the appearance of invasive American signal crayfish (*Pacifastacus leniusculus*) in this waterbody. In an effort to shed light on the potential role of these crayfish in this loss of diversity, research was conducted using an intact species pair from Paxton Lake, BC to determine if crayfish exert meaningful impacts on sticklebacks through the disruption of i) male stickleback reproductive behaviours and/or ii) juvenile stickleback growth rates. The results of reproductive behaviour trials demonstrated that limnetic male reproductive behaviour frequency was suppressed to a greater degree than that of benthic males in the presence of crayfish. This result suggests that crayfish disruptions may reduce conspecific mating opportunities for limnetic females, thus leading to increases in introgressive hybridization between benthic males and limnetic females. The results of the juvenile growth rate trial, on the other hand, demonstrated that crayfish do not disrupt growth rates of juveniles; however, significantly higher stickleback mortality levels were detected in the presence of crayfish. I conclude that invasive

American signal crayfish have likely facilitated introgressive hybridization between the Enos Lake species pair, but other factors may have contributed to this loss of biodiversity. My study highlights the sensitivity of recently differentiated species to disruption by invasive species. By identifying important sources of ecological disruption and significant threats to biodiversity, such studies are critical for guiding conservation efforts in Canada and worldwide.

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Chapter 1. General Introduction

1.1. Background

Understanding the origin, persistence, and threats to biodiversity has been a growing area of research in the scientific community in recent years. High profile projects, such as the *Barcode of Life* project at the University of Guelph in Ontario, Canada, have drawn international attention to the documentation of the biodiversity of life on Earth (Ward et al. 2009). Biodiversity is important to our quality of life as humans, as it provides us with many essential services, from food and medicine to aesthetics and general health (Cooney 2005). In recent centuries, however, human activities have had significant impacts on biodiversity, causing extinction rates of plants and animals to increase by orders of magnitude above baseline levels (Millennium Ecosystem Assessment 2005).

In aquatic habitats, human-facilitated impacts such as over-exploitation and habitat destruction threaten the persistence of aquatic biodiversity (Seaman 2007). According to the Food and Agriculture Organization of the United Nations (FAO), 18% of major marine fish stocks are being over-exploited (FAO 2002) and top fisheries researchers warn that global commercial fisheries are targeting lower trophic level species as larger-bodied fish species decline (Pauly et al. 1998). Meanwhile, aquatic habitats are being impaired through human activities such as the dredging and damming of rivers, increased eutrophication, and the destruction of coastal seagrass beds (Seaman 2007).

Aside from habitat loss and degradation, the greatest concern for the conservation of aquatic species in Canada is the threat of invasive species, especially in freshwater ecosystems (Dextrase and Mandrak 2006; Rosenthal et al. 2006). Invasive species threaten more than half of the freshwater fish species in Canada assessed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) (Dextrase and Mandrak 2006) and are believed to be responsible for more than 25 North American fish species extinctions in the 20th century (Miller et al. 1989; Kupferberg 1997). Invasive species have been shown to exert negative impacts on native species in their invaded ranges through various pathways, including predation (Kupferberg 1997), competition for space or resources (Whitfield et al. 2007), and the spread of parasites and novel diseases (Torchin et al. 2002; Miller and Vincent 2008; Freeman et al. 2009).

Another important threat to the biodiversity of aquatic species in Canada is the loss of species through introgressive hybridization (Bettles et al. 2005; Taylor et al. 2006). When hybrids between two species are fertile, a hybrid swarm can be produced through backcrossing and mating between hybrids, thus potentially displacing their parental taxa. This irreversible loss of unique parental allele combinations is called genomic extinction and represents an important source of biodiversity loss (Allendorf and Luikart 2007).

Although the scientific community is aware of the important threats to biodiversity that are posed by introgressive hybridization and invasive species, respectively, the possible links between these factors remain poorly understood. The purpose of my thesis was to gain an understanding of how the American signal crayfish (*Pacifastacus*

leniusculus) may have influenced the genomic extinction of a sympatric species pair of threespine stickleback, *Gasterosteus aculeatus*, (Taylor et al. 2006).

1.2. Threespine Sticklebacks Species Pairs

The threespine stickleback (*Gasterosteus aculeatus*) is a small fish, ubiquitously distributed in coastal habitats throughout its Holarctic range. *G. aculeatus* populations can be found in a wide range of aquatic environments, from fully marine to isolated lakes and streams, but are rarely found more than 200 km inland from the sea (McPhail 1994; 2007). Along this spectrum of aquatic habitats, various ‘species pairs’ have arisen, each containing two derived species with divergent ecological preferences and corresponding adaptations (McPhail 1994; McKinnon and Rundle 2002; Hendry et al. 2009). Within the transitional zone from marine to freshwater, sticklebacks have often formed parapatric species pairs, with narrow contact zones (Hendry et al. 2009). In a very small number of instances, however, stickleback species pairs have evolved in which the two freshwater lacustrine species coexist in sympatry without any physical barrier (Larson 1976; McPhail 1993, 1994; Gow et al. 2008).

These rare sympatric stickleback species pairs, which are referred to as ‘benthic’ and ‘limnetic’ species, exhibit divergent characteristics reflecting their respective adaptations to distinct niches and food sources in their shared lake environment (McPhail 1994). The limnetic species has a streamlined body shape and is specialized for life in open water habitats where its high number of long gill rakers are associated with trophic specialization on planktonic invertebrates. The benthic species, on the other hand, has a larger, deeper body shape and is specialized for life in the littoral zone, where its relatively wide mouth and few, short gill rakers are associated with trophic

specialization on benthic invertebrates (Larson 1976; Bentzen and McPhail 1984; Hatfield and Schluter 1999; Vines and Schluter 2006).

The origin of sympatric stickleback species pairs is one of the best documented cases of ecological speciation thus far, and one of the most recent. The small, coastal lakes in which these pairs are found, in what is now the central Strait of Georgia of British Columbia, have only been in existence for roughly 13,000 years. They were formed when massive Cordilleran glacial ice sheet retreated at the end of the Pleistocene, and previously compressed earth subsequently rebounded upward out of the sea, creating many small basins of seawater (Schluter and McPhail 1992; McPhail 1994). Anadromous marine sticklebacks became isolated in many of these seawater basins and slowly evolved adaptations to a freshwater existence as the basins gradually turned into freshwater lakes (McPhail 1994). Independent selection regimes in each of a very small subset of these lakes led to the formation of comparable benthic and limnetic forms in each lake as parallel divergences in ecologically relevant characteristics allowed the exploitation of abundant, but ecologically disparate, resources (Larson 1976; McPhail 1994; Schluter and Nagel 1995; Taylor and McPhail 1999, 2000). A question of whether each sympatric species pair arose through multiple colonizations from the sea or through sympatric speciation in each lake has been debated amongst evolutionary biologists (McPhail 1993; Schluter and McPhail 1992), but presently the weight of evidence supports a multiple colonization scenario with subsequent ecological speciation (Taylor and McPhail 1999, 2000).

Sympatric species pairs of threespine stickleback have thus far been documented in only five watersheds, including Vananda Creek and Paxton Lake on Texada Island,

Enos Lake on Vancouver Island, Hadley Lake on Lasqueti Island, and Little Quarry Lake on Nelson Island (Taylor and McPhail 2000; Gow et al. 2008). Apart from the Little Quarry Lake species pair on Nelson Island which has not yet been assessed by COSEWIC, and the Hadley Lake species pair, which has since gone extinct, all stickleback species pairs are currently listed as endangered by Canada's *Species at Risk Act* (SARA) (COSEWIC 2002; National Recovery Team for Stickleback Species Pairs 2007).

1.2.1. Reproduction and Growth

1.2.1.1. Life History

The reproductive behaviour of benthic and limnetic sticklebacks is surprisingly similar to that described for sticklebacks in general, involving male provision of parental care while females are responsible only for the selection of a suitable mate and associated nesting site (Wootton 1976; Ridgway and McPhail 1984). During the breeding season, extending from mid-March to mid-June (National Recovery Team for Stickleback Species Pairs 2007), benthic and limnetic males both build their nests in the shallow littoral zone in relative proximity to each other. Using pieces of vegetation and substrate, all stickleback males cover shallow depressions that they excavate in the substrate with a dome-like roof and apply an adhesive secretion to their nest to increase structural integrity. Entrance and exit holes, located on opposite sides of the nest, allow both the male and female to swim through the nest during the climax of the reproductive behaviour sequence (Wootton 1976; Ridgway 1982; Bentzen et al. 1984; Ridgway and

McPhail 1984, 1987). Amongst sympatric species pairs of sticklebacks, benthic males tend to use densely vegetated sites for nesting, while limnetic males tend to nest in the open on unsheltered sandy substrates (Ridgway and McPhail 1984, 1987). Due to the relatively high vulnerability of their breeding sites to detection by rival males, limnetic males are known to react more strongly to trespassers than do benthic males (Ridgway and McPhail 1987).

Once the nest is completed, stickleback males characteristically exhibit a “creep-through” behaviour and subsequently begin to court nearby females (Ridgway and McPhail 1984). During this courtship period, males exhibit a complex array of highly stereotyped behaviours (reviewed in Wootton 1976; Ridgway and McPhail 1984) including i) approaching a gravid female using either a straight or a zigzag swimming pattern, ii) biting the female on her egg-filled belly or elsewhere on her body, iii) attempting to lead a female towards his nest using either a direct or meandering path, or iv) indicating the opening of his nest. Although these behaviours are stereotyped amongst stickleback populations, the sequence of delivery differs significantly between benthic and limnetic males, and even between species pairs from different lakes (Foster and Baker 1995) and thus may be important for effective mate recognition and assortative mating.

After courtship is completed, and eggs have been laid in a male stickleback’s nest, he fertilizes them and then proceeds to fan and defend the eggs until they hatch (Wootton 1976). For a period of a few days to as long as two weeks after hatching, the male goes to great efforts to keep his offspring nearby the nest, before allowing them to disperse for feeding and growth (Wootton 1976). Limnetic juveniles spend the

productive summer months in the pelagic zone, feeding on plankton, and only return to the littoral zone when they have reached sexual maturity and need to reproduce (Bentzen et al. 1984). Benthic juveniles, on the other hand remain in the littoral and benthic zones for their entire lives, foraging primarily on benthic invertebrates (Bentzen et al. 1984).

1.2.1.2. Reproductive Isolation

Amongst pairs of recently formed species such as stickleback species pairs, it is possible that divergence can be reversed through interbreeding because hybrid offspring are often capable of reproduction (McPhail 1984, 1992; Todd and Stedman 1989; Hatfield and Schluter 1999; Taylor et al. 2006). The production and survival of hybrid offspring in species pair lakes is normally minimized through reproductive isolation processes which evolved as a byproduct of phenotypic and ecological divergences between the pair (Schluter 2000). These processes can be categorized into two major classes: pre-zygotic and post-zygotic. Pre-Zygotic processes restrict reproduction between benthics and limnetics, while post-zygotic processes involve selection against hybrid phenotypes. Together, these pre- and post-zygotic barriers to hybridization have been important to the initial divergence and subsequent maintenance of species pairs in sympatry (Vines and Schluter 2006; Gow et al. 2007).

As benthic and limnetic sticklebacks continued to diverge from each other morphologically, behaviourally, and ecologically, stronger pre-zygotic reproductive isolation developed through re-enforcement of assortative mating barriers (Vines and Schluter 2006). Divergent male sexual characters such as body size and nuptial

colouration, along with correspondingly divergent female preferences, are known to contribute to this assortative mating, albeit to varying degrees (Ridgway and McPhail 1984; Boughman 2001; Boughman et al. 2005). Divergences between benthic and limnetic males with regard to breeding habitat choice and courtship behaviour are also thought to contribute to assortative mating, although their relative importance remains unclear (Ridgway and McPhail 1984; Boughman et al. 2005). If pre-zygotic isolation fails, any hybrid sticklebacks produced in these lakes are normally selected against post-zygotically, either through disruptive selection as juveniles (Schluter 1995; Rundle and Schluter 1998; Hatfield and Schluter 1999; Gow et al. 2007), or through negative sexual selection as adults (Vamosi and Schluter 1999; Albert et al. 2007).

Reproductive isolation in sympatric species pair lakes, however, is likely incomplete and thus inherently unstable (Gow et al. 2006; Taylor et al. 2006). For this reason, recently formed species are known to be highly susceptible to hybridization upon contact with closely related species (Todd and Stedman 1989). Many cases of hybridization between closely related freshwater fish species have been documented in nature, including ciscoes (*Coregonus* spp.) in Canada's Great Lakes and Pacific trout species (*Oncorhynchus* spp.) in small lakes and streams (Todd and Stedman 1989; Taylor 2004). The occurrence of low levels of hybridization (~1%, McPhail 1984) is common amongst sympatric stickleback species pairs; therefore, even the smallest perturbations to vitally important isolating processes that could either reduce the relative fitness of extreme benthic and limnetic phenotypes, or weaken the assortative mating system, may lead to a reversal of species divergence through the production of increased numbers of reproductively viable hybrids (Taylor et al. 2006; Behm 2009).

1.2.2. Importance of Sympatric Stickleback Species Pairs

Divergence of sympatric stickleback species pair populations, based on relatively recent adaptations to distinct and divergent ecological niches within each lake, is a prime example of ecological speciation (Hatfield and Schluter, 1999). As such, the importance of sympatric species pairs for the study of ecological speciation has been equated to that of Galapagos finches and cichlid fish assemblages of the east African rift lakes (McPhail 2007).

Sympatric stickleback species pairs are especially valuable to the study evolution because of their age: as constrained by the known timeline of the coastal lake formation, these species pairs originated no more than 13,000 years ago – an evolutionary blink of the eye (McPhail 1993). Because speciation is usually too slow to be observed in its entirety, it is only possible to gain useful insights into speciation by studying recently formed species, as they are still in the process of diverging (Schluter 2000). Furthermore, it can often be difficult to determine the relative importance of ecology and genetics in reproductive isolation during speciation; in recently formed species, however, neutral genetic differentiation is still relatively low, thus increasing the relative importance of selection on ecologically relevant genetic traits in their divergence and persistence in sympatry (Taylor and McPhail 1999, 2000). Insights gained from the study of such traits are driving exciting new avenues of research (Barrett 2010).

Besides their great value to the advancement of evolutionary biology, it is also important to recognize the inherent value of sympatric stickleback species pairs as a contribution to biodiversity in the northern hemisphere. Stickleback species pairs

represent just one example of a diverse assemblage of sibling species that have arisen in north temperate freshwater habitats since the retreat of Pleistocene glaciations (Taylor 1999).

1.2.3. Collapse of the Enos Lake Stickleback Species Pair

Of the five sympatric stickleback species pair populations documented to date, however, two populations have recently been effectively lost, representing a significant loss of such biodiversity. The Hadley Lake species pair was driven to extinction in the late 1990s due to the introduction of the predatory brown bullhead, *Ameiurus nebulosus* (Hatfield 2001), while the Enos Lake pair has collapsed into a hybrid swarm, beginning in the 1990s, due to asymmetric introgressive hybridization between benthic and limnetic species (Kraak et al. 2001; Gow et al. 2006; Taylor et al. 2006). Many hypotheses have been posed to explain the cause of the Enos Lake collapse, but the factors responsible for this genomic extinction remain unconfirmed.

One of the leading factors implicated as a cause of the Enos Lake collapse is the American signal crayfish (*Pacifastacus leniusculus*), which was first sighted in Enos Lake in the late 1980s, shortly before the species pair began to collapse (Taylor et al. 2006). *P. leniusculus* is a large freshwater crayfish that has been known to reach a maximum adult carapace length (CL) of 57 mm, measured from the rear of the eye socket to the end of the carapace (Usio et al. 2001; Harrison et al. 2006). *P. leniusculus* has the most widely distributed native range of any species in the *Pacifastacus* genus, extending from central California, USA to southern British Columbia, and bordered by

the Rocky Mountains to the east and the Pacific Ocean to the west (Lewis 2002). Human-facilitated introductions of signal crayfish for use as bait, or as substitutes for collapsed crayfish fisheries, have expanded its range in North America and have allowed it to colonize much of Europe and Asia in the latter half of the twentieth century (Lewis 2002; Bondar et al. 2005). Studies in Enos Lake have demonstrated that signal crayfish are capable of significantly reducing species richness and density in benthic invertebrate and aquatic plant communities (Rosenfeld et al. 2008), a result that is consistent with several other studies documenting the disruptive effects of invasive crayfish (Hirsch and Fischer 2008; Kuhlmann et al. 2008; Pintor and Sih 2009).

The impacts of crayfish on the sticklebacks of Enos Lake, however, are currently poorly understood. The purpose of my thesis was to investigate what role, if any, *P. leniusculus* may have played in the asymmetric collapse of the Enos Lake stickleback species pair. I designed experimental treatments to test if these crayfish could have had a differentially disruptive effect on benthic and limnetic sticklebacks by interfering asymmetrically with male reproductive behaviour during the breeding season or by influencing juvenile growth rates of juveniles.

Chapter 2. Disruptive Effects of American Signal Crayfish

2.1. Introduction

Invasive species have been a growing concern for the conservation of native aquatic species in Canada (Dextrase and Mandrak 2006; Rosenthal et al. 2006). Second only to habitat loss and degradation, invasive species threaten more than half of the freshwater fish species in Canada assessed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) (Dextrase and Mandrak, 2006). Another important threat to the biodiversity of aquatic species in Canada is the collapse of coexisting ecotypes through introgressive hybridization (Bettles et al. 2005; Taylor et al. 2006). Possible links between these two important factors, however, remain poorly understood. The purpose of this study was to gain a better understanding of how the invasive American signal crayfish (*Pacifastacus leniusculus*) may have facilitated introgressive hybridization in a sympatric benthic-limnetic threespine stickleback species pair (*Gasterosteus aculeatus* Complex) in Enos Lake, British Columbia.

Sympatric stickleback species pairs are unique to a handful of small coastal lakes in southwestern British Columbia, Canada, and are highly valued for the insights that they afford studies of ecological speciation, an evolutionary process of fundamental interest to science (McPhail 2007). Each pair independently evolved from a marine ancestor, following their isolation in newly formed coastal lakes upon the retreat of the cordilleran ice sheet less than 13,000 years ago (Taylor and McPhail 2000). All known

sympatric species pairs consist of a larger, bottom-feeding 'benthic' species and a smaller, plankton-feeding 'limnetic' species, which diverged from each other with respect to behavioural, morphological, and ecological traits as they adapted to these disparate niches in each lake (McPhail 1994; Hatfield and Schluter 1999). As a byproduct of these adaptations to opposite ecological extremes, assortative mating barriers developed within each species pair, strengthening reproductive isolation between them (Ridgway and McPhail 1984; Vines and Schluter 2006).

Such pre-zygotic isolating barriers between recently diverged species, however, are often incomplete and hybridization can therefore occur relatively easily when these species encounter each other in nature (Todd and Stedman 1989). Sympatric species pairs of sticklebacks, in particular, are susceptible to hybridization because males of both species establish nesting territories in close proximity to each other in the littoral zones of small lakes where they coexist (McPhail 2007; National Recovery Team for Stickleback Species Pairs 2007). Perturbations to delicate pre-zygotic barriers between these close neighbours can thus quickly lead to increased production of hybrids during the breeding process when males of both species are responsible for building nests, attracting conspecific females, and providing parental care to their offspring (McPhail 1992, 2007; Taylor et al. 2006; Behm 2009).

Of the five known sympatric species pairs of sticklebacks identified in the past 40 years, two have already been effectively lost (Gow et al. 2008). One of these pairs, found in Enos Lake, Vancouver Island, collapsed into a hybrid swarm due to introgressive hybridization between the benthic and limnetic ecotypes in the lake (Taylor et al. 2006). The first evidence of an impending collapse in this system was reported by

Kraak et al. (2001), who found that the hybrid phenotype, intermediate to that of either the benthic or limnetic form, represented 12% of their morphological samples. This disturbing result came less than 20 years after McPhail (1984) had described a well-differentiated species pair, containing very low levels of hybrids (~1%). The collapse was subsequently confirmed by Gow et al. (2006) and Taylor et al. (2006) through a combination of morphological and molecular analyses, further demonstrating that the collapse took place asymmetrically through benthic-biased introgressive hybridization, resulting in a hybrid swarm characterized by a dominance of benthic alleles and a paucity of limnetic alleles.

Researchers have since been at a loss to determine what could have caused such a rapid and asymmetric reversal of speciation in Enos Lake. Some have suggested that the invasive American signal crayfish (*Pacifastacus leniusculus*) may have been a major factor in the collapse of the Enos Lake species pair (COSEWIC 2002; Taylor et al. 2006; Behm et al. 2010). It is thought that these large freshwater crayfish were introduced to Enos Lake (or dispersed there naturally from adjacent waterbodies) in the late 1980s, shortly before the lake's species pair started to collapse (Taylor et al. 2006; Peterson 2008; Rosenfeld et al. 2008). In addition, the rough-skinned newt (*Taricha granulosa*) that was native to Enos Lake has disappeared since the first appearance of the American signal crayfish (E.B. Taylor, Dept. of Zoology, UBC, pers. comm.). This coincidence of timing, combined with the fact that none of the other sympatric species pairs coexist with crayfish, has provided the basis for such hypotheses (Behm et al. 2010). Crayfish are voracious omnivores, known to inflict severe damage on aquatic vegetation in invaded habitats, and outcompete native

species for food and shelter (Hirsch and Fischer 2008; Kuhlmann et al. 2008; Pintor and Sih 2009). Crayfish are even known to prey on fish when the opportunity arises (Guan and Wiles 1998). Considering these facts, I hypothesized that *P. leniusculus* may have promoted increased hybridization between benthic and limnetic sticklebacks in Enos Lake by disrupting pre-zygotic assortative mating barriers in two important ways: 1) by influencing male stickleback reproductive behaviour during the breeding season, and 2) by impacting growth rates of juvenile sticklebacks during the growing season.

2.1.1. Reproductive Behaviour Effects (Hypothesis 1)

The behaviour of threespine stickleback on the breeding grounds is well documented and has been shown to be remarkably similar between populations, including sympatric species pairs (Wootton 1976; Ridgway and McPhail 1984). During the breeding season, stickleback males are known defend their territories from predators and conspecific males that may eat or steal eggs from their brood (Whoriskey and FitzGerald 1994). As benthic omnivores, crayfish roam the littoral zone of Enos Lake in search of food and probably trespass on stickleback nesting territories. Given that breeding stickleback males have a finite amount of time and stored energy to expend on nesting, courtship, and territorial defense, it is possible that allocating too many of these resources to repelling an intruding crayfish may cause them to suffer reduced reproductive success relative to unperturbed males.

Early studies of the Enos Lake stickleback species pair collapse by Gow et al. (2006) indicated that the collapse likely took place through asymmetric hybridization,

removing limnetic genes from the population much more quickly than benthic genes, thus creating a hybrid swarm dominated by benthic genotypes. Based on this observation, Gow et al. (2006) suggests that interspecific matings likely took place between benthic males and limnetic females. Furthermore, studies of nesting male stickleback courtship behaviours in Enos Lake have demonstrated that limnetic males react more strongly to intrusions by rival males than do benthic males, likely due to the relatively high vulnerability of their unsheltered breeding territories (Ridgway and McPhail 1987). Considering that limnetic males exhibit a heightened sensitivity to trespassers, I predicted that limnetic males would similarly exhibit more territoriality in the presence of crayfish than would benthic males, thus leaving limnetic males with fewer resources to allocate to nesting and courtship, and perhaps making them more likely to experience nesting failure. In Enos Lake, such a reduction in limnetic nesting effort could have resulted in the production of a disproportionately small number of limnetic nests on the breeding grounds. Assuming that such an effect could have resulted in a shortage of viable limnetic male nests in Enos Lake during the early years of the crayfish invasion, the likelihood of interbreeding may have increased.

2.1.2. Juvenile Growth Rate Effects (Hypothesis 2)

Increased metabolic costs are known to impoverish energy supplies that could otherwise be allocated to growth, which unfortunately can lead to a disruption in growth rate (Slos and Stoks 2008) and could ultimately have implications for juvenile survival and adult fecundity. This effect has been well documented in insects (Slos and Stoks 2008) and

amphibians (Steiner and Van Buskirk 2009). Many prey organisms, ranging from mammals to birds and fish, experience such increases in metabolic costs either in preparation for, or as the result of, 'fight or flight' responses (Barreto et al. 2003; Hawkins et al. 2004; Slos and Stoks 2008; Steiner and Van Buskirk 2009). Because crayfish are known to be aggressive competitors with, and predators of, fish (Guan and Wiles 1998; Pintor and Sih 2009) I predicted that juvenile stickleback growth rates would be suppressed in the presence of crayfish in captivity. Such a result would be particularly interesting if the growth rates of benthic and limnetic sticklebacks are affected differentially by crayfish. One way in which such an effect could have caused increased benthic-limnetic hybridization is if benthic juveniles, who share the littoral zone with crayfish, were to suffer lowered growth rates in the presence of crayfish (see Chapter 3 for an elaboration of these potential effects). If such asymmetric impacts were to take place, affected benthic juveniles might mature at a smaller size, which could increase the likelihood of hybridization with limnetic females, given that adult male stickleback body size is known to be an important cue for assortative mating in stickleback species pairs (Boughman et al. 2005).

2.2. Materials and Methods

2.2.1. General Approach

To assess the likelihood of asymmetric crayfish-mediated effects on benthic and limnetic sticklebacks during the breeding and juvenile growth stages of their life cycle, I recorded metrics of adult male reproductive behaviour frequency and juvenile growth rate during laboratory-based behavioural and growth trials in the presence and absence of crayfish. I used benthic and limnetic sticklebacks from Paxton Lake, Texada Island (British Columbia, Canada) in this study because this species pair is still intact and divergent, is known to readily exhibit natural behaviours in captivity (T. Vines, Dept. of Zoology, UBC, pers. comm.), and are evolutionarily and ecologically naïve to crayfish. Perhaps most importantly, this naïveté to crayfish made the Paxton Lake species pair a realistic model of the pre-collapse, pre-crayfish Enos Lake species pair, allowing me to replicate the effects imposed in Enos Lake following the initial crayfish invasion in the late 1980s.

2.2.2. Source of Animals and Animal Care

Adult benthic and limnetic sticklebacks for the reproductive behaviour trials were collected from Paxton Lake (Texada Island, British Columbia) from mid-April to early May, 2009, ($N_{\text{benthic}} = 40$; $N_{\text{limnetic}} = 83$). Juvenile benthic and limnetic sticklebacks for the juvenile growth trial were collected in September and October of 2009. To minimize impacts on the wild population, limnetic juveniles were collected from research ponds on the University of British Columbia (UBC) campus in September of 2009 ($N_{\text{limnetic}} = 100$).

These juveniles were the second generation offspring from wild Paxton Lake limnetic adults released in the research ponds in mid-April, 2008. Benthic juveniles in the UBC research ponds, however, were much older and larger than their limnetic counterparts. To avoid any confounding effects of body size differences between benthics and limnetics in the growth trial, however, efforts were made to achieve size-matching between benthic and limnetic juveniles. To achieve this, benthic juveniles were collected from Paxton Lake in late October, 2009, and only the smaller individuals, of a similar size to the limnetic juveniles collected in the UBC research ponds, were retained ($N_{\text{benthic}} = 60$). American signal crayfish were collected from Enos Lake during several collecting trips between June 2008 and May 2009, using prawn traps baited with dry cat food. All collection activities were permitted by the BC Ministry of Environment (permit #: NA/SU 09-52085) and the Department of Fisheries and Oceans' Species At Risk Act (permit #s: SECT 08 SCI 027 and SARA 121).

Stickleback and crayfish were transported to UBC in aerated coolers, and were held in 189 L aquaria in two separate walk-in, temperature-controlled environmental chambers. To avoid premature breeding, adult sticklebacks were separated by sex and species, and held at a late springtime temperature and photoperiod regime to induce breeding (17°C; 16h:8h light: dark photoperiod), while juvenile sticklebacks were separated by species and held at an early springtime temperature photoperiod regime to simulate conditions at the start of the post-winter growing season (15°C; 12h:12h light: dark photoperiod). Water quality in all fish aquaria was monitored for harmful nitrogenous wastes, and maintained at an approximate pH of 8.0 to reflect conditions in Paxton Lake (C. Ormond, Dept. of Zoology, UBC, pers. comm.). Fish were fed at least

once every two days with frozen bloodworms (*Chironomidae* larvae) or *Daphnia*. Crayfish were fed regularly with wafers of *Spirulina* algae. All fish were cared for and disposed of according to UBC animal care protocol (permit # A07-0378).

2.2.3. Reproductive Behaviour Effects (RBE)

2.2.3.1. RBE - Experimental Setup

Four behavioural observation arenas were set up in an environmental chamber. Two arenas were designated for use with limnetic males; the other two, for benthic males. Arenas were constructed from 189 L glass aquaria, measuring 47 cm x 92 cm x 48 cm. Three sides of each aquarium were covered with opaque plastic sheets to limit external stimuli, leaving the front side open for observation. A black plastic canopy was constructed over this side of the arena, allowing the observer to sit in darkness and record observational data. Each aquarium was illuminated from above with full-spectrum fluorescent light to prevent subjects in the arena from seeing the observer on the dark exterior of the aquarium. Each observational arena (Fig. 2.1) consisted of a nesting dish (diameter approx. 25 cm) filled with filter sand and topped with Douglas Fir needles and Java moss (*Vesicularia dubyana*) for use as nesting material; ten pieces of limestone cobble (diameter approx. 5 cm) and a piece of PVC tube (diameter approx. 3 cm; length approx. 5 cm) for use as shelter by fish or crayfish; and two 500 ml clear glass jars, to be used for confining crayfish and female stickleback, respectively, during certain treatments (see below). Confinement of crayfish or female stickleback allowed me to visually expose male sticklebacks to a potential mate and/or a live crayfish,

without allowing physical contact, thus differentiating between the effect of visual and tactile exposure. The female confinement jar was suspended in the water column, just below the water's surface in a central location in the back of the arena, while the crayfish confinement jar was placed on the floor of the arena on the opposite end from the nesting dish. The jars were positioned such that the female stickleback and crayfish could be kept in fixed positions, distant from the nesting dish and from each other, thus minimizing ambiguity in male behaviours when approaching either the crayfish or female stickleback. Each jar was covered with a lid of fiberglass screen, held in place by a PVC ring. A length of string tied to the lid of each jar allowed the observer to remotely release the confined individuals with minimal disturbance.

2.2.3.2. RBE - Protocol

The experimental protocol for the behavioural trials involved the observation of male stickleback reproductive behaviour during a sequence of treatments, including one baseline and four manipulated experimental treatments (described in Table 2.1) for each male. A sequential design was used to identify crayfish effects at varying degrees of exposure and at different stages of courtship to evaluate the degree to which crayfish disrupted courtship and nesting behaviour. To create a gradient of increasing crayfish disruption and reproductive intensity, the various treatments in this experiment involved sequentially confining and releasing both the female stickleback and crayfish.

At the start of each sequence, one sexually receptive stickleback male was selected, based on the expression of bright red and blue colouration and dominant territorial behaviour. Each male was added to an arena, and given sufficient time to

begin tending a nest (usually 24-48 hours). To determine a baseline level of male reproductive activity, each male was given visual access to a gravid conspecific female stickleback confined to a glass jar in the arena, while the crayfish enclosure remained vacant (Fig. 2.1). To determine the visual effects of crayfish on male reproductive behaviour, a medium-sized crayfish (18.1 ± 0.6 g) was added to a glass jar in the arena during Treatment 1, while the female stickleback remained confined. At the start of Treatment 2, the crayfish was released from its confinement jar to determine the additive effects of a free-roaming crayfish on male reproductive behaviour. Next, to measure crayfish effects on a full courtship scenario in Treatment 3, the female stickleback was released from her enclosure. Finally, to assess whether there was a return to baseline reproductive behaviour in the absence of crayfish, Treatment 4 involved the removal of the crayfish from the arena with a dip net, leaving the male and female sticklebacks relatively undisturbed.

2.2.3.3. RBE - Data Analyses

In all, behavioural observations were conducted on 19 benthic (60.7 ± 0.96 mm) and 16 limnetic (53.0 ± 0.61 mm) male sticklebacks, for a total of 35 observation sequences. Each observation treatment period lasted for ten minutes, divided into a total of 120 five-second time blocks. The ten-minute duration was selected to maximize the likelihood of observing different behaviours, while minimizing the time that each male was exposed to a potential stress. During each observational period, I assigned a positive score (+1) to each time block in which a stickleback male exhibited nest tending or courtship behaviours (as described in Wootton 1976; Ridgway and McPhail 1984) as these

behaviours contribute to successful reproduction. No scores were assigned to time blocks during which the male stickleback oriented towards the crayfish in the arena or exhibited no orientation towards either the nest or the female, as these behaviours do not contribute to successful reproduction. Between treatments, a minimum 'settling down' time of 5 minutes was to minimize carry-over effects from the previous observational period (see Liang and Carriere 2010).

Reproductive behaviour frequencies (proportion of total time blocks scored as positive) were tabulated for each ten-minute observational period and analyzed using R (R Development Core Team 2009). For statistical analysis, these proportional data were normalized using the following formula:

$$[\text{Normalized data} = \arcsin \sqrt{\text{data}}]$$

Paired t-tests were used to compare the means of pairs of observational periods to test for changes in reproductive behaviour frequency upon the introduction and removal of crayfish, respectively: a) Baseline versus Treatment 1; b) Treatment 3 versus Treatment 4. To test for differences in crayfish effects between benthic and limnetic males, Welch's two-sample t-tests were used to compare benthic and limnetic data sets in each treatment. Before these two-sample t-tests were carried out, behavioural frequencies of each benthic and limnetic male were subtracted from each male's respective baseline frequency to correct for differences in initial activity between benthic and limnetic males. The resulting behavioural frequencies reflect changes in benthic

and limnetic male behavioural frequencies, which could then be compared using the two-sample *t*-tests described above.

Fitting data to linear models

Because limnetic males are generally smaller than benthic males, it is conceivable that any differences in crayfish effects observed between them could be attributable simply to body size, rather than intrinsic differences in behaviour. To confirm that this was not the case, baseline-corrected reproductive behaviour frequencies from the initial introduction of crayfish (Treatment 2) were fit to a linear model with male total length (MTL) and species (benthic or limnetic) as the independent variables. The model's formula is as follows:

$$\text{[Behaviour frequency = slope (MTL) + slope(species) + intercept]}$$

Twelve of the behavioural sequences (six benthic, six limnetic), were extended by an additional two treatments to determine if crayfish size influenced the severity of crayfish effects on stickleback reproductive behaviour. Following observations of reproductive behaviour in the presence of the medium crayfish (Treatment 3) in each of these sequences, the medium-sized crayfish was removed and one small (5.0 ± 0.28 g) and one large (36.1 ± 1.89 g) crayfish were sequentially released into the arena, observed for ten minutes, and subsequently removed from the arena, such that only one crayfish was present in the arena at any time. The order in which the two sizes of crayfish were introduced was determined by a coin toss.

A linear model was also used to determine if the severity of crayfish impacts in each of these sequences were related to crayfish size. To create this model, baseline-corrected reproductive behaviour frequency values from the three phases of Treatment 3 were fit to a linear model with crayfish mass (CM) and species (benthic or limnetic) as the independent variables. The model's formula is as follows:

$$[\text{Behaviour frequency} = \text{slope}(\text{CM}) + \text{slope}(\text{species}) + \text{intercept}]$$

Testing assumptions

Parametric statistics were preferentially used for analysis due to their greater statistical power (Whitlock and Schluter 2009). Consequently, several tests were carried out to satisfy the assumptions of parametric statistics. To test for normality, the Shapiro-Wilk test statistic was used on each *t*-test data set, as well as the residuals of each of the linear models used in my analyses. To test for homogeneity of variance, however, two different methods were used: Fisher's *F*-tests were conducted on each pair of *t*-test data sets, while each of the linear models was tested by plotting a regression of the absolute values of its residuals against its independent variable. Transformed data sets that did not conform to the parametric assumptions were analyzed using less powerful non-parametric statistics (Wilcoxon matched pairs signed-rank test in the place of paired *t*-tests; Wilcoxon rank-sum test in the place of two-sample *t*-tests).

To determine the effects of crayfish size on behaviour suppression, each of these twelve replicates was extended by approximately 25 minutes. Inclusion of these multiple

crayfish replicates in the general analyses implicitly assumes that the delays and additional manipulations associated with these sub-treatments did not affect the recovery of reproductive behaviours in Treatment 4. To test that this assumption was valid, I assessed male reproductive behaviour recovery (Treatment 3 versus Treatment 4; benthic and limnetic combined), both including (N=35) and excluding (N=23) the additional sub-treatments, using a paired t-test.

2.2.4. Juvenile Growth Rate Effects (JGRE)

2.2.4.1. JGRE - Experimental Setup

During the juvenile growth rate trial, 60 limnetic and 60 benthic juveniles were distributed into 12 identical aquaria, resulting in a density of five benthics and five limnetics in each aquarium. Each aquarium contained a sponge filter, several pieces of limestone cobble (approximate diameter = 5 cm) for structure and a small piece of PVC tube for use as shelter. Visual barriers were placed between adjacent aquaria to eliminate interactions between them.

2.2.4.2. JGRE - Protocol

Mass and length were collected from each fish both at the start and at the end of the 90-day growth period (see Table 2.2) in order to establish growth rates of juvenile sticklebacks both in the presence and absence of crayfish. At the beginning of the experiment, each fish was uniquely marked using elastomer tags (Northwest Marine Technology, Inc., Shaw Island, Washington, USA) allowing comparisons to be made between start and end metrics for each fish. After allowing fish to become acclimatized

to their new surroundings for the first three weeks of the growth period, one crayfish was added to each of six randomly assigned aquaria, while the six remaining tanks were maintained as control replicates. Crayfish used in this experiment ranged in size from 11 to 41 g, and consisted of four males and two females (See Table 2.2).

Feeding was carried out daily, using frozen cubes of chopped and rinsed blood worms (*Chironomidae* larvae), each containing a specific ration of food. To keep up with increases in body size, food rations were set at 1.4g/tank/day during the first six weeks, and increased to 2.1g/tank/day during the following three weeks, and finally to 2.8g/tank/day during the final three weeks of the growth period. To maintain fish densities, mortalities occurring over the course of the experiment were immediately removed and replaced with conspecific individuals. Only the fish that survived the entire growth period ($N_{\text{benthic}}=54$; $N_{\text{limnetic}}=54$) were used in the final analysis of growth rates.

2.2.4.3. JGRE - Data Analyses

Data collected in the growth trial were analyzed using R (R Development Core Team 2009). Growth rates were calculated by dividing mass gains by the total number of days for which fish were grown (90 days for 11 aquaria; 67 days for 1 aquarium). Mean growth rates of the groups of benthics and limnetics from treatment and control tanks were compared using Welch's two-sample *t*-tests to determine if 1) there were any differences in growth rate in the presence and absence of crayfish and 2) if benthic and limnetic juveniles were affected differently from each other.

2.2.4.4. JGRE - Other Analyses

In addition to analyses to determine if there were any sub-lethal crayfish effects on juvenile growth rate, mortalities of juveniles over the course of the experiment were documented and analyzed to determine if crayfish had any lethal effects. Mortality rates were calculated for each aquarium (percentage of original fish lost during the experiment) and these rates were compared between treatment and control tanks using a conservative non-parametric Wilcoxon Rank Sum test.

2.3. Results

2.3.1. Reproductive Behaviour Effects

Following the successful construction of a nest by the stickleback male, and the subsequent introduction of a female stickleback to her confinement jar, the stickleback male almost immediately began performing alternating courtship and nesting behaviours. Upon the introduction of a confined crayfish to the arena, sticklebacks tended to continue performing reproductive behaviours, while occasionally approaching and inspecting the crayfish confinement jar. In the presence of a freely roaming crayfish, however, stickleback males tended to spend a great deal of time inspecting the crayfish and, in some cases, aggressively attacked it, especially when it came within approximately 20 cm of the male's nest. With the exception of defensive responses exhibited during the occasional attack from a stickleback male, crayfish generally, appeared to pay little to no attention to the sticklebacks with which they shared the arena and did not seem to be attracted to stickleback nests. Out of a total of 37 males that were tested, two limnetic males were discarded due to inactivity on the part of either the male stickleback or its crayfish counterpart.

The results of the reproductive behaviour trials demonstrated that the frequency of limnetic male reproductive behaviours were significantly suppressed by the introduction of a crayfish into their breeding territories, while those of benthic males were not altered significantly. The results of paired t-tests indicated that limnetic males experienced a significant suppression of reproductive behaviour upon the initial

introduction (Baseline versus Treatment 1) of a crayfish to the observational arena (-27%; $t = 4.39$; $df = 15$; $p < 0.001$), while benthic males did not (-6%; $t = 0.94$; $df = 18$; $p = 0.36$) (Fig. 2.2). Limnetic males also experienced a significant recovery in reproductive behaviour upon the initial removal (Treatment 3 versus Treatment 4) of the crayfish from the arena (+24%; $t = -4.67$; $df = 15$; $p < 0.001$), while benthic males did not (+12%; $t = -1.63$; $df = 18$; $p < 0.12$) (Fig. 2.2). While the magnitude of suppression of behaviour upon the initial introduction of the crayfish differed significantly between limnetic (-27%) and benthic (-6%) males ($t = 2.51$; $df = 31.8$; $p = 0.02$), the magnitude of recovery of behaviour upon the initial removal of the crayfish did not differ significantly between limnetic (+24%) and benthic (+12%) males ($t = -1.48$; $df = 32.8$; $p = 0.15$).

Comparisons of reproductive behaviour frequency between benthic and limnetic males confirmed that benthic and limnetic males indeed react differently to the presence of crayfish. During baseline observations in the absence of crayfish, limnetic males exhibited significantly higher (+33%) initial frequencies of reproductive behaviour than did benthic males ($t = -2.17$; $df = 32.4$; $p = 0.04$; Fig. 2.3). In the subsequent three treatments, limnetic male reproductive behaviour was suppressed, relative to baseline levels, to a greater extent than that of the benthic males (Fig. 2.4). Based on the results of two-sample t-tests, differences in suppression of reproductive behaviour between males (limnetic vs. benthic) were found to be significant upon the introduction of the confined crayfish (Treatment 1; -38% vs. -12%; $t = 2.47$; $df = 32.6$; $p = 0.02$), the subsequent release of the crayfish from its confinement (Treatment 2; -65% vs. -40%; $t = 2.67$; $df = 32.2$; $p = 0.01$) and, finally, the release of the female from its confinement (Treatment 3; -58% vs. -29%; $t = 3.14$; $df = 32.6$; $p < 0.01$) (Fig. 2.4). In Treatment 4,

when the crayfish was permanently removed from the arena, limnetic male reproductive behaviour frequency was still suppressed to a greater extent than that of the benthic males, although the suppression of behaviour for both species was minimal, and the difference between them was no longer significant (-24% vs. -7%; $t = 1.25$; $df = 32.4$; $p = 0.22$) (Fig. 2.4). Overall, limnetic males experienced greater suppression of reproductive behaviour than benthic males in all treatments involving crayfish.

The results of linear model-fitting confirmed that differences in suppression of reproductive behaviour were not simply attributable to body size. Using male total length (MTL) and Species (benthic or limnetic) as independent variables, Species was found to be a significant predictor of reproductive behaviour suppression ($p = 0.003$), while MTL was not ($p > 0.38$). Linear model-fitting results also indicated that crayfish size did not significantly influence the severity of male stickleback reproductive behaviour suppression. Neither the linear model for benthic males ($r^2 = 0.06$, $p = 0.31$), nor that for limnetic males ($r^2 = 0.22$, $p = 0.06$) was significant.

Analyses of reproductive behaviour suppression data both with and without the twelve 'multiple crayfish size' replicates confirmed that any additional disturbance associated with time delays and apparatus manipulation during these extra treatments did not influence the overall results of the final treatment. Paired t -tests comparing the recovery of male reproductive behaviour frequencies (Treatment 3 versus Treatment 4, benthic and limnetic data pooled) yielded similar results when the additional sub-treatments were included ($N=35$, $p = 0.006$) and excluded ($N=23$, $p = 0.007$).

2.3.2. Juvenile Growth Rate Effects

Juvenile sticklebacks did not appear to alter their behaviour in the presence of crayfish; in fact, they were observed to spend considerable time in the vicinity of the crayfish. When fed, the sticklebacks consumed their food rapidly, finishing it before it reached the bottom of the aquarium. Crayfish did not have an opportunity to eat the stickleback food, and tended to be inactive when observed during the day, and did not appear to exhibit any aggressive behaviour toward the sticklebacks.

My juvenile growth experiment demonstrated that the proximity of signal crayfish for extended periods of time did not influence the growth rate of juvenile benthic or limnetic sticklebacks. There were weak, but statistically non-significant, trends indicating increased growth rates amongst benthic juveniles (+5%; $t = -0.69$; $df = 7.3$; $p = 0.51$) and decreased growth rates amongst limnetic juveniles (-8%; $t = 0.33$; $df = 9.9$; $p = 0.75$). in the presence of crayfish (Fig 2.5).

Although crayfish had no significant sub-lethal effects on growth rates of sticklebacks, the Wilcoxon Rank Sum test demonstrated that mortality rates were significantly higher in the presence ($n=10$) than in the absence ($n = 0$) of crayfish ($p = 0.01$), indicating that there was a lethal effect of crayfish on stickleback juveniles, although its mechanism was not obvious. Equal numbers of benthic and limnetic juveniles died during the experiment. Because of this mortality, treatment data sets for both benthic and limnetic juveniles contained fewer individuals ($n=24$) than did control data sets ($n=30$).

2.4. Discussion

In the small number of lakes that still contain sympatric species pairs of *G. aculeatus*, strong reproductive isolation constrains hybridization. Pre-Zygotic isolating barriers such as divergence in body size and breeding colouration (Boughman 2001; Boughman et al. 2005); and courtship behaviour (Shaw et al. 2007) normally limit opportunities for hybridization between sympatric stickleback species pairs to varying degrees. Furthermore, post-zygotic barriers maintain species pairs by reducing the fitness of interspecific hybrids, relative to their pure benthic and limnetic neighbours, thus removing them from the population before they have a chance to reproduce (Schluter 1995; Hatfield and Schluter 1999; Gow et al. 2007). In Enos Lake, however, Behm et al. (2010) recently demonstrated that post-zygotic ecological barriers have been weakened or removed, probably due to perturbations of lake ecology, leaving hybrid stickleback individuals with a fitness level no different from that of limnetics and benthics.

The rapid collapse of the Enos Lake species pair in less than 20 years, however, suggests that there must have also been an increased production of hybrids in Enos Lake, suggesting that pre-zygotic isolation barriers have also been disrupted. My study provides useful insights into what may have contributed to this disruption. Evidence implicating *P. leniusculus* as a factor in the collapse of the Enos Lake species pair has thus far been only circumstantial. Although crayfish are known to have negative effects on fishes (Guan and Wiles 1998; Hirsch and Fischer 2008) in other areas, no direct effects of crayfish on a species pair of sticklebacks have been observed until now. I successfully tested hypotheses of potential crayfish-induced disruption of pre-zygotic

reproductive isolation between benthic and limnetic sticklebacks. My goal was to determine if crayfish might promote hybridization through disruptions at two critical phases of the stickleback life cycle: during the breeding season, and during juvenile growth.

2.4.1. Reproductive Behaviour Effects

In the first part of my study, I tested the hypothesis that crayfish differentially disrupt the reproductive behaviour of benthic and limnetic sticklebacks, potentially leading to decreased limnetic reproductive success and abundance. I demonstrated that crayfish negatively impact the reproductive activities of limnetic males to a greater extent than benthic males. My results suggest a process by which the frequency of interbreeding may have increased, potentially leading to the collapse of the species pair in Enos Lake.

In my experiments, limnetic males spent significantly less time on positive reproductive behaviours (see Ridgway and McPhail 1984) when in the presence of crayfish, while benthic males were not as severely perturbed. The results of the linear model fitting suggest that there must be differences between benthic and limnetic males, other than size, that influence their vulnerability to distraction during the breeding season. My observation of elevated baseline levels of reproductive behaviour in limnetic males suggests one explanation for this difference: limnetic males are more behaviourally active than benthic males and may thus be more likely to investigate and/or defend against intrusions into their breeding territories by other species. This

conclusion is consistent with a study by Ridgway and McPhail (1987) which demonstrated that limnetic male sticklebacks tended to invest more of their time and resources on territorial defense than benthic males did when confronted with an intrusion by a rival male. It has been suggested that the tendency of limnetic males to defend their territories more vigorously than benthic males is a behavioural adaptation to compensate for the fact that open nest sites are more vulnerable to predation and intrusion by rival males (see Ridgway and McPhail 1987). Assuming limnetic males have evolved such adaptations while maintaining normal levels of reproductive success, it is unlikely that such behaviours translate into any fitness disadvantages for limnetic males.

Another interpretation of my results, however, is that such disruptions of reproductive activities by crayfish, in addition to the ever-present threats imposed by rival males and predators, could be a greater than average burden on limnetic males, and could lead to significant delays in nesting. Given that male sticklebacks have finite time and energy resources to draw from during their relatively short breeding season, such time delays could easily result in decreases in limnetic nesting success. Assuming that the pattern observed in captivity holds true in a natural environment, it is possible that the overall nesting success of limnetic males in Enos Lake was decreased, relative to that of benthic males, upon the introduction of crayfish in the 1980s. Assuming fewer limnetic males were capable of producing viable nests in the presence of crayfish in a given breeding season, it is possible that the Enos Lake breeding grounds during these early years of crayfish invasion would have contained a shortage of limnetic nesting sites from which limnetic females could choose.

Research by Todd and Stedman (1989) and Willis et al. (2004) demonstrated that, in general, members of a pair of sympatric species are much more likely to hybridize with each other when there is a shortage of conspecific males of one species (see review by Wirtz 1999). Assuming that benthic nests would have been more common than those belonging to the more defensive limnetic males in a crayfish - impacted environment, I suspect that limnetic females in Enos Lake would have been more likely to be courted by benthic males than by conspecific males, which would perhaps have led to increased levels of hybridization. This scenario represents a case in which a demographic imbalance, caused by an invasive crayfish, has influenced pre-zygotic reproductive barriers and rendered them less effective: in the presence of crayfish, it appears that the reproductive barriers that played such an important role in the divergence and subsequent maintenance of the Enos Lake pair may have been insufficient to prevent interbreeding, in the presence of increased benthic - limnetic encounter rates (Gow et al. 2006; Vines and Schluter 2006).

Considering that several lines of evidence support the idea that asymmetric introgression has occurred in Enos Lake, the results of my study, and my conclusion that introgression occurred primarily between benthic males and limnetic females, are particularly relevant. Early studies of the Enos Lake species pair collapse suggested that asymmetric introgression between the pair favoured the production of benthic-like sticklebacks during the crayfish invasion (Gow et al. 2006; Taylor et al. 2006). Morphological and genetic data demonstrated that the population contained an abundance of benthic alleles, but contained few limnetic alleles and was virtually devoid of pure limnetic or benthic genotypes (Gow et al. 2006; Taylor et al. 2006).

In species that exhibit female choice during reproduction, asymmetric hybridization usually occurs between females of a rare group and males of a common group (Wirtz 1999). Given that sticklebacks exhibit female choice during reproduction (Wootton 1976), the fact that the benthic genotype now predominates in Enos Lake (Gow et al. 2006; Taylor et al. 2006), and my demonstration that limnetic males exhibit a disproportionate reduction in reproductive behaviour in the presence of crayfish, it is possible to conclude that the introgressive collapse of the Enos Lake species pair took place preferentially between benthic males and limnetic females through the scenario hypothesized above.

Wootton's (1976) review of stickleback reproductive behaviour sheds further light on the hybridization in Enos Lake; he noted that stickleback nests are capable of holding multiple clutches of eggs at one time. This capability could allow benthic males to obtain matings with benthic females while also hybridizing with limnetic females. Assuming that limnetic males were investing more of their time on repelling crayfish, they would meanwhile likely be fertilizing fewer clutches of eggs overall, and contributing less to the pool of offspring. If this was the case in Enos Lake, this behaviour of multiple clutch rearing helps to explain the pattern of collapse that was ultimately observed. Asymmetric introgression over several generations, exacerbated by the continuation of benthic-benthic breeding and preferential backcrossing between pure benthics and F1 hybrids, could have conceivably resulted in an under-representation of the limnetic genotype, and a gradual increase in the frequency of benthic alleles, effectively swamping out limnetic alleles in Enos Lake. Molecular evidence collected by Gow et al. (2006) and Taylor et al. (2006) agrees that this is likely what took place. The

consistency of these several lines of evidence support the hypothesis that crayfish disruption of male reproductive behaviours could produce the kind of hybrids that are now being observed in Enos Lake (Gow et al. 2006).

Although the results of my reproductive behaviour study supported my hypothesis, the interpretation discussed here is based on several speculative assumptions. There are likely many possible explanations for the patterns observed in my data. The hypotheses tested in this study barely scratch the surface of possible explanations for how crayfish may have influenced introgression in Enos Lake. Crayfish may have facilitated the collapse in various ways, and alternative hypotheses abound. For example, it is possible that benthic juveniles in Enos Lake became less naïve to crayfish in the years following their introduction to the lake, and may have thus developed a fear of crayfish. If so, benthic juveniles could have experienced depressed growth rates when encountering crayfish in the littoral zone, resulting in a reduction in size at maturity (see Chapter 3 for a more detailed description of this hypothesis). With a smaller adult size, limnetic females might have been more likely to mistake a benthic male for a conspecific, given the importance of body size as an assortative mating cue (Boughman et al. 2005), and higher rates of introgressive hybridization could have resulted. In a related hypothesis, competition with crayfish for food could have led to a similar stunting of growth in benthic juveniles, also leading to increased hybridization. Crayfish may also have encouraged hybridization between Enos Lake benthics and limnetics by disrupting their respective breeding habitats – another possible assortative mating cue – or by altering the selective regime in the lake, thus favouring hybrid phenotypes.

In addition to these hypotheses focussing on male sticklebacks, future studies should aim to determine if and how crayfish effects on females may have contributed to increased hybridization in Enos Lake. Female threespine sticklebacks exhibit a wide range of reproductive behaviours as well, and are responsible for making the final decision about whether or not to mate with a male (Wootton 1976; Ridgway and McPhail 1984). Given the role female sticklebacks play in choosing a mate, it is conceivable that the presence of crayfish could alter their behaviour in such a way as to promote increased introgression, perhaps by making them less discriminate.

2.4.2 Juvenile Growth Rate Effects

In the second part of my thesis, I tested the hypothesis that crayfish differentially suppress the growth rates of juvenile benthic and limnetic sticklebacks. Past studies have suggested that prey growth rates can be suppressed in the presence of a predator. In studies by Barreto et al. (2003) and Hawkins et al. (2004), both tilapia (*Tilapia* spp.) and Atlantic salmon (*Salmo salar*) fry have been shown to hyperventilate at the sight of a predator, even though they had never seen the specific predator species before. Even without an actual attack by a predator, a phenomenon called 'prey intimidation' can have strong effects on prey species, including suppressing growth rates in many cases (Slos and Stoks 2008). Some animals may even be threatened by the introduction of a harmless, but novel, object (Sneddon et al. 2003). Knowing that crayfish are potential predators on fish (Guan and Wiles 1998) or, at the very least, should represent a novel

object (as in Sneddon et al. 2003), it is reasonable to hypothesize that crayfish would have exerted an impact on juvenile growth in sticklebacks.

By conducting a controlled growth trial, I demonstrated that the presence of crayfish in captivity did not exert sub-lethal effects on the juvenile growth rates of either limnetic or benthic sticklebacks, at least for the duration of my study. Although there were weak trends of decreased limnetic, and increased benthic, growth rates in the presence of crayfish, this trend was not significant. Ultimately, my results were inconsistent with my hypothesis, suggesting that if crayfish have influenced the collapse of the Enos Lake species pair at all, their influence was not exercised through a reduction in juvenile growth rates.

Studies of growth rate in the presence of predation threats are unpredictable, however, and often yield mixed results. For example, Abrams and Rowe (1996) presented results for two species of frogs that responded in completely opposite ways in the presence of a predator; one species grew more quickly and the other grew more slowly. There are several factors that may have led to the null effect observed in this experiment while, under different conditions, an effect may have been detected. The first involves the known tendency of fish to habituate to a potentially harmful stimulus. Using rainbow trout (*Oncorhynchus mykiss*), Sneddon et al. (2003) demonstrated that fear of a novel object diminished over time, as the fish became accustomed to its presence. Perhaps in my study, the negative crayfish effects occurred, but diminished too quickly to be detected as decreased growth rate. In nature, a greater density of crayfish, and more sporadic exposure of these crayfish to juvenile sticklebacks may decrease the likelihood of habituation.

It is equally possible that juvenile sticklebacks simply did not identify crayfish as a threat in my experiment. Although naïve tilapia and salmon fry in studies conducted by Barreto et al. (2003) and Hawkins et al. (2004) exhibited a fear of the experimentally introduced predators, the species used were known to be natural predators of tilapia and salmon fry, respectively. It is probable, then, that the prey species used in these studies have had sufficient time to evolve an instinctive response to these predators. Crayfish, on the other hand, have never been predators of sticklebacks in Paxton Lake; therefore, the juveniles used in our study likely lack an instinctive response to crayfish. As was alluded to earlier, benthic juveniles in Enos Lake may have lost their naïveté to crayfish over time and, unlike the Paxton Lake fish used in my study that retain this naïveté, the Enos benthic juveniles might have suffered a suppression of growth rate in the presence of crayfish, with important implications for increased hybridization. This alternative hypothesis is discussed in greater detail in chapter 3. Furthermore, crayfish inactivity during the juvenile growth trial may have contributed to a smaller effect than may have been observed in a more natural environment.

Finally, it is possible that a significant crayfish effect was not detected due to a lack of power in my experimental design (Whitlock and Schluter 1999). Power could be improved by increasing sample size and effect size (by using more fish and/or increasing the duration of the experiment, respectively).

Although crayfish did not have any sub-lethal effects on juvenile sticklebacks, there was clear evidence of lethal crayfish effects. The fact that every mortality that took place in my study occurred in the presence of a crayfish is difficult to dismiss, given that tanks were kept clean and levels of harmful waste products were closely monitored.

Guan and Wiles (1998) demonstrated that *P. leniusculus* occasionally prey on fish, and that it was mainly the larger crayfish (> 33 mm carapace length [CL]) that did so. These data are consistent with my results; 80% of the mortalities that occurred were in tanks with crayfish that exceeded 33 mm CL. Anecdotal observations from my study provide further evidence of lethal effects: 1) of the ten mortalities, only two were uneaten by the crayfish and 2) one freshly killed fish was found floating at the surface of the aquarium water, with its head missing. Since equal numbers of benthic and limnetic sticklebacks were included in the mortalities, these facts do not lend support to my initial hypothesis that crayfish disrupt juvenile limnetics more than juvenile benthics. This evidence of lethal crayfish effects, however, certainly raises questions about how crayfish may have influenced the collapse of the Enos Lake species pair. Vamosi (2003) suggested that variation in predation pressure among lakes may explain why some potential species pair lakes contain only solitary populations. He argued that high rates of mortality in a stickleback population can have various effects. For example, mortality may either reduce a population to such a small size that divergent selection is weakened, or it may prevent the utilization of specific habitats. Further research is required to determine if crayfish-induced mortality could have had such an influence in Enos Lake.

2.4.3. Conclusions

Considering the results of my behavioural trials, I conclude that differential crayfish effects on stickleback reproductive behaviour is a plausible mechanism for contributing to the collapse of the Enos Lake species pair by disproportionately disrupting limnetic

male reproductive activity. By contrast, the results of my growth trial suggest that naïve juvenile benthic and limnetic sticklebacks do not suffer any significant or differential suppression of growth rate in the presence of crayfish in captivity, although a weak trend in this direction exists, especially for limnetics. Furthermore, I conclude that both benthic and limnetic juveniles are susceptible to lethal effects of crayfish. It remains unclear, however, whether crayfish intentionally prey on juvenile sticklebacks, or if mortality events may be better explained by other hypotheses. Moreover, it remains unclear how such lethal crayfish effects could have led to the collapse of the Enos Lake species pair. As there are other plausible factors that may have contributed to the collapse, further research is required to gain a complete understanding of this phenomenon.

Having demonstrated that the American signal crayfish is a possible promoter of hybridization between sympatric stickleback species pairs, we are now one step closer to ensuring that the remaining sympatric species pairs are left unperturbed. Some have suggested that eradicating crayfish from Enos Lake may allow the Enos stickleback species pair to re-diverge. It is unclear, however, if enough of the parental limnetic genome is salvageable and it is unclear if the task of ensuring all crayfish are removed is achievable. Recent efforts in Enos Lake removed nearly 7,000 crayfish from the lake over the course of a 28 day period. Mark-recapture data from this effort determined that Enos Lake currently contains more than 100,000 crayfish (Peterson 2008), suggesting that removal of the entire crayfish population may require sustained effort. Freeman et al. (2009) found that similar removal methods had only moderate success in invaded habitats in the United Kingdom, suggesting that such methods may not be practical on their own.

A recent eradication study carried out in Norway tested the use of a pharmaceutical called BETAMAX VET.® on ponds containing *P. leniusculus* (Sandodden and Johnsen 2010). According to Sandodden and Johnsen (2010) the results of this eradication effort appear to be promising, although it is too early to determine if it was successful. Furthermore, as the protocol in the Sandodden and Johnsen (2010) study involved the draining of the pond, this method would probably not be practical in the case of Enos Lake, as it would require the temporary relocation of the sticklebacks, there is a risk of recolonization if crayfish remain in adjacent waterbodies. The results of other work suggests that eradication of crayfish may require a variety of site-dependent approaches, but further research is required to determine a safe and effective strategy (Freeman et al. 2009).

Although a full scale effort to eradicate American signal crayfish from Enos Lake may be impractical, there are many other things that can be done to ensure that similar invasions do not occur elsewhere. Tighter restrictions on the transportation of non-native or potentially invasive species, for example, should be implemented to protect sensitive and rare species. American signal crayfish are of particular concern to those interested in the conservation of the remaining sympatric stickleback species pairs, as the native range of these crayfish is separated from the remaining species pair habitats by only the relatively thin barrier of water imposed by the Strait of Georgia (Lewis 2002). Efforts should also be made to reduce the disruption of stickleback breeding grounds in species pair lakes during the breeding season.

Resource agencies and conservation authorities should be mindful of the fragility of barriers that allow the maintenance of recently evolved species, and realize that no amount of perturbation to fish habitat can be discounted as harmless.

Table 2.1. Summary of experimental treatments carried out in the portion of my thesis focusing on the disruption of threespine stickleback (*Gasterosteus aculeatus*) male reproductive behaviour by American signal crayfish (*Pacifastacus leniusculus*). Observation periods listed here were carried out in sequence on one male stickleback at a time.

Observation Period	Free-roaming subjects	Restrained subjects
Baseline	Stickleback Male	Stickleback Female
Treatment 1	Stickleback Male	Stickleback Female Crayfish
Treatment 2	Stickleback Male Crayfish	Stickleback Female
Treatment 3	Crayfish Stickleback Female	none
Treatment 4	Stickleback Male Stickleback Female	none

Table 2.2. Masses and lengths (mean \pm standard error) of benthic and limnetic juvenile threespine sticklebacks (*Gasterosteus aculeatus*), before and after the implementation of a 90-day growth trial investigating the effect of American signal crayfish (*Pacifastacus leniusculus*) on growth rate ($n_{\text{before}} = 60$ B, 60 L; $n_{\text{after}} = 54$ B, 54 L). Masses and lengths (mean \pm standard error) of crayfish ($n = 6$: 4 male, 2 female) from before and after the trial are also listed.

	Before (mean \pm se)		After (mean \pm se)	
	Mass (g)	Length (mm)	Mass (g)	Length (mm)
Control Limnetic	0.55 \pm 0.03	40.5 \pm 0.77	0.88 \pm 0.05	49.3 \pm 0.83
Control Benthic	0.72 \pm 0.02	42.4 \pm 0.58	1.71 \pm 0.07	56.6 \pm 0.87
Treatment Limnetic	0.53 \pm 0.04	40.4 \pm 0.99	0.81 \pm 0.05	48.3 \pm 0.82
Treatment Benthic	0.72 \pm 0.02	42.2 \pm 0.39	1.71 \pm 0.08	56.4 \pm 0.93
Crayfish	26.3 \pm 5.25	33.0 \pm 2.50	25.9 \pm 5.20	33.3 \pm 2.68

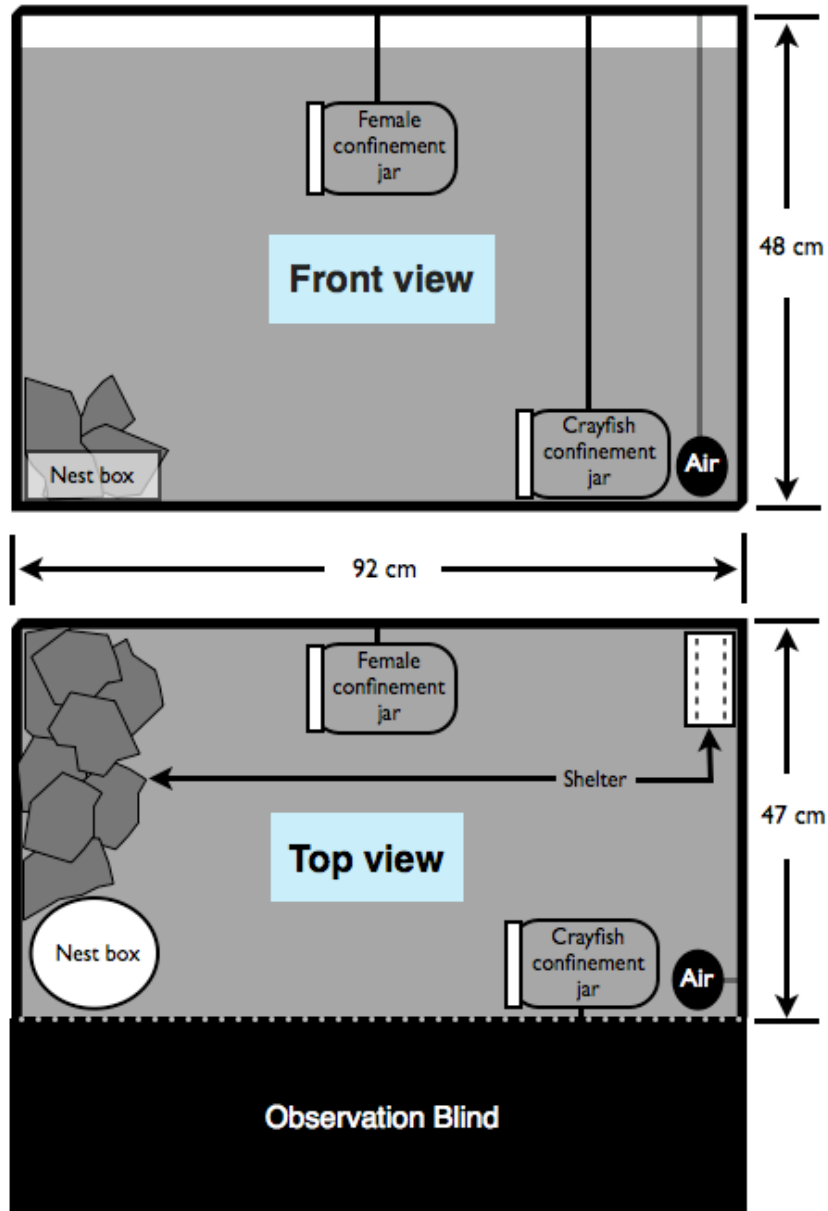


Fig. 2.1. Apparatus set up for threespine stickleback (*Gasterosteus aculeatus*) behavioural trials, indicating the layout of observation arena contents. The top view of the arena shows the orientation of the observation blind where the observer was located during observation periods, allowing one-way viewing of sticklebacks in the arena.

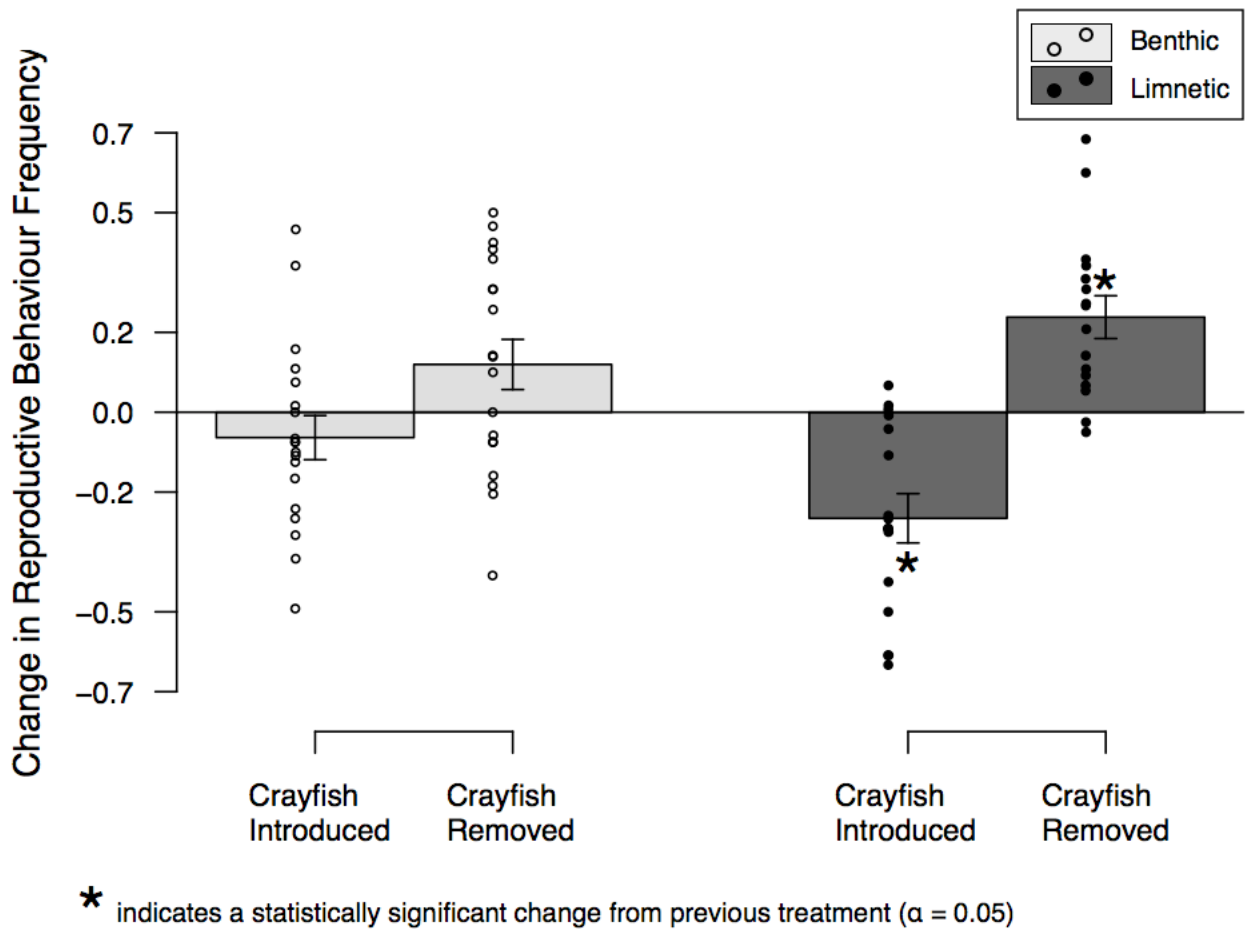
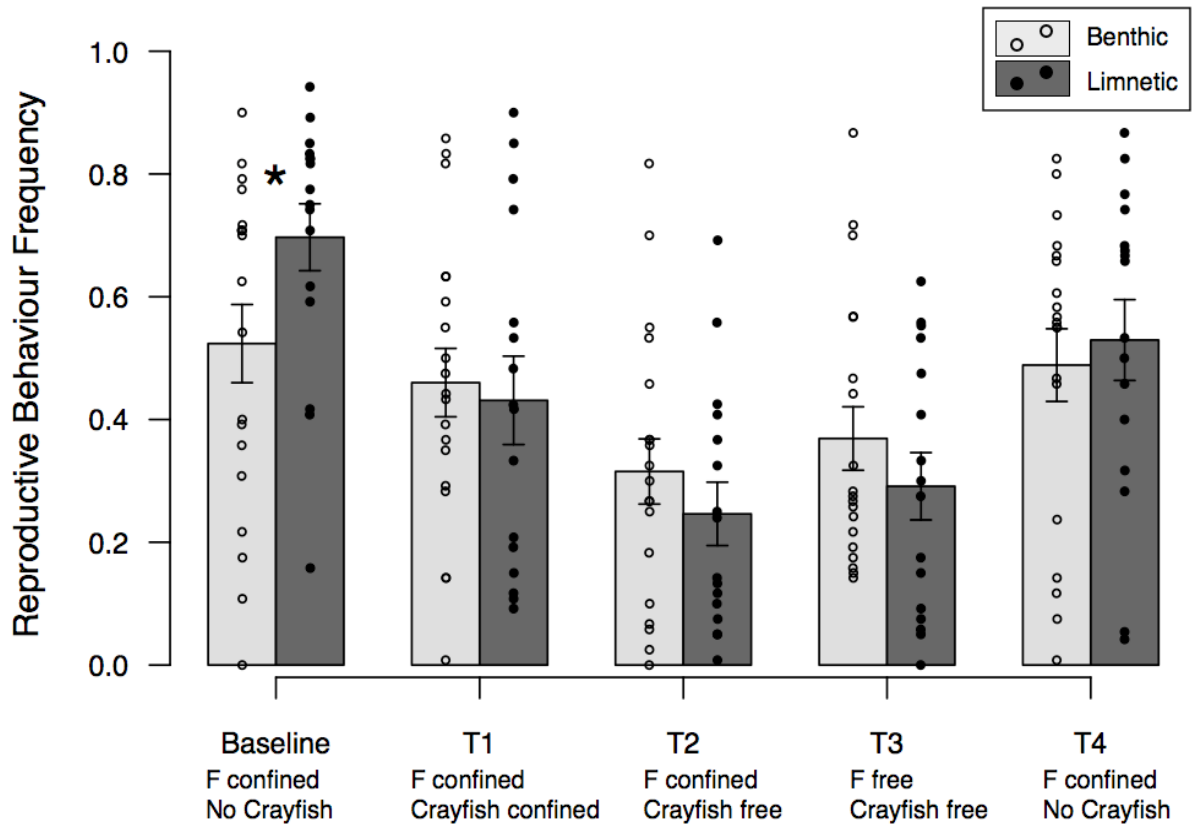


Fig. 2.2. Changes in male threespine stickleback (*Gasterosteus aculeatus*) reproductive behaviour frequencies (mean \pm standard error) upon the initial introduction, and initial removal, of American signal crayfish (*Pacifastacus leniusculus*) from the observational arena. Crayfish introduction means reflect changes in behavioural frequency between Baseline and Treatment 1. Crayfish removal means reflect changes in behavioural frequency between Treatment 3 and Treatment 4.



* indicates statistically significant difference between benthic and limnetic males ($\alpha = 0.05$)

Fig 2.3. Frequencies of male threespine stickleback (*Gasterosteus aculeatus*) stickleback reproductive behaviour (mean \pm standard error) during baseline and treatment observational periods of an experiment testing the effects of American signal crayfish (*Pacifastacus leniusculus*) on stickleback reproductive behaviour.

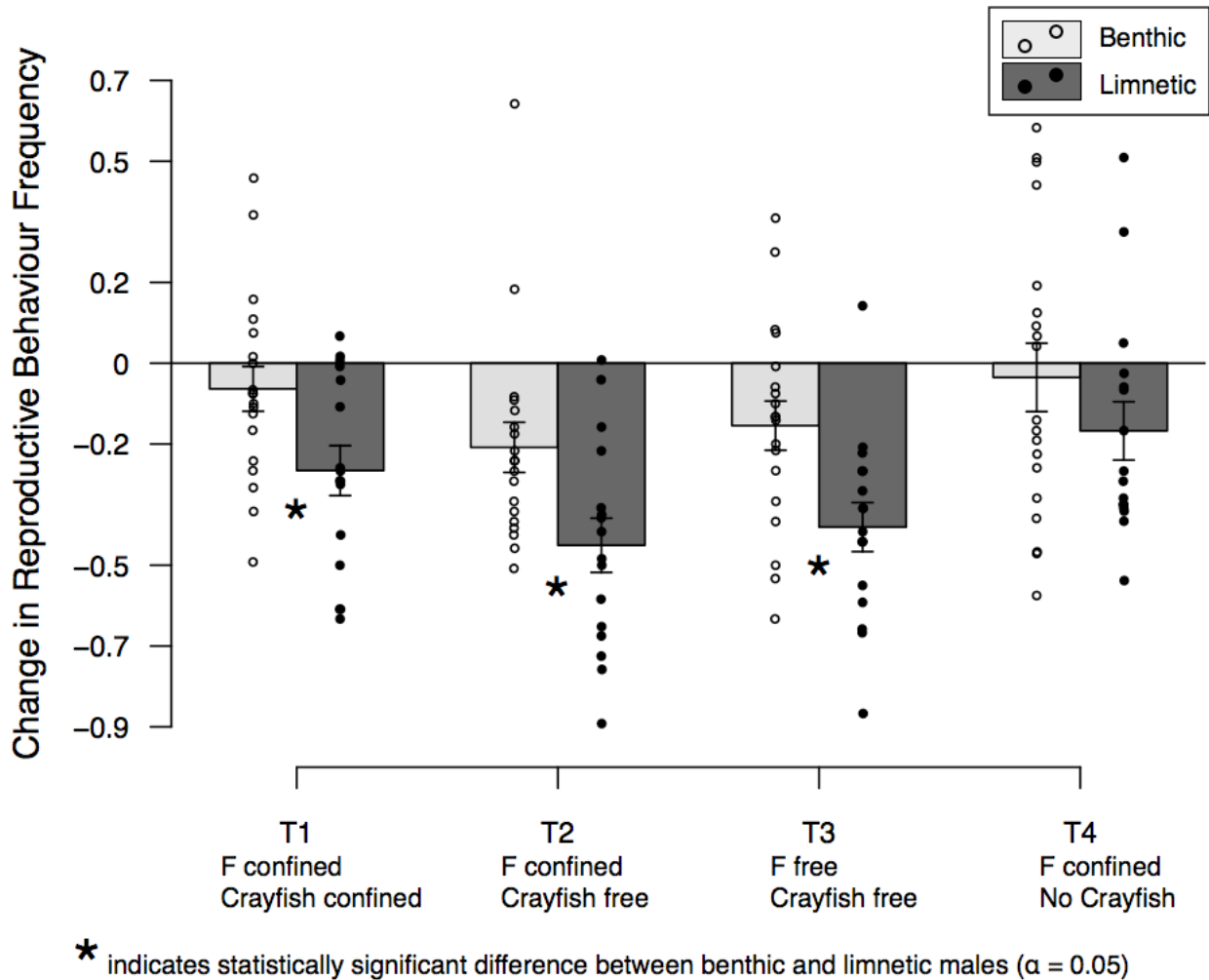


Fig 2.4. Frequencies of male threespine stickleback (*Gasterosteus aculeatus*) reproductive behaviour, relative to initial baseline measures (mean \pm standard error), for four treatment observational periods of an experiment testing the effects of American signal crayfish (*Pacifastacus leniusculus*) on stickleback reproductive behaviour. Negative values indicate a suppression of reproductive behaviour.

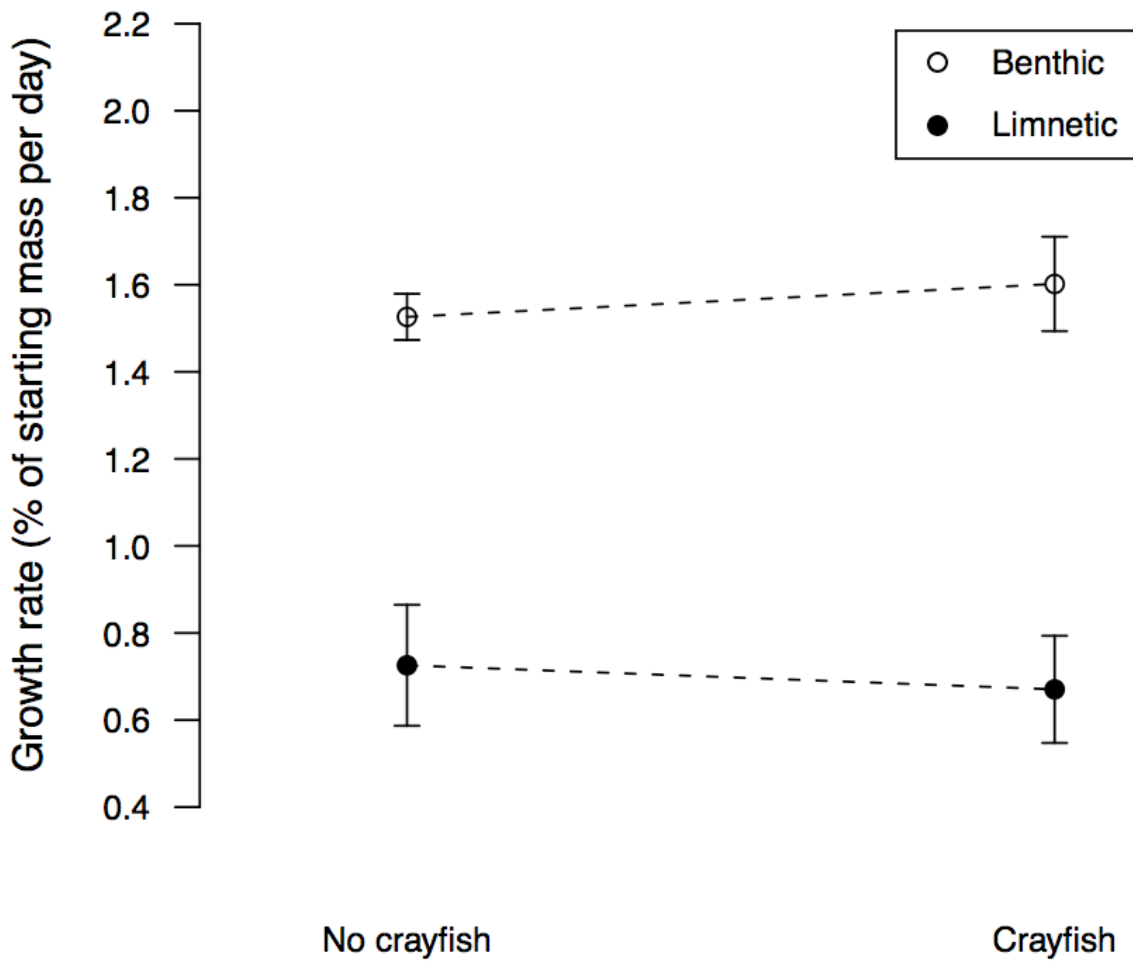


Fig. 2.5. Growth rates (in % of starting mass / day; mean \pm standard error) of juvenile threespine stickleback (*Gasterosteus aculeatus*) in the presence and absence of American signal crayfish (*Pacifastacus leniusculus*). Means and standard errors for each of the four treatment groups were based on tank means.

Chapter 3. General Conclusion

Hypotheses of crayfish-induced hybridization mechanisms tested in my thesis research investigated how the American signal crayfish (*Pacifastacus leniusculus*) may have disrupted threespine stickleback (*Gasterosteus aculeatus*) in Enos Lake both during the mating season and the growing season. One hypothesis, which was supported by the results of a behavioural study, suggested that biased disruption of limnetic male reproductive behaviour by crayfish may have led to a demographic effect, potentially increasing the occurrence of limnetic females depositing their eggs in nests belonging to benthic males. The other hypothesis, which was not supported by the results of my growth experiment, suggested that juvenile sticklebacks might suffer a suppression of growth rate, perhaps affecting the two species differentially, due to intimidation by crayfish. As reasonable as these hypotheses may have been, they are likely not the only valid hypotheses that may help explain the collapse of the Enos Lake species pair. In fact, many alternative mechanisms have been hypothesized in recent years as potential explanations for this phenomenon, and it is possible that it was a combination of these that contributed to this loss of biodiversity. The majority of the alternative hypotheses discussed below focus on additional ways in which crayfish impacts may have contributed to a disruption of stickleback breeding interactions; however, some other, more direct, anthropogenic impacts are also discussed.

3.1. Alternative Hypotheses

3.1.1. Alternative Crayfish Impacts

As demonstrated in the current study, juvenile benthic and limnetic stickleback, raised in captivity in identical aquaria, suffered equal levels of mortality in the presence of crayfish. During the growing season in the wild, however, limnetic and benthic juveniles are known to occupy different areas of the lake: limnetic juveniles feed on plankton in the pelagic zone while benthics feed on invertebrates in the benthic and littoral zones (Bentzen et al. 1984). In nature, benthic juveniles are thus more likely to encounter crayfish as juveniles, and may thus suffer greater losses than limnetics, despite their equal levels of susceptibility. After spending several generations in the presence of crayfish, it is possible that Enos Lake benthic sticklebacks lost their naïveté and developed a stronger response to crayfish than limnetics. Based on this assumption, it can be hypothesized that these juvenile benthic sticklebacks would have suffered a suppression of growth rate when encountering the increasingly common crayfish in the littoral zone. Meanwhile, juvenile limnetics, feeding unexposed to crayfish in the open water, would not have been subject to the same interactions with crayfish. Growth-suppressed benthics may have reached maturity at a smaller size, similar to that of a mature limnetic male. Since body size is one of the cues that female sticklebacks use for assortative mating (Nagel and Schluter 1998), this removal of size differences could conceivably have promoted interbreeding between benthic males and limnetic females.

It is also possible that crayfish may have suppressed the growth rates of benthic juveniles through competition for food sources. Benthic sticklebacks and crayfish both prey on benthic invertebrates in the littoral zone, but crayfish are known to be strong competitors (Carpenter 2005; Rosenthal et al. 2006). Assuming crayfish reduce the abundance of benthic invertebrates in the littoral zone, it is possible that benthic sticklebacks may mature at a smaller size, potentially leading to increased hybridization, as described in the previous hypothesis.

A third alternative hypothesis is that *P. leniusculus* has imposed disruptive effects on the Enos Lake species pair by altering the composition of breeding habitat. Crayfish, in general, are known to be voracious omnivores and strong competitors in their invaded ranges, and have a reputation for out-competing native species, while reducing the richness of prey species, including aquatic plants (Guan and Wiles 1998; Stenroth and Nystrom 2003; Carpenter 2005; Dorn and Mittlebach 2005; Rosenthal et al. 2006; Ilhéu et al. 2007). It is well known that benthic and limnetic males have contrasting preferences for nesting habitat, with benthics preferring sheltered, densely vegetated areas, and limnetics preferring open areas (Ridgway and McPhail 1984). If aquatic vegetation plays an important role in the segregation of benthic and limnetic nests in the littoral zone, the removal of this vegetation by crayfish may promote hybridization. Rosenfeld et al. (2008) demonstrated that the diversity and density of aquatic vegetation in Enos Lake was significantly decreased in crayfish enclosures, relative to crayfish exclosures. It is plausible that such an effect may have contributed to the disruption of assortative mating in Enos Lake.

Finally, a fourth alternative hypothesis involving crayfish impacts suggests that *P. leniusculus* may have effectively reshaped the ecosystem in Enos Lake, thus changing the balance of selection amongst the stickleback residents such that it now favours hybrids. It is known that species are, through the maintenance of ecological preferences, capable of shaping their own ecosystems, which in turn can have evolutionary implications for the species (Bassar et al. 2010). Given that stickleback species pairs are highly specialized to life in their divergent habitats, perturbations to these ecological conditions can have consequences for inherently unstable groups such as sympatric species pairs (Behm 2009). Crayfish are known to be highly disruptive to their invaded habitats (Guan and Wiles 1998) including Enos Lake (Rosenfeld et al. 2008). Consequently, it is not unreasonable to suspect that they too are capable of shaping the ecology of their surroundings. Based on mark-recapture data, American signal crayfish have an estimated population size in Enos Lake in excess of 100,000 individuals (Peterson 2008) and crayfish are known to be capable of altering ecological variables, such as food sources, in the littoral zone of the lake (Rosenfeld et al. 2008). Changes in divergent ecological resources can dramatically alter adaptive landscapes, removing the fitness advantage of resource specialization by benthic and limnetic sticklebacks, and the fitness disadvantage that presumably selects against hybrid offspring (Gow et al. 2007; Behm et al. 2010). The apparent generalist nature of Enos Lake stickleback hybrids would have given them a competitive advantage in a reshaped Enos Lake lacking divergent trophic resource axes. Behm et al. (2010) demonstrated that stickleback hybrids in Enos Lake are, in fact, no longer being selected against, although the reasons underlying this change remain unclear. This hypothesis suggests

a possible explanation for a breakdown in post-zygotic isolation mechanisms in Enos Lake; a lack of ecological selection against hybrid offspring.

3.1.2. Alternative Anthropogenic Impacts

Anthropogenic impacts may also have influenced the collapse of the Enos Lake species pair. The Great Lakes of eastern Africa are home to one of the most iconic explosions of speciation in the world: until recently, Lake Victoria was home to at least 500 species of haplochromine cichlids (Seehausen et al. 1997). As with sympatric species pairs, these cichlids have diverged from each other to fill empty niches in their habitats, but this divergence is maintained through mate preferences for specific colouration (Seehausen et al. 1997). Seehausen et al. (1997) discovered that recent declines in diversity of cichlid species in Lake Victoria, due to increased introgression between species, has been caused by reduced clarity of the lake's water which has made colour-based mate recognition difficult. Anthropogenic influences such as agriculture and deforestation have been blamed for this disruption of the cichlid assortative mating system (Seehausen et al. 1997).

Like cichlids, assortative mating in threespine stickleback species pairs is partly maintained through the use of visual signaling, and the preferences of stickleback females are finely tuned to the frequencies of colour displayed by conspecific males (Boughman 2001). Changes in the clarity of Enos Lake water, either through increased turbidity or eutrophication, could conceivably lead to decreased efficacy of visual signaling in these fish. No evidence exists, however, to suggest that there have been any changes to the clarity of the water in Enos Lake in the past few decades. Also, the

importance of nuptial colouration as a contribution to assortative mating is considered to be relatively weak (Boughman et al. 2005) so whether Enos Lake has suffered the same fate as Lake Victoria is uncertain.

Eutrophication, however, is not the only anthropogenic influence that could have potentially led to the Enos Lake collapse. It is more likely that development in the surrounding community of Nanoose Bay, which has increased in recent decades, may have altered the ecology of Enos Lake in other ways that may have promoted hybridization. Water level fluctuations in Enos Lake in the last 60 years due to increased water use by local businesses and damming activities in the Enos Lake may have had adverse effects on the ecology of the lake (COSEWIC 2002). Damming of the lake increased the water level, and may have led to influxes of dissolved organic carbon (D.O.C.) from the surrounding landscape. D.O.C. can potentially have similar effects on water clarity as those described for eutrophication. Water level fluctuations can also potentially affect niche availability and/or egg clutch survival: Enos Lake has a large, shallow littoral zone, where stickleback males prefer to nest, although benthic and limnetic males utilize slightly different parts of this habitat (COSEWIC 2002; Ridgway and McPhail 1984). If Enos Lake water levels in the 1980s fell by a sufficient amount, some of the potential breeding sites would have no longer been submerged, and would thus have been eliminated. Any limnetic males tending to clutches of eggs in shallow areas during a rapid draw-down of lake water might be forced to abandon their nests, thus resulting in decreased limnetic reproductive success during that season. Furthermore, such a drop in water level may have caused limnetic males to nest closer to benthic males, thus increasing the likelihood of introgression (COSEWIC 2002).

3.2. Summary

The research carried out in my thesis represents a first attempt at testing hypotheses of how crayfish may have led to the collapse of the Enos Lake stickleback species pair and perhaps more questions have been raised than answered. A great deal of further research will be required to determine the exact causes of this collapse. Unfortunately, very few ecological data exist from studies completed in Enos Lake prior to the species pair collapse. Aerial photography has been inconclusive in determining prior aquatic plant densities (R. Peterson, Nanoose Naturalists, pers. comm.) and much of the impression that we have of what Enos Lake looked like before the 1980s has been gathered from anecdotal observations. This lack of pre-impact data makes it difficult to infer what changes in Enos Lake may have caused such a rapid collapse of the species pair. One of the only facts we are certain of is the coincidence of timing between the crayfish introduction and stickleback collapse. It seems clear that studies of crayfish effects are of ultimate importance in understanding what happened in Enos Lake.

Invasive species are known to threaten the persistence of native species in their invaded habitats through factors such as predation (Kupferberg 1997), competition (Whitfield et al. 2007), and the spread of disease (Miller and Vincent 2008). According to Dextrase and Mandrak (2006) introductions of invasive species to aquatic environments is one of the greatest threats to freshwater species at risk in Canada. As a result of the research carried out in this study, we now have evidence in favour of plausible processes by which crayfish could have played an important part in the collapse of the Enos Lake species pair through disruption of pre-zygotic isolation. The results of my

thesis provide further justification for crayfish removal efforts in invaded habitats. Given the ineffectiveness of crayfish eradication efforts once established, however, conservation priorities should perhaps focus on preventing the transportation of crayfish between water bodies through better public education and increased monitoring. My results also indicate that sympatric stickleback species pairs may be most vulnerable to disturbance by crayfish during the breeding season, suggesting that crayfish removal efforts in Enos Lake, if continued, should be carried out in the late winter, thus temporarily relaxing the disruptive pressure of crayfish when breeding is taking place.

Overall, my research highlights the sensitivity of recently formed species to perturbation by invasive species. New species are immensely valuable for the advancement of our understanding of evolutionary biology, and thus must be protected. Through research programs such as this one, restoration and conservation efforts may be more efficiently focussed such that our goal of the conserving biodiversity in Canada and worldwide can be achieved.

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Appendix A



THE UNIVERSITY OF BRITISH COLUMBIA

ANIMAL CARE CERTIFICATE

Application Number: A07-0378

Investigator or Course Director: [Eric B. Taylor](#)

Department: Zoology

Animals:

Sticklebacks 600

Start Date: June 1, 2007

Approval Date: October 2, 2007

Funding Sources:

Funding Agency: British Columbia Ministry of Environment
Funding Title: Assessing critical habitat and causes of hybridization in stickleback species pairs

Unfunded title: N/A

The Animal Care Committee has examined and approved the use of animals for the above experimental project.

This certificate is valid for one year from the above start or approval date (whichever is later) provided there is no change in the experimental procedures. Annual review is required by the CCAC and some granting agencies.

A copy of this certificate must be displayed in your animal facility.

Office of Research Services and Administration
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