BIOLOGICAL INVASIONS AS FORTUITOUS EXPERIMENTS IN NATURE: ECOLOGY, EVOLUTION, AND PHENOTYPIC PLASTICITY OF NON-NATIVE BROWN TROUT (*Salmo trutta*) IN NEWFOUNDLAND, CANADA

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Biological invasions as fortuitous experiments in nature: Ecology, evolution, and phenotypic plasticity of non-native brown trout (*Salmo trutta*) in Newfoundland, Canada

By

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For Finn Michael who loves eeeshh

Abstract

Biological invasions represent under-utilized research opportunities to gain insight into fundamental evolutionary and ecological questions. I focused on the invasion of brown trout to Newfoundland, Canada, as a case study and conducted meta-analyses of published literature, field sampling, laboratory, common-garden, and reciprocal transplant experiments to understand what can be learned by embracing the fortuitous research opportunities afford by this invasion.

In the first chapter I conducted a meta-analysis of published rates of phenotypic change to assess the contribution of invasive versus native species in revealing the rate and form of phenotypic change in wild populations. I found that invasive species have disproportionately contributed to published rates of phenotypic change, but most of these estimated rates are based on extensive studies in a few species. Results in Chapter One suggest that invasive and native species both exhibit evidence of abrupt phenotypic change and suggest an important role of the environment in driving trait change in wild populations.

In Chapter Two I examined the dynamics of the brown trout invasion in Newfoundland by assembling a presence-absence database to investigate the physical environmental correlates associated with population establishment at the watershed-scale. I found that relatively large and productive watersheds are more likely to be successfully established, but that all watersheds in Newfoundland are susceptible to invasion and population establishment.

In Chapter Three, I quantified among-population differences in a suite of phenotypic traits (e.g. growth rates, body shape and size, colour patterns) and correlated this diversity

with environmental features. On the whole, phenotypic variation was predictable given habitat use, suggesting either phenotypic plasticity or adaptive evolution in maintaining this association.

In the final chapter, I assessed the contribution of genetics and environmental effects on the population differentiation detected in Chapter Three, along with the associated fitness consequences of these phenotypic differences. With a combination of common-garden and reciprocal transplant experiments, I quantified the role of plasticity in facilitating survival in novel environments and revealed patterns not predicted by theory. Specifically, results suggested that plasticity in functional morphology – while common – did not occur in the direction favoured by natural selection.

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Introduction and Overview

Present day distributions of organisms on earth represent a temporal snapshot of the dynamic interplay between local extirpation and colonization. Species are regulated in time and space through a host of biotic and abiotic factors including the intrinsic capacity to disperse. Patterns of species dispersal have wide-reaching impacts and, among other things, influences metapopulation and metacommunity dynamics, species interactions, population viability, and biogeography (reviewed by Bullock et al., 2001). Humans, intentionally and unintentionally, have bridged the barriers to dispersal and greatly facilitated the spread of organisms around the globe. For example, in Hawaii the estimated rate of species introductions has increased from ~1 species every 100,000 years prior to Polynesian settlement, to ~1 every 20 years following post-European settlement (Lockwood et al., 2007).

The majority of introduction events fail to establish self-sustaining populations, though the rates of failure and successful invasions are difficult to quantify and subject to error. Williamson (1996) estimated that only 10% of all introduced species succeed in establishing viable populations, and that only 10% of those survivors will become invasive (i.e., spread and compete with native species). Caution is warranted; however, as this so called 'tens-rule' does not apply consistently among taxa. The 'tens-rule' may generally categorize rates of invasion success and failure in plants, and indeed it was formulated with data on plants, but it seems to miss the mark in vertebrate species. Jeschke & Strayer (2005) provided a compelling argument that vertebrates, and especially birds and fishes, have a high probability of successfully establishing populations upon introduction (often > 70%).

Regardless of the precise rates, vast amounts of time and money are spent responding to the effects of species invasions, and the economic losses to commerce are correspondingly high. In the United States, for example, cumulative costs of 'harmful non-native invaders' were estimated to exceed $100 billion per annum (Knowler & Barbier, 2000). Furthermore, invasive species are associated with declining ecosystem 'health' in many areas (Hassan et al., 2005) and are often the primary cause of native species declines or extinction, especially on islands (e.g. Fritts & Rodda, 1998).

However, in this adversity rests opportunity. Multiple introduction and colonization events represent serendipitous "natural experiments" from which ecological and evolutionary questions can be investigated. Among these questions are: What is the rate and form of natural selection in nature? What factors (e.g., evolutionary history of the invaders, physical habitat conditions of the new environment, and the structure of the biological community of the new environment) determine invasion successes and failures? Are adaptations of exotic species and affected native species predictable or chaotic? Do reduced genetic variability and population bottlenecks limit the capacity for adaptation to novel conditions? How does adaptive phenotypic plasticity influence the evolutionary trajectory of populations?

In this introductory chapter of my thesis I review: 1) my derivation and definition of 'invasive', 2) a brief history of non-native invasive species as natural experiments, 3) the historical roots of the brown trout invasion to Newfoundland set within the context of the time period, 4) phenotypic plasticity, the 'Baldwin effect' and their importance to invasion, and 5) conclude with a brief overview and synopsis of each manuscript (chapters 1-4). While reading this thesis you may note a change in pronouns (from 'I' to 'We') between chapters 1,

and the remainder of chapters. This was intentional and correctly represents the intellectual contribution to the work; either it was entirely my own, or a true collaboration with others. For a complete explanation of authorship and citations of publications that have already resulted and are intended to result, see the included co-authorship statement.

Invasive species defined

Before proceeding too far, it is wise to clarify my usage of the term 'invasive non-native' species. Adventives, alien, established, exotic, naturalized, noxious, pest, waif, and weed are just a few of the terms that researchers have affixed to their description of non-native species. In a recent attempt to review the subject and provide an all-encompassing definition, Valery et al. (2008) failed to provide additional clarity and posited a cumbersome definition with enough necessary stipulations to ensure a highly conservative classification of invasive (Table 1). I tabulated definitions from some of the more often cited works on invasive species and conclude that the most apparent similarities among definitions of invasive species included: 1) species occur outside their native or natural range, 2) humans played a role in them getting there, 3) the organisms acquire or maintain a competitive advantage over native species, and 4) their actions result in economic and/or ecological damage.

Table1. Tabulated definitions of 'invasive' species

Reference	Definition of Invasive
Elton (1959)	No formal definition given: You know an alien species when you see one
Chung Kim and McPheron (1993)	Wide reaching definition of 'pest': Any organism with a destructive troublesome tendencies
Williamson (1996)	Invasion occurs when an organism, any sort of organism, arrives somewhere beyond its previous range
Mooney and Hobbs (2000)	Invoke invasive in their definition of invasive species: Alien species that not only take hold in their new foreign habitat but also become aggressive or invasive
Booth et al. (2003)	Definition of weed: A native or introduced (alien) species that has a perceived negative ecological or economic effect on agricultural or natural systems
Cox (2004)	No formal definition given: Apparently used to characterize organisms dispersed by humans which have some level of ecological impact and includes organisms who colonize areas on their own volition following human mediated disperal
Sax et al. (2005)	Non-native, exotic, alien, and introduced used interchangeably to refer to those species that are not indigenous to a region in question and reserve the use of the term 'invasive' to mean species that cause ecological or economic damage
Lockwood et al. (2007)	Adopt a neuteral terminology: use 'non-native' to describe species that were moved outside their normal geographic ranges via human actions regardless of their impact on native ecosystems Use the term 'invasive' to describe species that have a demonstratable ecological or economic impact, but admit to occasionally interchanging terms if nothing else to avoid redundancy
Sax et al. (2007)	Adopt a general definition: a species that hs been introduced or has otherwise been established there because of human activities
Valery et al. (2008)	A biological invasion consists of a species' acquiring a competitive advantage following the disappearance of natural obstacles to its proliferation which allows it to spread rapidly and to conquer novel areas within recipient ecosystems in which it becomes a dominant population
Westley (2009; present review)	Here I use the term invasive and non-native interchangably and apply the term only to species or populations of species that have spread outside their natural range either directly or indirectly via human mediated dispersal Thus, for example, I consider brown trout in Newfoundland invasive (introduced source) even though they are spreading on their own volition. However, I do not consider brown trout colonizing recently deglaciated rivers in Iceland 'invasive' as the founders come from a natural source

In this thesis, I use the term 'invasive' for species that have spread outside their natural geographic range via direct or indirect human mediated dispersal and that have established self-sustaining populations. These species may or may not acquire competitive dominance over native species and need not result in ecological or economic damage to qualify. Direct human mediated dispersal is used synonymously here with intentional or unintentional introductions. Examples of intentional introductions include the transport of species for biological control (e.g., *Gambusia affinis* for mosquito control) and unintentional introductions include transport of organisms unknowingly (e.g., plant invasions initiated through soil ship ballast, see Mack (2003) for a review). Indirect human mediated dispersal is the spread of a non-native species on its own accord following establishment from direct human dispersal (e.g., the spread of introduced rabbits in Australia following release of 24 individuals; Williams and Moore (1989)).

How should managers tasked with species conservation and ecosystem functioning view invasive species? This is increasingly difficult in a world where the divides between native and non-native invasive are blurred by time and societal perceptions. Indeed, in a recent paper Davis et al. (2011) argued that species should be judged on their ecological roles in new ecosystems rather than their origins. For the remainder of the thesis I set aside the important question of how to deal with the clear and present threat of biological invasions and focus on what can be learned about ecology and evolution from invasive species.

A brief history of non-native invasive species as experiments in nature

Capitalizing on the introduction and spread of invasive species as experiments is not a novel concept. The historical legacy of invasive species in ecological and evolutionary

inquiry began simply with opportunistic observations and developed through time into elaborate experiments designed to explicitly examine factors surrounding invasion. Following a unusually severe storm in the winter of 1898 Herman Bumpus, professor at Brown University in Rhode Island, noticed 'scattered about the ground dead or exhausted, a large number of English sparrows (*Passer domesticus*) from the colony wintering in the vines of the old athenaeum'(Bumpus, 1899). In a biography written by his son, the elder Bumpus was 'quick to see that here before his eyes was an experiment in nature' (Bumpus, 1947). He collected 136 birds and examined and compared traits among those that lived and those that died. Bumpus demonstrated what is now referred to as stabilizing selection; individuals close to the population mean survived at a higher rate than individuals at the extremes of the distribution (Bumpus, 1899), but also revealed that selection can act differently on correlated traits. Bumpus' paper in 1899 has become a classic and the resulting dataset has been the subject of extensive reanalyses in the primary literature (for example see Janzen & Stern, 1998). Perhaps most importantly, this fortuitous natural experiment with introduced sparrows represents the first demonstration of the capacity to observe and quantify natural selection in nature.

In addition to being the first models for investigating natural selection, introduced house [aka English] sparrows were among the first species to be examined for rapid population divergence. In the summer of 1917 Joseph Grinnell joined colleagues from the Museum of Vertebrate Zoology of the University of California to 'collect' specimens in the Inyo region of south-eastern California. To his surprise he encountered house sparrows, which were likely colonizing descendants of individuals introduced to New York City during the period 1860-1864 and those examined by Herman Bumpus in the winter of 1899. He,

like Bumpus, argued that this was a natural experiment to examine, in real time, the divergence of populations. However, no obvious differences were discernible in the new colonists (Grinnell, 1919). Grinnell presumed this lack of differentiation resulted from insufficient time since colonization of the new habitat. This explanation, as it turns out, was likely correct: approximately 50 years later Johnston and Selander (1964) expanded on Grinnell's ground work and reported the rapid evolution of 'races' of introduced house sparrows throughout North America.

Herman Bumpus and Joseph Grinnell, though pioneers in the use of invasive species to examine ecological and evolutionary questions, were precursors to the development of invasion biology as a defined scientific discipline. What Rachel Carson was to the ecological effects of pesticides, Charles Elton was to the ecology of animal and plant invasions. With astounding foresight, Elton (1958) articulated the primary questions that still largely constitute the base of current invasion biology. Among these questions were: what characteristics make some species invasive and others not? What causes the lag in time between introduction and population explosion? What characteristics of the ecosystem facilitate or resist invasion? Many of his hypotheses, such as native species diversity as a repellent force to invasion, have been embraced by many contemporary invasion biologists (Lockwood et al. 2007 and references therein), but some of his other insights have proven incorrect (or simplistic). For example, Elton observed that environments with higher degrees of anthropogenic disturbance were more likely invaded than more pristine areas. However, this led him to erroneously surmise that the disturbance *per se* was the important causal mechanism. Current research suggests that disturbance is a correlate of other more important large scale drivers such as propagule pressure[7] (Lockwood et al., 2005).

Regardless, Charles Elton's pioneering work on invasive species marks the emergence of invasive biology as a defined discipline.

Enter the contemporary invasion biologists. A dizzying amount of literature on invasion ecology and biology has been written in the past decades (reviewed by Lockwood et al. 2007), and much of it has focused on the control and spread of perceived 'pest' and 'weed' species of plants and animals (Myers & Bazely, 2003, Coombs et al., 2004, Williamson, 1996). An emergent subset of researchers has capitalized on the opportunity to use invasive species as convenient model organisms to examine ecological and evolutionary questions. Recent reviews of ecological and evolutionary insights gained from studying invasive species (Sax et al., 2005, Cox, 2004, Sax et al., 2007) provided a convenient launching point for the following thesis.

Brown trout in Newfoundland: historical roots of a serendipitous research programme

The establishment of brown trout in Newfoundland is a small sentence in an epic global story of salmon and trout (family Salmonidae) introductions. Efforts to introduce these fish around the globe were daunting. Present day ecological consequences of these introductions notwithstanding (McDowall, 2006), the effort to introduce salmon and trout can be understood as passionate attempts to create the comforts of familiarity by homesick expatriates. Indeed, Wilson's (1879) chronicle *Salmon at the Antipodes* described his attempts to bring Atlantic salmon and brown trout to Australia as a 'labour of love.' In a similar chronicle, Maitland meticulously describes the process of construction and operation of Howietoun Fishery, among the first commercial salmon and trout hatcheries in Europe. For

example, Maitland describes the processes of brood stock collection and spawning, and describes (below) packaging of fertilized ova in containers of ice-moistened-moss for shipments around the globe (Fig. 1.):

"Each tray has four holes cut in the sides to admit air freely to the moss and to facilitate adjusting between the fillets. A large ice-tray rests on the top of the ova trays and is bevelled outwards so as to entirely close the inside of the outer box, the lid of which is merely fastened by a wooden pin passing through a stable, so that crushed ice may be easily supplied as described. A cleverly designed drain is fitted in the bottom of the box to carry off the melted ice. In one of these boxes ova can be safely transported during a period of sixty days."

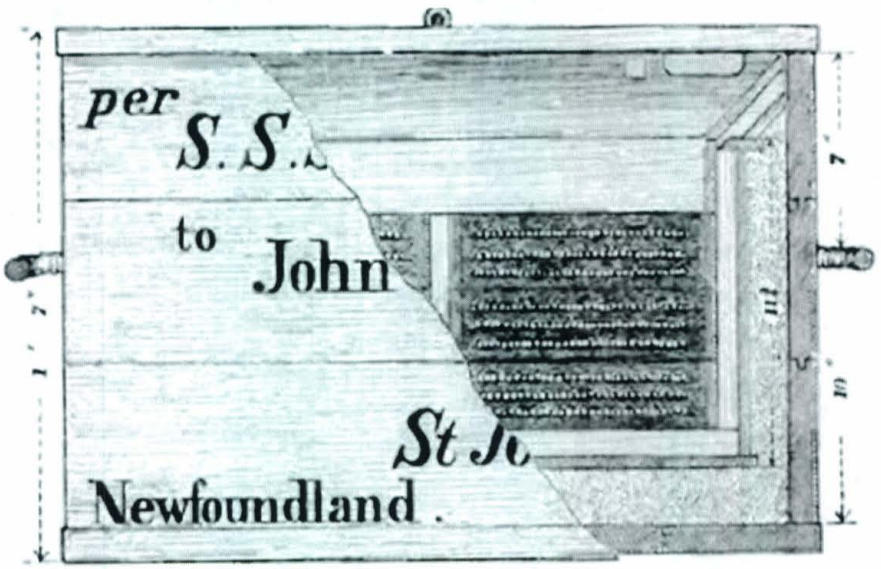

Fig. 1. Wooden shipping container used to send fertilized ova from the Howietoun Hatchery to locations around the globe (described in the quote above). This image from page 42 in Maitland (1887) was drawn to convey the scale and layout of this custom made containers, but by coincidence, shows the container destined to John Martin in St. John's, Newfoundland. Maitland likely chose Newfoundland as an example, at least in part, to boast about his success sending brown trout overseas but currently serves to highlight Newfoundland's place in a larger global story.

In addition to wanting the familiar sport fish of home, brown trout were also likely imported because St. John's – a city that by the end of the 19th century had been settled for approximately 400 years – was apparently suffering from the effects of over-population, pollution, and overfishing of native Atlantic salmon and brook trout. Evidence for this comes in a letter written in 1885 to the local St. John's newspaper by a citizen in favour of government support for stocking of brown trout (as cited by Hustins 2007):

"The angler might thrash the same waters for a week now and not kill a good fish. The same observations apply in a somewhat lesser degree to the Petty Harbor Ponds and to all the ponds within a radius of a dozen miles of St. John's. These ponds are fished out."

Regardless of precise motives, the first definitive evidence of brown trout importation to Newfoundland was in 1883 at the request of John Martin, a civil engineer with the Water Works of the capital city of St. John's and president of the Newfoundland Game Fish Protection Society (Hustins 2007). This precedes the frequently cited 1886 date provided by Andrews (1965), but supports the first date of importation reported by others (Frost 1940; Scott and Crossman 1964; van Zyll de Jong et al. 2004). Preserved correspondence by John Martin in Maitland (1887) indicates that early introductions of Loch Leven (a Scottish lake near Edinburgh) strain brown trout were highly successful:

ST. JOHN'S, JUNE 8TH,1886

MY DEAR SIR,– I am glad to say the Lochleven trout ova has done well - in fact, I may say, it was a perfect success, not five percent of loss on the whole lot. In fact, all the ova I got from you was the same - no loss worth speaking of. The first I got is three years old now, and fine fish. I think they spawn this year, as they are the size of herring now, and very fat. The water supply for my new hatchery is first-class, and plenty of it, so that is the main thing. I hatched 900,000 last winter, and all did well with me.– Yours truly,

J. Martin

This correspondence is especially illuminating for two reasons. First, it provides evidence that indeed the first importation was of Loch Leven brown trout in 1883 (his first fish were three years old and the letter dated 1886). Second, it conveys the scale of the hatchery operation that John Martin had operating at Long Pond, the headwaters of the Rennie's River/Quidi Vidi watershed. Unfortunately the records of the numbers of trout produced and stocked to local waters are, at best, incomplete.

In addition to Loch Leven brown trout, two other strains, so called 'German' and 'English', were also introduced to Newfoundland. However, the precise origins of these latter strains of trout are not entirely clear. It is likely that the German von Behr strain originated in rivers near Hamburg and was used for importation to other European watersheds, North America, and Chile (Smiley, 1884, Frost, 1940), whereas the English strain may have been propagated from brood stock originating from chalk streams in the English Midlands or conceivably from mixed strains originating from Germany or Denmark (A. Ferguson, Queen's University of Belfast, personal communication, January 2008). The second chapter of this thesis, summarizes the available information regarding stocking numbers and locations and concluded that at least 156,000 hatchery raised brown trout were introduced to 16 watersheds. Moreover, the results suggest that over 90% of the stocked fish were Scottish Loch Leven descendants.

Since their first introduction in 1883, brown trout populations have volitionally spread throughout eastern Newfoundland. Humans may have accelerated or facilitated the patterns of population expansion through stocking, but most watersheds have probably been successfully colonized by straying anadromous trout. In the second chapter we go into more detail, but suffice it to say here that similar patterns of straying and colonization are observed

in other systems. For example, a contemporary brown trout invasion is occurring in the sub-Antarctic Kerguelen Islands (Davaine, 1997, Launey et al., 2010), where straying fish have rapidly spread to many watersheds after first introduction. Ultimately, the route of introduction – either natural straying or human stocking – is inconsequential for understanding how the environment shapes the phenotypes of the surviving colonizers and the role of phenotypic plasticity in facilitating survival in novel conditions.

The Baldwin effect, adaptive phenotypic plasticity, and the successful colonization of novel environments

Plasticity describes the ability of organisms to respond to environmental conditions by modifying their phenotype within their life time. This formal concept was first popularized in plants by Bradshaw (1965) and modernized by researchers such as Sonia Sultan (e.g. Sultan, 1987), Massimo Pigliuccci (e.g. Pigliucci, 2001), and Trevor Price (e.g. Price et al., 2003). Here I adopt a wide reaching definition of phenotype and consider any quantifiable trait in an organism's morphology, physiology, behaviour, or life history to be part of the phenotype. The essentially infinite number of potential phenotypes that an individual can express across environmental gradients has led some to suggest the utility of thinking about the observed phenotype as just one possibility in an individual's 'phenome' (e.g., West-Eberhard 2003). Phenotypic plasticity is ubiquitous in nature; however, not all plasticity is adaptive (i.e., plasticity that allows individuals to have higher fitness than it would were it not plastic; sensu Ghalambor 2007). Phenotypic plasticity has attracted recent attention and controversy with regards to its role in adaptive evolution of populations (reviewed by West-Eberhard, 2003); with the debate whether plasticity impedes or advances

evolution being especially contentious. I set this evolutionary controversy aside and here focus on the relationship between plasticity and biological invasion to novel environments.

What does the empirical evidence reveal about the role of adaptive plasticity in biological invasions? Interestingly, the role of plasticity for survival in novel environments has a historical legacy. Baldwin (1896) proposed the concepts of 'organic selection' and 'orthoplasy' collectively referred to as the 'Baldwin Effect' (reviewed by Crispo, 2007), which predicts that plasticity will facilitate survival and reproduction in novel environments (organic selection) and steer the evolutionary trajectory in the direction of the plastic response (orthoplasy). It is not surprising that contemporary invasion biologists frequently suggest that plasticity facilitates invasion via the expression of advantageous phenotypes in a broad range of novel environments (e.g., Daehler, 2003, Davidson et al., 2011). However, recent models suggests that phenotypic plasticity may actually serve to oppose invasion by steepening the fitness landscape and thereby making invasion more difficult even by plastic invaders (Peacor et al., 2006). While frequently cited as important in biological invasion (e.g., Rejmanek et al., 2005), the extent and thoroughness of the discussion of plasticity and invasion is often limited. For example, recent book reviews of biological invasion either make no reference (Cox, 2004, Sax et al., 2005) or passing reference (Kim & McPheron, 1993, Lockwood et al., 2007) to phenotypic plasticity. Similarly, West-Eberhard's (2003) review of developmental plasticity and evolution made no explicit reference to non-native invasive species.

The first empirical demonstration of the Baldwin was made by Georgii Gause, a Russian biologist best known for his articulation of the "exclusion principle" in ecology and his work on antibiotics. In a series of experiments summarized in a 1942 publication, Gause

demonstrated that the salinity tolerance of clones in *Paramecium caudatum* (a typically freshwater species, which sometimes occur in brackish environments) increased markedly after exposure to hyper-saline conditions (Gause, 1942).

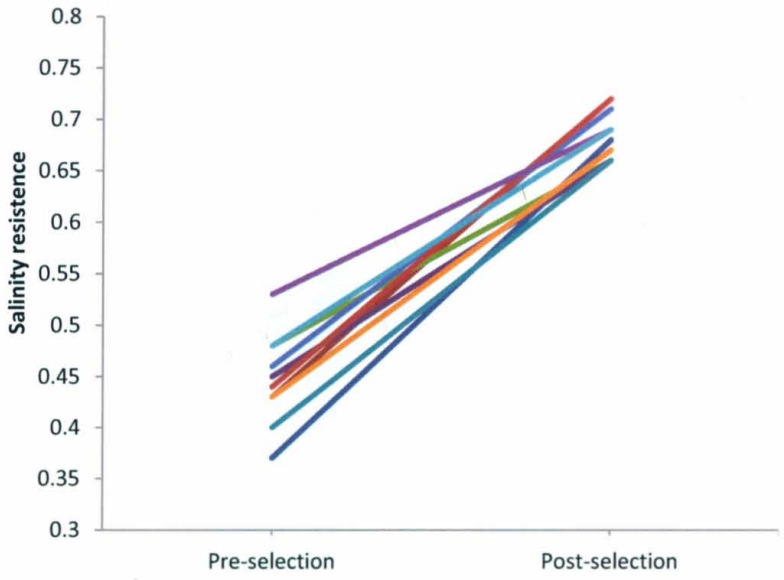

Fig.2. Change in salinity resistance (expressed as the concentration of salt killing 50% of individuals in 24 hours) in clones of Paramecium caudatum (each line represents a clonal strain) prior to and post acclimation to 0.36% salinity. Data plotted from Table 1 in Gause (1942).

Patterns of plastic change in salinity resistance clearly showed a consistent increase in tolerance and an apparent convergence of level of resistance (Fig. 2). This suggests that clones exposed to higher salinity responded via phenotypic plasticity in a direction that facilitated survival. These results demonstrated, in the laboratory, the first critical stages in the Baldwin effect. Though he did not quantify the evolutionary significance of his findings, Gause concluded that the adaptive modifications he observed "prepare the way for the subsequent evolutionary advance."

In addition, the Gause experiments provide a convenient springboard to introduce the concept of reaction norms (interchangeably called norms of reaction). Phenotypic plasticity is often mathematically and graphically described by a norm of reaction, a function (usually linear but not necessarily so) that expresses how phenotypic values among group of organisms change along environmental gradients (Schmalhausen, 1949, Schlichting & Pigliucci, 1998, Hutchings, 2004). Plasticity is evidenced by reaction norms with non-zero slopes whereas the absence of plasticity is inferred when trait values do not change with the environment. Reaction norms that run parallel suggest that the response to environmental change is similar among organisms, while crossing reaction norms suggest genetic variation in plasticity or so called genotype x environment (G x E) interactions. If this genetic variation is additive, natural selection could drive evolutionary change in the shape of the reaction norm. The norms of reaction depicted in Fig. 2. demonstrate both plasticity (non-zero linear slopes) and G x E interactions. Two general approaches to modelling plasticity are taken in the literature; the *character state* or the *polynomial* (sometimes confusingly referred to as the reaction norm) approach (discussed in detail by Via et al., 1995). In the character state approach, norms of reaction are modeled as the value of a phenotypic character that would be expressed by genotypes or groups of organisms as a function of the environment. The polynomial approach models the reaction norm as a polynomial function of the phenotypic values across environments. Underpinning these two approaches rests the idea that phenotypic plasticity is a phenotypic trait *per se*, separate from the phenotypic value of a given trait with its own separate genetic control. In this thesis I adopt a character state approach as it is appropriate when examining the response of organisms to discrete environments but do consider plasticity and character trait values as separate traits, each

capable of incurring and responding to selection. Moreover, in discrete environments, polynomial and character state approaches are mathematically equivalent (De Jong, 1995). Plasticity can be adaptive or non-adaptive both of which have consequences for contemporary evolution in novel environments (Ghalambor et al., 2007). I consider plastic responses that either occur in the direction consistent with selection or that facilitate survival to be adaptive. For a thorough review on the differences between adaptive plasticity and plasticity as an adaptation see Gotthard and Nylin (1995).

Taken as a whole, few studies have explicitly examined the role of plasticity in invasion beyond the controlled conditions of a laboratory or greenhouse. In the following paragraphs I briefly discuss two examples that provide insight into plasticity and invasion in nature.

Fountaingrass *Pennisetum setaceum*, a C_4 perennial grass, was introduced for its ornamental appeal in the early 1900s to the Hawaiian Islands. Fountaingrass has subsequently invaded a wide range of habitats of varying altitude from sea level to nearly 3000 m. Sites differ dramatically in temperature (mean winter temperature 2°C in sub-alpine site; 17°C in coastal site) and timing of rainfall. Correspondingly, plants show adaptive divergence in morphological, physiological, and reproductive traits. In a reciprocal transplant experiment with clones of plants from three populations (coastal, middle, sub-alpine), Williams et al. (1995) reveal no genetic influence on observed differences in phenotypes indicating that plasticity has maintained the diversity of form in nature. Plasticity has apparently done such a good job of maintaining adaptive phenotypes in a given environment that there is insufficient heritable material on which selection can act, thereby precluding local adaptation. Alternatively, Williams et al. (1995) suggested that introduced fountaingrass

has not evolved local adaptation because genetic variation was limited by a genetic bottleneck. Reductions in genetic variability are often cited as hindering local adaptation in introduced species; however, empirical evidence suggests that adaptation is possible even when very small numbers of individuals are introduced (salmonids: Koskinen et al., 2002, *Drosophila*: Huey et al., 2005, mammals: Williams & Moore, 1989).

Additional evidence for an important role of plasticity and the establishment of novel environments comes from the colonization of low-land habitats by dark-eyed juncos (*Junco hyemalis*) in California. Juncos are native to North America and in the early 1980s, a small population colonized the coastal environment surrounding the University of California, San Diego (UCSD) campus from a nearby mountainous area. Yeh and Price (2004) investigated the influence of variation in breeding season length (a reportedly 'classically plastic trait') on population persistence in both derived and ancestral populations. They reported a markedly longer breeding season length in the new population, resulting in higher offspring production and recruitment compared to the ancestral population. Interestingly, the new population has shown virtually no change in population size through six years of intense monitoring, thereby suggesting that increased reproductive output is necessary to compensate for higher juvenile to adult mortality rates in the colonized range. Without the compensatory effect of lengthened breeding season it was estimated (while controlling for immigration, density-dependent offspring recruitment, and habitat carrying capacity) that the new population would decline by approximately 20% per year and quickly go extinct. The mechanism(s) underlying the failed recruitment from fledglings to adults are unclear as movement by juveniles from the study area was indistinguishable from mortality. To date, this example provides the strongest and clearest quantitative support of Baldwin's

idea of organic selection (plasticity mediated survival and reproduction in novel environments). In addition, more recent evidence suggests that these plastic responses have influenced trait evolution in this system. Price et al. (2008) reported that lengthened breeding season has directed the evolutionary trajectory of a heritable sexually selected trait, white tail feathers, in this population.

These empirical examples provide evidence for an important role of adaptive plasticity in biological invasions, but fail to provide key insight during the first critical stages of colonization and introduction. No study that I am aware of has attempted to explicitly track the fitness consequences of phenotypically plastic traits of transplanted individuals from the earliest stages of introduction. Indeed, few studies have been fortunate to monitor the first few generations of natural colonization (though see Anderson & Quinn, 2007, Anderson et al., 2008). This research gap is echoed by Ghalambor et al. (2007) who suggested that 'If an identifiable subset of individuals that possess a particularly favourable combination of plastic traits are found to be the successful colonizers of new environments, such evidence could show an important role of plasticity in facilitating adaptation.' As highlighted here, evidence suggests adaptive plasticity can facilitate invasion and population persistence in novel environments; however, the role of plasticity in the earliest stages of invasion and whether it represents a prominent driving force toward local adaptation in nature is unclear. Furthermore, it remains to be seen whether phenotypic plasticity will hinder or promote adaptive responses of native and invasive species to the rapidly changing global climate (Chown et al., 2007).

Thesis overview and rationale

The chapters that follow are my attempts to understand the ecological and evolutionary consequences of the biological invasion by brown trout in Newfoundland, and in doing so aim to address some of the outstanding questions surrounding the role of the environment and phenotypic plasticity in shaping the outcome during the first stages of an invasion.

I set the stage for this work in **Chapter One**, where I asked a broad and general question concerning what we have learned about the rate and form of phenotypic change in populations via the study of invasive species. To do so, I expanded an existing database of available rates of phenotypic change in 90 species of plants and animals and showed that the majority of our inferences about population divergence and evolution are based on invasive species. Moreover, I show that in spite of presumably strong selection pressures, native species are evolving as quickly as invasive species along similar temporal trajectories. However, I do reveal an important role of phenotypic plasticity in explaining phenotypic change and suggest that differences may exist in the plastic potential between invasive and native species.

In **Chapter Two** I turn my attention to brown trout in Newfoundland and ask, in general terms: Who are these invaders? Where did they come from? Where and how many were introduced? Where have they gone and why? Where are they going? Though brown trout has been established in Newfoundland for over a century, surprisingly little had been done to address these questions. Chapter two expands on the history presented in this

introduction and includes the numbers and locations of first introductions. We also assembled a large database of watershed that had either been or not been successfully invaded by brown trout to understand the landscape factors that may explain and predict population establishment. We conclude that populations are not distributed randomly across the landscape but seem to occur in watersheds that are relatively large and productive compared to watersheds where brown trout are absent. Curiously, this pattern mirrors the patterns of distribution by brown trout *within* watersheds: brown trout are typically found in the lower, more productive reaches of watersheds both in their native European (Korsu et al., 2007) and introduced North American (Budy et al., 2008) range.

The observation of populations established among a range of physical environments sets the stage for **Chapter Three**, where we investigate the relationship between adaptive phenotypic traits (i.e. likely linked to fitness) and environmental factors in 16 trout populations. We reveal differences among populations in a suite of traits (e.g. body shape and colour) and find significant correlations with habitat features. Specifically, we show that large and presumably faster flowing streams are associated with individuals with relatively robust body shapes compared to smaller, slower flowing streams. Similarly, dark environments with greater canopy cover correlate with darker pigmentation in trout.

Chapter Four combined a common-garden and a reciprocal- transplant experiment to simultaneously address questions concerning local adaptation, the adaptive significance of observed phenotypes, the underlying genetic influence on phenotypes, and the role of plasticity in facilitating survival in the early stages of an invasion. Results suggest that, at least in the three populations examined, local adaptation (based on survival and growth of tagged individuals) to environmental conditions is likely. The populations displayed significant

differences in swimming and feeding related morphology even when reared in different environments, providing strong evidence of underlying genetic control. Moreover, morphology was plastic and varied across environments in a manner consistent with patterns observed in the field. That is, large rivers tend to induce larger more robust body shapes. However, counter to predictions, plasticity in morphology was often counter to the direction of natural selection. Overall, attempts to predict the plastic responses of organisms to novel environments may be more complicated than previously appreciated.

Co-authorship statement

Along with the general introduction and discussion, Chapter One was written and conceived entirely by myself. Chapters 2-4 were co-written with my advisor, Ian Fleming. The third chapter was also co-authored by Corinne Conway who greatly contributed to the intellectual development and execution of the paper. All authors deserving authorship have been included. In all cases, I suggest readers refer to and cite the peer-reviewed versions of these chapters.

Publications (published, or anticipated) and authorship arising from this thesis:

Chapter One:

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Chapter Two:

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Chapter Three:

Westley, P. A. H., Conway, C. & Fleming, I. A. Novel environments shape phenotypic variation in recently established brown trout (*Salmo trutta*) populations. In review, *Evolutionary Ecology Research.*

Chapter Four:

Westley, P. A. H. & Fleming, I. A. Testing predictions of the Baldwin effect in nature: does phenotypic plasticity facilitate survival in novel environments? Journal still in consideration.

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Chapter 1: What invasive species reveal about the rate and form of contemporary phenotypic change in nature

Abstract

Biological invasions represent opportunities to gain insight into fundamental evolutionary questions as abrupt changes in selection pressures and reproductive isolation are likely to lead to rapid evolutionary change. Here I formally investigate the role of invasive species in revealing the rate and form of contemporary phenotypic change in wild populations. To do so, I expand and utilize a database of over 5,500 evolutionary rates of phenotypic change from 90 species of plants and animals. On an absolute basis, invasive species have disproportionately contributed to the available evolutionary rates; however, the preponderance of these rates is the consequence of extensive study in a small number of individual species. Invasive species are more often examined with experimental designs suited to elucidating divergence among populations rather than change within populations. Contrary to expectations, I found mixed evidence to support the hypothesis that phenotypic change is positively associated with amount of time of divergence depending on whether interpretation is based on change measured in darwins (phenotypic change per year) or haldanes (standard deviations of change per generation). Results suggest that both invasive species and native species provide evidence that phenotypic change can be markedly abrupt as observed changes during short time intervals were often as great as those seen in longer time intervals. Finally, results here reveal a potentially important role of the environment and by extension phenotypic plasticity to drive trait change in wild populations, though the potential for plasticity to influence evolutionary trajectories remains unclear. Thus future work should continue to seek an understanding of the mechanistic underpinnings –both genetic and environmental– of how phenotypic variation allows populations to adapt to rapidly changing global environments.

Introduction

Darwin's recognition of natural selection as the primary evolutionary force marks the beginning of a debate that still abounds today. At face value, the question of how quickly organisms evolve seems straight forward; however, elucidating a conclusion and reaching a consensus is anything but trivial (recently reviewed in Gingerich, 2009). The emergent voices in this debate generally align in one of the following three archetypical camps: *i*) evolution is necessarily gradual and slow (e.g. Darwin, 1859, Fisher, 1930), *ii*) evolution is punctuated, and thus is sometimes fast and sometimes slow but never in between (Elredge & Gould, 1972), and *iii*) evolution is often fast (Hairston et al., 2005, Palumbi, 2001). Inferences into the rate of evolution are drawn from investigations using the fossil record (Gingerich, 1993, Gingerich, 2009, Hunt et al., 2008), longitudinal studies tracking trait changes in laboratory (Lenski et al., 1991) and wild populations (Grant, 1999, Reznick & Ghalambor, 2001) or meta-analyses from literature (Hendry & Kinnison, 1999, Kinnison & Hendry, 2001, Darimont et al., 2009). A key insight is that the *rate* of evolution is inversely proportional to the temporal scale of observation, thus resulting in the observation that the *amount* of evolutionary change (e.g. mean trait change between two points in time) is essentially independent of time frequently reported (e.g. Hendry et al., 2008, Schluter, 1996, Gingerich, 1993, Gingerich, 2009). However, this seems difficult to reconcile with the typically high trait heritability (reviewed by Carlson & Seamons, 2008) and intense selection often observed in nature (Kingsolver et al., 2001, Kingsolver & Pfennig, 2007).

One potential explanation is that natural selection fluctuates in direction and intensity through time and thus may explain periods of both long-term stasis and short-term

abrupt evolutionary change (Bell, 2010, Siepielski et al., 2009). Selection is expected to be intense during extraordinary environmental changes (Lande, 2009, Reznick & Ghalambor, 2001), such as when populations are transplanted outside their native ranges or colonize newly accessible habitat (for an empirical example see Anderson et al., 2010). These species invasions thereby represent opportunities to gain insight into the evolutionary process over short timescales. Following in the pioneering footsteps of Joseph Grinnell (1919) and Charles Elton (1958), researchers are increasingly using biological invasions to investigate key ecological and evolutionary processes (Sax et al., 2005 and references therein). Insights into the mechanisms driving adaptation to novel environments have been illuminated from these opportunistic studies of invasive species (reviewed by Sax et al., 2007) as well as many empirical examples of contemporary evolution (i.e. the evolution occurring in the recent past of approximately 200 generations or fewer). For example, population bottlenecks and reductions in genetic diversity, rarely seem to limit the capacity for adaptive evolution in novel environments (Wares et al., 2005). Koskinen (2002) provide support for this via their study of small introduced grayling populations (*Thymallus thymallus*). In short, rapid phenotypic divergence occurred within 100 years even though very few individual fish were introduced to several alpine Norwegian lakes. Additionally, research derived from the paradoxical ability of invasive species to sometimes outcompete local species suggests that individuals are not always optimally adapted to their environments (Sax & Brown, 2000, Korsu et al., 2007); though only a small subset of species that are transplanted become successful invaders thereby suggesting the power of local adaptation (Williamson, 1996). Furthermore, research on phenotypic changes in populations of invasive species supports the hypothesis that adaptive evolution can occur in only a few to dozens of generations

(Huey et al., 2005, Reznick & Ghalambor, 2001, Palumbi, 2001). Indeed, some of the now iconic examples of contemporary evolution in nature are derived from studying invasive species such as mosquito fish (*Gambusia affinis*) in Hawaii (Stearns, 1983), salmon (*Oncorhynchus spp.*) in New Zealand (Quinn et al., 2001a) and Lake Washington, USA (Hendry et al., 2000), Trinidadian guppies (*Poecilia reticulate*) transplanted across predation barriers (Endler, 1988), and old world fruit flies (*Drosophila obscura*) introduced to North and South America (Huey et al., 2000).

However, it remains unclear whether recently invading species that are likely experiencing abrupt directional selection in their novel habitats and native species that are likely maintained around adaptive optima via stabilizing selection are evolving at similar rates and along similar temporal trajectories. Here I expand on an existing database of evolutionary rates (Kinnison & Hendry, 2001, Hendry et al., 2008, Hendry & Kinnison, 1999) to address this and other questions with the overarching objective of formally investigating invasive species' role in illuminating our understanding of contemporary phenotypic change in wild populations. Specifically, I ask the following questions:

i) What proportion of available rates of phenotypic change (influenced by both genetics and environment) is derived from the study of invasive vs. native species?

ii) What proportion of available rates provides evidence for population divergence from a common ancestor (i.e. synchronic experimental design, sensu Hendry and Kinnison 1999) vs. change through time within populations (i.e. allochronic design)? Similarly, what proportion of available rates are the results of common-environment or quantitative genetics studies, thereby indicating that trait change likely have a heritable basis?

iii) What is the relationship between trait changes and the amount of time of divergence (i.e. what is the shape of evolutionary trajectories between species)? Are invasive species and native species changing similarly through time and thus is there evidence of similar temporal evolutionary trajectories? Does the shape of the relationship between traits and time of divergence support the hypothesis of gradual or abrupt phenotypic change?

iv) What role does the environment and thus potentially phenotypic plasticity (i.e. the ability of individual genotypes to produce multiple phenotypes in different environments) play in our interpretation of phenotypic change in invasive and native species?

Materials and methods

To investigate the contribution of invasive species to our understanding of contemporary phenotypic change, I searched the ISI Web of Knowledge database (version 4.2; Thomson Reuter) with combinations of the following keywords: exotic species, invasive species, introduced species, contemporary evolution, rapid evolution, evolutionary rates, haldanes, and darwins. Additionally, I searched for the papers that had cited Hendry et al. (2008) assuming that researchers reporting rates of contemporary phenotypic evolution would cite this publication. My intention here is a broad-strokes attempt to investigate the role of invasive species compared to native species in illuminating the rate and form of evolution in nature (though see Cox, 2004, Reznick & Ghalambor, 2001), thus only papers that report evolutionary rates in either of two metrics, darwins and/or haldanes were included in the database (evolutionary rates were not calculated from data reported in publications).

In short, darwins represent rates of phenotypic change expressed on the logarithmic scale (base *e*) per million years and is calculated by taking the difference in natural logarithm of trait means (observed within a population through time, or across with a common ancestor) and dividing by the length of time in millions of years (Haldane, 1949, Gingerich, 1993). In contrast, the haldane represents the change in mean phenotype expressed in standard deviations per generation (Gingerich, 1993, Hendry & Kinnison, 1999), which facilitates comparisons among species with dramatically different reproductive systems and generation times (generation length, in years, ranged from 0.1-30 in the database). For further discussions concerning the derivation and interpretation of darwins and haldanes see Gingerich (1993), Hendry and Kinnison (1999), and Hendry et al. (2008), and Appendix 1-1.

To a previously published version of the database (Hendry et al. 2008), I was able to add a total of 305 rates of phenotypic change extracted from 10 papers (i.e. Seeley, 1986, Bone & Farres, 2001, Fisk et al., 2007, Gienapp et al., 2008, Hargeby et al., 2004, Haugen et al., 2008, Herrel et al., 2008, Michaud et al., 2008, Quinn & Adams, 1996, Eroukhmanoff et al., 2009). The result was an expanded database of 2,989 and 2,570 estimates in darwins and haldanes, respectively. The database includes rates from 90 species across a range of taxa: plants (n= 26 species), freshwater invertebrates (n=2), marine invertebrates (n=7), insects (n=4), reptiles (n=2), amphibians (n=2), fish (n=18), birds (n=18), and mammals (n=11).

Each rate was assigned to one of two descriptive groups, invasive or native. Definitions and terminology surrounding invasive species are often vague and contentious (Valery et al., 2008). I assigned the designation of 'invasive' to species that have been moved beyond their native range (defined by their intrinsic dispersal capacity) through obvious human activity, such as intentional introductions for sport fishing (e.g. Chinook salmon in

NZ, Kinnison et al., 2001), sport hunting (rabbits in Australia, Williams & Moore, 1989), bio-control (mosquito fish in Hawaii, Stearns, 1983), or research (guppies introduced above waterfalls in Trinidad, Endler, 1988). Additionally, the spread of species beyond the site of initial introduction via range expansion was included in the invasive species category. Alternatively, species were categorized as 'native' if they were evolving in locations within the confines of their own dispersal capabilities (e.g. the finch species complex in Galapagos, Grant, 1999). Furthermore, species were considered native that were colonizing new habitat in the absence of direct human assistance (e.g. aquatic isopods colonizing new habitat in Swedish lakes; Eroukhmanoff et al., 2009). This methodology resulted in four instances where species were categorized as both invasive and native depending on the context, such as sockeye salmon (*O. nerka*) which is native to the Columbia River (Quinn & Adams, 1996) and an invader to Lake Washington (Hendry et al., 2000), though the number of these instances were too small to allow a formal analysis. There is additional ambiguity in some designations such as whether brown trout (*Salmo trutta*) colonizing portions of a native watershed following passage around hydropower dams (Haugen et al., 2008) should be categorized as native or invasive (they were analyzed as natives). Echoing the sentiments of Hendry et al. (2008), I invite readers to reanalyze the evolutionary database (available at http://dx.doi.org/10.5061/dryad.8078) with the designations they feel are most appropriate.

Additional information was collected for each evolutionary rate such as details of the experimental design and whether a trait in question had a demonstrated genetic basis. Phenotypic change within a population through time was assigned an allochronic experimental design whereas between-population divergence through time was categorized as a synchronic experimental design (sensu Hendry and Kinnison 1999). Evolutionary rates

resulting from 'common-garden' experiments or from quantitative genetic studies were designated as 'genetic', and others were designated 'phenotypic.' This last categorization facilitated investigations of the potential role of environmentally induced phenotypic plasticity in divergence between native and invasive species.

I based analyses on the average amount of change at the species, genus, and family taxonomic levels to control for non-independence of evolutionary rate estimates (following Einum & Fleming, 2002). This non-independence, which arises from the disproportionate contribution of certain species to the database, is henceforth referred to as the 'Stearns Effect' (in honour of Steve Stearns' copious work on mosquito fish life history evolution). This hierarchical methodology assumes that higher level taxonomic groupings have a greater degree of evolutionary independence, thereby facilitating the interpretation of differences between invasive and native species while controlling for possible confounds of shared ancestry. Furthermore, evolutionary rates scale negatively with time due in part to spurious self-correlation (Hendry & Kinnison, 1999, Gingerich, 1983), thus analyses to investigate differences in the rate and form of phenotypic change were conducted on the averages of the absolute value of the numerator of darwins or haldanes as following Hendry and Kinnison (1999), to account for temporal effects.

A combination of visual inspection of evolutionary rates (representing all rate estimates) and binomial tests for equality of proportions (after correcting for the Stearns effect) were used to investigate differences in the contribution of invasive vs. native species to evolutionary studies (Crawley, 2002). Chi-square tests on number of species classified as invasive or native species that were derived from allochronic vs. synchronic experimental designs and from designs conducted in the field or common-environments. General linear

models with temporal covariates (ANCOVA) were fit to various taxonomic subsets of data (e.g. species, genus, family) to investigate differences in absolute rate of phenotypic change between invasive and native species and to test for evidence supporting an abrupt or gradual model of phenotypic change. I interpreted a lack of correspondence between the amount of phenotypic change and the time interval of observation as evidence for abrupt phenotypic change whereas positive relationships between trait change and time was interpreted as evidence of gradual change. That is, under the abrupt model of phenotypic change the amount of change during short time intervals is as great as during long intervals and under the gradual model the extent of phenotypic change increases with time interval of observation.

The database assembled here precludes a direct examination of the influence of environmental factors such as phenotypic plasticity on population divergence, thus I used an indirect measure by fitting ANCOVA models to subsets of data resulting from *i*) field studies; rates are 'phenotypic only', *ii*) common-rearing/greenhouse studies; 'genetic only', and *iii*) pooled datasets. I assume that the role of the environment and phenotypic plasticity is maximized and minimized in the 'phenotypic only' and 'genetic only' datasets, respectively, and that the pooled dataset integrates the role of genetics *and* the environment. Regardless of the dataset used, ANCOVA models were first fit to test interactions between the temporal covariates and fixed grouping term (invasive or native species classification). Non-significant interactions were removed and the models were refit to allow a direct examination of differences among species designations (i.e. invasive vs. native). All statistical analyses were conducted in R. v2.10.1 (R Development Core Team 2009). Finally, I refrain from referring to observed phenotypic divergence observed in the database as evolution except in select

cases (e.g. allochronic studies of heritable beak size evolution in *Geospiza fortis*). I take this conservative approach as inferring evolution from patterns of divergence has known problems (e.g. see Fig.1. in Hendry and Kinnison 1999). Ultimately, identifying the genetic basis of a trait does not in and of itself suggest evolutionary change as divergence of known heritable traits can be the result of phenotypic plasticity as well as adaptive evolution (discussed by Losos et al., 2001, West-Eberhard, 2003).

Data quality and potential biases

Comparisons of rate and form of phenotypic change were done while attempting to control for additional variation and potential data biases. I controlled for the pervasive influence of anthropogenic disturbance on wild populations by comparing evolutionary change in invasive species to native species in the absence of other obvious human perturbations. Analyses comparing evolutionary trajectories and the potential role of the environment and phenotypic plasticity in invasive species were based on rates from systems of 'invasion and range expansion after invasion' where as rates for native species were derived from systems labelled as 'in-situ natural conditions and natural range expansion' (i.e. minimal anthropogenic disturbance). Mounting evidence suggests that anthropogenic activity influences both the strength of selection and rate of evolutionary response in wild animal populations (Hendry et al., 2008, Darimont et al., 2009) and failing to control for such effects resulted in altered interpretation and obscured comparisons of invasive and native species (results not presented here).

Invasive species comprise a non-random small subset of species with traits that are likely conducive for establishing and invading new habitats (Kolar & Lodge, 2001, Kolar &

Lodge, 2002, Williamson, 1996) and are unrepresentative of most species (i.e. are biased). However, this bias is biologically pertinent and is not an artifact of data collection or publication partiality and thus differences in the rate or form of phenotypic change between invasive species and native species arising from this 'bias' is still of interest. In contrast, there could be a bias towards publication of dramatic or 'rapid' evolutionary change that may be more common in invasive species or individuals transplanted among habitats (e.g. Reznick et al., 1997) compared to native species. On the other hand publication of evolutionary rates using native species are often done during events where selection may be abruptly strong, such as climatic events like El Nino (Grant, 1999). Ultimately then, the discrepancy between comparing rates of invasive and native species due to publications biases alone may not be severe as it seems.

To investigate the role of the environment and by extension potentially phenotypic plasticity in influencing rates of divergence between invasive and native species I compared a subset of data from quantitative genetics studies and common rearing experiments ('genetic rates' where environmental effects should be minimized) to data collected from wild populations ('phenotypic rates' where divergence is due to genetics *and* environment). This comparison had the potential to lead to dubious interpretation arising from inherent experimental biases, specifically potential gene by environment interactions occurring in the 'genetic' dataset that is absent in the 'phenotypic' dataset. This interaction may occur in comparing divergence of F_1 lab raised offspring of wild parents which obviously have been reared in different environments. This gene x environment interaction is likely not an issue in quantitative genetics studies conducted in wild populations or in long-term laboratory studies on strains or clones. To investigate this potential bias I

categorized the genetic rates of evolution as the result of 'wild' (experiments done entirely in the wild), 'semi-wild' (experiments done on lab reared F_1 offspring of wild parents), or 'lab' (experiments based on F_2 or greater generations or cloned strains).

No potential bias was observed between experimental groups in either haldanes or darwins (interaction term of ANCOVA with temporal covariate $F_{2, 32} = 0.04$, $p = 0.97$). This suggests that 'wild', 'semi-wild', and 'lab' groups are diverging similarly through time and are directly comparable to the 'phenotypic' dataset as no potential gene x environment interaction was found. Ideally, one would compare rates of trait divergence from studies reporting both a phenotypic and genetic rate of evolution; however, I only found 14 studies that fit this criteria resulting in comparisons of a very small number of species (five invasive and four native) making interpretation difficult. Thus, the investigation of the role of the environment and potentially phenotypic plasticity utilized the entire 'genetic' and 'phenotypic' datasets while acknowledging these underlying caveats.

Results

Have invasive and native species contributed equally to the database of evolutionary rates?

Taken as a whole, 83% and 84% of the available rates (all rates combined, not controlling for non-independence of rates, 'Stearns Effect') in darwins and haldanes, respectively, were derived through the study of invasive species (Table 1-1). However, this dramatic skew in contribution is due mostly to the Stearns Effect (e.g. 1,100 rates in haldanes are contributed by Stearns personally) and controlling for species non-independence results in a greater proportion of species in the database classified as native

rather than invasive. Of the 90 species in the database, 33% were classified as invasive and 67% were classified as native, even though more individual rates are derived from the invasive species. This skew towards native species in the database is statistically significant (darwins, $X^2_{df=1} = 16.1$, $p<0.001$) indicating that over half of the species examined in studies of contemporary evolution were evolving within their native ranges.

Are invasive species typically investigated in the context of divergence from a common ancestor or within-population divergence and do rates of phenotypic change typically result from traits with a known genetic basis?

First examination of Table 1-1 suggests that invasive species reveal more about population divergence from a common ancestor rather than about within-population change, and more about overall phenotypic change than about change with a known genetic basis. Indeed, approximately 81% of the available estimates involving invasive species in both darwins and haldanes result from studies with synchronic experimental designs (Table 1-1). Similarly, 70-72% of the rates from invasive species results from observations of trait changes without confirmed genetic bases, and thus potentially results from environmental effects and includes a role of phenotypic plasticity (Table 1-1). Invasive species were significantly more likely to be examined within the context of between-population divergence (synchronic studies) rather than within-population change (allochronic studies) even after controlling for the effect of non-independence of available rates, the Stearns Effect ($X^2_{df=1} = 18.9$, $p<0.001$). In contrast, controlling for the Stearns Effect yielded statistically similar proportions of invasive and native species used in common-environment vs. field studies ($X^2_{df=1} = 0.06$, $p = 0.80$).

Are native and invasive species evolving along similar trajectories and do they support the hypothesis of gradual or abrupt phenotypic change?

Results with darwins as a rate metric suggest that invasive and native species are evolving along similar trajectories at all taxonomic levels, as inferred by insignificant interaction terms to fit ANCOVA models (Table 1-2). Moreover, the temporal covariate (in years) was not significant at any taxonomic level, indicating that invasive and native species are evolving over similarly flat trajectories (Fig. 1-1). Trait change that is independent of time interval of observation in both invasive and native species supports the model of abrupt phenotypic change and suggests that the magnitude of change occurring early in the time series is similar to magnitude of time occurring later. Finally, removal of non-significant interaction terms and non-meaningful covariates yields similar and non-significant (via ANOVA) estimates of mean phenotypic change between invasive and native species (Table 1-2), thereby suggesting that the both the rate and form of phenotypic change is similar among species.

In contrast, results with haldanes as the rate metric yield different interpretation among invasive and native species. Highly significant ($p<0.001$) interaction terms were detected between the temporal covariate (measured in generations) and the grouping classification term (invasive or native) at the species, genus, and family taxonomic levels (Table 1-2). This interaction was the result of differences in evolutionary trajectories whereby trait change in invasive species showed no relationship to time and trait change in native species varied positively and significantly with time ($p<0.001$ at all taxonomic levels, Fig. 1-1). The influence of three observations (advance in egg-laying date in *Sterna paradisaea* and *Sturnia phillippensis*, reported in Gienapp et al. (2008) and divergence of a suite of traits in

an aquatic isopod *Asellus aquaticus* Eroukhmanoff et al. (2009)) is apparently driving this interaction effect as removal of these observations yields non-significant interaction terms (generation*invasion status p=0.21). Implications for the underlying potential role of the environment and by extension perhaps phenotypic plasticity in this interaction are discussed subsequently.

Are environmental effects such as plasticity influencing our interpretation of divergence between invasive and native species?

To investigate the potential role of phenotypic plasticity, ANCOVA models were fit to subsets of data at the species taxonomic level that included studies that were 'phenotypic only' and 'genetic only.' Analyses at higher taxonomic levels yielded similar interpretation and are not reported here. Phenotypic only studies were the result of field observations or in instances where heritable bases for traits had not been determined. In contrast, genetic only studies were the result of studies done in common environments or the results of quantitative genetic research. Thus, it was assumed that the influence of the environment and potentially phenotypic plasticity would be most obvious in the phenotypic only studies and its influence minimized in genetic only studies.

A potentially important role of environmental effects such as phenotypic plasticity in driving species divergence emerged from two lines of evidence, both surrounding results from native rather than invasive species. First, invasive species and native species showed statistically similar rates and forms of divergence in all analyses where the potential role of the environment and plasticity were possible (datasets including phenotypic rates), but a highly significant ($F_{[1,24]}$=5.68, p=0.03, Table 1-2, Fig. 1-2) difference in the mean rate of change when controlling for the environment (Table 1-2, Fig. 1-2). Native species displayed

a larger phenotypic response in the dataset of genetic rates compared to their invasive counterparts, suggesting both a high evolutionary and plastic potential. However, these results also suggest a high evolutionary potential of invasive species despite clear evidence of them being extraordinarily plastic.

Second, results in haldanes reveal a significantly positive relationship observed in native species between absolute phenotypic change and time (in generations), which was the result of phenotypic studies, and particularly driven by the influence of observations of advancing egg-laying date in *Sterna paradisaea* and *Sturnia phillippensis*. Changes in breeding phenology in birds is often assumed to have a strong environmental component (Gienapp et al., 2008, Yeh & Price, 2004) and thus the marked changes in egg-laying date observed here may potentially be due to plastic responses to climatic change (Fig. 1-2). In contrast to the results discussed above in darwins, no significant difference in the mean magnitude of trait change was detected between invasive and native species (Table 1-2) when controlling for the potential influence of the environment and plasticity.

Discussion

Taken as a whole, results here suggest an important role of invasive species in revealing the rate and form of phenotypic change in wild populations. Both invasive and native species provide evidence for abrupt rather than gradual phenotypic change as change is typically independent of time. Results suggest that the apparent abrupt changes observed may be due in part to phenotypic plasticity. Counter to expectation, invasive species did not exhibit markedly greater phenotypic change compared to native species even though rapid reproductive isolation and marked changes in selection pressures following biological

invasions seem ripe for driving dramatic phenotypic change. However, several caveats emerged with regard to the utility of invasive species as models for illuminating evolution. First, the preponderance of individual rate estimates was derived from the study of invasive species, but the majority of these rates result from the extensive study of only a few species. Indeed, many studies using invasive species employ study designs to investigate trait changes in multiple populations derived from a recent common ancestor. Thus on an individual species level, native species rather than invasive species contribute disproportionally to the database of evolutionary rates. Second, the majority of individual rates extracted from invasive species are phenotypic only and do not have determined heritable components, thereby integrating both environmental and genetic effects. Caveats notwithstanding, results here highlight the role of invasive species as opportunistic models for examining population divergence and evolution.

Invasive species contribution to available estimates of phenotypic change

On an absolute basis, invasive species have disproportionately contributed to the database of available rates of phenotypic change, which is indicative of their suggested utility as excellent models for investigating contemporary evolution (Huey et al., 2005). However, the total number of invasive species investigated in studies of contemporary phenotypic change is relatively small compared to the number of native species used as models to examine evolution in nature. As a result, the number of estimates on a per species basis derived through the study of invasive species is large (~80/species) compared to native species (~10/species). This discrepancy is driven by the combination of large number of rates extracted from a relatively small number of invasive species used as models. The small number of invasive species used should perhaps not be a surprise as only a small fraction of

species that are introduced succeed in establishment and successful invasion (Williamson, 1996, Lockwood et al., 2007). Furthermore, the same species are introduced repeatedly to locations across the globe (Rahel, 2000), simultaneously representing their social value (e.g. use as sport or biological control) and inherent ability to successfully establish self-sustaining populations (Kolar & Lodge, 2002). The resulting global biotic homogenization likely has lasting ecological and evolutionary implications, though a refined understanding of the consequences of community homogenization is still emerging (Olden et al., 2004). At a more basic level, studies using invasive species usually employ multiple pair-wise comparisons of trait changes between populations derived from a single common ancestor thereby inflating the number of estimates derived from a single species. These caveats surrounding the use of invasive species as evolutionary models should not detract from their value as opportunistic natural experiments. Indeed, investigating the eco-evolutionary dynamics of the small, non-random, subset of species that become serial global invaders may well give insight into species invasibility and biological invasions in general (e.g. Kinnison et al., 2008).

Experimental design and genetic control of traits

Biological invasions lend themselves to synchronic experimental designs, which by definition serve to quantify phenotypic divergence through time. Correspondingly, results here suggest that invasive species are significantly more likely, even after controlling for the Stearns Effect, to be examined in this context rather than in allochronic studies that better serve to investigate within-population phenotypic evolution per se. In contrast, native species are used in both synchronic and allochronic experiments at statistically similar proportions. Synchronic studies are designed to provide insight into population divergence; however, they can also provide insight into evolution, albeit with careful interpretation. An

observed rate of population divergence is the product of multiple evolutionary trajectories, which may be indicative of similar or markedly different rates of evolutionary change. Thus, caution must be used when interpreting and inferring evolutionary change from observed divergence rates (discussed by Hendry & Kinnison, 1999). It is here that contemporary invasions of non-native species provide opportunities to disentangle divergence from evolution via tracking trait change in the early years of the invasion or colonization and ideally coupling with laboratory rearing studies. Thankfully, there are many excellent model systems to examine, such as fishes colonizing newly accessible habitat (Milner et al., 2008, Whiteley et al., 2009, Anderson et al., 2010) or passerine birds invading the campus of the University of California San Diego (Yeh, 2004).

Traditional definitions of phenotypic *evolution* are predicated on a determined genetic basis for traits in question (e.g. West-Eberhard, 2003). Thus, trait change with no determined heritable basis is potentially the result of environmental effects (e.g. maternal investment) and/or phenotypic plasticity, thereby limiting the utility for understanding evolution as such. This does not imply that invasive species are not excellent models for investigating evolutionary processes, but rather the lack of a clear genetic basis to traits fails to control for the influence of other important sources of trait variation. Results here reveal that on an absolute basis the majority of available rates derived from invasive species come from traits with no determined heritable component, which at face value suggests that invasive species provide less insight into phenotypic evolution. However, on a species level after correcting for the Stearns Effect, statistically equal proportions of rates in invasive and native species result from common garden experiments (environment controlled, determination of genetic basis possible) or wild experiments (environment not controlled, determination not

possible). This latter result confirms the intuitive prediction that both invasive and native species have potential to provide insight into phenotypic evolution in nature.

Are native and invasive species evolving along similar trajectories and do they support the hypothesis of gradual or abrupt phenotypic change?

Results here provide little evidence that invasive and native are evolving over separate trajectories and generally support the model of abrupt phenotypic change as trait change rarely varied as a significant function of time. These results are, by and large, analogous to the findings of Hendry et al. (2008) and Darimont et al. (2009) who report similar evolutionary trajectories (slope of phenotypic change regressions) between systems evolving under natural vs. anthropogenic contexts. In accordance with the findings here, both Hendry et al. (2008) and Darimont et al. (2009) report that phenotypic change was independent of the time interval of observation thereby supporting the model of abrupt phenotypic change. However, two notable distinctions emerged between the findings here and those analyses and prior work. First, a significantly positive relationship between phenotypic change and time was detected in native species when measured with haldanes. This pattern suggests that, in this case, native species support the model of gradual evolutionary change whereby the magnitude of phenotypic change increases as a function of time. Additionally, this emergent pattern supports unique evolutionary trajectories between native and invasive species; however, subsequent analyses suggest that environmental effects and potentially phenotypic plasticity maybe underpinning this result (see below). Furthermore, it is possible that this pattern arises because time in haldanes is based on generations, rather than years, which is more appropriate for comparing across species that

differ greatly in generation length (range = 0.1-30 years in the database). For this and several other reasons, Hendry and Kinnison (1999) conclude that rates measured in haldanes, compared to darwins, are more appropriate in studies of contemporary phenotypic change; however, inclusion of darwins in this analysis revealed insights into the potential role of plasticity that might otherwise have been overlooked.

Second, I found little evidence that the magnitude of phenotypic change differed between native and invasive species while controlling for the effect of time. In contrast, Hendry et al. (2008) and Darimont et al. (2009) show that, compared to natural systems, the magnitude of phenotypic change is greater in systems experiencing anthropogenic disturbance (including biological invasions) and especially high in animal populations subject to selective harvest. That no difference in the magnitude of phenotypic change in native and invasive species was detected here is curious given that abrupt changes to natural selection pressures are expected following introduction to novel habitats (Lahti et al., 2009) or during extraordinary environmental conditions (Lande, 2009). It is plausible that research involving native species is biased towards circumstances of similarly abrupt changes in selection, such as characters shifts in beak morphology of Darwin's finches following El Nino events (Grant, 1999) or advances in bird breeding phenology during periods of global climate change (Gienapp et al., 2008).

Furthermore, this result indirectly supports the increasingly reported pattern that human activity in natural systems acts as a powerful evolutionary force (Hendry et al., 2006, Palumbi, 2001, Darimont et al., 2009, Hendry et al., 2008). Here I report no difference in the magnitude of phenotypic change between species of human-mediated biological invasions and native species, thereby suggesting that the effect of anthropogenic activities reported

elsewhere must primarily arise from other mechanisms besides species invasions. The obvious mechanism likely underpinning dramatic phenotypic changes in anthropogenically disturbed systems is directed harvest and exploitation of wild populations. Recent empirical evidence based on long-term datasets of exploited populations reveals complex patterns of trait selection (Edeline et al., 2007, Kendall et al., 2009, Carlson et al., 2007, Siepielski et al., 2009) and the patterns of phenotypic change are correspondingly complex (Darimont et al. 2009).

How does the environment and potentially phenotypic plasticity influence our interpretation of divergence between invasive and native species?

Results here suggest that environmental effects such as phenotypic plasticity may be contributing to the emergent patterns of native and invasive species phenotypic change. This is inferred by two general sources of evidence using both darwins and haldanes as rate metrics, both emerging from results of native species. First, invasive and native species show similar magnitude of phenotypic change in contexts including a potential role of the environment and plasticity, but native species exhibit greater response in contexts minimizing the effect of the environment. This result is counter to the expectation that invasive species exhibit greater phenotypic plasticity compared to their native counterparts (Richards et al., 2006 and references therein). However, to date, empirical evidence that supports this expectation has been equivocal (Hulme, 2008). This equivocation likely stems from inherent difficulty in drawing comparisons as the results are contingent on the environments in which they are conducted (Williams et al., 2008), sensitive to source populations used (Colautti et al., 2009), and dependent upon the chosen metric of plasticity (Valladares et al., 2006). Similarly here, interpreting species differences in the potential to

exhibit phenotypic plasticity is dubious as suites of different traits are being compared among environments. Indeed the environmental influences on studies of plasticity make it exceedingly difficult to accurately predict responses to changing environments (Husby et al., 2010), thereby representing a daunting challenge in a rapidly changing global climate.

Furthermore, the magnitude of phenotypic trait change expressed by invasive species in common-garden environments was significantly less than native species in the same context. Theory predicts that invasive species are likely to experience loss of genetic variability following introduction (Lockwood et al., 2007), which in turn is expected to influence the capacity for expressing phenotypic variability (Allendorf & Luikart, 2007). However, empirical evidence suggests that newly established invading populations rarely show signs of reduced genetic variation (Wares et al., 2005) and correlations between genetic and phenotypic variability are often weak (Allendorf & Luikart, 2007). Thus, it seems plausible that the significantly reduced magnitude of phenotypic change by invasive species observed here is the result of additional, though not mutually exclusive, mechanisms. For example, it is possible that the common-garden environments chosen for examining the invasive species were too benign to elicit phenotypic responses in these species (Pujolar et al., 2006, Ghalambor et al., 2007) and that other common-gardens would have yielded different interpretations (Williams et al., 2008).

Second, results with haldanes as a rate metric provide complimentary evidence for the role of phenotypic plasticity in the divergence of native and invasive species. Native species displayed evidence for a gradual model of evolution via a significantly positive relationship between phenotypic change and time (as measured in generations). However, this relationship is non-significant when controlling for environmental effects (i.e. when

traits with determined genetic components are analysed separately) thereby implicating the role of plasticity and suggesting that native species are capable of exhibiting dramatic phenotypic responses in nature. The ability for plasticity to maintain continued trait change observed in native species is intriguing and worthy of additional investigation, but only seems plausible if plasticity itself was evolving through time. Interestingly, the potential for plasticity to evolve is supported by recent theoretical (Lande, 2009) and empirical findings (Crispo et al., 2010).

Recently, there has been rejuvenated interest in the role of phenotypic plasticity to influence the evolution of populations (reviewed by West-Eberhard, 2003) though debate and confusion surrounding its mechanisms and evolutionary role still abound (Via et al., 1995, Crispo, 2007, Pigliucci, 2007). Specifically, it is unclear whether plasticity serves to shield genotypes from selection or works as a mechanism to create novel variation on which selection acts and thus whether plasticity enhances or retards the rate of phenotypic evolution (Ghalambor et al., 2007). Results here support the hypothesis that plasticity underlies the phenotypic variation observed in wild populations, but it remains unclear whether these populations have genetically diverged. Meta-analyses confirm significantly positive correlations between indices of population differentiation for quantitative traits and neutral genetic markers, thereby suggesting that phenotypic divergence observed here may indeed be indicative of underlying genetic changes (Leinonen et al., 2008, Merila & Crnokrak, 2001). Thankfully, attempts to understand, in a holistic fashion, the mechanistic underpinnings –both genetic and environmental– of phenotypic variation are mounting, especially with regard to how phenotypic variation allows organisms to respond adaptively to novel or extreme environments (Ghalambor et al., 2007, Lande, 2009). This is especially

timely in an era of dramatic global change; however, it remains to be seen how abruptly changing interactions of phenotypic change, fitness, and population abundance (i.e. eco-evolutionary dynamics) influences the long-term sustainability and persistence of exploited or invading species (Kinnison & Hairston, 2007, Kinnison et al., 2008).

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Chapter One Tables

Table 1-1. Contribution of invasive species and native species to available estimates of evolutionary change in two rate metrics, haldanes and darwins. Proportions of total available rate estimates are shown within the context of experimental design (allochronic or synchronic) and whether the rate derived from a trait with (genetic) or without (phenotypic) a determined heritable basis.

Species designation		Experimental design			
		Allochronic vs. Synchronic		Genetic vs. Phenotypic	
Invasive	haldanes (N=2131)	0.03	0.81	0.13	0.70
	darwins (N=2450)	0.02	0.81	0.13	0.72
Native	haldanes (N=539)	0.07	0.09	0.06	0.11
	darwins (N=439)	0.09	0.08	0.06	0.11

Table 1-2. Results of statistical comparisons between invasive species and native species based on mean absolute phenotypic change observed at the species, genus, and family taxonomic levels using metrics of Darwins and Haldanes. ANCOVA models were fit to subsets of data based on traits with (genetic) or without (phenotypic) determined heritable components or pooled data (Pheno & Gene). Sample sizes (N) represent the number of species involved in comparisons and values are F-statistics for the effects of classification (invasive or native), time (years or generations), and their interaction. Means are least-square estimates of phenotypic change in invasive and native species while controlling for time. Values significant at P<0.05* or P<0.01**.

				ANCOVA with interaction			ANCOVA without interaction		Means	
Rate metric	Taxanomic level	Study design	N: invasive/native	Classification	Time	Interaction	Classification	Time	Invasive	Native
Darwins	Species	Pheno & Gene	32/29	0.49	0.22	1.00	0.49	0.22	0.13	0.20
Darwins	Genus	Pheno & Gene	29/29	1.02	0.45	0.53	1.03	0.46	0.16	0.27
Darwins	Family	Pheno & Gene	23/23	0.48	0.67	1.84	0.05	0.32	0.16	0.27
Darwins	Species	Phenotypic only	23/19	2.77	0.01	0.00	2.83	0.01	0.12	0.08
Darwins	Species	Genetic only	15/11	3.89	1.39	0.00	4.06	1.46	0.15*	0.58*
Haldanes	Species	Pheno & Gene	33/29	3.91	0.00	19.1**	na	na	na	na
Haldanes	Genus	Pheno & Gene	30/26	3.50	0.02	16.5**	na	na	na	na
Haldanes	Family	Pheno & Gene	23/20	2.90	0.02	11.4**	na	na	na	na
Haldanes	Species	Phenotypic only	24/25	10.8*	0.01	43.6**	na	na	na	na
Haldanes	Species	Genetic only	13/10	0.15	0.14	0.638	0.15	0.14	1.50	2.24

Chapter One Figures

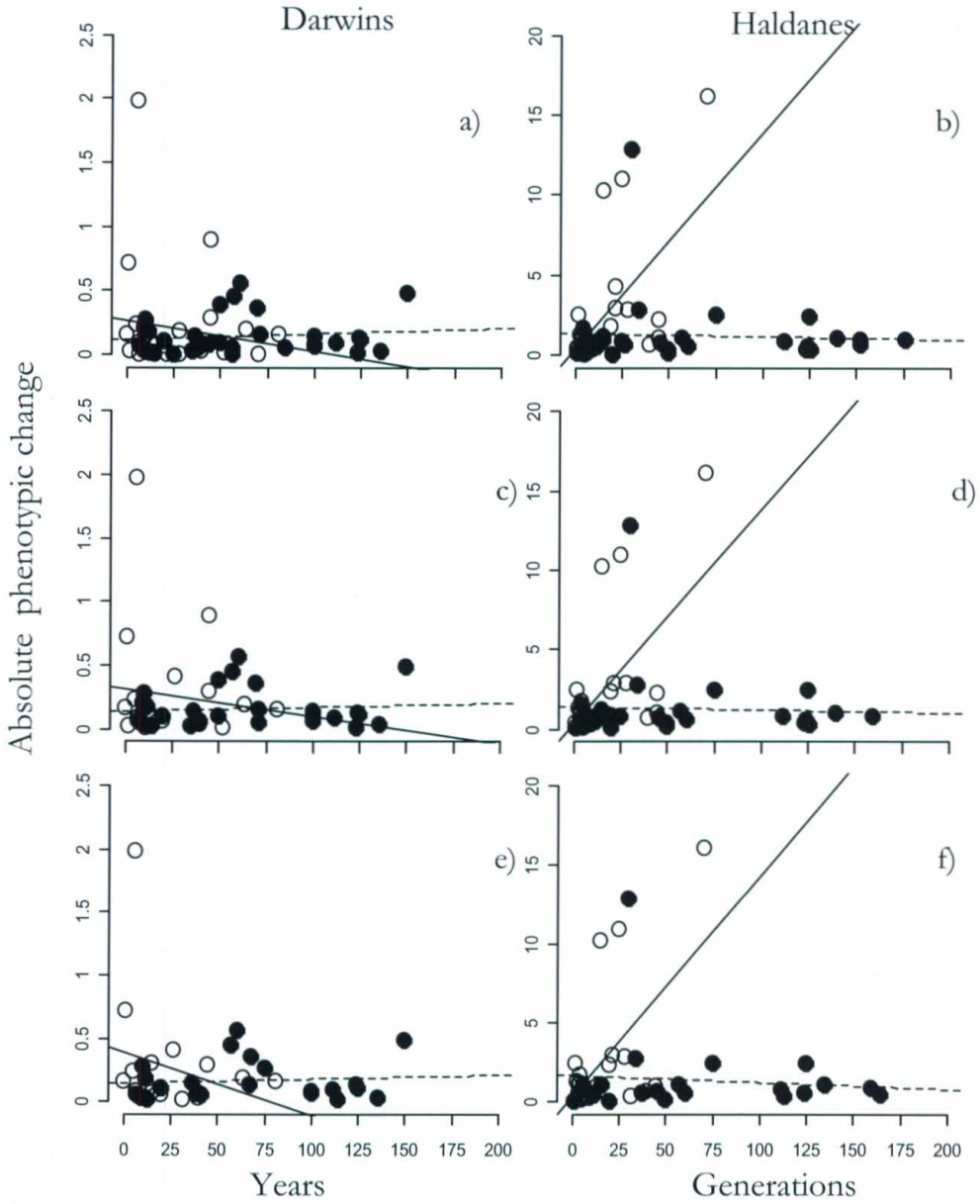

Fig. 1-1. Phenotypic changes in invasive species (filled points) and native species (open points) in darwins (left column) or haldanes (right column) expressed at the species (a,b), genus(c,d), and family (e,f) taxomomic level plotted as a function of time interval (years or generations for darwins or haldanes, respectively). Lines represent ordinary least squares regressions fit to averages from invasive species (dotted line) and native species (solid line).

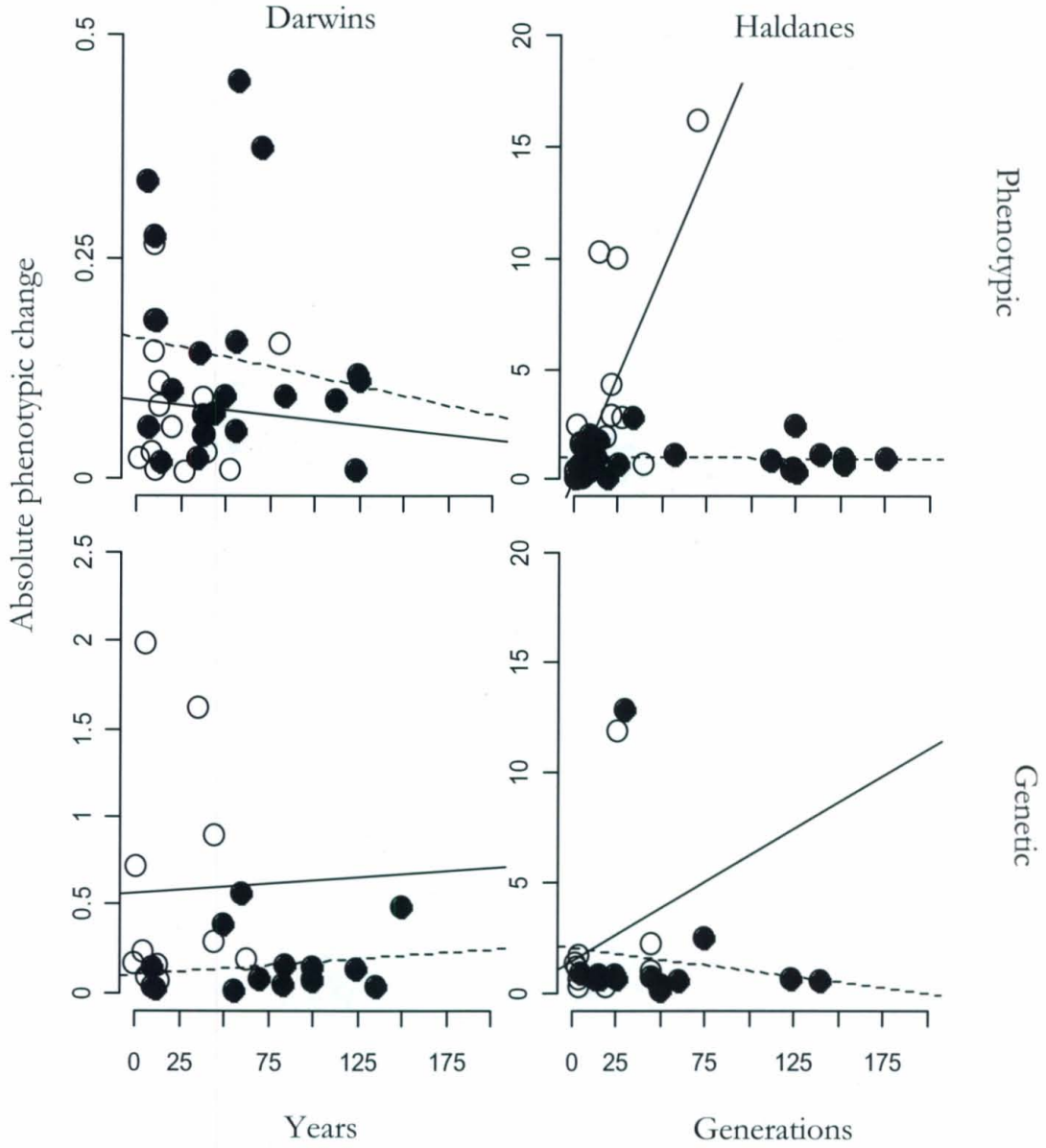

Fig. 1-2. Absolute phenotypic changes (darwin/haldane numerator) in invasive species (filled points) and native species (open points) in darwins (left column) or haldanes (right column) expressed at the species taxomomic level plotted and plotted as a function of time interval (years or generations for darwins or haldanes, respectively). Top panels represent 'Phenotypic' data where traits were measured in wild individuals and bottom panels are changes in traits measured in common-garden or quantitative genetic studies 'Genotypic'. Lines represent ordinary least squares regressions fit to averages from invasive species (dotted line) and native species (solid line).

Chapter 2: Landscape factors that shape a slow and persistent aquatic invasion— brown trout in Newfoundland 1883-2010

Abstract

Aim: We investigated watershed-scale abiotic environmental factors associated with population establishment of one of the 'world's 100 worst alien invaders' on a temperate Atlantic island. Within the context of the conservation implications, we aimed to quantify 1) the early history and demographics (numbers and origins) of human-mediated brown trout (*Salmo trutta*) introductions, 2) the current distribution of established populations, and 3) the indentify watershed-scale environmental factors that may resist or facilitate trout establishment.

Location: Island of Newfoundland, Canada.

Methods: We combined field sampling with historical and contemporary records from literature to assemble a presence-absence and physical habitat database for 312 watersheds on Newfoundland. Probability of watershed establishment was modelled with general additive ANCOVA models to control for non-linear effects of propagule pressure (i.e. the distance to and number of invasion foci within a biologically relevant range) and model performance based on AIC.

Results: Between 1883 and 1906, 16 watersheds were introduced with brown trout from the Howietoun Hatchery, near Stirling, Scotland. Since that time populations have established in 51 additional watersheds at an estimated rate of spread of 4 km yr^{-1} . We do not detect any obvious abiotic barriers to resist trout establishment, but show that for a given amount of propagule pressure that relatively large and productive watersheds are most likely to be established.

Main conclusions: Brown trout have successfully invaded and established in watersheds of Newfoundland and are currently slowly expanding on the island. Populations are more likely to establish in relatively large and productive watersheds, thereby supporting predictions of island biogeography theory. However, we suggest that all watersheds in Newfoundland are potentially susceptible to successful brown trout invasion and that abiotic factors alone are unlikely to sufficiently act as barriers to population establishment.

Introduction

Island biogeography theory (IBT) predicts that distributions of organisms are maintained by a dynamic balance between local extirpation and colonization (MacArthur & Wilson, 1967). According to classic IBT, the asymptotic number of species (i.e. species richness) should increase with increasing size of an island or habitat fragment and decrease with greater distance from a colonization source. IBT is elegant in its simplicity, has been supported empirically by iconic experimental manipulation of whole islands (Simberloff & Wilson, 1969) as well as contemporary research in fragmented landscapes (e.g. Leach & Givnish, 1996), and has been influential to the fields of conservation and invasion ecology (Losos *et al.*, 2009).

Humans frequently bridge the barriers to dispersal thereby facilitating the spread of organisms around the globe (Lockwood *et al.*, 2007) representing large-scale replicated experiments to test tenets of IBT (Sax *et al.*, 2005). One of the emerging insights of these imperfectly planned experiments is that ecological systems rarely show signs of saturation and that establishment of non-native species into novel environments is common (Sax *et al.*, 2007). However, the ability to successfully invade varies among taxa (Williamson, 1996, Jeschke & Strayer, 2005) and is context dependent (Korsu *et al.*, 2007) making predictions of which species will become invaders especially elusive. What is becoming increasingly clear is that vertebrates are exceptionally successful invaders, and once introduced have a high potential to become established (Jeschke & Strayer, 2005).

Freshwater fishes species of the genera *Micropterus* (Warner, 2005), *Oncorhynchus* (Crawford & Muir, 2008), *Salvelinus* (Dunham *et al.*, 2002), and *Salmo* (MacCrimmon &

Marshall, 1968) are successful global invaders, having been repeatedly spread through intentional introductions for recreational fishing and aquaculture. Recent years have shown a substantial increase in our understanding of the ecological risk factors (e.g. diet and niche breadth, temperature tolerances, and life history strategies) that likely underpin the success of these species (Kolar & Lodge, 2002, Olden et al., 2006, Ruesink, 2005). Additionally, recent work has highlighted the important role of propagule pressure, the societal motives behind the original introductions, and interactions with abiotic environmental features associated with invasion success (reviewed by Ruesink, 2005, Lockwood et al., 2005, Moyle & Marchetti, 2006). Taken together, these tools have greatly enhanced our ability to understand the patterns and processes behind the successful invasions of these freshwater fish species; however, continued work remains vital as members of these species are often implicated in the decline or extirpation of local species (McDowall, 2006) and the disruption of ecosystems (Schindler *et al.*, 2001).

Among the most successful freshwater fish invaders is brown trout (*S. trutta*). Brown trout has the ominous distinction as one of the '100 worst invasive alien species' by the Invasive Species Specialist Group (Lowe *et al.*, 2000), and is a current conservation concern in many regions including New Zealand (McDowall 2006), the Falkland Islands (McDowall *et al.*, 2001), the Patagonia region of South America (Pascual, 2007), and North America (Waters, 1983, van Zyll de Jong et al., 2004). One of the first sites of brown trout introduction to North America was to the island of Newfoundland in the late 19th century (Scott & Crossman, 1964, Andrews, 1965, Hustins, 2007 Fig. 2-1). The introduced trout, which were descendants of non-anadromous (i.e. freshwater resident) ancestors, quickly established self-sustaining populations and, as they have in other regions (e.g. Launey *et al.*,

2010), spread to new locations presumably by anadromous (i.e. sea-going) dispersers. However, very little is known about the current distribution of brown trout on the island and the physical environmental factors associated with establishment of watersheds are entirely unknown. Moreover, recent declines in populations of native salmonids (Atlantic salmon, *S. salar* and brook charr, *S. fontinalis*) in Newfoundland (DFO, 2006) mirror patterns of species displacement and competitive exclusion shown elsewhere (Waters, 1983, Korsu et al., 2007), thus a better understanding of the brown trout invasion is urgently needed for planning for conservation of native fishes.

The overarching goal of this paper is to quantify the watershed-scale factors associated with brown trout population establishment with the aim of informing future conservation plans for the long-term persistence of native fish. To meet this objective, we 1) document the early history and demographics (numbers and origins) of human-mediated brown trout introductions, 2) determine the current distribution of established populations, and 3) identify abiotic environmental variables associated with presence of trout populations in an attempt to elucidate the factors facilitating or impeding establishment. Here we combine field sampling and data assembled from literature and existing government databases to test predictions generated from IBT and invasion theory that probability of watershed establishment is positively associated with watershed size and productivity while controlling for distance to invasion sources (a surrogate for propagule pressure).

Materials and methods

Species description

Brown trout is a polytypic species with a native Eurasian distribution, which in the course of approximately 90 years (ca.1852-ca.1938) became a successful global invader via extensive intentional introductions (MacCrimmon & Marshall, 1968, Elliott, 1994). The life history of brown trout varies markedly among populations and among individuals within populations, but in general involves fall spawning by mature individuals in flowing waters, parental care by females in the form of egg burial, protracted embryonic development and use of small streams by juvenile trout (Baglinière & Maisse, 1999). Brown trout exhibit two alternative life history strategies, a complete life-time in freshwater (freshwater residency) or temporary feeding migrations to sea (anadromy) followed by homing to natal streams for reproduction (Stuart, 1957, Crisp, 2000, Jonsson & Jonsson, 1993). Brown trout are capable of highly accurate homing (Armstrong & Herbert, 1997); however, a small proportion of individuals either fails or 'decides' not to home and stray to other systems to breed. Straying by anadromous brown trout thereby represents a mechanism for invasion of suitable habitat (Launey *et al.*, 2010).

Invasion origins

The brown trout invasion process to Newfoundland follows the archetypical pattern of all successful biological invasions (Kolar & Lodge, 2001): *i*) *transport* of propagules and survival upon introduction, *ii*) *establishment* of populations, *iii*) *spread* to novel areas, and *iv*) ecological *impact*. A detailed history of brown trout importation and introductions goes beyond the scope and objectives of this paper so we only provide a brief overview here.

Shipments of trout embryos from the Howietoun hatchery in Stirling, Scotland began in 1883 and were followed by other importations in 1884, 1892, 1905-1906 (Frost, 1940, Andrews, 1965, Scott & Crossman, 1964, Hustins, 2007). The majority of imported trout were 'Scottish' strain, though latter shipments were comprised by 'English' and 'German' strains (Hustins, 2007). Imported trout survived well upon introduction and established populations in watersheds in the surrounding vicinity of St. John's (Maitland, 1887). Brown trout escaped into a watershed with a route to the sea in 1884 representing the first potential source of anadromous colonizers. Subsequent watersheds were established presumably by straying anadromous fish, though the timing and order of watershed invasion and establishment are unknown. Ecological impacts of the brown trout invasion are not well understood, but likely include competition and displacement of native fish (van Zyll de Jong et al., 2004, Gibson & Cunjak, 1986) and hybridization with Atlantic salmon (Verspoor, 1988, McGowan & Davidson, 1992). Readers should refer to Hustins (2007) and Fig. 2-2. for additional details.

Data sources and quality

Database of population establishment

We used multiple sources of data to address the invasion origins, distribution of established populations, and watershed factors associated with brown trout establishment. Data to investigate the historical origins and demographics of early introductions were compiled from Maitland (1887) and Hustins (2007 and references therein). We assembled a database of watersheds that are established or unestablished by brown trout populations from the Department of Fisheries and Oceans (DFO) Newfoundland Freshwater Salmonid

Inventory, which was initiated to provide base line data on all river systems on the island of Newfoundland[1]. We combined these records with historical data from Maitland (1887), Hustins (2007), and the reported distribution of brown trout by DFO's Angler Guide (DFO, 2010), which lists brown trout watersheds managed for sportfishing. In doing so, we recognized that the numbers and locations of historical stocking, as well as DFO's data on the current distribution of brown trout are conservative and have associated caveats. Uncertainty in the current known distribution of brown trout arises from a host of complicating factors such as angler effort in certain areas or habitats (e.g. estuaries or salt ponds), misidentifications with the closely related Atlantic salmon, and lack of reporting.

Thus in an attempt to address the uncertainty in the assembled presence-absence database we used data from independent field sampling in 2008 and 2010. We selected watersheds to sample within and near the edge of the presumed dispersal range of brown trout (Fig. 2-3). Our choice of watersheds reflects a balance in time and large distances to cover as well as objectives of other on-going complimentary projects concerning the trout invasion. We employed single pass upstream electrofishing with a backpack electrofisher for a minimum of an hour of active shocking time. We focused our sampling in the lower sections (~ 5km from the mouth) of watersheds assuming that if populations are established individuals are most likely detected in these parts of the watershed (for empirical examples of this pattern see Korsu et al., 2007, Budy et al., 2008). Moreover, we focused our efforts in habitats associated with brown trout, such as pools, cut banks, and side-channels (Armstrong et al., 2003, Westley et al., 2011). Taken together, we are confident that our sampling

[1] Brown trout presence absence data compiled from:
http://public.geoportal-geoportail.gc.ca/dfoGeoPortal/

protocol is sufficient in detecting thoroughly established populations as single pass electrofishing is frequently used to accurately assess trout populations in streams (Kruse *et al.*, 1998).

We managed to sample a total of 24 watersheds by electrofishing during 2008 and 2010. There was a strong concordance (96%) between the assembled presence-absence database and our field sampling. We found 100% agreement between our sampling and the database for seven systems reportedly absent of trout and brown trout were encountered in all but one of 17 reportedly established watersheds. Given subsequent evidence to support the presence of brown trout in this watershed (e.g. it is a managed brown trout system) we retained the record in the presence data. Furthermore, we returned to 10 watersheds in 2010 that had been surveyed in 2008 and again found evidence of established populations in all of those systems, indicating certain establishment.

(b) Abiotic environmental factors and propagule pressure

The DFO online database also contained two classes of watershed-scale environmental variables for 312 watersheds, measures of watershed size and water chemistry. The specific variables were: watershed area (km^2), watershed width (km), watershed length (km), watershed perimeter (km), watershed relief (m), length of mainstem flowing water (km), total length of flowing waters (km), number of tributaries, pH, hardness (ppm), conductivity ($\mu S\ cm^{-1}$ at 25°C), turbidity (J.T.U), alkalinity (ppm), calcium (ppm), chloride (ppm), bicarbonate (ppm). For more information on the collection and measurement of these variables, see (Porter *et al.*, 1974). Unfortunately, data on obstructions in watersheds were not sufficiently available for incorporation into our analyses. However, obstructions are only likely to be important when they form a complete barrier at the mouth

of a watershed as brown trout are apparently pre-adapted to establishing the lower sections of watersheds (Budy et al., 2008, Korsu et al., 2007). Environmental data were not available for 23 locations of established populations shown in Fig. 2-3. The locations with missing data result mainly from original stockings into landlocked ponds that were not surveyed by DFO and multiple sites of known populations within watersheds (e.g. six sites within the Rennie's watershed, Table 2-2) rather than inherent biases in how the database was assembled.

We attempted to elucidate the association between watershed establishment and physical environmental factors while controlling for distance to and number of nearby invasion foci (a surrogate for propagule pressure). We modelled propagule pressure as the interaction of the distance (km) of the mouth of each watershed to the mouth of the closest source watershed (i.e. established with brown trout) *and* the total number of sources within a 100-km radius of each watershed. We based the 100-km radius on the typical distance an anadromous brown trout may travel at sea (Klemetsen *et al.*, 2003 and references therein). Distances were calculated using the least cost distance tool in ArcGIS, v. 9.2 (ESRI), which provides a consistent and realistic framework for estimating distance through the ocean. That is, our estimates represent the shortest distance of a watershed to the source by excluding travel through land, thereby estimating the shortest distance a sea-going colonist would have to travel from the source to a potential invasion site. This surrogate measure of propagule pressure was applied and incorporated in our models as a smooth non-linear term following the general approach and logic of Rouget & Richardson (2003).

Data analysis of watershed-scale factors associated with establishment

We investigated the factors associated with watershed establishment in several steps. First, the relationship between presence and absence of brown trout was investigated using correlation and principal components analysis (PCA) on continuous physical environmental variables that were standardized to account for order of magnitude differences in watershed characteristics such as watershed area. PCA was used to distil a highly correlated set of 16 habitat variables data into a less-correlated data set for subsequent use in explaining brown trout establishment. The number of principal components used in interpretation was based on deviations from the broken-stick distribution as described by (Peres-Neto *et al.*, 2003).

We then used variables from this informative and less correlated data set to investigate brown trout presence and absence using a linear modelling information-theoretic framework. We formulated three a priori candidate models and assessed the weight of support of each model using (ΔAIC) as our selection metric which simply represents the difference between the AIC value of a candidate model to the AIC value of the candidate model with the lowest AIC value. We interpreted models with ΔAIC scores of 0-3 to have substantial empirical support, scores of 4-7 to have markedly less support, and scores of greater than 7 to have very little support (Burnham & Anderson, 2002). Additionally, we calculated AIC weights as a measure of modelling selection uncertainty. We interpret AIC weights as the probability of selecting a candidate model as the best model if the modelling procedure was done many times (Hobbs & Hilborn, 2006). We fit binomial ANCOVA models, with binomial error and logit link, using the GAM function in the 'mgcv' library in R v. 2.10.1 to account for auto-correlation and non-linearity in our covariate surrogate for propagule pressure (Crawley, 2007).

Results

Invasion demographics

The records we have compiled indicate that at least 156,000 juvenile brown trout were introduced across 21 locations in the immediate vicinity of St. John's and to adjacent communities (Table 2-1, Fig. 2-1.). These records also indicate that the preponderance (93%) of the 156,000 brown trout introduced to Newfoundland waters were of the Scotish Loch Leven-strain originating from the Howietoun hatchery. In contrast, only 7% of the originally introduced trout were of the German von Behr-strain. Unfortunately no records of numbers of stocked English-strain brown trout are known for the stockings that did occur.

Current distribution

The number of watersheds established by brown trout increased four-fold from 16 in 1883 (Table 2-1) to 68 in 2009 (Table 2-2). Brown trout populations are currently established in watersheds on the Avalon, Burin, and Bonavista peninsulas (Fig. 2-3).

Environmental factors associated with establishment

The pattern of watershed establishment in (Fig. 2-3.) and initial analyses including all 312 watersheds suggested dispersal limitation by brown trout. Only our measure of propagule pressure had any power to predict establishment (results not shown) and inclusion of these systems obscured the role of environmental factors associated with establishment elsewhere. Thus, for the remainder of the study we focused on elucidating environmental factors associated with established (n=45) or unestablished (n=68) watersheds within the presumed trout dispersal range (Fig. 2-3).

Forty percent of the watersheds examined were established by brown trout (45 established/113 total) and abiotic environmental variables varied markedly (Table 2-3) between these watersheds; however, many variables were highly correlated (Table 2-4). Thus, a principal components analysis (PCA) facilitated the distillation and interpretation of these highly correlated habitat characteristics for quantifying presence or absence of established brown trout populations. The first two axes of the PCA explained 69% of the total variance in the data (Table 2-5) and were the only axes interpreted based on the broken-stick method. The first axis described a gradient of increasing watershed area, width, length, perimeter, length of mainstem river, total length of flowing waters, and number of tributaries. The second axis described a gradient of watersheds with increasing pH, hardness, conductivity, alkalinity, calcium, and bicarbonate.

We modelled the importance of watershed area (representing PCA axis 1) and conductivity (PCA axis 2) on predicting brown trout presence or absence while controlling for the non-linear effect of propagule pressure. We chose to use these important variables from the two axes of the PCA rather than principal component scores in our subsequent modelling to facilitate direct interpretation and to correspond to predictions of biogeography (e.g. larger watersheds should be more likely to be established than smaller ones). Watershed area and conductivity values were logarithmically transformed prior to modelling to meet parametric assumptions. We chose to model the importance of conductivity because conductivity correlates with important biological processes in Newfoundland (Adams, 2006) and has been used elsewhere as a surrogate for watershed productivity (Copp, 2003, Ryder, 1982).

Brown trout establishment was positively associated with both watershed area and conductivity. We found strong evidence in favour of a model containing watershed area and conductivity as parametric predictors and a measure of propagule pressure as a smoothed term covariate. This model explained 80% of the observed deviance and received virtually indisputable support based on the model's AIC weight (w_i =0.99). In contrast we found little support for models containing only conductivity (deviance explained = 66%, ΔAIC =15, w_i = 0.01) or watershed area (deviance explained = 25%, ΔAIC =31, w_i ~0) again while controlling for non-linear effects of propagule pressure.

Discussion

Brown trout have successfully invaded and established populations are slowly expanding on the island of Newfoundland. The initial roots of the trout invasion trace their origins to the Howietoun Hatchery in Stirling, Scotland and were predominately descendants of non-anadromous Loch Leven broodstock. Approximately 125 years since their first introduction, brown trout have spread from 16 watersheds of introduction to invade and establish populations in at least 51 additional watersheds. Our results suggest that the brown trout invasion is a contemporary process, as new populations have continued to establish over the past two decades. Modelling the presence-absence of established brown trout populations in watersheds of Newfoundland indicates that for a given measure of propagule pressure large and productive watersheds are more likely to be established relative to unestablished watersheds. Taken as a whole, these results suggest an important role of watershed area and productivity in the dynamics of establishment by brown trout in

Newfoundland watersheds, but also suggest that no watershed is inherently immune to establishment.

Invasion origins and current distribution

Documenting the history and demography of a species' introduction is an important first step towards understanding the dynamics of a biological invasion. Our documentation of the history surrounding the invasion of brown trout to the island of Newfoundland yield several salient points. The majority of introduced trout were descendants of non-anadromous (i.e. freshwater resident) Loch Leven parents (Hustins, 2007) though anadromous (sea-going) populations of brown trout are currently common in Newfoundland watersheds (van Zyll de Jong *et al.*, 2004). Hatchery propagation of brown trout ceased by the beginning of the 20th century, which makes the current distribution of brown trout in watersheds of Newfoundland particularly striking. We suggest, as others have (Bradbury et al., 1999, van Zyll de Jong et al., 2004), that the majority of watersheds have been established by straying anadromous trout, a pattern documented in other brown trout invasions (e.g. Launey *et al.* 2010). Fish with anadromous life histories are difficult to transplant outside the native range (Quinn et al., 2001a) and anadromy is often implicated in the failure of transplanted species to establish (Quinn, 2005). Brown trout in Newfoundland are thus a rare exception where anadromy and subsequent straying are primary drivers of invasion success.

The Newfoundland brown trout invasion is apparently an on-going contemporary process. By combining data sources with our field surveys, we confirmed several systems to be established within a twenty year period. For example, our electrofishing surveys confirmed the presence of an established population in the Southeast Placentia River (Fig. 2-

3), which apparently had not been established when (Verspoor, 1988) thoroughly sampled this river. Similarly, the presence of an established population in Bonavista Bay (Fig. 2-3) is likely now acting as a source of colonists to slowly expand the range westward.

Unfortunately the data assembled here do not reveal information on the founders of established watersheds and underlying interactions between founders and landscape factors are possible as three 'strains' of trout were originally imported and introduced to Newfoundland waters. It is possible that watersheds in Newfoundland have been colonized by 'favoured founders' who represent non-random pre-adapted subsets of potential colonists (sensu Quinn *et al.* 2001). In a recent empirical example, Launey et al. (2010) show that by combining microsatellite information to assess founder origins with demography they are able to better understand the processes by which brown trout introduced to three rivers on the Kerguelen Islands have successfully colonized 16 additional rivers in approximately 40 years.

Landscape factors associated with establishment

Watershed establishment was positively associated with watershed area and conductivity (a surrogate for productivity) while controlling for the influence of propagule pressure. Large watersheds are more likely to receive colonizers based on chance alone (MacArthur & Wilson, 1967), but may also attract roaming potential colonizers, thereby increasing the propagule pressure experienced by these watersheds. This is possible given the general pattern that some watersheds serve as 'magnets' to straying salmonid species, though why some rivers are more attractive than others is not known (reviewed in Quinn, 2005). However, our analysis attempted to control for the effect of propagule pressure and thus suggests that large watersheds are easier to establish relative to smaller watersheds. The

positive association between watershed size and establishment is in general agreement with predictions of island biogeography theory and corroborates patterns found in translocated cutthroat trout (*O.clarki*, Haring & Fausch, 2002) and brown trout (Marchetti et al., 2004, Launey et al., 2010) populations. The positive relationship between watershed area and trout invasion are consistent with species saturation and biological resistance to invasion at small scales (Levine, 2000). However, the mechanisms underpinning invasion success may vary at fine (e.g. within habitat segments of a river) or coarse (e.g. watershed) spatial scales and are thus difficult interpret. The ability of a biological community to resist invasions varies among scales (Shea & Chesson, 2002, Levine & D'Antonio, 1999) and have received particular attention in plant species where spatial heterogeneity of resources appear to explain this scale dependence (Davies *et al.*, 2005). In California, watersheds with the most invasive species also contain the most native species (Marchetti et al., 2004, Moyle & Marchetti, 2006) though biotic interactions, such as predation, may enhance community resistance to invasion within river segments of these watersheds (Harvey *et al.*, 2004).

Watershed productivity, which we modelled with water conductivity as a surrogate (Ryder, 1982), was also an important factor associated with establishment. This result supports the Subsidized Island Biogeography hypothesis (sensu Anderson & Wait, 2001) that suggests an important role of allochthonous inputs in predicting species diversity on small islands or habitat fragments. The low productivity and high acidity of Newfoundland watersheds (Table 2-3) likely provides a proximate explanation for the relatively slow growth by stream-dwelling Atlantic salmon compared to their European counterparts (Hutchings & Jones, 1998) and have been used to predict fish assemblages in Newfoundland lakes (Van Zyll de Jong *et al.*, 2005). It is possible that low productivity reduces the probability of

successful establishment by brown trout via increased inter and/or intra-specific competition for limited food resources (Elton, 1958). Additionally, productivity often correlates with other potentially important variables such as disturbance (Lockwood *et al.*, 2007). Anthropogenic sources of disturbance in watersheds of Newfoundland are minor except for those containing large human populations, such as those near St. John's where streams have been channelized and flow regimes altered (Gibson & Haedrich, 1988). Paradoxically, growth of salmonids in these disturbed city rivers is exceptionally high compared to other watersheds on the island, presumably due to high nutrient input (Gibson & Haedrich, 1988). Thus, productivity and disturbance appeared correlated in some Newfoundland systems, though data deficiencies preclude a formal evaluation of these ideas. Curiously, disturbance does not appear a necessary condition for successful salmonid establishment, thereby suggesting a role of productivity per se. For example, brown trout are associated with relatively undisturbed watersheds in California (Marchetti *et al.*, 2004) and Chinook salmon have invaded the virtually pristine region of Patagonia (Correa & Gross, 2008).

Future outlook and conclusions

Brown trout have successfully invaded the island of Newfoundland and in approximately 125 years established populations in a range of watersheds; however, their apparent rate of spread is comparably slow relative to other documented salmonid invasions. In twenty-five years, Chinook salmon invaded a large portion of South America (14 degrees of latitude) at a rate of approximately 54km/yr (Correa & Gross, 2008) and in New Zealand Chinook invaded at a rate of approximately 13km/yr (Unwin & Quinn, 1993). Similarly, pink salmon (*O. gorbuscha*) have rapidly spread throughout the vast Great Lakes Basin since their introduction into the Current River, a tributary of Lake Superior, in 1956 (Mills *et al.*, 1993).

In contrast, brown trout on the Island of Newfoundland have established in watersheds within 500 km of the primary introduction sources near St. John's, which translates to a modest 4km/yr invasion rate. Assuming this rate remains constant the most distant watersheds in Newfoundland would not be expected to be established until the 24th century. Managers should take caution in this latter assumption, however, as many invading species exhibit periods of slow population growth followed by dramatic non-linear rates of establishment and spread after variable amounts of lag time (Facon *et al.*, 2006).

Previous work on the biology of anadromous brown trout in Newfoundland suggests at least two mechanisms to explain this relatively slow invasion rate. First, O'Connell (1982) reports small distance (typically less than 50 km) marine migrations by anadromous trout in Newfoundland, which led him to suggest that these short migrations reduced the probability of fish straying into suitable watersheds. Second, O'Connell (1982) reported a high proportion of upstream migrating adults that were not maturing in a given season (i.e. skip spawning), thereby slowing the rate of population growth, slowing the time to habitat saturation, and potentially reducing the number of strays produced. Brown trout exhibit highly variable life history traits such as age and size at maturity and skipped spawning between seasons appears common in populations (Klemetsen *et al.*, 2003); however, it is not clear how skipped spawning may alter population dynamics and in turn how this may affect the rate of dispersal by brown trout or other invading species (Kot *et al.*, 1996).

In conclusion, our analyses suggest that all watersheds in Newfoundland are susceptible to trout invasion as abiotic environmental factors substantially overlap between established and unestablished systems (Table 2-3). That is, we detected no obvious abiotic

factors acting as strong barriers to establishment. However, we do show that trout are more likely to establish in relatively large and productive watersheds after controlling for the effect of propagule pressure (i.e. the distance to and the total number of potential nearby sources) and thus it seems likely smaller and less productive watersheds will become established given sufficient time and propagule pressure. Indeed, we provide evidence of a dispersal boundary that suggests it is only a matter of time, albeit potentially a long time, before distant watersheds beyond this boundary receives invaders. Therefore, we suggest extra vigilance to detect early invaders in these especially susceptible systems as results presented here coupled with global patterns suggest that establishment is likely in brown trout invasions.

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Chapter Two Tables

Table 2-1. Watershed, waterbody (location within watersheds), destination of water from watersheds or landlocked if water does not drain to sea, geographical coordinates (degrees, minutes, decimal seconds) of waterbody locations, year and number of individuals introduced (when available), and source strain of brown trout introduced to the island of Newfoundland. Data compiled from Hustins (2007).

Watershed	Location	Destination	Latitude	Longitude	Year (number introduced)	Source strain
Bauline	Whiteway's	Landlocked	47 39 52.23	52 45 55.74	1892, 1896 (1,000)	German
Brigus	Hodgewater Pond	Conception Bay	47 30 27.73	53 16 17.24	1892	German
Clement	Clement's Pond	Landlocked	47 30 58.28	52 55 31.50	1905-1906	English
Cove Pond	Cove Road Ponds	Landlocked	47 25 02.28	53 09 00.70	1886 (10,000)	Loch Leven
Dildo	South Dildo Pond	Trinity Bay	47 29 46.97	53 32 46.90	1889 (10,000)	Loch Leven
Lee's	Lee's Pond	Conception Bay	47 24 30.92	53 11 35.91	1896 (4,000)	German
Lee's	Lee's Pond	Conception Bay	47 24 30.92	53 11 35.91	1905-1906	English
Lower Island	Lower Island Ponds	Landlocked	48 00 13.02	52 59 46.28	1888 (10,000)	Loch Leven
Mundy's	Mundy's Pond	Landlocked	47 33 06.30	52 44 22.10	1886 (5,000)	Loch Leven
Murray's	Murray's Pond	Landlocked	47 36 51.69	52 49 13.01	1905-1906	English
Petty Harbour	Petty Harbour Ponds	Atlantic (eastern Avalon)*	47 27 07.41	52 42 35.68	1888 (10,000), 1889 (3,000)	Loch Leven
Rennies	Long Pond	St.John's	47 34 40.99	52 44 00.74	1888 (40,000)	Loch Leven
Rennies	Quidi Vidi	St.John's	47 34 52.53	52 41 23.77	1886 (10,000)	Loch Leven
Rennies	Rennie's River	St.John's	47 34 40.45	52 42 57.34	1884	Loch Leven
Rennies	Upper Long Pond	St.John's	47 34 16.08	52 45 46.64	1886 (20,000)	Loch Leven
Rennies	Virginia Lake	St.John's	47 36 24.39	52 42 07.18	1886 (1,000)	Loch Leven
Robin's	Robin's Ponds	Landlocked	47 39 25.87	52 45 42.90	1892 (1,000)	German
Rocky	Hodge Water Cat Hills	St.Mary's Bay	47 24 46.72	53 31 59.86	1896 (4,000)	German
Rocky	Ocean Pond	St. Mary's Bay	47 27 23.13	53 37 45.18	1892	German
Topsail	Topsail Road Ponds	Conception Bay	47 32 03.92	52 56 39.64	1886 (15,000), 1889 (2000)	Loch Leven
Trinity	Trinity Bay Ponds	Trinity Bay	48 22 20.32	53 23 22.84	1889	Loch Leven
Windsor	Windsor Lake	St.John's	47 35 55.07	52 47 34.00	1883 (5,000), 1884 (5,000)	Loch Leven

*Currently landlocked due to impassable hydropower plant

Table 2-2. Characteristics of established brown trout systems in Newfoundland. Established watersheds (presented in alphabetical order), waterbody (location within watersheds), geographic coordinates, source strain (if known), and applicable reference.

Watershed	Waterbody	Latitude	Longitude	Source strain	Reference
Aquaforte	Aquaforte River	47 00 17.86	52 59 10.07	Natural colonization (source unknown)	DFO (2008)
Avondale	Avondale River	47 26 07.09	53 12 23.99	Natural colonization (source unknown)	DFO (2008)
Bauline	Whiteway's	47 39 52.23	52 45 55.74	German	Hustins (2007)
Bauline	Whiteway's River	47 41 05.59	53 28 13.50	Natural colonization (source unknown)	DFO (2008)
Biscay Bay	Biscay Bay River	46 47 01.46	53 16 43.93	Natural colonization (source unknown)	DFO (2008)
Brigus	Hodgewater Pond	47 30 27.73	53 16 17.24	German	Hustins (2007)
Cape Broyle	Cape Broyle River	47 05 35.41	52 58 38.33	Natural colonization (source unknown)	DFO (2008)
Chance Cove	Chance Cove Brook	47 38 38.07	53 48 39.73	Natural colonization (source unknown)	DFO (2008)
Chapel Arm	Chapel Arm River	47 31 07.57	53 42 09.20	Natural colonization (source unknown)	DFO (2008)
Clement	Clement's Pond	47 30 58.28	52 55 31.50	English	Hustins (2007)
Colinet	Colinet river	47 13 15.60	53 32 56.26	German	Hustins (2007)
Colliers	Colliers Bay River	47 35 16.04	53 42 37.94	Natural colonization (source unknown)	DFO (2008)
Colliers	Colliers River	47 27 17.76	53 14 07.10	Natural colonization (source unknown)	DFO (2008)
Come by Chance	Come by Chance River	47 50 48.23	53 58 54.65	Natural colonization (source unknown)	DFO (2008)
Cove Pond	Cove Road Ponds	47 25 02.28	53 09 00.70	Loch Leven	Hustins (2007)
Dildo	South Dildo Pond	47 29 46.97	53 32 46.90	Loch Leven	Hustins (2007)
Dildo	South Dildo River	47 32 50.77	53 31 38.99	Natural colonization (source unknown)	DFO (2008)
Green's harbour	Green's Harbour River	47 37 37.20	53.29 36.14	Natural colonization (source unknown)	DFO (2008)
Harbour Main	Gallows Cove	47 27 14.39	53 05 26.14	Natural colonization (source unknown)	DFO Salmonid Fish Inventory
Harry's	Harry's Pond	47 46 53.25	53 11 00.34	Natural colonization (source unknown)	DFO (2008)
Heart's Content	Heart's Content Brook	47 52 39.88	53 20 28.96	Natural colonization (source unknown)	DFO (2008)
Heart's Content	Musquash Pond*	47 52 26.15	53 22 05.66	Loch Leven	Hustins (2007)
Heart's Delight	Heart's Delight River	47 46 10.72	53 27 01.85	Natural colonization (source unknown)	DFO (2008)
Holyrood	Holyrood Pond	46 49 35.72	53 36 27.08	Natural colonization (source unknown)	DFO Salmonid Fish Inventory
Hopeall	Hopeall River	47 36 06.82	53 30 35.12	Natural colonization (source unknown)	DFO (2008)
Indian Pond	Indian Pond	47 27 15.21	53 05 25.17	Natural colonization (source unknown)	DFO (2008)
Island Pond	Island Pond Brook	47 43 59.14	53 13 50.18	Natural colonization (source unknown)	DFO (2008)
Kelligrews	Kelligrews River	47 29 40.21	53 00 32.78	Natural colonization (source unknown)	DFO (2008)
Lee's	Lee's Pond	47 24 30.92	53 11 35.91	German	Hustins (2007)
Lee's	Lee's Pond	47 24 30.92	53 11 35.91	English	Hustins (2007)
Little Salmonier	Little Salmonier River	47 02 43.23	53 44 10.37	Natural colonization (source unknown)	DFO Salmonid Fish Inventory
Lower Gullies	Lower Gullies River	47 28 27.36	53 01 48.30	Natural colonization (source unknown)	DFO (2008)
Lower Island	Lower Island Ponds	48 00 13.02	52 59 46.28	Loch Leven	Hustins (2007)
Manuel	Manuels River	47 30 59.72	52 46 30.97	Natural colonization (source unknown)	DFO (2008)
Mobile	Mobile River	47 15 12.06	52 53 06.83	Natural colonization (source unknown)	DFO (2008)

Table 2-2. Continued

Watershed	Waterbody	Latitude	Longitude	Source strain	Reference
Mozzen	Mozzen Pond	47 52 26.15	53 22 05.66	Natural colonization (source unknown)	DFO (2008)
Mundy	Mundy's Pond	47 33 06.30	52 44 22.10	Loch Leven	Hustins (2007)
Murray	Murray's Pond	47 36 51.69	52 49 13.01	English	Hustins (2007)
NE Placentia	NE Placentia River	47 13 37.19	53 52 30.66	Natural colonization (source unknown)	DFO (2008)
NE River	NE River	46 45 15.81	53 16 47.27	Natural colonization (source unknown)	Verspoor (1988)
New Harbour	New Harbour River	47 34 38.55	53 32 32.52	Natural colonization (source unknown)	DFO (2008)
North Arm	North Arm River	47 23 34.44	53 09 27.80	Natural colonization (source unknown)	Gibson and Cunjak (1986)
North Harbour	North Harbour River	47 10 55.10	53 37 47.84	German	Hustins (2007)
North River	North River	47 32 27.60	53 18 39.74	Natural colonization (source unknown)	DFO (2008)
Northwest River	Northwest River	46 45 52.76	53 21 05.91	Natural colonization (source unknown)	DFO (2008)
O'Donnells	O'Donnells	46 45 05.12	53 36 10.66	Natural colonization (source unknown)	DFO Salmonid Fish Inventory
Old Shop	Old Shop	47 32 00.40	53 35 47.40	Natural colonization (source unknown)	DFO Salmonid Fish Inventory
Petty Harbour	Petty Harbour Ponds	47 27 07.41	52 42 35.68	Loch Leven	Hustins (2007)
Piper's	Pipers Hole River	47 55 24.89	54 16 26.16	Natural colonization (source unknown)	DFO (2008)
Point Verde	Point Verde	47 13 31.39	54 00 48.75	Natural colonization (source unknown)	DFO Salmonid Fish Inventory
Portugal Cove South	Stoney River	46 47 01.46	53 16 43.93	Natural colonization (source unknown)	Enders et al. 2007
Princeton	Princeton Brook	48 39 33.36	53 06 56.66	Natural colonization (source unknown)	DFO (2008)
Renews	Renews River	46 56 33.03	52 58 32.11	Natural colonization (source unknown)	DFO (2008)
Rennies	Long Pond	47 34 40.99	52 44 00.74	Loch Leven	Hustins (2007)
Rennies	Quidi Vidi	47 34 52.53	52 41 23.77	Loch Leven	Hustins (2007)
Rennies	Quidi Vidi River	47 34 52.53	52 41 23.77	Natural colonization (source unknown)	DFO (2008)
Rennies	Rennie's River	47 34 40.45	52 42 57.34	Loch Leven	Hustins (2007)
Rennies	Upper Long Pond	47 34 16.08	52 45 46.64	Loch Leven	Hustins (2007)
Rennies	Virginia Lake	47 36 24.39	52 42 07.18	Loch Leven	Hustins (2007)
Rexton	Robin Hood Pond	48 23 42. 28	53 19 32.12	Natural colonization (source unknown)	DFO (2008)
Robin's	Robin's Ponds	47 39 25.87	52 45 42.90	German	Hustins (2007)
Rocky	Ocean Pond	47 27 23.13	53 37 45.18	German	Hustins (2007)
Rocky	Rocky River	47 13 57.03	53 33 22.01	German	Hustins (2007)
Rocky	Hodge Water Cat Hills	47 24 46.72	53 31 59.86	German	Hustins (2007)
Salmon Cove	Salmon Cove River	47 46 55.43	53 10 30.50	Natural colonization (source unknown)	DFO (2008)
Salmonier	Salmonier	47 10 25.84	53 39 47.84	German	Hustins (2007)
SE Placentia	SE Placentia River	47 13 10.96	53 55 13.49	Natural colonization (source unknown)	DFO (2008)
Seal Cove	Seal Cove River	47 27 59.53	53 04 11.72	Natural colonization (source unknown)	DFO (2008)
Shearstown	Shearstown River	47 35 26.05	53 18 15.23	Natural colonization (source unknown)	DFO (2008)
Shoal Harbour	Shoal Harbour River	48 11 36.66	54 00 58.52	Natural colonization (source unknown)	DFO (2008)
South River	South River	47 32 13.73	53 16 27.39	Natural colonization (source unknown)	DFO (2008)
Spread Eagle	Spread Eagle River	47 31 50.73	53 36 56.52	Natural colonization (source unknown)	DFO (2008)
Stone Ducky	Stone Ducky Brook	47 19 46.39	52 49 14.84	Natural colonization (source unknown)	DFO (2008)
Topsail	Topsail River	47 31 35.38	52 54 19.92	Natural colonization (source unknown)	DFO (2008)
Topsail	Topsail Road Ponds	47 32 03.92	52 56 39.64	Loch Leven	Hustins (2007)
Trinity	Trinity Bay Ponds	48 22 20.32	53 23 22.84	Loch Leven	Hustins (2007)
Waterford	Waterford River	47 32 24.86	52 43 39.12	Natural colonization (source unknown)	DFO (2008)
Windsor	Windsor Lake	47 35 55.07	52 47 34.00	Loch Leven	Hustins (2007)
Witless	Pierre's Brook	47 15 08.18	52 51 40.21	Natural colonization (source unknown)	DFO (2008)

*Evidence for the strain of origin is based on an unsubstantiated historical letter cited in Hustins (2007)

Table 2-3. Factors associated with watershed unestablishment (absent) and establishment (present) by brown trout in Newfoundland. Values represent the mean ± standard deviation (SD) of each (n) watershed. See text for description of variables and units of measure.

	Absent (n=68)	Present (n=45)
Distance to nearest source	18 (20)	11 (14)
Number of sources	13 (8)	14 (7)
Watershed area	72 (86)	98 (123)
Watershed width	4 (2)	5 (3)
Watershed length	14 (8)	16 (8)
Watershed perimeter	44 (27)	54 (32)
Watershed relief	243 (53)	259 (60)
Length of mainstem river	12 (10)	13 (9)
Total length of flowing water	64 (76)	66 (81)
Number of tributaries	18 (14)	17 (13)
pH	6.2 (0.4)	6.4 (0.4)
Hardness	7.8 (6.5)	6.8 (3.1)
Conductivity	31.2 (9.4)	40.4 (42.4)
Turbidity	1.4 (1.0)	1.1 (1.0)
Alkalinity	2.9 (1.9)	2.7 (1.4
Calcium	1.4 (1.4)	1.4 (1.0)
Chloride	7.4 (2.4)	7.8 (6.4)
Bicarbonate	4.3 (2.3)	3.5 (1.7)

Table 2-4. Pearson correlation values of 16 habitat characteristics used in modelling brown trout establishment in watersheds on the island of Newfoundland. Correlations between variables greater than 0.5 are highlighted in grey. See text for description of variables and units of measure.

	area	width	length	perim	relief	main_len	tot_len	num_tribs	pH	hard	conduct	turb	alk	cal	chl	bicarb
drainage area	1															
axial width	0.85	1														
axial length	0.86	0.73	1													
perimeter	0.94	0.84	0.85	1												
relief	0.47	0.57	0.4	0.52	1											
mainstem length	0.71	0.48	0.76	0.75	0.29	1										
total length of flowing water	0.88	0.81	0.74	0.85	0.49	0.6	1									
number of tributaries	0.61	0.56	0.62	0.68	0.42	0.66	0.61	1								
pH	0.05	0.2	0.09	-0.06	0.13	-0.14	0.01	-0.16	1							
hardness	0.12	0.27	0.26	0.05	0.34	0.01	0.09	-0.08	0.65	1						
conductivity	-0.16	-0.08	-0.07	-0.23	0.1	-0.19	-0.19	-0.27	0.57	0.64	1					
turbidity	0.19	0.19	0.26	0.23	0.57	0.26	0.22	0.26	0.08	0.3	0.35	1				
alkalinity	0.07	0.19	0.06	0.02	0.29	-0.05	0.05	-0.08	0.66	0.8	0.58	0.24	1			
calcium	0.08	0.26	0.21	0.01	0.35	-0.08	0.04	-0.13	0.75	0.89	0.79	0.36	0.8	1		
chloride	-0.31	-0.33	-0.32	-0.33	-0.16	-0.22	-0.29	-0.24	0.11	0.05	0.7	0.18	-0.01	0.21	1	
bicarbonate	0.08	0.23	0.23	-0.02	0.25	-0.09	0.04	-0.11	0.76	0.89	0.63	0.24	0.87	0.92	-0.04	1

Table 2-5. Results of a principal components analysis (PCA) on environmental variables of Newfoundland watersheds. See text for description of variables and units of measure.

	PCI	PCII
Eigenvalues	2.47	2.24
Cummulative % variance	38	69
Eigenvectors		
drainage area	0.369	-0.105
axial width	0.357	-0.025
axial length	0.359	-0.055
perimeter	0.367	-0.143
relief	0.267	0.069
mainstem length	0.290	-0.149
total length of flowing water	0.347	-0.111
number of tributaries	0.278	-0.166
pH	0.080	0.345
hardness	0.150	0.375
conductivity	-0.003	0.381
turbidity	0.159	0.119
alkalinity	0.117	0.364
calcium	0.132	0.411
chloride	-0.131	0.154
bicarbonate	0.130	0.393

Chapter Two Figures

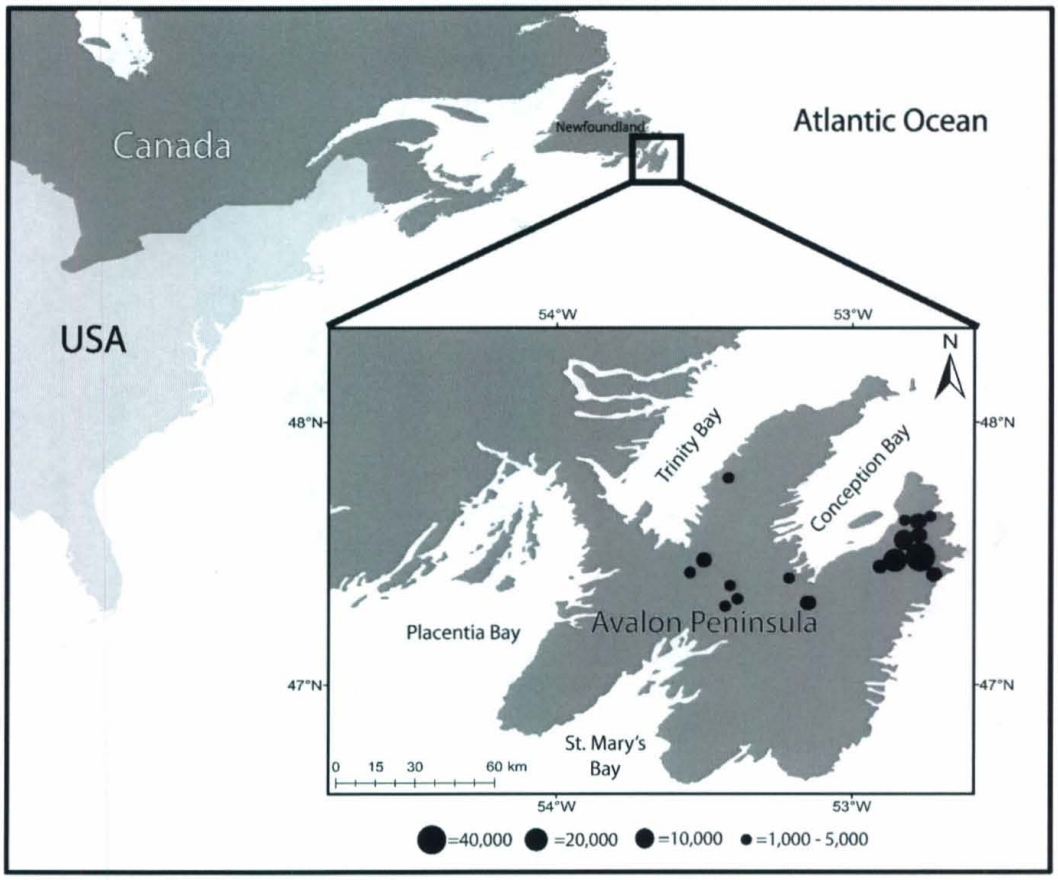

Fig. 2-1. Island of Newfoundland showing watersheds (denoted by filled circles) of brown trout introductions on the Avalon Peninsula, where the size of the circle is roughly proportional to the numbers of trout introduced to a given watershed. See Table 2-1 for additional information.

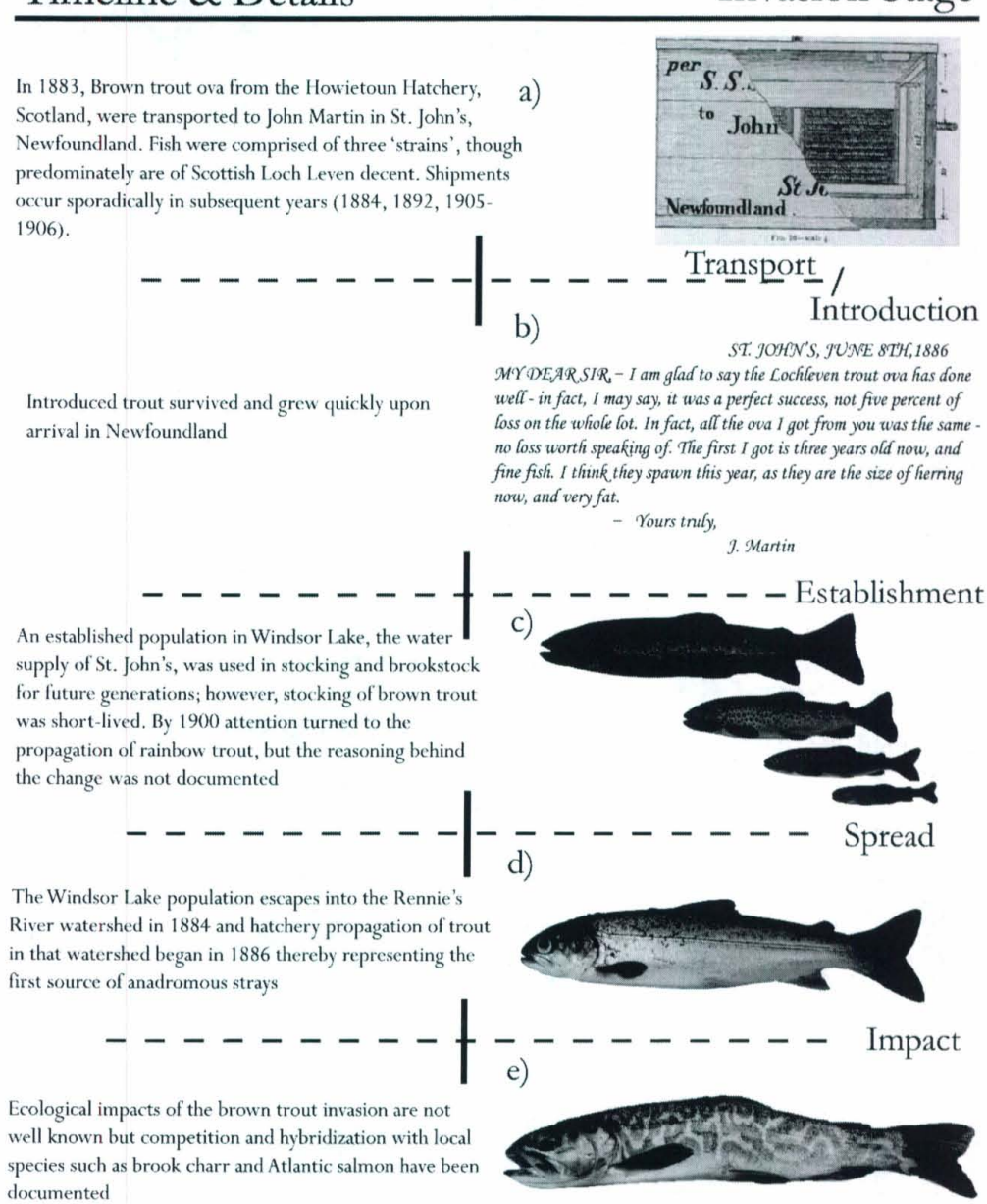

Fig. 2-2. An annotated timeline of the brown trout invasion process to the island of Newfoundland. Discrete stages in the invasion process follow the logic of (Lockwood et al., 2007, Kolar & Lodge, 2002) and are denoted by dashed horizontal lines. Important dates and details of the invasion are provided on the left side of the figure and supporting images at each stage are provided on the right a) image of the original shipping container for transporting brown trout ova showing its intended destination to John Martin and St. John's, Newfoundland (Maitland, 1887), b) excerpt from a letter by John Martin where he proclaims successful importation of brown trout (Maitland, 1887), c) images of representative size and age classes of brown trout, which we take as evidence for establishment, d) image of a 115 mm potential anadromous colonizer, and e) a hybrid between a brook trout and brown trout sampled in a St. John's river. Photographs by the authors.

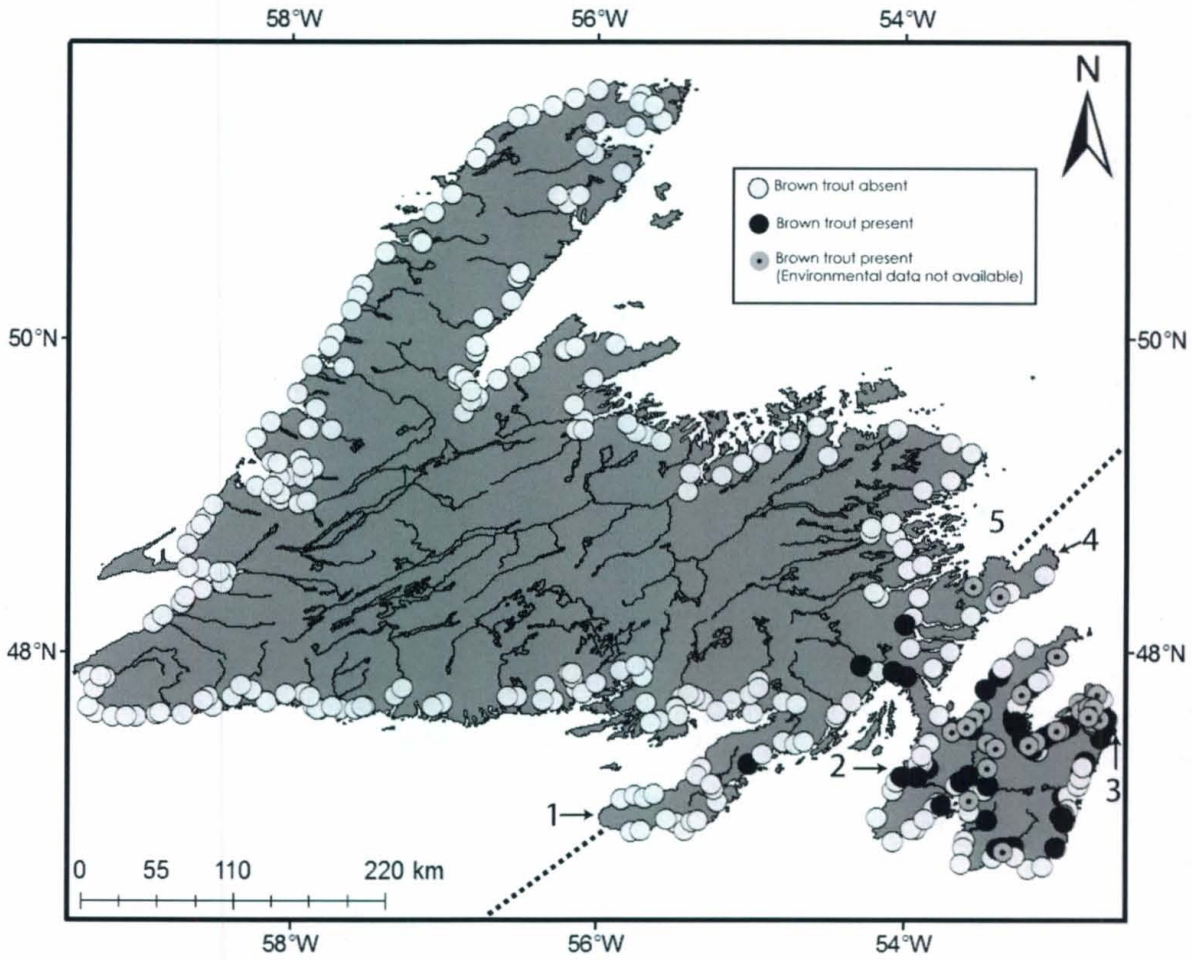

Fig. 2-3. Current distribution of watersheds established by brown trout populations on the island of Newfoundland. The dashed lines denote the apparent dispersal boundary and thus only watersheds to the right of the boundary were included in our analyses (see text). Numbers represent locations mentioned in the text: 1) Burin Peninsula, 2) Southeast Placentia River, 3) St. John's, 4) Bonavista Peninsula, and 5) Bonavista Bay. See Fig.1. for other important locations.

Chapter 3: Novel environments shape phenotypic variation in recently established brown trout (*Salmo trutta*) populations

Abstract

Species translocations represent excellent opportunities to investigate the early stages of adaptive radiations. Abrupt changes in selection regimes and exposure to novel environmental conditions can lead to phenotypic divergence in contemporary time. Brown trout were transplanted from Europe to Newfoundland in 1883 and subsequently spread from sites of original introductions to found established populations in watersheds differing in abiotic habitat features. We quantified phenotypic variation among 16 populations of brown trout in Newfoundland to test the hypothesis that populations have differentiated in a suite of morphological, meristic, and growth traits in no more than 130 years. Additionally, we tested whether the observed variation among populations reflected characteristics of novel abiotic environmental features. Discriminant function analysis based on size-adjusted traits assigned individuals to population of origin at rates much greater than chance alone. Moreover, multivariate analysis of variance MANOVA detected significant differences in principal component scores based on size-adjusted traits, corroborating the results of the discriminant function analysis. Results revealed a potentially important role of the environment, and thus likely phenotypic plasticity, in explaining the observed variation. Body shape of individuals, as quantified by geometric morphometrics, differed markedly among populations and was correlated with habitat features such as river size and flow. In addition to body shape, we detected significant correlations between similarity in suites of phenotype traits and habitat characteristics using Mantel's tests. However, the strength and importance of the correlations between specific habitat features and phenotypes differed among size and age classes of fish suggesting ontogenetic changes in selection pressures or microhabitat use. While the genetic role in the expression of the traits examined is not known, it is likely that many have at least some heritable basis and thus the observed difference among populations may represent genetic adaptation to divergent regimes of selection. Alternatively the patterns reported here may result from adaptive phenotypic plasticity that has likely facilitated persistence in novel environments.

"And 't is so with many kinds of fish, and of trouts especially, which differ in their bigness, and shape, and spots, and colour."— Izaak Walton (1653) from *The Compleat Angler*

Introduction

Adaptive phenotypic divergence can occur rapidly in populations exposed to abrupt environmental change and divergent regimes of natural selection (Schluter, 2000, Hendry & Kinnison, 1999, Ghalambor et al., 2007). Empirical examples of this 'contemporary' phenotypic divergence are replete in the literature derived from a range of taxa, including fruit flies (Huey *et al.*, 2000) aquatic isopods (Eroukhmanoff et al., 2009), reptiles (Losos, 2009), fishes (Haugen & Vollestad, 2001, Stearns, 1983), birds (Johnston & Selander, 1964), and mammals (Williams & Moore, 1989). Systems experiencing anthropogenic disturbance, such as species invasions can reveal how abrupt shifts in selection pressures can drive contemporary phenotypic change in wild populations (Westley, 2011, Hendry et al., 2008). Indeed, some of the most compelling examples of contemporary adaptive change are derived from the opportunistic natural experiments represented by biological invasions (Huey et al., 2005, Sax et al., 2007).

Repeated global translocations of fishes such as salmon, trout, and charr (family Salmonidae) afford excellent research opportunities to examine patterns and processes of phenotypic divergence (e.g. Crawford & Muir, 2008). Salmon and trout are renowned for their remarkable diversity in behaviour, ecology, morphology, and life history both among species and among populations within species (Quinn, 2005, Groot & Margolis, 1991, Elliott, 1994, Klemetsen et al., 2003, Fleming, 1998). This diversity is typically thought to reflect local adaptation to environmental conditions experienced by individuals and populations during rearing and spawning (Taylor, 1991, Garcia de Leaniz et al., 2007).

Throughout much of the native salmonid range, this diversity has evolved within 5,000-15,000 years following the end of the last glacial epoch (Hendry & Stearns, 2004). Translocations allow refinement of the time-scales over which adaptive change can arise, as the precise age of populations are often known from stocking records and history (Ayllon et al., 2006, Quinn et al., 2001a, Hendry et al., 2000). Moreover, salmonid transplants allow investigation into the role of the environment and phenotypic plasticity (i.e. ability of an individual to respond to environmental conditions) in shaping and maintaining biological diversity among newly founded populations (Hutchings, 2011). The interpretation of the mechanisms giving rise to phenotypic variation among transplanted populations is frequently complicated by continued artificial propagation of multiple mixed genetic pools following initial introductions (e.g. continued stocking of exotic salmonids to the Great Lakes, Mills *et al.*, 1993). However, stocking of individuals from a *single* common gene pool into different environments affords the opportunity to investigate the environmental and genetic architecture underlying fitness related phenotypic traits (Reed et al., 2010a). By extension, the establishment of non-native populations from a common source imported into novel environments represents an analogous serendipitous research situation.

Here we use brown trout (*Salmo trutta*) introduced to the island of Newfoundland, Canada, as such a model system. Brown trout were imported for sport fishing to Newfoundland beginning in the late 19th century (reviewed by Hustins, 2007), survived upon introduction, established self-sustaining populations, and spread to new areas without human assistance. Populations are currently established in at least 70 watersheds that differ in various environmental factors (Westley & Fleming, 2011). In this paper we address the question of how recently established populations have responded to novel environments

across a gradient of abiotic environmental factors. The primary goal of this study was to quantify variation in a suite of morphological, meristic, and growth traits known to be, or likely, linked to fitness among16 brown trout populations established within 130 years. The objectives were to assess variation in body shape and fin sizes, colouration and pigmentation patterns, and growth rates of individuals within and among populations, and correlate this suite of phenotypic traits to habitat characteristics such as river size, water chemistry, and a measure of isolation (i.e. distance of watersheds to their nearest neighbour). To the extent that phenotypes are shaped or selected by the environment, we predicted that populations exhibiting similar suites of phenotypic traits would also inhabit rivers with similar habitat features. Specifically we predicted: 1) a positive association between river size and body shape such that relatively large and deep rivers would correlate with the expression of deep-bodied individuals, whereas small shallow streams would be associated with shallow-bodied fish presumably to aid in streamlining, 2) colouration patterns would be inversely related to extent of canopy cover and riparian vegetation where relatively drab and dark colouration patterns would be expressed in relatively dark environments parallel to the patterns observed among colouration and canopy cover in Trinidadian guppies, *Poecilia reticulata* , 3) water conductivity (a surrogate for productivity) would mediate trait expression indirectly through growth, and 4) that populations in close spatial proximity would be phenotypically similar resulting from either environmental similarity (watersheds close in space may be similar) or founder effects as spatial distance is interpreted as a proxy for time since population establishment (populations near the putative source are assumed to be older than populations near the edge of current range).

Methods

Brown trout and population history

Brown trout is a member of the family Salmonidae native to Europe, North Africa and western Asia. Their distribution has rapidly expanded via intentional introductions around the globe and currently populations of trout are established on every continent, except Antarctica (MacCrimmon & Marshall, 1968, Elliott, 1994). Brown trout display dramatic variation in morphology, colour, and life history patterns, which has been recognized by astute naturalists such as Izaak Walton since at least the 17th century (Walton, 1653). The high level of within-species variability caused considerable taxonomic confusion and historically nearly 50 distinct species were described based on ecomorphs and subpopulations of brown trout before being recognized as one polytypic species in 1911 (reviewed by Behnke, 1986, Elliott, 1994). More recently, this variation has been revealed to reflect interactions between genetics and environmental factors (Ferguson, 1989, Ferguson & Taggart, 1991, Ferguson & Mason, 1981, Hutchings, 2011, Bernatchez et al., 1992).

The founding trout in Newfoundland were primarily comprised of non-anadromous (freshwater resident) Loch Leven strain from the Howietoun Hatchery in Scotland. Individuals from two other strains were also purportedly introduced, but apparently in much smaller numbers (Hustins 2007). Thus, it is likely that the preponderance of established trout populations were founded by common ancestral gene pool. The trout survived well upon introduction to lakes, established self-sustaining populations, and spread to novel rivers and watersheds via anadromous (sea-going) dispersal (Westley & Fleming, 2011).

Sample collection

To quantify phenotypic differentiation among populations, we sampled a total of 1677 brown trout during June-September 2008 from 16 watersheds in eastern Newfoundland (Fig. 3-1). These watersheds were selected to represent a variety of habitats along a gradient of increasing distance from the putative source population near St. John's (Fig. 3-1) and to cover a range of habitat types. Fish were collected with single-pass upstream electrofishing (Smith-Root LR-24 backpack shocker) and with beach seines and gillnets (3mm mesh size) in deeper pools of rivers where electrofishing was ineffective. We collected fish throughout river sections (mean section length; 860m, range 82-6800 m) to reduce the potential of sampling related individuals. Additionally, we attempted to collect across the size and age classes available in each site (~100 individuals were targeted) and therefore we sampled microhabitat associated with different age classes of fish (Armstrong *et al.*, 2003).

Fish processing and data collection

Fish were anesthetised in clove oil (0.25mL/L), measured (fork-length, nearest mm), weighed (0.1 g), and photographed with a 12.1 mega-pixel Canon digital camera (PowerShot A650 IS) using a low compression JPEG format. Each photograph included a unique identifying label, a scale bar to allow standardization among photos taken at different heights (necessitated because fish ranged in size from 30 - 362 mm), and an X-Rite mini colour checker card (X-Rite Inc., Grand Rapids, MI).). This colour card contains vignettes designed to express a known colour according to RGB digital colour space (Red-Green-Blue) allowing for standardization of lighting among images (see Bergman & Beehner, 2008, Whiteley et al.,

2009 for examples of colour standardization). Salmonids, like other fish species, can change melanin-based colour rapidly during periods of stress but adapt their colouration to environmental conditions over periods of days or weeks (Sugimoto, 2002, Sumpter et al., 1985, Donnelly & Dill, 1984). To limit the effect of our handling on physiological colour change we minimized the time from capture to photographing, and used standardized white-coloured storage containers and consistent concentration of anaesthetic. After photographing, a sample of scales was collected for subsequent ageing and the adipose fin was removed and stored in 95% ethanol for future genetic analyses. Removal of the adipose fin also provided an external mark to avoid resampling individuals between days. Fish were allowed to recover and released.

Growth data

Scales were mounted on microscope slides and digitally photographed with a Lumenra Infinity 2 camera affixed to an M420 1.25 x compound microscope under 10x -20 x magnification. Scales were assigned to year class where the number designation corresponded to the number of winter marks on the scale. We then calculated specific growth rates for each individual by dividing the log difference in length-at-capture and length-at-emergence by the number of growing days, which we defined as the number of days from emergence to capture. This approached allowed us to assess growth of fish collected over a 9 week span of the summer growing season. For simplicity, and because individual values of size and day of emergence were not available, we assumed that all fish emerged at 25 mm on May 15th of their first spring. These values are based on laboratory and field observations with these populations (Westley and Fleming unpublished data) and from literature (Elliott, 1994, Klemetsen et al., 2003).

Body shape data

Differences in body shape among populations were assessed with geometric-morphometrics (Adams *et al.*, 2004). Two-dimensional body shape was quantified by placing 14-homologous landmarks on digital images in the program tpsDig2, Version 2.12 (Rohlf, 2005). The landmarks (Fig. 3-2) were based on Michaud et al. (2008). Landmark data were aligned to a single consensus shape configuration using Procrustes superimposition using the program tpsRelw, Version 1.46 (Rohlf, 2006). After alignment, Relative Warp scores (analogous to Principal Component scores) were calculated for each individual. Interpretation of how each Relative Warp contributed to body shape was based on visualizations of thin-plate spline transformations generated in tpsRelw.

Phenotypic suite data

We conducted a separate and complementary analysis based on a suite of 11 morphological, meristic, and growth traits, similar to the approach by Michaud et al. (2008). Direct linear measures of six traits were measured from photographs using ImageJ, Version 1.42q (freely available at: http://www.rsbweb.nih.gov/ij/). Traits measured included: 1) surface area of the eye (mm^2), 2) surface area of the head (mm^2), 3) body depth (mm), 4) length of the pectoral fin (mm), 5) length of the caudal fin (mm), and 6) depth of caudal peduncle (mm). Measurements were taken by the same person to reduce variability and a haphazardly-selected sample of 100 fish was re-measured to assess error in data collection. Repeatability for all traits was excellent with r^2 values of ~0.99 between duplicate measurements on the same individuals.

Additionally, we recorded the number of pigmented spots and quantified two metrics of colouration on each fish as such patterns are often used to differentiate among

brown trout populations (Aparicio *et al.*, 2005), are herititable (Blanc *et al.*, 1994), and apparently linked to fitness (Wedekind *et al.*, 2008). We counted spots irrespective of their colour on the left flanks of each fish ignoring spots on fins as placement and position of fins were not standardized sufficiently among photographs to always allow counts. To assess colouration, we first standardized all photographs to a common colour vignette to account for differences in lighting conditions during photographing (reviewed by Stevens *et al.*, 2007). Overall amount of red colouration was then measured as the percentage of pixels where the red value of the RGB colour space fell above a threshold of 50 points (~20%) above both the green and blue pixel values. Next, we extracted the mean value of pixels in the red, green, and blue spectrum and used these values to interpret the overall lightness or darkness of body colouration from individuals. This is justified as higher mean RGB values are associated with bright colours and lower mean values associated with darker colours. Colour analysis was conducted using the Image Processing Toolbox of Matlab © and automated with custom written routines, which are available upon request.

Habitat sampling

We quantified five abiotic habitat variables from each sampling location that we predicted may influence morphology of individuals among populations. Habitat surveys were conducted within a two week span during periods of similar water conditions. At a total of three haphazardly chosen sites corresponding to the downstream, middle, and upstream end of the sample section we measured: *i*) the ratio of wetted width to depth, *ii*) stream gradient, *iii*) the extent of riparian canopy cover using the categories: 1 = no cover or only grasses, 2 = alders and willows along banks, branches encroached the stream, 3 = large alders and

conifers present, branches and woody debris in channel, 4 = alders, conifers, and deciduous species present, large amount of wood in the channel, and little light reaching stream, *iv*) water transparency using a standard 1.3 m transparency tube (Dahlgren *et al.*, 2004), and *v*) water conductivity with an Accumet ® AP 85 handheld meter.

To these five variables we added a measure of spatial distance (km) from the mouth of each sample watershed to the mouth of the Rennie's River watershed, which we identified as the putative source of the original invasion in Westley & Fleming (2011). We assigned negative distances to locations *south* of the invasion source and positive values to locations *north*. In doing so, we obtained pairwise distances between the mouths of each sampling watersheds. We calculated distances using the least-cost distance tool in ArcGIS with an approach that provided a realistic distance that a fish would have to swim between locations, thereby capturing the dynamics of potential gene flow and colonization (see Westley & Fleming 2011 for details). The distances between sample locations are interpreted as potential gene flow while the distance from the source represents a surrogate for time since colonization assuming a stepping-stone type dispersal process.

Statistical analysis

Shape (warp scores) and morphological and meristic traits (linear measures, spot counts, and colouration) were size-adjusted prior to statistical analyses. This was necessary as traits such as body shape can change markedly during ontogeny (Loy *et al.*, 1998), complicating interpretation among individuals of different sizes and ages. Indeed, exploratory data analysis revealed distinct patterns of allometry among fish sizes and supported a division of our samples among the three primary size and age classes observed

(small: <60mm, intermediate: 60-150mm, and large: >150mm, Fig. 3-3a). We then conducted separate analyses using these subsets of data (henceforth referred to as 'size groups'). We measured morphological and meristic traits from a common number of fish per population and size group, which was set by the population where the minimum number of fish in each group was sampled ($n_{<60mm}$=17, $n_{60\text{-}150mm}$=19, $n_{>150mm}$=15, Table 2). We only sampled individuals from populations and size groups if sample numbers were greater than the number of variables we were extracting (n=14 for the geometric morphometrics and n=11 for the suite of other variables).

All morphological and meristic traits varied significantly with size, thus each trait was corrected to the mean length of each size group (45, 100, and 200 mm, respectively) using common within-group allometric coefficients (Reist, 1986, McCoy et al., 2006). Allometric coefficients for each trait represent the slope coefficient of analysis of covariance (ANCOVA) on log (x+1) transformed trait and body length values. We verified common within-group slope by testing for significant body size*population interaction terms of ANCOVA for each trait. Within group allometry for all traits was statistically similar (homogeneity of slopes), but differed markedly among groups supporting the presence of size-dependent allometric effects. We chose to standardize to a common body length rather than centroid size to aid in biological interpretation, and because preliminary analyses suggested a strong linear relationship between these two covariates (length = 0.7575 * centroid + 7.0728, $r^2 = 0.998$).

To test for differences in body shape among populations we used one-way ANOVA with warp scores as the dependent variable and sampling location as a factor. Comparisons and interpretation were done by visual inspection of means and Tukey HSD post-hoc tests.

To test for differences in the suite of phenotypic variables we used linear discriminant function analysis (DFA) using a jackknife – leave one out procedure – to assess reclassification rates of individuals to populations based on discriminant functions of size-adjusted morphological and meristic traits. Variables used in DFA were: growth rate (not size adjusted), weight, body depth, caudal depth, pectoral length, caudal length, head surface area, eye surface area, number of spots, amount of red colouration, and overall brightness of colour. Brightness values for each individual represent the first principal component scores extracted from a separate principal components analysis on RGB data (Whiteley *et al.*, 2009). We followed the DFA with principal components analyses (PCA) of size-adjusted variables to reduce dimensionality and to account for correlation among traits. We based the number of principal component axes for interpretation and inclusion in subsequent analyses on the broken stick model (Peres-Neto *et al.*, 2003). Scores of retained principal components axes were used as dependent variables to test for population differentiation with MANOVA. Analyses were conducted separately among size groups.

We visualized morphological and habitat similarity (based on Euclidean distances) among populations with non-metric multidimensional scaling (NMDS) plots fitted using the ecodist package in R v.2.10.1 (R Core Development Team, 2009). Coordinates on the first and second dimension represent the positions in multivariate space that best maintained the order in the original similarity matrix (i.e. minimum stress) after 100 random starting configurations. To aid interpretation of morphological and habitat similarity, we overlaid vectors where lengths represent the correlation strength with each variable.

Ordinary least squares regression (OLS) was used to test the hypothesis that body shape (Relative Warp scores) was significantly associated with the environment. For each

size group, we fit seven *a priori* regression models to assess the weight of evidence to suggest population-specific average body shape was influenced by environmental factors. Specifically, we hypothesized that aspects of river size (i.e. width-depth ratio), stream flow (i.e. width-depth ratio plus gradient), distances between sample locations (i.e. potential founder effects), or productivity (i.e. conductivity) might explain observed variation in body shape. We used ΔAIC values as measures of evidence, where we interpreted ΔAIC values < 4 to provide substantial support for a candidate model (see Westley & Fleming 2011 for a similar approach).

We employed the BIO-ENV routine (Clarke & Ainsworth, 1993) to assess the correlative relationship between phenotypic similarity in the suite of morphological, meristic, and growth variables with environmental similarity. In short, this routine calculates a similarity matrix of phenotypic values (based on retained PCA axes scores), selects all possible subsets of environmental variables, calculates Euclidean distances for this subset, and finds the correlation between the matrix of phenotype and the matrix of environmental variables for each subset. Mantel's tests (Legendre & Legendre, 1998) were then used to test the significance of the correlations generated for each size group from the BIO-ENV routine. Mantel statistics (R_m) and probabilities based on 999 permutations were calculated in the vegan package of R (Team, 2009b). We included all six recorded environmental factors to assess similarity, as we hypothesized each may be influencing some aspect of the observed phenotypes. Specifically, we hypothesized that stream size and flow may influence features of body shape (body and caudal depth and fin sizes), water clarity and riparian cover may influence colouration patterns, and stream conductivity may relate to growth, which may mediate other trait expression.

Results

Population differentiation

Body shape

The first Relative Warp explained 43% of the variation in shape and described a decreasing relative size of the head and deepening of the body and caudal areas (Table 3-1). The second and third warps explained 14% and 8% of the variation in shape, respectively, and suggested dorsal-ventral bending of the fish during photographing. This 'arch effect' has been described elsewhere and attributed to error in placement of specimens during photo capture (Valentin et al., 2008, Michaud et al., 2008). Furthermore, the 21 remaining warps individually explained little variation and were not retained for subsequent analyses. Thus, we limited our analyses of shape to scores of the first Relative Warp.

Overall, the first Relative Warp (body shape) varied significantly and non-linearly with body size (Fig. 3-3a). However, three distinct *linear* allometric trajectories were detected corresponding to dominant size and age classes. Size- and age-specific allometric coefficients motivated our approach to divide samples into distinct size groups as populations differed markedly in frequency of fish sizes encountered (Fig. 3-3b). As a result, not all populations are included in each size category (Population $\mathbf{n}_{<60\ mm} = 13$, $\mathbf{n}_{60\text{-}150mm} = 16$, $\mathbf{n}_{>150\ mm} = 7$). In general, shape changed most rapidly in fish less than 60 mm (linear coefficient of shape vs. body size = 0.07), intermediate in fish 60-150mm (coefficient = 0.04), and slowest in fish larger than 150 mm (coefficient = -0.01, Fig. 3-3c). Moreover, the sign of coefficient changed from significantly positive (a trend toward smaller heads and deepening body) in the first two size groups to significantly negative (larger heads and streamlined bodies) in the largest size group (see Fig. 3-3 for thin-plate spline visualizations).

Significant differences among populations were detected (via ANOVA) after adjusting shape variables using within-size-group common allometric coefficients and mean group body size (Table 3-1). Shape of the <60 mm size group differed markedly among sampling locations ($F_{13,604} = 34.9$, $p < 0.001$) with Parker's Pond Brook and Savage Creek populations having the smallest and largest size-adjusted shape values, respectively (Table 3-1). Intermediate sized fish (60-150 mm) also differed significantly in adjusted shape among populations ($F_{15,716} = 48.8$, $p < 0.001$). Fish from the Chance Cove, Renews River, and Parker's Pond Brook populations had the smallest mean shape values, whereas the Savage Creek and Torbay populations had the largest values (Table 1). Finally, and similarly, body shape of individuals in the largest size group differed among populations ($F_{6,240} = 34.9$, $p < 0.01$). Rennie's River and Virginia River populations were not different from each other in body shape (relatively small warp values), but differed significantly from the remaining populations, which had relatively large warp values (Table 3-1).

Suite of Phenotypic traits

Analyses of size-adjusted morphological, meristic, and growth variables provided additional evidence of population differences (Appendix table 3-1). Results of linear discriminant function analysis (DFA), based on size-adjusted traits on average, assigned 69%, 54%, and 57% of individuals correctly to populations in the small, intermediate, and large size groups, respectively. These values are much greater than what is expected by chance alone (6.3-14.3%; Table 3-2).

Principal components analysis (PCA) on the same traits resulted in the retention of four significant axes for all size groups. These four axes explained ~65% of the variation in each size group (Table 3-3). The first principal component in the smallest size group

explained an inverse relationship between growth rate and caudal fin length, head size, eye size, red colouration, and number of spots (Table 3-3a). The second axis described an increasing gradient in body depth, weight, and caudal depth. Colouration values loaded heavily on the third axis, while pectoral length and growth loaded positively on the fourth axis.

Pectoral length, caudal length, head size, and eye size of intermediate sized fish loaded heavily and inversely to growth on PC1 (Table 3-3b). The second axis described a gradient of body depth and caudal depth and the third axis described a positive association between growth, red colouration, and spots. The final PC axis retained for intermediate sized fish described an inverse relationship between weight, spots, and caudal length.

In contrast to the previous size groups, the first PC axis for the largest size group described measures of overall size, such as body depth, weight, caudal depth, pectoral length, and eye size (Table 3-3c). The second axis suggested an inverse relationship between body depth, caudal depth, and eye size, while the third axis described an inverse relationship between overall colour, red colour, and growth. Caudal length, caudal depth, and spots loaded heavily and positively on the fourth axis.

Principal component scores from the four retained axes were significantly different among populations for the small size group (MANOVA, Wilks $\lambda_{12,210} = 0.05$, $p < 0.001$), intermediate group (Wilks $\lambda_{15,309} = 0.16$, $p < 0.001$), and large group (Wilks $\lambda_{6,106} = 0.27$, $p < 0.001$). Moreover, all PC variables in MANOVA were significantly different in each size group ($p < 0.001$).

Non-metric multidimensional scaling (NMDS) plots facilitated visualization of differences among populations (Fig. 3-4) and placed populations in multivariate space while maintaining original similarities (i.e. low stress, range 0.10- 0.13) for small (Fig. 3-4a), intermediate (Fig. 3-4b), and large size groups (Fig.3-4c).

Habitat similarity

Physical habitat features varied markedly among sampling locations (Appendix Table 3-2). For example, systems ranged between 1.7 m to 16.9 m in average width and 12 cm to 38.1 cm in depth. Similarly, conductivity ranged widely between a low of 33.1 μS in Chance Cove to a high of 299.3 μS in the Waterford River.

Nonmetric multidimensional scaling placed populations in two dimensions while maintaining original similarity relationships (stress = 0.03) and thus represents an accurate visualization of similarity in habitat features. Results reveal a cluster of systems (i.e., Virginia River, Rennie's River, Waterford River, Savage Creek, and Topsail) that are close to the putative source of invasion and to each other, have high conductivity, and relatively low water clarity (Fig. 3-5). Parker's Pond Brook which feeds the protected city water supply of St. John's is an exception and is characterized by high gradient and small width/depth ratio in addition to low conductivity. The remaining populations generally aligned on a north-south distance axis (Fig. 3-5).

Environment-phenotype correlations

We detected the influence of abiotic environmental factors on body shape, based on Relative Warp scores; however, the strength and specific environmental variable most

influential on shape varied among size groups. Overall, environmental factors explained little of the variation in the small size group (2-12%) and the most favoured model contained the sole effect of conductivity, though it only explained 10% of the variation. Models with combinations of distance, stream size (width-depth ratio), stream flow (size+gradient), and conductivity all received substantial support based on ΔAIC values, yet they explained little of the observed variation (Table 3-4). Relative Warp scores were positively related to conductivity and distance (increasing dorsal ventral and caudal axis, declining head size) and inversely related to stream size (increasing streamlining).

In contrast, markedly more variation in body shape was explained by environmental factors in the intermediate and largest size groups. The most favoured model contained the individual effect of distance for both the intermediate ($r^2 = 0.26$) and large ($r^2 = 0.25$) size groups. However, all other models tested also received substantial support ($\Delta AIC < 4$), indicating that combinations of distance between watersheds, stream size and flow, and conductivity may influence shape (Table 3-4). Body shape of intermediate size fish responded similarly to distance, stream size, and conductivity as small fish (Table 3-4), while the largest size group revealed a different pattern. Among population variation in body shape of fish >150 mm was positively related to distance (similar to other groups) and stream size but negatively related to conductivity.

Analyses based on population similarity in a suite of morphological, meristic, and growth traits (visualized in Fig. 3-4) also revealed significant correlations in similarity of environmental features, but were again dependent on size group (Table 3-5). Similar to the results based on body shape alone, phenotypes among populations based on fish < 60 mm were not significantly correlated with environmental variables. The BIO-ENV routine

suggested that the strongest correlation was between phenotype and the combined effects of riparian cover, stream size, stream gradient, and water clarity ($R_m = 0.17$); however, this correlation was not statistically significant ($p = 0.122$). In contrast, population phenotypes based on the intermediate size groups were strongly and significantly correlated with all the variables extracted from the BIO-ENV routine. The strongest correlation between phenotype and environment was the result of distance between watersheds, riparian cover, and stream size ($R_m = 0.47$, $p < 0.005$). Population phenotypes based on the largest size group was best correlated with riparian cover and conductivity ($R_m = 0.38$), though the correlation was not significant ($p = 0.06$).

Discussion

We found evidence to support the hypothesis that brown trout populations in Newfoundland currently differ in a suite of phenotypic traits no more than 130 years, or approximately 32 generations, after first introduction. Body shape of individuals varied significantly with habitat occupancy, consistent with the prediction that steeper, faster-flowing streams select for more streamlined morphology whereas relatively small but productive streams select for deeper bodied fish. Consistent with predictions, overall population phenotypes based on 11 morphological, meristic, and growth variables correlated significantly with 6 habitat variables, though the effects were more pronounced in larger fish. Taken as a whole, populations displaying similar suites of phenotypes tended to inhabit rivers characterized by similar suites of habitat features. It currently is unclear whether the phenotypic differences among populations have resulted from local selection on a common

population of founders, recurrent phenotypic plasticity as the same pool of genotypes have been exposed to differences in environmental conditions during development, or some combination of processes. In summary, we suggest that the established non-native trout populations in Newfoundland display levels of phenotypic variation on par with that observed in the native range (e.g., Karakousis et al., 1991, Pakkasmaa & Piironen, 2001) and that this variation, acknowledged by Izaak Walton in 1653, has arisen in contemporary time. However, the mechanisms and processes that have driven and maintained this phenotypic variation are currently unclear.

Body shape

We detected distinct, non-linear allometry in body shape based on geometric morphometrics and Relative Warp analysis. Allometric relationships between shape and size were linear *within* each size group but strikingly non-linear *among* the range of sizes observed. To our knowledge this is the first report of such non-linear allometry in juvenile salmonids, though such patterns have been reported elsewhere in species that exhibit marked shifts between larval and juvenile morphologies (Loy *et al.*, 1998). The pattern of allometry observed in this study serves as a poignant example of the danger in assuming linear, common-group allometries when comparing morphological traits among populations and environments (reviewed by McCoy *et al.*, 2006). We controlled for this underlying allometry by size-adjusting body shape values using within-size-class allometric coefficients and mean body sizes.

Taken as whole, these patterns in body shape among populations are presumably maintained by a combination of phenotypic plasticity and adaptive evolution. At least some

populations of salmonids display adaptive plasticity with regards to body shape, where plasticity acts in the direction thought to be favoured by selection (Pakkasmaa & Piironen, 2000, Haas et al., 2010, Franssen, 2011). Additionally, body shape in juvenile salmonids has significant levels of underlying additive genetic variance, suggesting that evolutionary responses in shape to natural selection are likely (Hard *et al.*, 1999).

Population variation in a phenotypic suite

In addition to differences in geometric morphometric analysis of body shape, we detected significant interpopulation variation in a suite of morphological, meristic, and growth traits of individuals. Overall, discriminant function correctly reassigned individuals to populations based on this suite of traits at a rate much greater than predicted by random chance. Principal components analysis (PCA) followed by multivariate analysis of variance on retained principal component axes scores corroborated these among population differences and yielded some salient points. Traits representing the highest loadings on the retained components varied among the three size groups of fish and correlations among traits also varied with size. For example, we detected negative allometry between growth rate and eye size and head size as reported elsewhere (McDowall & Pankhurst, 2005). In contrast, eye and head size were independent of growth among fish in the large size group. Similarly, we detected size-specific relationships between growth and amount of red colouration. Growth was negatively correlated with red colouration in the small and large size groups, and positively correlated in the intermediate size groups. We interpret these patterns to indicate that the factors underlying expression of these traits vary with size and are presumably related to ontogenetic shifts in habitat and resource use and availability (Nicieza,

1995, Michaud et al., 2008, Bisson et al., 1988). Furthermore, these findings support the complex pattern of allometry revealed in geometric morphometric analyses on body shape. The suite of phenotypic traits, which we reduced to principal component, axes scores, differed significantly among populations. Thus we suggest that the similarity in phenotypes visualized by non-metric multidimensional scaling plots (Fig. 3-4) represents statistically and biologically significant differences among populations.

Environment-phenotype correlations

Body shape

Environmental features explained little of the among population variation (~10%) in body shape of fish in the small size group. We suggest that the lack of association between environmental factors and body shape may arise from environmental factors that were not quantified or did not represent the appropriate spatial scale of the rearing dynamics in the smallest fish. Alternatively, little differentiation in the small size group may reflect an insufficient amount of time for plasticity and selection to act. Some combination of these explanations are likely as previous work has demonstrated the influence of rearing environments to drive body shape in salmonids of similar size and age (Pakkasmaa & Piironen, 2000, Pavey et al., 2010). Future work that seeks to precisely quantify microhabitat use of small fish in these populations with the intention of predicting body shape may be illuminating.

In contrast, markedly more variation in body shape was explained among populations based on the intermediate and largest size groups. For both groups, a trend of increasing deepening of the body and caudal area was detected on a south to north gradient, such that populations south of the invasion source were characterized by thinner bodies and

populations to the north displaying more robust shapes. Distance was weakly and non-significantly correlated with the other environmental variables predicted to influence body shape, suggesting that this is not simply a result of underlying correlations between factors. This pattern in body shape may have arisen from founder effects as it is plausible that watersheds to the south and north of the original invasion source have been established by different subsets of colonizers. On-going research to understand another contemporary brown trout invasion in the Kerguelen Islands is illustrative as patterns of genetic diversity in recently established populations were primarily understood by introduction history and founder effects (Launey *et al.*, 2010). Interestingly, research in the Kerguelens also suggests that landscape environmental factors mediate the rate and direction of trout migrations and ultimately structure the genetics of colonizing populations.

In addition, an aspect of river size (i.e. width-depth ratios), gradient, and conductivity were also important explanatory factors of body shape variation. Populations based on intermediate (60-150mm) sized fish, rearing in streams characterized by relatively high gradient and large width-depth ratios displayed narrower and more streamlined morphology. This pattern is consistent with other studies that report a general pattern of stream-lined morphology in lotic habitats (flowing water) and robust body shape in lentic (stillwater) habitats (Keeley et al., 2007, Pakkasmaa & Piironen, 2000, Pavey et al., 2010, Haas et al., 2010, Franssen, 2011). Curiously, body shape based on the largest size group (>150 mm) was inversely related to conductivity and positively related to stream size. That is, high conductivity apparently shaped or selected for large heads while streams with large width-depth ratios were associated with deep-bodied individuals. While the underlying mechanisms of this are unclear, it is plausible that the water flow and stream size exert different shaping

and selection pressures in smaller fish and that growth beyond a critical size allows ontogentic shifts to different habitats (e.g. pools and riffles; Nicieza, 1995, Bisson et al., 1988) or prey items (Michaud et al., 2008, Denton et al., 2009), which likely correspond to changes in selection pressures.

Across Newfoundland, colonizing brown trout are more successful in establishing populations in productive rather than unproductive watersheds (Westley & Fleming, 2011) and the result here suggests that water chemistry and productivity influence also influence body shape. Water chemistry may be both directly (e.g. developmental plasticity) and indirectly (e.g. shape mediated by growth differences in response to productivity) influencing body shape though mechanisms that can be complicated, and frequently unclear (Crispo & Chapman, 2011).

Phenotypic suite of traits

As predicted, our results suggest that populations displaying similar suites of phenotypic traits tend to inhabit rivers with similar abiotic environments. Not all abiotic environmental variables were important correlates of phenotypes; however, and analogous to the analysis of body shape, the importance of particular variables differed among size groups of fish. Correlations between phenotype and environment were weak and non-significant in the smallest size group (<60 mm), strong and significant in the intermediate size group (60-150mm), and marginally significant in the largest size group (>150 mm). We focus the remainder of the discussion on the results of the intermediate size group as all populations were represented and correlations with environmental features were strongest.

Phenotypic similarity was best correlated with three environmental predictors: distance between watersheds, the extent of riparian cover, and stream size (i.e., width-depth ratio). We interpret the importance of distance among watersheds and stream size to explain aspects of body shape, such as body depth, caudal depth, and head size, following our previous logic. Moreover, we detected positive correlations of size-adjusted pectoral and caudal fins with stream size, supporting the observation that these traits, critical for swimming performance, can be plastic depending on rearing environments (Pakkasmaa & Piironen, 2000, Bisson et al., 1988, Imre et al., 2002).

Riparian cover was an important correlate of phenotypic similarity among populations, which we attribute to the effect of riparian cover on resource availability and growth patterns, which in turn, influences trait expression. In addition to providing physical habitat structure, riparian cover determines the amount of light reaching streams and thus influences photosynthesis and primary productivity. In Trinidad, canopy cover explains 93% of the variation observed in guppy growth rates by influencing the standing crop of algae and food availability (Grether *et al.*, 2001). Furthermore, riparian cover limits the potential for algal pigments, such as chlorophylls and carotenoids, to be assimilated into the food chain, which in turn, influence patterns of sexual colouration in guppies (Grether *et al.*, 1999). The brown and black spots on the sides of brown trout are melanin-based and can be synthesized by the animal directly, whereas the orange and red spots and fin colouration contain high concentrations of carotenoids accumulated via the environment (Fig. 3-2., Stevens, 1948, Wedekind et al., 2008). Similar to the patterns reported by Grether *et al.* (1999, 2001), the extent of riparian cover was negatively correlated with growth ($r_{14} = -0.34$), extent of red colouration ($r_{14} = -0.16$), total number of pigmentation spots ($r_{14} = -0.16$), and

overall brightness of colouration (r_{14} = -0.07) in our study. The principal component analysis revealed that slow growing fish tended to have fewer overall spots and less red colouration compared to their faster growing counterparts. This suggests that colour and pigmentation patterns are mediated by growth and pigment availability, which in turn, is affected by riparian cover. Moreover, we detected a positive correlation (r_{14} = 0.40) between eye size and extent of riparian cover. Is unclear, however, whether dark environments select for the expression of large eyes or again reflect patterns in growth as slow growing fish, which often inhabit dark environments, tend to have large eyes.

The success of brown trout to establish populations around the globe is often attributed to their wide-environmental tolerances and the ability to respond plastically to environmental change (Elliott, 1994). Indeed, the success of invasive species as a whole is frequently linked to adaptive phenotypic plasticity (Davidson *et al.*, 2011). Plasticity can allow persistence in novel environments (Yeh & Price, 2004, Ghalambor et al., 2007) and is an important route towards genetically-determined local adaptation (Chevin & Lande, 2011a, Chevin et al., 2010, Lande, 2009, West-Eberhard, 2003). The similarity between phenotype and environmental features, combined with the observation that many traits are correlated with growth, strongly implicates the underlying influence of phenotypic plasticity in shaping brown trout populations in Newfoundland. This does not, however, preclude the possibility that the phenotypic variation observed represents underlying genetic architecture. For example, established populations of brown trout in the Kerguelen Islands have, as inferred from neutral microsatellite loci, rapidly evolved in less than 20 years (Ayllon *et al.*, 2006). Moreover, correlations between phenotype and environment may arise from the successful colonization by a subset of pre-adapt individuals to certain conditions ('favoured founders'

sensu Quinn et al., 2001a). Minor changes in trait values can have disproportionately large influences on fitness and population vital rates, irrespective of whether the changes result from environmentally-induced plasticity, genetic adaptation, or combinations of the two (Kinnison *et al.*, 2008). Changes in vital rates and fitness can feedback on the potential for species to spread and colonize new environments and thus, understanding the realized fitness consequences of the observed phenotypic variation in Newfoundland brown trout populations is an important next step towards testing for local adaptation and assessing their potential to further invade.

Acknowledgements

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Chapter Three Tables

Table 3-1. Average ± SD size adjusted shape values based on the first relative warp scores. Visualizations depict extreme values observed in each size group, exaggerated two times to aid interpretation of differences.

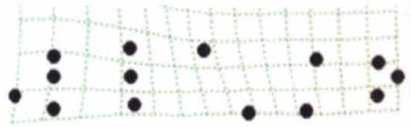
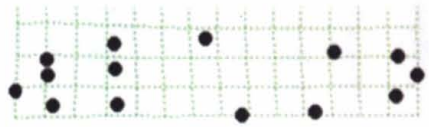
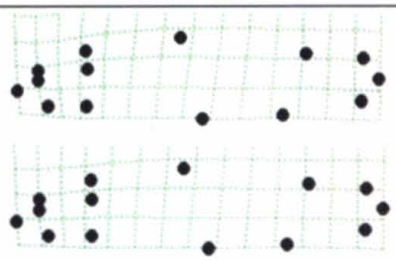
Size group	Population	n	Size adjusted shape	Shape visualization
<60 mm	Avondale	41	-0.019 ± 0.011	
	Chance	23	-0.022 ± 0.010	
	Chapel	77	-0.014 ± 0.009	
	Parkers	88	-0.039 ± 0.010	
	Raymonds	50	-0.032 ± 0.011	
	Renews	21	-0.030 ± 0.008	
	Rennies	46	-0.016 ± 0.010	
	Rexton	34	-0.022 ± 0.014	
	Salmon Cove	46	-0.015 ± 0.013	
	Savage	17	-0.010 ± 0.007	
	SE Placentia	40	-0.019 ± 0.008	
	Topsail	62	-0.022 ± 0.010	
	Witless	60	-0.029 ± 0.011	
60-150 mm	Avondale	57	0.007 ± 0.010	
	Chance	74	-0.002 ± 0.011	
	Chapel	24	0.018 ± 0.007	
	Parkers	19	-0.001 ± 0.011	
	Raymonds	20	0.016 ± 0.009	
	Renews	72	-0.001 ± 0.010	
	Rennies	33	0.013 ± 0.010	
	Rexton	42	0.015 ± 0.009	
	Salmon Cove	20	0.021 ± 0.010	
	Savage	77	0.024 ± 0.008	
	SE Placentia	40	0.002 ± 0.010	
	Topsail	37	0.018 ± 0.008	
	Torbay	63	0.023 ± 0.008	
	Virginia	57	0.020 ± 0.009	
	Waterford	51	0.017 ± 0.010	
	Witless	46	0.007 ± 0.008	
>150 mm	Rennies	22	0.018 ± 0.012	
	Salmon Cove	25	0.025 ± 0.010	
	Savage	26	0.027 ± 0.009	
	Topsail	15	0.026 ± 0.009	
	Torbay	61	0.022 ± 0.011	
	Virginia	48	0.017 ± 0.010	
	Waterford	30	0.022 ± 0.011	

Table 3-2. Reclassification rates based from linear discriminant function analysis based on 11 size-adjusted morphological traits in 16 brown trout populations. Discriminant functions and reclassifications were conducted separately in three size groups. Percentages are based on n=17, n=20, n=15 fish per population in the <60 mm, 60-150mm, and >150mm size groups.

	% correctly assigned		
Population	<60 mm	60-150 mm	>150 mm
Avondale	47	65	—
Chance Cove	82	68	—
Chapel Arm	65	75	—
Parker's	77	75	—
Raymond's	71	60	—
Renews	82	53	—
Rennie's	53	65	47
Rexton	76	40	—
Salmon Cove	82	30	73
Savage	75	60	75
SE Placentia	65	65	—
Topsail	65	67	47
Torbay	—	50	75
Virginia	—	38	43
Waterford	—	30	38
Witless	65	20	—
prior probability	7.7	6.3	14.3

Table 3-3. Loadings from the first four principal component axes based on growth and size-adjusted morphological and meristic variables for three size groups of fish a) <60 mm, b) 60-150 mm, c) >150 mm. Significant loadings are highlighted in grey.

Variable	PC1	PC2	PC3	PC4
a) <60mm				
growth	-0.326	0.258	-0.286	0.384
body depth	0.078	0.646	-0.176	-0.021
weight	-0.14	0.47	0.36	-0.21
caudal depth	0.227	0.385	-0.093	0.043
pectoral length	0.270	0.083	0.305	0.643
caudal length	0.412	-0.001	-0.085	0.290
head size	0.332	0.288	-0.148	-0.294
eye size	0.433	-0.024	-0.044	-0.352
coloration	0.120	-0.155	-0.751	0.026
red coloration	0.391	-0.043	0.065	0.259
spots	0.330	-0.184	0.236	-0.180
Eigenvalue	3.17	1.63	1.21	1.02
% of total variance	28.9	14.8	11.0	9.3
% cumulative variance	28.9	43.7	54.7	64.0
b) 60-150 mm				
growth	0.349	-0.215	0.303	-0.237
body depth	-0.035	-0.648	-0.021	0.056
weight	-0.076	-0.292	0.048	0.604
caudal depth	-0.144	-0.590	-0.033	-0.039
pectoral length	-0.479	0.036	0.073	-0.168
caudal length	-0.340	-0.039	0.184	-0.384
head size	-0.444	-0.154	-0.126	0.010
eye size	-0.488	0.140	-0.045	-0.069
coloration	0.204	-0.127	0.224	-0.211
red coloration	-0.093	-0.022	0.781	-0.104
spots	-0.141	0.192	0.434	0.581
Eigenvalue	2.71	1.85	1.25	1.16
% of total variance	24.7	16.8	11.3	10.6
% cumulative variance	24.7	41.4	52.8	63.4
c) >150mm				
growth	0.131	-0.284	0.313	-0.068
body depth	0.460	-0.334	-0.113	0.010
weight	0.314	-0.307	-0.274	-0.291
caudal depth	0.324	-0.448	-0.105	0.317
pectoral length	0.443	0.216	0.063	0.012
caudal length	-0.088	-0.056	0.072	0.762
head size	0.471	0.336	0.017	-0.140
eye size	0.275	0.523	-0.021	0.146
coloration	-0.062	-0.053	-0.616	0.210
red coloration	-0.006	0.248	-0.590	0.069
spots	0.252	0.111	0.258	0.373
Eigenvalue	2.55	1.81	1.47	1.33
% of total variance	23.1	16.4	13.4	12.1
% cumulative variance	23.1	39.6	52.9	65.0

Table 3-4. Variation (r^2) in body shape values (relative warp 1 scores from geometric morphometrics) of 16 brown trout populations in Newfoundland. Models incorporating combinations in conductivity, stream size (width-depth ratio), stream gradient, and distance to putative ancestral source in explaining variation in shape that received substantial support (ΔAIC scores <4) are shown in bold. Analyses were conducted separately in three size groups to account for differences in allometry.

Size group	k	Predictors	r^2	AIC	ΔAIC	Coefficient
	2	**conductivity**	0.1	-87.99	0	positive
	2	**distance**	0.05	-87.12	-0.87	positive
	2	**size**	0.03	-86.92	-1.07	negative
<60 mm	3	**flow (size + gradient)**	0.02	-85.03	-2.96	
	4	**flow (size + gradient), conductivity**	0.1	-84.06	-3.93	
	4	distance, flow (size + gradient)	0.04	-83.3	-4.69	
	5	distance, flow (size + gradient), conductivity	0.12	-82.42	-5.57	
	2	**distance**	0.26	-106.31	0	positive
	2	**size (w.d)**	0.25	-106.1	-0.21	negative
	4	**distance, flow (size + gradient)**	0.29	-105.6	-0.71	
60-150mm	5	**distance, flow (size + gradient), conductivity**	0.3	-105.31	-1	
	2	**conductivity**	0.16	-104.41	-1.9	positive
	3	**flow (size + gradient)**	0.19	-104.27	-2.04	
	4	**flow (size + gradient), conductivity**	0.16	-103.01	-3.3	
	2	**distance**	0.25	-55.91	0	positive
	3	**flow (size + gradient)**	0.17	-54.83	-1.08	
> 150 mm	4	**distance, flow (size + gradient)**	0.15	-54.59	-1.32	
	2	**conductivity**	0.23	-54.5	-1.41	negative
	2	**size (w.d)**	0.09	-53.36	-2.55	positive
	5	**distance, flow (size + gradient), conductivity**	0.18	-53.12	-2.79	
	4	**flow (size + gradient), conductivity**	0.09	-52.89	-3.02	

Table 3-5. Environmental factors correlated with population phenotype of Newfoundland brown trout. Correlates represent the best combinations of variables associated with phenotype and significance of the Mantel correlation (Rm) is based on Mantel's tests.

Size group	k	Correlates	R_m
	1	size	0.11
	2	cover,size	0.15
<60 mm	3	cover, size, gradient	0.16
	4	cover, size, gradient, clarity	0.17
	5	distance, cover, size, gradient, clarity	0.05
	6	distance, cover, size, gradient, clarity, conductivity	-0.02
	1	distance	0.31***
	2	cover, size	0.38***
60 - 150mm	3	distance, cover, size	0.47****
	4	distance, cover, size, gradient	0.41***
	5	distance, cover, size, gradient, clarity	0.34***
	6	distance, cover, size, gradient, clarity, conductivity	0.23**
	1	conductivity	0.3
	2	cover, conductivity	0.38*
>150 mm	3	distance, cover, conductivity	0.33
	4	distance, cover, gradient, conductivity	0.31
	5	distance, cover, size, gradient, conductivity	0.23
	6	distance, cover, size, gradient, conductivity, clarity	0.15

*p<0.1, **p <0.05, ***p<0.01,****p<0.005

Chapter Three Figures

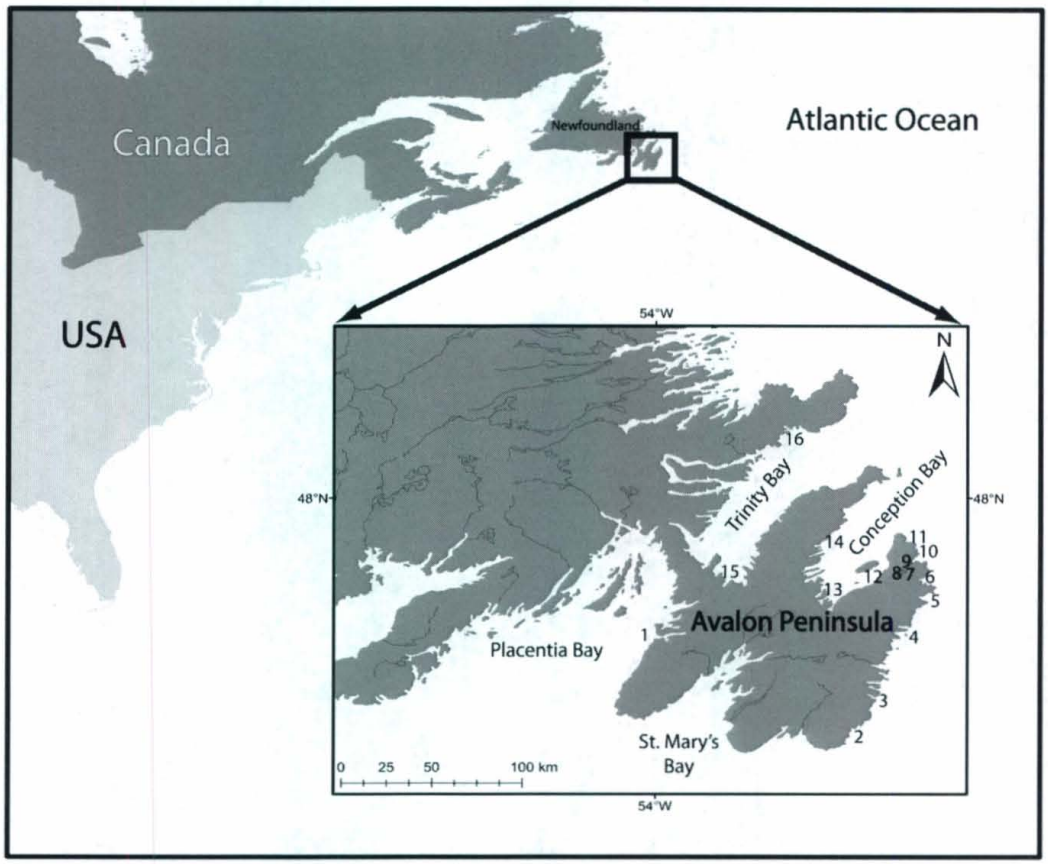


Fig. 3-1. Approximate locations of sampled brown trout populations on the island of Newfoundland, Canada. Numbers correspond to the following: 1) Southeast Placentia River, 2) Chance Cove River, 3) Renews River, 4) Witless Bay/Pierre's Brook, 5) Raymond's Brook/Petty Harbour, 6) Waterford River, 7) Rennie's River, 8) Parker's Pond Brook/Windsor Lake, 9) Virginia River, 10) Savage Creek, 11) Torbay River, 12) Topsail River, 13) Avondale River, 14) Salmon Cove River, 15) Chapel Arm River, 16) Port Rexton River. For coordinates of these systems see Table 2 in Westley and Fleming (2011). The city of St. John's is located in the vicinity of numbers 7, 8, and 9.

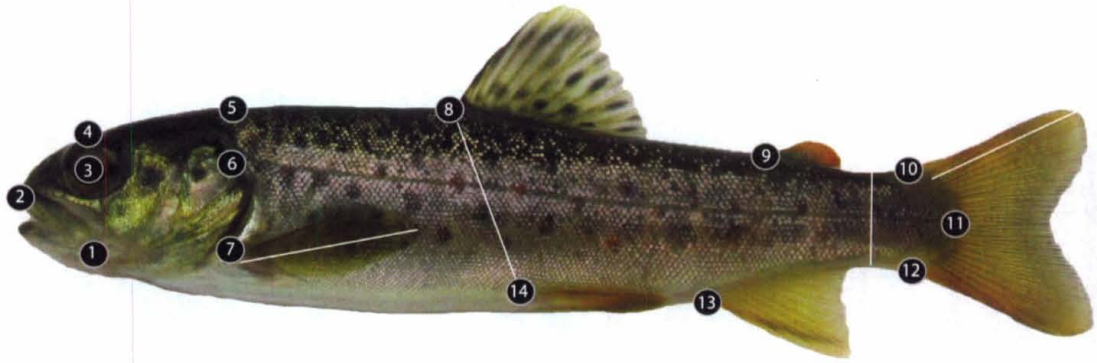


Fig. 3-2. Location of homologous landmarks and linear measures used to quantify shape differences in Newfoundland brown trout populations. Surface area of the head and eye were also quantified using the Freehand polygon tool in ImageJ. Count of pigment spots and quantification of red and overall colouration were also completed from photographs (see methods).

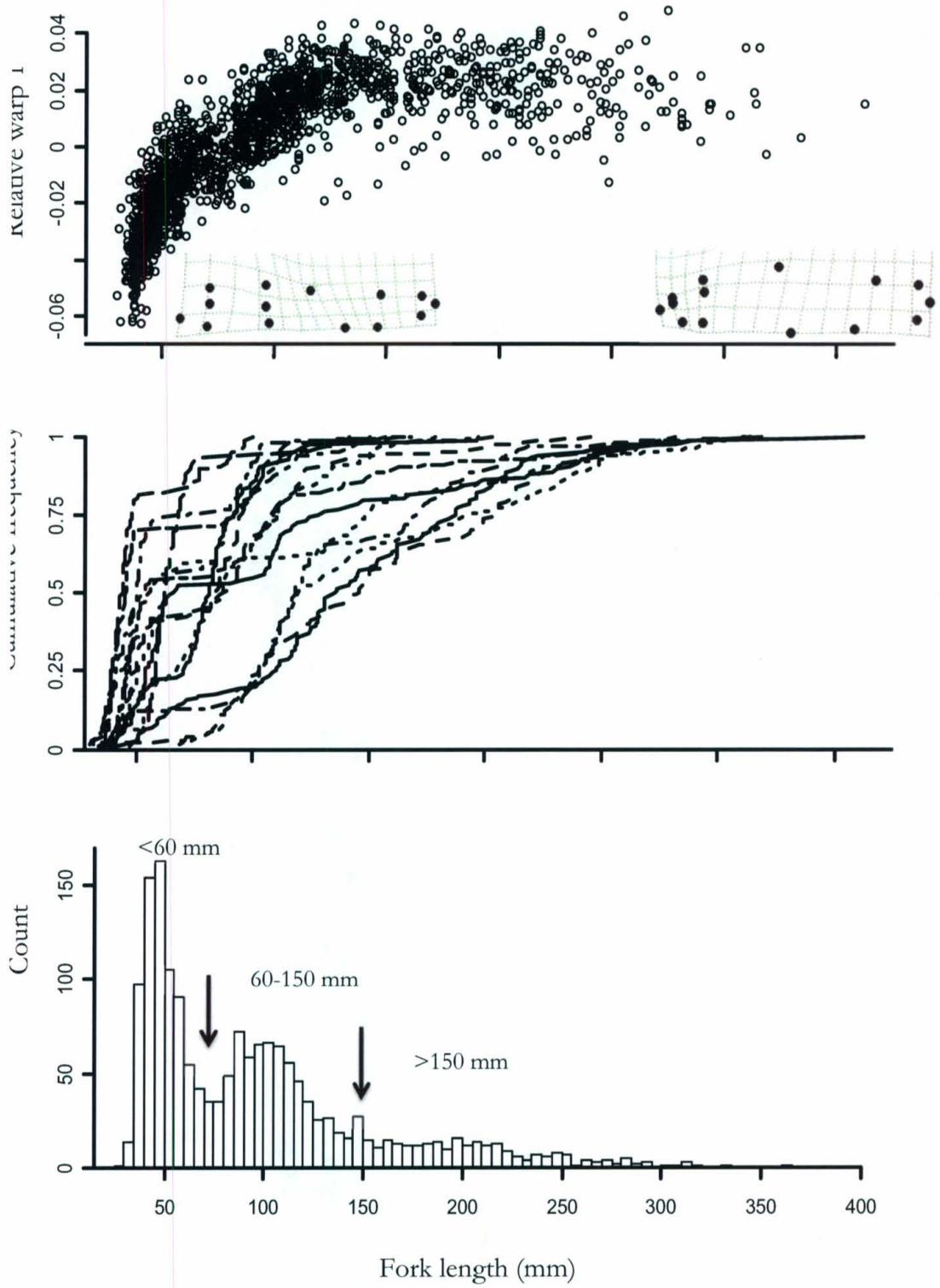


Fig. 3-3. Relative warp scores and thin-plate spline visualizations depicting the extreme values of the first relative warp (a), cumulative frequency of total sampled fish in 16 trout populations based on fork length (b), and histogram of fork lengths pooled across populations, arrows denote breaks corresponding to size groups in statistical analyses (c).

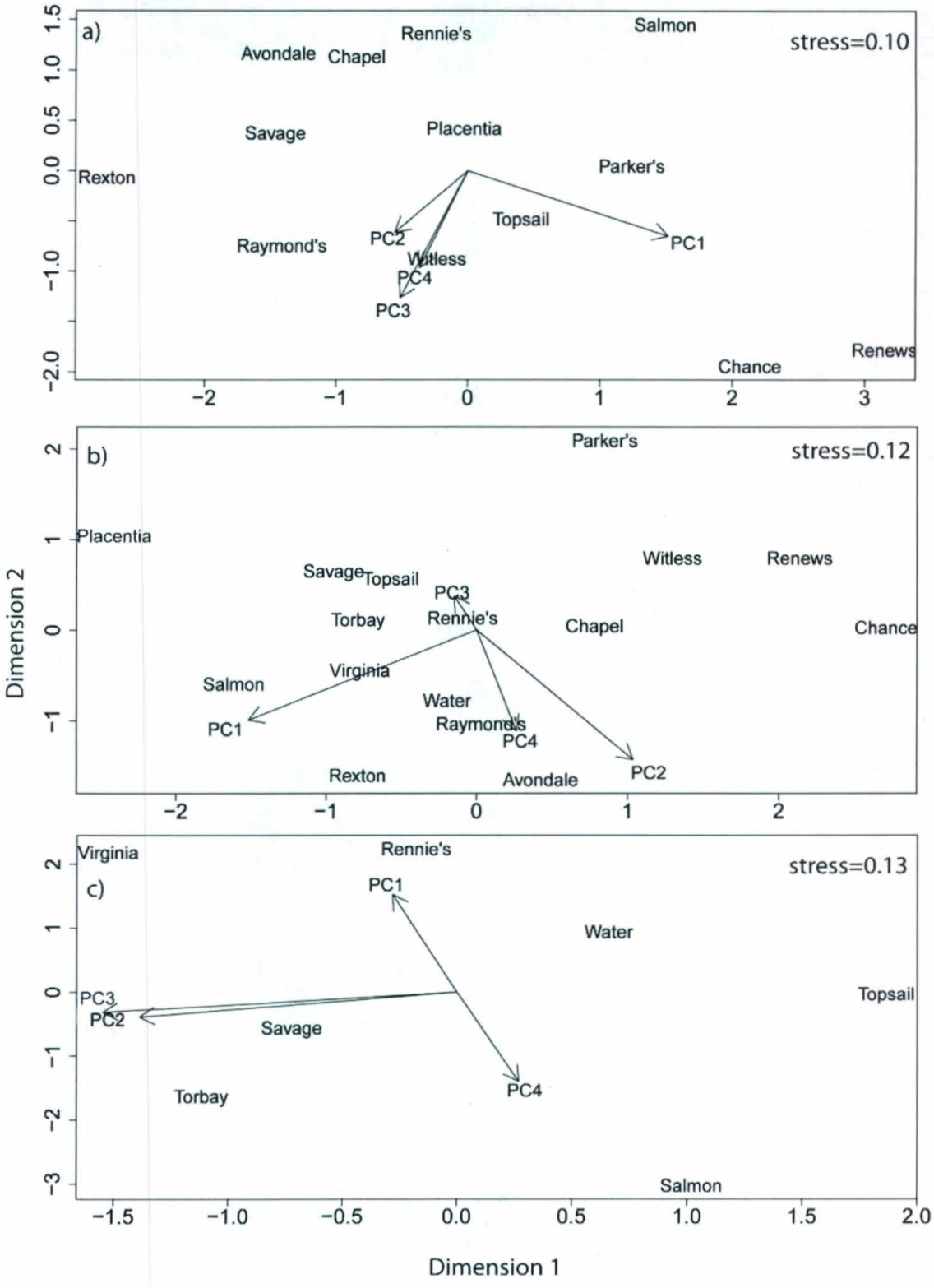


Fig. 3-4. Non-metric multidimensional scaling plots to visualize phenotypic differentiation of brown trout populations in Newfoundland, based on fish <60 mm (a), 60-150 mm (b), and >150mm (c). Similarity represents Euclidean distance based on four retained principal component axes scores on 11 phenotypic variables. See methods and results for more details.

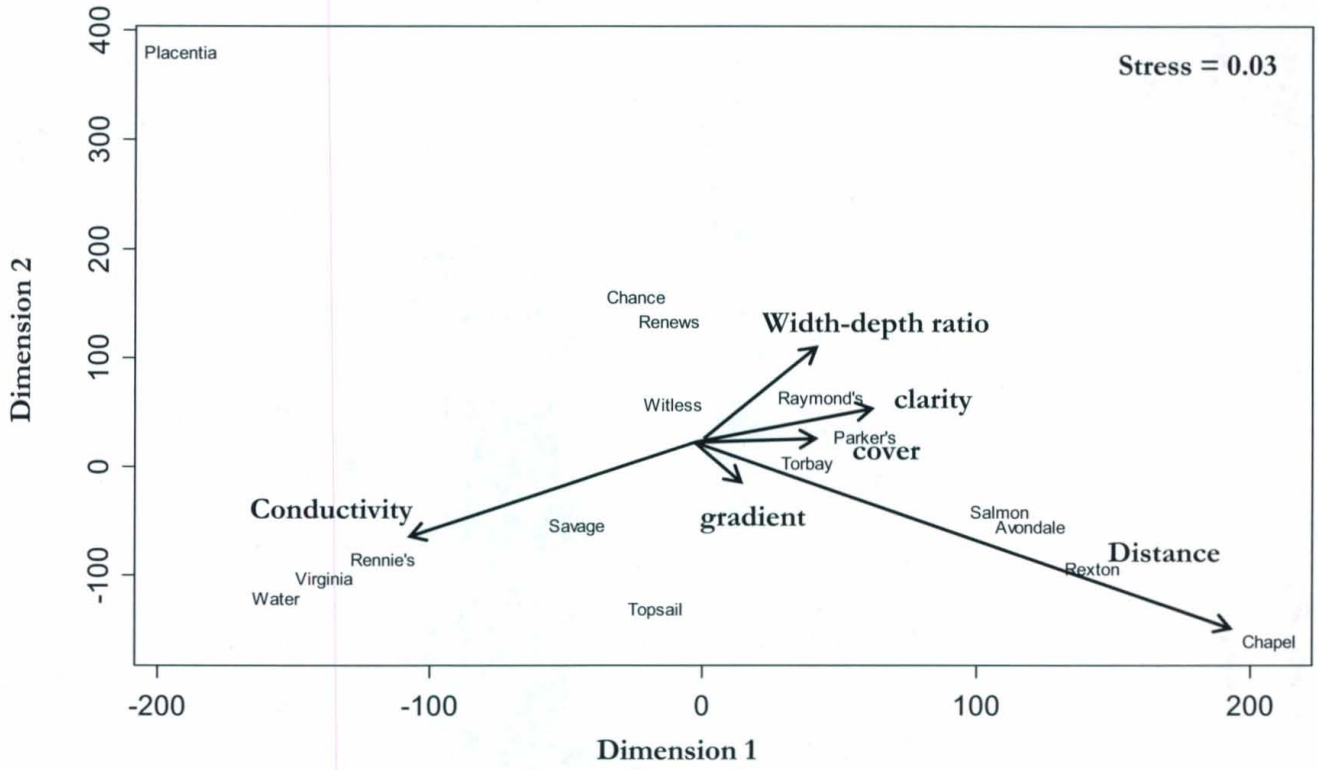


Fig. 3-5. Non-metric multidimensional scaling plots to visualize differentiation of watersheds in Newfoundland based six environmental variables: conductivity, extent of riparian cover (cover), water clarity (clarity), distance to the putative ancestral source, stream gradient, and wetted-width to depth ratio. See methods and results for more details.

Chapter 4: Testing predictions of the Baldwin effect in nature—does phenotypic plasticity facilitate survival during the introduction stage of invasion?

Abstract

Phenotypic plasticity – the ability of an organism to respond to an environmental stimulus with a change in state, form, movement, or behaviour – is increasingly thought to represent a mechanism for populations and species to cope with abruptly changing environmental conditions. Over a hundred years ago, J. M. Baldwin proposed a 'new factor' in evolution whereby plasticity could facilitate survival in new environments and allow selection to act on the survivors in the direction of the plastic response. These predictions encapsulate the first vital stages of the 'Baldwin effect' whereby environmental induction of traits can shape the course of subsequent evolution; however, few studies have attempted to empirically test these predictions in nature. In this paper, we combine common-garden and reciprocal-transplant experiments along with formal quantifications of natural selection to test the hypotheses that phenotypic plasticity acting body size, growth rate, and functional morphology in juvenile brown trout (*Salmo trutta*) should allow individuals to persist when introduced to novel environments and that plasticity should be predictable based on patterns of natural selection. To do so, we raised individuals from three populations in common laboratory conditions until large enough to tag and track in the wild. We detected marked plasticity in swimming morphology, specifically the depth of the head and body, after approximately two months of rearing in three wild streams. Populations that survived introduction were generally consistent in their plastic responses, though we did detect evidence of population-specific plasticity suggesting underlying genetic variation. Counter to predictions, the plasticity we observed was frequently in the *opposite* direction from selection even though it moved in a direction generally *assumed* to be adaptive (i.e. small rivers seemed to plastically induce shallow-bodies and vice versa in large rivers). We did detect evidence of greater survival and growth of individuals reared in their local environments compared to when reared in foreign locations, suggesting local adaptation has evolved in these populations recently descended from common ancestors. Overall, our results suggest that plasticity may shape phenotypes in unpredictable ways and that attempts to forecast the response of populations to rapidly changing global environments may be prone to failure.

Introduction

Theory predicts that phenotypic plasticity – the ability of an organism to respond to an environmental stimulus with a change in state, form, movement, or behaviour – should facilitate survival during periods of abrupt environmental change (Lande, 2009, Ghalambor et al., 2007). Research to understand the role of phenotypic plasticity as an evolutionary pathway has surged in recent decades (see reviews by West-Eberhard, 2003, Pigliucci, 2001) after a long period of disfavour among many evolutionary biologists resulting, in part, from the perception that plasticity is merely a 'proximate' rather than an 'ultimate' explanation of the phenotype (e.g. Mayr, 1961). It is currently, and has been at least since the time of Darwin, widely acknowledged and accepted that the environment shapes individual phenotypes (Darwin, 1859, DeWitt & Scheiner, 2004). What remains controversial, however, is whether plasticity serves to shield individuals from selection or produces novel variation on which selection can act, and thus whether plasticity accelerates or retards the rate of phenotypic evolution (Paenke et al., 2007, Via et al., 1995). The desire to predict how species and populations will respond to large-scale climate change and anthropogenic disturbance has helped fuel the resurgent interest in phenotypic plasticity (Crispo et al., 2010, Charmantier et al., 2008), with a primary goal to understand whether plastic responses will be sufficient to allow persistence (Reed et al., 2011, Chevin & Lande, 2011b). Theoretical studies (i.e. mathematical modelling and simulation, see Chevin et al., 2010) have greatly outpaced empirical research and many of the predictions from classical, as well as contemporary work on plasticity remain to be tested in nature.

Biological invasions and invasive species (i.e. species that are transplanted and established beyond their native range) are serendipitous research systems to explore the importance of phenotypic plasticity in the successful colonization of novel habitats (Westley, 2011). Brief bursts of directional selection are predicted and observed to occur during the early stages of colonization and invasion (Reznick & Ghalambor, 2001), and plasticity is frequently postulated as a means for individuals to withstand abruptly divergent patterns of selection (for an empirical example in barnacles see Neufeld & Palmer, 2008). The hypothesis that plasticity should facilitate survival in an altered environment or during periods of stress is far from new. In 1896, J.M. Baldwin formulated a process now referred to as the *Baldwin effect*, by which response and accommodation to environmental inputs (i.e. plasticity) would permit survival and allow time for selection to act on the traits of the survivors, and by doing so, shapes the course of future evolution and adaptation (Baldwin, 1896). The Baldwin effect is related to, yet separate from, *genetic assimilation* (sensu Waddington, 1961); the process where an environmentally acquired character, through selection, becomes genetically determined and canalized (for clarification see Crispo, 2007).

Phenotypic plasticity that results in greater individual fitness to a current environment (i.e. adaptive plasticity) represents a fundamental component of the Baldwin effect and was historically referred to as *organic selection.* In a series of laboratory experiments, Gause (1942) demonstrated patterns in plasticity to salt resistance in clones of *Paramecium* sp. consistent with organic selection and the Baldwin effect (though Gause did not cite Baldwin). Specifically, surviving clones all adjusted their tolerance to salt not only in the *same* direction but in the direction that apparently *allowed survival.* Though these early controlled experiments suggest the potential for species to respond predictably to an abruptly changed

environment, it is less clear whether we can observe, in natural settings, plasticity shifting the phenotypes of invading and colonizing species in similarly predictable ways?

Three biological examples suggest that adaptive phenotypic plasticity helps determine the success of invasion and have potentially shaped the course of future evolutionary change. The first empirical quantitative support of Baldwin's prediction that plasticity can facilitate survival during the early stages of colonization comes from dark-eyed juncos (*Junco hyemalis*) that established a population on the University of California, San Diego campus approximately 30 years ago. Here plasticity in breeding length has allowed population persistence as increased reproductive effort appears necessary to compensate for high mortality experienced in the novel environment (Yeh & Price, 2004). The second line of evidence comes from experimental introductions of *Anolis* lizards to a series of small Bahamian islands (reviewed by Losos, 2009). After 15 years the subset of individuals that successfully colonized displayed morphological diversification in hind limb size beneficial to particular habitat use (perch height and diameter in vegetation). Additionally, phenotypic plasticity during early development was revealed to shape hind limb morphology in the direction predicted by selection and habitat use and appears to foreshadow the adaptive differentiation that evolves over additional generations (Losos et al., 2000). Finally, in a recent review Badyaev (2009) suggests that novel adaptations observed in populations of colonizing house finch (*Carpodacus mexicanus*) are consistent with the Baldwin effect. Here it was concluded that novel environments experienced by expanding populations induced developmental variation, that this variation was phenotypically accommodated and that the induced developmental outcomes, favoured by selection, were transferred across generations. Though these examples combine to provide a compelling case for plasticity to

influence colonization and invasion, they all take (out of necessity) a retrospective approach rather than catching the Baldwin effect 'in the act'. Indeed it is possible for important processes to move so quickly as to pass us by (Pigliucci & Murren, 2003).

In this paper we examine the importance of phenotypic plasticity to influence body shape morphology and potentially facilitate survival of colonizing brown trout (*Salmo trutta*) during the *introduction stage* of an invasion. We take a prospective approach and predict: 1) that plastic responses to abrupt environmental change should move phenotypes of populations and individuals within populations in similar directions and, if the traits are largely environmentally induced, lead to phenotypic convergence toward the wild-type and presumably optimal phenotype, 2) that plastic responses should be predictable and adaptive, that is, phenotypic plasticity should act in the direction favoured by natural selection, and 3) natural selection acting on colonizer phenotypes would be *directional* and *strong*. We addressed these questions through the combination of laboratory 'common-garden' and reciprocal-transplants experiments into natural river systems of three non-native brown trout populations that shared common ancestors approximately 130 years (~30 generations) ago. The complementary nature of the common-garden and reciprocal transplants allowed simultaneous testing for the presence of fine-scale local adaptation by comparing the performance of local- and wild-reared individuals against foreign groups and facilitated assessment of the genetic underpinnings of body morphology and growth rates. Throughout this experiment, we assume that phenotypic differentiation among populations reared in common conditions is the result of underlying genetic differentiation.

Methods

The study system

Brown trout is a member of the family Salmonidae that exhibits marked variability in its biology and ecology (but see Jonsson & Jonsson, 2011, Elliott, 1994). Great variability notwithstanding, an archetypical life history of brown trout includes fall spawning in streams and rivers followed by protracted embryonic and larval development in gravel substrate. Young fish, termed fry, emerge to claim and maintain feeding territories in the spring, representing an important period of selection (Einum & Fleming, 2000). After a variable amount of time rearing in small rivers fish, now termed parr, often migrate to larger habitats, such as lakes, mainstem rivers, or the ocean to complete their juvenile rearing before the majority of the population returns to natal sites for reproduction. This fine-scale homing of brown trout and other salmonids to natal areas promotes reproductive isolation and local adaptation of populations in response to site-specific regimes of natural and sexual selection (Hendry et al., 2004). However, a small percentage of brown trout populations (~2-5%) may stray to non-natal sites representing a mechanism for both gene flow and the colonization of new habitat (Jonsson & Jonsson, 2011 and references therein).

The native range of brown trout is Eurasian, but beginning in the late 19th century, brown trout's renown as a game fish motivated its wide-spread introductions around the globe (MacCrimmon & Marshall, 1968). The island of Newfoundland was among the first North American locales to receive shipments of brown trout embryos from Europe in 1883 (Maitland, 1887). The fish were first planted in a land-locked water body, Windsor Lake, in the capital city of St. John's. The fish survived well upon introduction to Windsor Lake and

in the following year of 1884, fry from Windsor Lake escaped to the Rennie's River watershed and subsequently propagated there in a hatchery until the early 1900s, when all hatchery production of brown trout ceased (Hustins, 2007). The introduction of fish to the Rennie's River watershed is notable as it represents the first location in Newfoundland with a traversable connection to the ocean and thus represents the first potential colonizing source for other watersheds. The Waterford River watershed, along with at least 50 other watersheds differing in a host of environmental features, have been successfully colonized, presumably by straying anadromous individuals (Westley & Fleming, 2011).

Field observations reveal population-specific differences in a suite of phenotypic traits likely important for fitness and the observed variation correlates predictably with environmental features, such as river size and riparian canopy cover (Westley et al., Chapter three.). This pattern of environmental matching with phenotype is suggestive of genetic adaptation, phenotypic plasticity in response to local conditions, or a combination of mechanisms. However, the extent to which individual traits, such as body shape and growth rates are genetically controlled, and thus may evolve in response to selection, is not known in these populations. Moreover, the fitness consequences of observed differences in size, growth rate, and body shape among populations have yet to be determined. In addition, on-going microsatellite analyses on the study populations indicate significant pair-wise genetic differentiation and large F_{st} values at many loci (O'Toole et al., in prep), suggesting limited gene flow among populations, and thus potential for local adaptation.

Experimental animals

During October and November 2008, mature brown trout were collected in Parkers Pond Brook (a tributary of Windsor Lake), the Rennies River, and the Waterford River with electrofishing, dipnets, and gillnets (Fig. 4-1). A total of 23 families were successfully created by crossing eight unique sires and dams at the Parkers and Rennies locations and seven unique parents at the Waterford location. An eighth family originally created from the Waterford suffered 100% mortality during early embryonic development. We measured the fork length (mm), and mass (g) of the dams, along with wet mass (mg) of five eggs/female (Table 4-1). Crosses were conducted on site at the Parkers and Rennies locations while adults from the Waterford were transferred back to the laboratory and held in common circular tanks as many of the females, though visually maturing at the time of capture, were not sufficiently ripe to spawn in the field. Crosses were made in the laboratory when females were ready to spawn (determined when females expressed eggs under light abdominal pressure). We assume that the parents selected for crossing were a random sample of spawning individuals and representative of the three populations.

Fertilized ova were incubated at the family level in standard hatchery trays on a common source of flow-through water at ambient temperatures. Dead individuals (eggs and larvae) were quantified and removed three times per week during the incubation phase. Except for the one family from the Waterford that experienced 100% mortality, survival was extremely high (>98% in all families). Hatching began on 21 December and was completed by mid-January. Fry were removed from incubator trays (i.e. 'emerged') when their yolk sac had been nearly absorbed and they exhibited free-swimming behaviour. All families had emerged by mid-March 2009. Newly emerged fry were housed temporarily as separate

families to monitor potential mortality during this critical emergent stage and introduced to feed (live brine shrimp *Artemia sp.* mixed with 0.5 mm standard dry salmonid food, Corey Feed Mills, Fredericton, NB, Canada). After the fish had acclimated to dry food, families from each population were combined into communal 1 m^2 diameter tanks (one tank per population) as space limitations precluded separate rearing of families. Fish were kept at ambient photoperiod, shared a common flow- through ambient water source and were fed *ad libitum* 4-8 times daily with commercial dry salmon feed.

In late July and early August 2009 we collected, through electrofishing, approximately 100 wild young-of-year individuals from each of Parkers, Rennies, and Waterford populations and transported them to the laboratory for phenotypic sampling and tagging (see next section). Wild fish were measured and tagged the day after capture and allowed to recover fully before being returned into their home river with other experimental groups (see subsequent).

Phenotypic measurements and tagging prior to release

In mid- July, fish from all populations had obtained sufficiently large size (~50 mm total length and 1 g wet weight) to be implanted with unique passive integrated transponder (PIT) tags (length 8.4 mm, weight 64 mg, frequency 134.2 kHz, Biomark, Boise, Idaho). Three hundred haphazardly chosen individuals from each of the three laboratory populations and 100 wild individuals collected from each location, were lightly anaesthetized with MS-222, weighed to the nearest 0.0001g on an analytical balance, and photographed with a Nikon D9000 and 50 mm micro lens. Fish were photographed in a standardized position on their right side following the procedures of Westley et al. (Chapter three.), under

5200K true daylight fluorescent lights. Fish were then implanted with a PIT tag through a small vertical incision in the abdominal cavity and allowed to recover in common $1m^2$ circular tanks. Mortality as a result of tagging was very low (<2%) and there was no signs of tag-loss. In most cases, fish were feeding and behaving normally within 24 hours. In early September 2009, 50 additional individuals from the Parkers, Rennies, and Waterford laboratory populations were measured and PIT tagged to facilitate the tracking of individual growth in the laboratory environment. All measurements, tagging, and housing of the experimental animals were done in accordance with the guidelines provided by the Canadian Council on Animal Care and with approval of Memorial University's Institutional Animal Care Committee.

Experimental design— reciprocal transplants

Release

To test the prediction that phenotypic plasticity should shape phenotypes similarly in the direction favoured by selection, we reciprocally planted four experimental groups into three natural rivers, differing in environmental features (Table 4-2). Relative to the other locations, Parkers was smaller in length, width, and depth, slower flowing, less productive (conductivity as an indicator of productivity, Copp, 2003), cooler on average, and had greater levels of canopy cover. In contrast, the Rennies and Waterford rivers were longer, larger, faster flowing, highly productive, warmer, and had less canopy cover. Rivers differed in biotic variables such as conspecific density and the presence and abundance of potential fish predators like the American eel, *Anguilla rostrata*, and larger, older conspecifics (Table 4-2). All rivers would have historically contained brook trout (*Salvelinus fontinalis*) but currently are

dominated by brown trout, presumably resulting from competitive exclusion (Westley et al., 2011). Additionally, each release site was situated approximately 40 m downstream of complete obstructions in the form of a perched culvert at Parkers (no brown trout detected above the culvert, Westley & Fleming personal observations), a 3 m waterfall at Rennies (a barrier even to full size adult trout, Robbins, 2001) and an 18 m section of chutes and rapids at the Waterford River where water velocities exceeded 1.6 ms^{-1}, approximately twice the prolonged swimming capacity of juvenile brown trout (0.6-0.8 m/s; Bull, 2010). Thus the upstream limit of the release locations were marked by complete obstacles to fish movement and the downstream limits were taken to be where Parkers and Rennies entered large lakes (Windsor and Quidi Vidi lakes, respectively) and where the west branch of the Waterford entered the mainstem of the river. Small fish are virtually absent in this mainstem stretch of river, which we infer is a reflection of poor habitat for young of year trout (Westley et al., Chapter three.).

At each location we released individuals of three laboratory raised F_1 populations (i.e. one local and two foreign at each site) along with individuals from the local wild-born population (referred to as 'wild'). Individuals from the laboratory groups were haphazardly assigned to a release and rearing location. All groups were released in approximately equal proportions (~100 in each group, slight deviations resulted from counting errors and mortality during transit to release locations). Thus for example at the Rennies release location; 100 local (laboratory-raised), 99 local (wild-born), 100 foreign Parkers (laboratory-raised), and 102 foreign Waterford (laboratory-raised) individuals were released. The wild fish from Parkers Pond Brook are the one notable exception to the balanced design as only 65 individuals were released. At the time of collection in late July, many of the young of year

trout were less than the threshold size for tagging and as a result 48% of the 65 tagged wild fish from Parkers were relatively large (>65 mm) yearlings. We chose not to tag additional large wild fish in order to avoid further confounding of the effect of population origin and size.

Recapture

We conducted sampling to recapture tagged individuals beginning in early October (~70 days post release) and continued through mid-November (for dates see Table 4-2). Our goal was to search the length of the experimental rivers in their entirety in order to maximize tag recovery and to accurately gauge relative survival between groups. To that end, we employed the following recapture protocol on the rivers: each location was divided into sections based on habitat characteristics (e.g. pool or riffle) and sampling began at the upper most section and sequentially proceeded downstream. In the following year we initiated our sampling at the bottom and worked sequentially upstream because we hypothesized that greater numbers of fish may have dispersed to downstream areas over the winter. Each section was shocked in an upstream direction with electrofishing. Captured fish, regardless of size, were removed and temporarily stored in aerated containers until the upstream part of the section was reached. All fish, including potential predators (large brown trout and eels), were then scanned for PIT tags with handheld readers (Pocket Reader, Biomark, Boise, Idaho). We conducted additional upstream electrofishing passes when tagged individuals were recovered and repeated the sampling of sections until subsequent passes yielded no additional tags. In this manner, we systematically worked throughout the entire length of the experimental river.

Tagged individuals were recorded by river section, anesthetized with clove oil, weighed (0.1 g), and photographed in the field using the same protocol and equipment employed prior to release. Fish were released near their site of capture at the end of the sampling day. Landmarks were placed on the photographs of recaptured individuals and again used to calculate Relative Warp scores following the methods used prior to release. Doing so facilitated investigations of shape-change plasticity of individuals and populations reared in different locations.

Analytical approach and data analysis

Population-specific body shape in a common laboratory environment

We used a multivariate approach, geometric morphometrics, to quantify body shape among populations reared under common laboratory conditions. This allowed us to compare the shape of fish raised in the laboratory versus wild environments, and to investigate plastic changes in shape among environments (Adams et al., 2004). Two-dimensional body shape was quantified by placing 14-homologous landmarks on digital images in the program tpsDig2, Version 2.12 (Rohlf, 2005). The landmarks were modified from Westley et al. (Chapter three) to include points on the posterior and anterior insertion of the orbit as to capture changes in eye size (Fig. 4-2). Landmark data were aligned to a single consensus shape configuration using Procrustes superimposition using the program tpsRelw, Version 1.46 (Rohlf, 2006). After alignment, Relative Warp scores (analogous to Principal Component scores) were calculated for each individual. Interpretation of how each

Relative Warp contributed to body shape was based on visualizations of thin-plate spline transformations generated in tpsRelw.

We tested for significant relationships between body size and shape values and when significant relationships were detected we adjusted shape values to a common mean length using the equation (Hendry et al., 2002, Quinn et al., 2001b, Reist, 1986, Fleming & Gross, 1989):

$$Shape_{adj} = Shape_{obs}(Length_{mean} / Length_{obs})^{b} \qquad \text{Eq.1}$$

where $Shape_{adj}$ is the adjusted shape, $Shape_{obs}$ is the observed shape, $Length_{mean}$ and $Lengh_{obs}$ are the mean and observed lengths, respectively, and *b* is the common within-group regression slope between shape ($\log_e + 1$) and length ($\log_e + 1$) from ANCOVA without an interaction term. Shape variables were adjusted to a common body length rather than to the centroid value to aid in direct biological interpretation, which was justified as body length and centroid size were strongly related (length = 0.7575 * centroid + 7.0728, $r^2 = 0.998$).

We used analysis of variance (ANOVA) followed by Tukey post-hoc tests, corrected for multiple comparisons, to test the hypothesis that populations reared in common conditions differed in mean size-adjusted body shape.

Apparent survival

We used recapture information (recaptured or not) as a proxy for survival to test for differences in the *relative* performance among experimental groups released at each of the three rearing locations. This approach has been used successfully elsewhere and deemed highly appropriate for experimental designs, such as ours, that employ few recapture bouts

(Nosil & Crespi, 2006). Individuals that survived until fall 2009 were assigned a value of 1, which we determined by either recapturing the individual in the fall or at any subsequent time. That is, individuals that we did not recapture in the fall of 2009, but did recover in 2010 must have been alive in 2009 (similar logic employed by Carlson et al., 2004, Hendry et al., 2003).

We modeled the proportion of fish surviving (number survived/number released) until fall using a generalized linear model with binomial error and population origin and rearing location as fixed categorical factors (Agresti, 2007). To assess the relative importance of population origin, location, and the interacting effects of the two on survival we compared four *a priori* models using AICc (Akaike Information Criterion corrected for small sample size). Specifically, we fit models corresponding to the following hypotheses: 1) survival varied solely as a function population origin (i.e. includes wild- reared and laboratory raised-groups), 2) survival varied solely among rearing locations, 3) survival was higher among populations in their local environment (i.e. population x rearing location interaction), and 4) survival was completely random with regards to location or population (i.e. a null model). The use of AICc was appropriate as we detected no appreciable overdisperson (ratio of residual deviance: residual df = 0.8-1.09).

Growth

Specific growth rates were estimated for individuals that survived and were recaptured in the fall. Growth information was not estimated for individuals known to survive the fall but were not recaptured until the following summer. Organism growth rate

varies as a function of size, thus we used a standardized mass specific growth rate (Ω) following Ostrovsky (1995) to quantify growth among groups differing in size:

$$\Omega = \frac{M_2^{\tau} - M_1^{\tau}}{\tau * time} \qquad \text{Eq.2}$$

where M_1 and M_2 are body mass (g) at the beginning and end of the experiment, respectively, time is the number of days in the between observations, and τ is the species-specific allometric coefficient for the relationship between growth rate and body mass. We set τ = 0.308 as the value is well established in brown trout (see Elliott et al., 1995 and references therein).

We again employed linear models in a selection framework to test for differences in growth among populations and among rearing locations. Four ANOVAs with the following parameters (set as fixed factors) were fit to individual growth data: 1) population origin only (this includes wild produced and laboratory raised groups), 2) location only, 3) population x location, and 4) null model. Growth data were examined for normality and met parametric assumptions of ANOVA prior to fitting. We interpret the importance of the population term as evidence of genetic control over growth, the location term to be the role of environmental forcing on growth, and the interaction between population origin and location as evidence of underlying genetic control on growth norms of reaction (i.e. populations grow fast in some environments and slow in others).

Shape plasticity

We tested for the presence of population specific-patterns in phenotypic plasticity of morphological shape and the potential for population x environment interactions using similar logic to analyses of survival and growth. Again, four ANOVA models were fit to two measures of average shape after individuals had reared for approximately 70 days in three wild environments. We interpret the ANOVA with only a population term to examine the sole effect of population-level *genetic* control on shape, the ANOVA with location as the only predictor to test for the sole effect of the *environment*, and the ANOVA with a population x location interaction term to test for *genetic x environment interactions* (i.e. norms of reaction). The weight of evidence to support each model was again assessed with AICc.

To test for evidence of phenotypic convergence of populations in each environment, we fit ANOVA models to shape data of individuals at release and again at recapture with population as a sole categorical predictor. Specifically, if convergence of phenotypes as a result of plasticity was complete we predicted that the ANOVA with a population term would fare no better in model selection than that of the null model, again based on AICc. In contrast, if population phenotypes remained distinct after rearing in common environments we predicted continued support of the ANOVA model with a population term compared to the null model.

Finally, we examined patterns in plasticity at the individual level by plotting observed head and body shape values of individuals at time of release (i.e. their shape after rearing in a laboratory environment) and then again at recapture in the fall (their shape after rearing in natural environments). This 'reaction norm' approach provided a visual representation and examination of individual variation in phenotypic response. We interpreted zero slopes (i.e.

horizontal lines) as lack of plasticity, non-zero slopes as evidence for the presence of plasticity, and crossing lines among individuals as genetic differences in plastic response.

Quantifying natural selection and correlations with plasticity

To test the prediction that plasticity would move in the direction favoured by selection, we first quantified the strength and shape of natural selection acting on, body size and two metrics of body shape. Following standard procedures (reviewed by Brodie, 1995), we standardized traits to a mean of zero and standard deviation of one based on group and release location values. Additionally, we quantified location-specific selection by pooling all groups within a rearing location. By definition, survival is described by a dichotomous process (0 or 1) so we used logistic regression with a binomial error structure (Janzen & Stern, 1998) to calculate selection gradients and selection differentials. Selection gradients represent the strength of selection acting directly on each trait –excluding indirect selection acting through other traits– whereas selection differentials represent the *total* strength of selection – both direct and indirect selection– acting on a given trait (Lande & Arnold, 1983). Linear selection gradients (β) were estimated by regressing survival (0 or 1) against standardized body size (fork length, mm), standardized body shape (first Relative Warp derived from TPSrelW), and standardized head shape (fourth Relative Warp). Linear selection differentials (i) were estimated by individually regressing survival against standardized body size, standardized size-adjusted head shape, and standardized body shape.

We estimated non-linear selection gradients (γ) and differentials (j) by adding squared standardized trait terms to the previously specified models. Negative and positive non-linear

coefficients were interpreted as evidence of stabilizing and disruptive selection, respectively. We report doubled estimates of γ and *j*, following Stinchcombe et al. (2008).

The procedures outlined above were implemented to generate parameter estimates and not to assess the 'significance' of the estimates. Rather, we assessed the strength of selection observed acting on body size and shape by comparing linearly converted selection coefficients (Janzen & Stern, 1998) against values in the Kingsolver et al. (2001) selection database available at: http://www.bio.unc.edu/faculty/kingsolver/lab/. Specifically, we compared the absolute values of our selection estimates against absolute values of linear gradients and differentials. To compare the strength of non-linear selection, we first multiplied the values of γ and *j* in the Kingsolver database by two, as many of the values have likely been reported incorrectly as half their true value (Stinchcombe et al., 2008).

To visualize the shape of the selection function acting on body size, body shape, and size-adjusted body shape we constructed univariate cubic splines (Schluter, 1988). Splines were fit for each population group at each release location using the GAM function with binomial error in the 'mgcv' library in R (R Development Core Team, 2009a).

We regressed group average change in head shape and body shape in each release location against group-specific selection, as measured in gradients and differentials, to test the hypothesis that traits move plastically in the direction favoured by selection. In addition, we used the sign (positive or negative) of the selection differentials and gradients generated above as predictors of individual plastic change in head shape and body shape. Specifically, we predicted that surviving individuals would shift their phenotype in the direction favoured by directional selection. For example, if we detected positive directional selection acting on

head shape (i.e. larger values of head shape increased probability of survival) then we would expect individuals to shift their head shape toward positive values. In contrast, we would expect the opposite given a negative selection differential or gradient. To test the hypothesis that individual plastic responses were predictable based on the direction of selection, we tallied the number of individuals in each population and rearing location that moved in the predicted direction. We assume that each observation of individual plasticity is independent of others. The number of fish moving in the predicted direction versus those that did not was then compared to a binomial distribution with binomial tests.

Results

Population-specific body shape in a common laboratory environment

Relative Warp 1 and Relative Warp 4, which explained 23.7% and 5.3% of the variation in shape , respectively, were retained for subsequent analyses. Relative Warp 1 described a gradient of increasing relative head and eye size, whereas Relative Warp 4 revealed a gradient of increasing body and caudal depth. Henceforth, we refer to Relative Warp 1 as 'head shape', and Relative Warp 4 as 'body shape.' Relative Warps 2 and 3 were interpreted as an arching of the body due to minor differences in placement of fish during photographing and were thus not retained for analyses. This 'arch effect' has been observed elsewhere and may be especially common in fish subjects (Michaud et al., 2008, Westley et al., Chapter three., Valentin et al., 2008). The remaining warps explained little additional

variation, had no obvious biological interpretations, and were not retained for further analysis.

We detected a significant relationship between body size (FL, mm) and head shape ($F_{1,1158} = 591$, $p < 0.0001$, $r^2 = 0.34$) but no relationship between body size and body shape ($F_{1,1158} = 3.4$, $p = 0.06$, $r^2 = 0$). As a result, we adjusted observed values of head shape to a common body size of 53.7mm (grand mean length of all fish, following Eq. 1). As no relationship between size and body shape was detected, we used observed values.

Significant differences in head shape and body shape were detected with ANOVA between populations grown in laboratory versus wild environments, even after controlling for the effect of size. Laboratory-raised fish had relatively large heads and eyes compared to their wild- raised counterparts ($F_{1,1158} = 608$, $p < 0.0001$, Fig. 4-3a). We also detected differences in body shape among environments; wild-raised individuals were more streamlined in shape (i.e. thin, shallow-bodied) compared to the relatively deep-bodied laboratory-raised fish ($F_{1,1158} = 7.3$, $p = 0.007$, Fig. 4-3b).

We also detected population-level differences in both head shape ($F_{5,1154} = 163$, $p<0.00001$, Fig. 4-4a) and body shape ($F_{5,1154} = 5.07$, $p < 0.0001$, Fig. 4-4b). All groups differed (Tukey post-hoc, corrected $p < 0.05$) in size-adjusted head shape ($p < 0.05$) except for the wild populations from the Rennies and Waterford Rivers, which displayed statistically similar head shapes ($p = 0.99$, Fig. 4a). Populations were more similar in body shape than head shape, yet significant differences were still detected. The laboratory-raised Rennies population was significantly deeper-bodied than the laboratory-raised and wild-reared Parkers populations ($p < 0.005$) and the wild-raised Waterford population ($p < 0.005$). All

other population-level comparisons in body shape were not significantly different ($p > 0.05$, Fig. 4b).

Phenotypic plasticity in functional morphology

Brown trout displayed marked plasticity in both head shape and body shape after ca. 70 days of rearing in three wild environments (Fig. 4-5a,b). On average, populations displayed larger heads and eyes relative to their body size when reared in the Parkers location and smaller heads and eyes when reared in the Rennies and Waterford locations (Fig. 4-5a). Similarly, populations tended to be more streamlined in body shape in the Parkers location and deeper bodied in Rennies and Waterford (Fig. 4-5b). Though reaction norms were generally parallel among populations and locations, we did observe cases of crossing reaction norms. This visual result was confirmed by strong evidence of population x location interactions in head shape and body shape suggesting some degree of genetic control of reaction norms (Table 4-3). Models with a population x rearing location interaction term were heavily favoured over the next best model fit containing only the effect of location. However, this interaction emerged in only a subset of populations and locations and differed between shape traits. For example, the direction and magnitude of the plastic response in head shape was generally similar among all populations in the Parkers and Rennies environment (parallel reaction norms towards lower values of head shape, Fig. 4-5a). In contrast, the Parkers population exhibited a lack of plasticity between the Rennies and Waterford locations, while the other populations showed a response towards smaller average head and eye size. With regard to body shape, Parkers and Waterford populations exhibited a more similar response to each other compared to the Rennies population. The Rennies

population which diverged in shape in the Rennies location and converged with the other laboratory reared populations in the Waterford (Fig. 4-5b).

Curiously, populations tended to respond similarly by shifting their shapes in the same directions but showed little evidence of phenotypic convergence. In each rearing location, models with a population grouping term fit to head shape and body shape of individuals recaptured after approximately 70 days of rearing were favoured (i.e. $\Delta AICc > 4$) over models with no population variable (Appendix 4-1). This result emerged, at least in part, from the maintained difference between wild and laboratory-raised populations. In each rearing location the laboratory-raised fish were consistently deeper-bodied and had larger heads and eyes compared to their shallow-bodied, small-headed wild counterparts (Fig. 4-5 a,b).

Shape and strength of natural selection

Patterns of natural selection acting on body size and two aspects of shape varied among locations and among populations within locations. For example, selection differentials – which integrate direct and indirect effects – on body size were positive (large fish favoured) for laboratory-raised groups reared in the Parkers location, negative for the same groups in Rennies, and variable in the Waterford (Table 4-4). Similarly, size-adjusted head shape was negative (i.e. fish with small eyes and heads favoured in Parkers), positive in Rennies, and variable in Waterford. Curiously, the wild-raised Parkers and Rennies groups experienced patterns of selection acting on body size in the opposite direction than that of the laboratory-raised groups. Selection gradients – which represent selection on a trait while

controlling for indirect selection acting through correlated traits – generally yielded the same interpretations suggesting independence between variables (Table 4-5).

Counter to the prediction that directional selection should be strong during the early stages of an invasion to new environments, we detected weak directional selection based on both standardized differentials and gradients compared to estimates of selection strength for other organisms (Kingsolver et al., 2001). Only one estimate of directional selection (body size of wild fish in the Parkers location) was greater than the 50th percentile in the Kingsolver database, 10 were greater than the 25th percentile, 14 were greater than the 10th percentile, and the remainder were less than the 10th percentile. In contrast, we detected the presence of nonlinear patterns of selection acting on body size and shape (Fig. 6) and the strength of nonlinear selection was strong. For example all but one selection gradient was greater than the 25th percentile, 21 were greater than the 50th percentile, nine were greater than the 80th percentile, and three exceeded the 95th percentile (Table 4-5). Patterns of selection were complex among locations and populations (Fig. 4-6), but in general we detected stabilizing selection on body size, disruptive selection on head shape, and variable selection on body shape (stabilizing in the Parkers environment and disruptive in Rennies and Waterford).

Selection-plasticity correlations

Counter to our predictions, the patterns of plasticity in head shape and body shape of brown trout reared in three wild environments were not predictable based on the direction of natural selection. At the group level, plasticity in both head shape and body shape was inversely related to the experienced selection pressures; however, the observed relationship between selection and plasticity (taken as the difference in mean trait size

between release from the laboratory environment and at recapture) was not statistically significant (Fig. 4-7, $p > 0.05$). Interpretation of the relationship between plasticity and selection was similar based on gradients (direct selection on the individual traits) and differentials (direct and indirect selection on traits), though more of the variation in plasticity was explained by selection gradients (Fig. 4-7).

We detected marked variation in plasticity of head shape and body shape within individuals at each location (Fig. 4-8 to Fig. 4-13); however, individual change in phenotype was not predictable by the patterns in natural selection. This was perhaps most striking in the Parkers location (Fig. 4-8), where 92% of 85 individuals were observed to shift their head shape in the positive direction even though selection acted in the opposite direction (favoring smaller values of head shape). Assuming a binomial process, the probability of seeing 92% of individuals move in the same direction is 2.79×10^{-16}. Individual plasticity in body shape also trended towards positive values in the Parkers location, but was less consistent than head shape. On a whole, body shape of 65% of individuals moved in the positive direction, which again was counter to predictions of selection. Plasticity in head shape and body shape in the Rennies and Waterford locations was less consistent among individuals compared to the Parkers location. Except for two cases, the direction of individual phenotypic change was significantly different from expectations of a binomial process (50% moved positively, 50% moved negatively). Interestingly, the head shape and body shape of wild fish in the Rennies and Waterford, respectively, moved in consistent positive directions and was consistent with the direction of natural selection experience in these locations (positive values of head shape and body shaped favoured).

Apparent Survival

The number of recapture events (a proxy for survival) differed among populations and locations (Table 6). Of the 1166 total fish released, we recovered 198 individuals in the fall of 2009 and another 26 new individuals (i.e. those not recovered in the fall but must have been alive) in the summer of 2010. Thus on a whole, 19.2% of released fish survived until the fall. We detected evidence of differential survival among locations and an interaction between population and location (Table 4-3). Support for the model containing a population x location interaction term is consistent with the hypothesis of local advantage. Overall, only one foreign group (laboratory-raised Waterford fish reared in the Rennies) fared better than a local group (laboratory-raised Rennies fish reared in the Rennies). Evidence for local advantage was most obvious in the Parkers rearing environment, where the local individuals (both laboratory and wild-raised local groups) were recovered at markedly higher rates compared to the foreign groups (Table 4-6). Local advantage was less apparent, but still present, in the Rennies and Waterford rearing locations. Moreover, we detected a consistent advantage of the local wild-raised individuals over all other groups reared in the same environments. In each location, we recovered the highest proportion of released wild-raised individuals suggesting highest survival by these groups.

The pattern in survival observed among populations in the fall persisted until the following summer in the only population where a number of recaptures were made, Parkers (60% of all recaptures), and is suggestive of a continued local advantage (Table 4-6).The small sample sizes from the two other locations of release necessitate caution in interpretation thus necessitate our focus on results obtained in the fall of 2009.

Growth

We observed large differences in growth among environments; fish from all populations grew over twice as fast when rearing in the Rennies and Waterford locations compared to the Parkers or Laboratory environments (Fig. 4-14). In addition, populations exhibited different patterns of growth among locations as inferred from ANOVA where the strongest model contained a population x location interaction term (Table 4-3). This is consistent with the prediction that local populations would grow faster in their home environment and slower in foreign locations. For example, Parkers individuals when reared in Parkers grew faster than Rennies individuals, but grew slower than Rennies fish when reared in the Rennies environment (Fig. 4-14). Moreover, Parkers fish tended to grow faster in the laboratory environment than the Rennies or Waterford populations. Finally, growth of wild fish within the Rennies and Waterford rearing locations were *lower* than the growth observed by the other groups providing a potential explanation of the higher observed recovery rates of wild fish in these environments. Indeed this was most apparent in the Rennies location where laboratory raised fish of all populations grew, on average, nearly twice as fast as the local wild Rennies population (Fig. 4-14).

Discussion

To the best of our knowledge, the approach taken here is a novel attempt to test, in wild settings, the predictions derived from classical (Baldwin, 1896) and contemporary theory (e.g. Lande, 2009). This theory suggests that phenotypic plasticity should facilitate survival in new environments, move individuals in a consistent direction, and be predictable

based on the direction of selection. We found evidence consistent with the prediction that plasticity would shift phenotypes in consistent directions among environments. Populations – based on mean values of head shape and body shape – typically tracked each other among rearing locations, although we did detect marked variability in population-level and individual-level plastic responses, as evidenced by crossing of reaction norms and significant population x location interaction terms in ANOVA. This suggests genetic differences in plastic capacity and, if this variation is additive, the potential for an evolutionary response to selection on reaction norms. In spite of marked plasticity in head shape and body shape, we detected little evidence of phenotypic convergence suggesting strong underlying genetic control on shape. Counter to predictions, we did not find clear evidence that observed plastic responses were adaptive, as change in head shape and body shape was consistently *independent* and in some cases *opposite* to the direction favoured by selection. Thus, it was not evident that plasticity abetted survival but rather individuals apparently survived despite their potentially mal-adapted plastic responses. Moreover, the form and strength of natural selection on body size and shape was non-linear and strong, counter to the prediction that selection would be directional and strong when organisms are introduced to foreign environments. We did; however, detect evidence of local advantage in performance, based on both survival and growth, suggesting that local adaptation can evolve quickly in populations descended within approximately 130 years from common ancestors.

Phenotypic plasticity in shape without convergence

Brown trout, as a species, displays remarkable phenotypic plasticity in life history and morphology (reviewed and discussed by Jonsson & Jonsson, 2011). True to this general

pattern, we observed marked influence of the environment on the shape of juvenile trout reared in three wild streams. These findings are consistent with other recent demonstrations of morphological plasticity in juvenile salmonids and other fishes (Pakkasmaa & Piironen, 2000, Pavey et al., 2010, Parsons et al., 2011, Franssen, 2011). As we predicted, plasticity worked in consistent directions among populations and locations. On average, fish from all populations exhibited larger heads and eyes (adjusted for body size) when reared in the Parkers location and displayed relatively small heads and eyes when reared in the Rennies and Waterford locations. Similarly, populations were characterized by streamlined (shallow) body shapes in the Parkers and Waterford environments and more robust body shapes in the Rennies River. Although plasticity typically acted in concert, we did detect evidence of population-specific patterns of plasticity in both aspects of shape. Head shape varied among populations and locations consistently, save for a distinct difference in the Parkers population, which revealed a canalized response when reared in the Rennies and Waterford locations. That is, the Parkers populations responded like the other populations, with a shift towards relatively large head and eyes in the Parkers location and smaller head and eyes in the Rennies, but differed from the other populations by showing no change in shape between the Rennies and Waterford (other populations revealed a further reduction in head and eye size between these locations). Patterns of plasticity in body shape again generally followed the same trend among populations and locations (shallow bodies in Parkers, deeper bodies in Rennies, and shallow bodies again in Waterford), but here the Rennies population showed a differential response, with a greater change in their home environment compared to the other groups. These results combine to suggest that inferences of plasticity among populations are sensitive to the traits under consideration and are context specific; that is,

different traits in different populations in different common-gardens lead to different interpretations (Williams et al., 2008). Population-specific responses in morphological shape to different rearing environments suggest a heritable component to the reaction norms (Hutchings, 2011) and highlight the potential for an evolutionary response to selection (West-Eberhard, 2003). To be clear, we consider the *values* of shape and *plasticity* in shape observed among environments as *separate traits* each independently subject to selection.

Despite considerable plasticity in head shape and body shape, we detected equivocal evidence of phenotypic convergence in any of the three natural stream environments. We base our interpretation on both visual examinations of trait means across environments and results of ANOVA, where models fit to head and body shape data provided as much support for a population grouping effect after individuals had reared for ca. 70 days in different rivers as at their time of release. These patterns are counter to observations made in other systems. When transplanted from areas of relatively calm wave action to rough environments, barnacles (*Balanus glandula*) shift –with phenotypic plasticity – their feeding and reproductive appendages such that individuals originally from calm and rough environments come to display similar morphology within a common environment (Neufeld & Palmer, 2008). Moreover, the direction of the plastic change observed was consistent with predictions of what would be favoured by natural selection (though selection was not quantified per se). By way of another example, Williams et al. (1995) reciprocally transplanted clones of introduced fountaingrass (*Pennisetum setaceum*) across a gradient of elevations on Hawai'i and by doing so demonstrated the power of plasticity to shape phenotypes. Here, plasticity was so good at producing suitable phenotypes that clones moved to common environments displayed similar values in a suite of traits. These results

suggest that phenotypic plasticity shielded genotypes from selection perhaps masking any underlying genetic adaptations. In contrast, our results are consistent with recent work to understand the potential for domestication to alter growth response reaction norms in Atlantic salmon (*Salmo salar*). In this example, researchers showed that wild, farmed, and hybrid (crosses between wild and farmed fish) differed in the altered the height (y-intercept) of the linear reaction norm but not the slope (Morris et al., 2010), and that this difference may have resulted from domestication. Like our results, all groups displayed marked growth plasticity but no evidence for convergence of growth phenotypes.

A lack of phenotypic convergence despite plasticity suggests strong underlying genetic control on head and body shape. This is consistent with the interpretation of Hard et al. (1999) and Keeley et al. (2007) who reveal genetic control on morphology in Chinook salmon (*Oncorhynchus tshawtyscha*) and ecotypes of rainbow trout (*O. mykiss*). Moreover, the maintained phenotypic differences we observed among populations in common environments contributes to the mounting evidence that morphology can evolve quickly in introduced populations (Kinnison et al., 2003, Westley, 2011).

Is plasticity in shape predictable or chaotic?

Phenotypic plasticity yielded patterns of morphological shape in brown trout populations consistent with frequently made predictions based on river size and presumed flow regimes (Pakkasmaa & Piironen, 2000, Pavey et al., 2010, Bisson et al., 1988, Haas et al., 2010, Franssen, 2011). We detected a trend toward more robust morphology (e.g. body and caudal depth) of fish reared in the relative large and fast flowing Rennies River, compared to more streamlined morphology of fish reared in the relatively smaller and more placid Parkers

and Waterford environments. Additionally, we detected a marked increase in relative eye size of fish reared in the over-grown and dark Parkers environment compared to fish reared in the relatively open and bright Rennies and Waterford Rivers. These patterns are often interpreted as an adaptive response to environmental conditions (e.g. Pakkasmaa & Piironen, 2000); however, to our knowledge no study has simultaneously quantified body shape and natural selection in juvenile salmonids to formally test this assumption (though see Carlson et al., 2009 for an example in adult sockeye salmon).

Our results suggest that plastic changes in morphology, though coincident with general predictions of what should be adaptive, are *not predictable* or explainable given patterns of quantified natural selection. At the population-level, we detected an inverse relationship between selection and plasticity, where plasticity moved traits in the direction opposite to that favoured by selection. A similar pattern was observed within individuals among environments where plastic change was either independent of selection or in the opposite direction. In general, individual responses were varied in strength and form and opposite to observed plastic responses. How can these results be reconciled? In the following paragraphs we propose four non-mutually exclusive explanations.

First, body morphology may be pleiotropically linked to other traits that are also targets of selection. Body size is the most immediate candidate for such a linkage; however, we observed weak correlations between size and shape, and in addition, adjusted shape to remove the effect of size prior to analyses. Moreover, correlative fitness relationships between body size and shape – either positive or negative – were present but not consistently so. Perhaps more likely, body shape values were inversely influenced by growth rate (Fig. 4-15). Slow growth was associated with a plastic change in morphology toward

large heads and eyes and streamlined bodies, whereas rapid growth was correlated with deeper bodies and smaller heads and eyes. To be clear, these changes are independent of size. That is, individuals of the same size that have grown at different rates displayed different patterns of plasticity and morphology. This pattern is frequently revealed, but rarely discussed, in other studies comparing morphology of populations among environments. For example, in a recent study, populations of cyprinids (*Cyprinella lutrensis*) exhibited plastic shifts towards deep-bodied morphology in reservoirs compared to their stream-dwelling ancestors (Franssen, 2011). Though it was interpreted that these morphological shifts are adaptive and reflective of rearing conditions, it also seems plausible that the change in morphology reflects different growing conditions in lake versus stream environments. At a mechanistic level, Devlin and colleagues (in revision) report that growth rate greatly influences the allometry of somatic traits (e.g. eyes) in contrast to neural traits (e.g. brain tissue). Fast growth can be favoured by selection in wild salmonid populations (e.g. Carlson et al., 2004), but may come at the cost of inducing morphology poorly-suited to environmental conditions. We detected an inverse relationship between survival and positive trait values of head shape and body shape (i.e. deep-bodied, small headed and eyed fish were favoured) despite observed consistent plasticity in the opposite direction. This could be explained by selection favoring the faster growing, deep-bodied and small headed subset of individuals. In contrast, we detected selection favoring the more shallow-bodied individuals in Rennies and Waterford suggesting rapid growth was selected against in these environments. This is a plausible scenario because despite growing slower, the wild fish were recovered at much higher rates than the faster growing laboratory-raised populations.

Unfortunately, the nature of our experimental design – specifically few recapture bouts – does not allow for a formal quantification of selection acting on growth.

Second, temporal variation in the strength and form of selection may underpin the apparent discrepancy between plastic response and natural selection. It is widely accepted that selection varies in *space* among environments (Kawecki & Ebert, 2004) and increasingly clear that selection varies in *time* within environments (Bell, 2010, Siepielski et al., 2009, Siepielski et al., 2011). We quantified selection acting on aspects of morphological shape during the introduction stage of an invasion and found that selection during this period was frequently opposite of general predictions based on swimming capacity (i.e. shallow fish favoured in small streams, and robust fish favoured in large streams) and opposite to plasticity. Extensive work on Galapagos finches clearly demonstrates that selection varies through time, where robust bill sizes are favoured during periods of drought and the opposite during periods of precipitation (reviewed by Grant, 1986). Analogously, selection may have changed in direction or strength over a longer time frame of observation. Conditions during the winter are often harsh for stream-dwelling salmonids (Huusko et al., 2007) and it is possible that increased flow regimes during the winter may have selected for the morphology we observed. Thus, the plastic responses we detected may have foreshadowed selection acting over longer timescales. Few fish were recaptured following the winter, limiting our ability to directly assess this potential. Furthermore, it is possible that alternative or additional fitness measures would have yielded different interpretations of how selection acts on morphological shape. In a recent meta-analysis, Siepielski et al. (2011) suggest that selection acting on fitness measures of reproductive success or fecundity is stronger and less variable in direction than selection based on survival. We based

interpretations on a proxy for survival – recapture probability – as following tagged groups through their entire life history was not practical. However, doing so may undoubtedly have yielded additional and different insights.

Third, the ability for plasticity to yield adaptive phenotypes rests on the assumption that environmental cues are reliable and perceivable. This assumption was formally investigated recently by Reed et al. (2010b), who showed that plasticity can buffer populations from environmental stochasticity and facilitate persistence when optimal trait values and cue reliability are tightly correlated. Previous experimental work suggests that salmonids are capable of perceiving environmental cues and can respond with a change in morphology (Donnelly & Dill, 1984, Pakkasmaa & Piironen, 2000). We observed a response in morphology when fish were reared among environments differing in size and flow; however, it remains unclear whether the plastic response was truly mal-adaptive (Ghalambor et al., 2007), adaptive over a longer period of selection (Siepielski et al., 2009) or whether the cues were poorly correlated with the optimal morphological values (Reed et al., 2010b).

Fourth, counter to predictions the strength of directional selection was weak and strong non-linear selection prevailed. This result runs counter to theory (Reznick & Ghalambor, 2001, Chevin & Lande, 2011a) and empirical work (Anderson et al., 2010) that propose that directional selection during the early stages of invasion should be strong. In a recent review, Kingolver and Diamond (2011) aimed to better understand the factors that limit directional selection, with particular emphasis on the potential effects of fitness trade-offs, and indirect and fluctuating selection. Their results were inconsistent with the hypothesis that trade-offs among different fitness components or indirect selection (except perhaps for body size) would limit total directional selection on phenotypic traits. Similarly,

they suggest that temporal fluctuation in selection, though apparently wide-spread in nature, has limited capacity to influence total directional selection observed in most systems. Putting our results into this context, we suggests that the morphology of introduced fish was sufficiently close to the optimal values in each environment and that directional selection may not be the appropriate predictor of plastic responses.

Local adaptation

We detected higher relative survival and growth of individuals when reared in their home environment than when reared in foreign conditions, suggesting that these three populations – recently established from common ancestors – have quickly evolved adaptations to local conditions. This is in general agreement with results from other systems where salmonids have revealed the propensity to evolve quickly in new environments (Quinn et al., 2001a, Hendry & Stearns, 2004, Taylor, 1991, Garcia de Leaniz et al., 2007) and across a range of spatial scales (Fraser et al., 2011, Meier et al., 2011). Evidence supporting this interpretation emerges from two general patterns. First, ANOVA models including a location x population interaction terms received the most weight of evidence based on AIC_c suggesting that populations did well (based on survival and growth) in some locations and poorly in others. It is important to note, however, that a model including only the sole effect of location also received substantial support in explaining survival(ΔAIC_c = 3.3). This suggests that we cannot rule out the possibility that survival did not vary among populations but solely among environments. Second, wild local individuals in each location survived at higher rates than any of the other introduced groups, and grew at markedly

different rates (relatively fast in Parkers and slow in Rennies and Waterford compared to the other groups).

We acknowledge the potential that wild groups may have had higher performance based on prior experience in the streams, and thus the advantage of the wild-raised groups may reflect environmental and not genetic effects. It is post plausible that a prior advantage to wild fish was present in the form of territorial acquisition and maintenance (Gibson, 1993, Einum & Fleming, 2000); that is, the wild fish presumably would have already established feeding territories at the time of capture and tagging, giving them an advantage over other groups upon release (Rhodes & Quinn, 1998). In addition, having pre-existing territories may have limited the tendency for wild fish to move, which may have increased the likelihood of recapture. Unfortunately, in this study, as in many capture-mark-recapture experiments, survival is confounded with emigration and we cannot rule out the possibility that individuals that were not recaptured and presumed dead simply moved outside of the experimental sections. We purposefully chose experimental stream sections with upstream limits to movement in the form of velocity barriers, but controlling for downstream emigration was not feasible. We did; however, assess the potential for individuals to move out of Parkers downstream to Windsor Lake during the spring of 2010 with a fykenet set to fully span the mouth of the stream, something that was not feasible in the other rivers. Overall we detected little movement of tagged individuals (n=3) into Windsor Lake and left the fyke net in place until stream-wide electrofishing surveys had been completed. Ultimately, we assume that if individuals were displaced downstream it was the result of competitive inferiority (Chapman, 1966, Elliott, 1994) maintaining the validity of relative performance among released groups.

The differential patterns of growth observed among populations and locations also suggest local adaptation. This was most evident in the Parkers population, which relative to the other populations grew faster in environments where growth was poor and slower in environments where growth was favorable. Specifically, Parkers individuals grew faster in their own wild environment and the laboratory environment than in the Rennies and Waterford, where growth was rapid. In contrast, the other groups – especially the Rennies population – grew relatively slow in Parkers and the laboratory and rapidly in the other locations. Local adaptation to thermal regimes, as may have occurred here, has been reported elsewhere in brown trout (Jensen et al., 2008, Jensen et al., 2000) and evidence from introduced grayling populations reveals that adaptation can evolve quickly (Haugen & Vollestad, 2001, Haugen & Vollestad, 2000). Curiously, we observed that growth among individuals raised entirely in the laboratory environments was slower than that of individuals released into two wild environments, the Rennies and Waterford. One explanation is that relaxed selection (Lahti et al., 2009) in the laboratory setting may have allowed slow growing individuals to persist, serving to lower the average growth rate. If this were the case, we would expect to see much greater variation (i.e. error) around the observed mean growth of laboratory fish compared to fish reared in the wild. In fact, we detected the opposite pattern with greater variation in growth observed in wild-rearing individuals, though some of this may be the effect of different sample sizes among environments (greater sample sizes for laboratory-reared populations). Alternatively, the relatively slow growth in the laboratory may be more about the conditions in the Rennies and Waterford than in the laboratory per se. We observed remarkably fast growth in the Rennies and Waterford (many individuals at least doubled in weight in approximately 70 days), corroborating the results of Gibson &

Haedrich (1988), who reported 'exceptional' growth of experimental groups of Atlantic salmon released into the same rivers.

Conclusions

The capacity of individuals to adaptively respond to a changing environment is frequently invoked to explain how populations may persist in a rapidly changing world (Reed et al., 2011, Charmantier et al., 2008). Our results reveal that plasticity, while prevalent and in the direction often *assumed* to be adaptive, may not necessarily be favoured by selection. We detected a potential trade-off between growth and morphology, whereby growth consistently shaped individuals in ways that may not be advantageous in all environments. Ultimately, the plasticity in morphology we observed was not predictable based on patterns of selection, suggesting that attempts to predict the plastic responses of populations and species may be exceedingly difficult and prone to error. Over a hundred years after J.M. Baldwin suggested a 'new factor' in evolution, few studies have attempted to empirically test predictions derived therein (Badyaev, 2009). We show here that the first stages leading to the Baldwin effect – plasticity facilitating survival and selection in the direction of the plastic response – are more complicated and varied than predicted by theory.

Chapter Four Tables

Table 4-1. Number of families and biological information for the dams used in creating experimental fish.

	Parkers	Rennies	Waterford
Families created (n)	8	8	7
Fork length (mm)	376 (290-450)	272 (195-468)	428 (290-600)
Weight (g)	572.1 (250-942)	230.8 (67-910)	826.3 (221-1931)
Egg wet weight (mg)	131.6 ± 18.6	107.2 ± 16.7	109.8 ± 31.8

Table 4-2. Abiotic and biotic characteristics of Parker's Pond Brook, Rennies River, and Waterford River, Newfoundland.

Variable	Location		
	Parkers	Rennies	Waterford
Release coordinates	47°36'06.44"N, 52°46'18.21"W	47°34' 34.04"N, 52°42'46.98"W	47°31'30.11"N, 52°44'49.58"W
Date of release	7/31/2009	8/4/2009	8/7/2009
Dates of recapture	10/7/2009, 10/14/2009, 6/23/2010, 6/24/2010, 7/29/2010, 8/4/2010	10/5/2009, 10/6/2009, 10/12/2009, 10/23/2009, 11/11/2009, 6/25/2010, 7/27/2010, 8/6/2010, 8/7/2010	10/11/2009, 10/13/2009, 10/22/2009, 10/23/2009, 6/26/2010, 7/28/2010, 8/4/2010, 8/5/2010
Section length (m)	386	935	725
Width (m)	1.8 (0.81)	6.5 (1.9)	5.6 (0.30)
Depth (cm)	7.5 (0.14)	23.2 (4.2)	11.9 (20.2)
Flow (m/s)	0.12 (0.04)	0.29 (0.21)	0.24 (0.08)
Conductivity (µS/cm)	43.6 (0.47)	246.3 (17.1)	299.3 (30.8)
Temperature	10.3 (1.5-21)	11.7 (1.2-20)	11.3 (0.9 - 21)
Canopy cover	3.33	1.67	1
Conspecific density (g/m^{2-})	2.59 (2.5)	2.21 (1.6)	4.6 (2.6)
Eels (*Anguilla rostrata*)	absent	present (0.018 fish/m^2)	present (0.021 fish/m^2)
Brown trout age distribution	0-2	0-5+	0-6+

Table 4-3. Results of ANOVA models fitted to survival, growth, and two aspects of morphology in brown trout.

Variable	Model	Parameters	AICc	ΔAICc
Survival	Location + population + interaction	11	1125.68	0
	Location	2	1128.94	3.3
	null	0	1139.9	14.2
	Population	5	1142.21	16.5
Growth	Location + population + interaction	14	-173.84	0
	Location	3	-156.43	17.4
	Population	5	143.87	317.7
	null	0	153.59	327.4
	Location + population + interaction	11	-1301.2	0
Head shape	Location	2	-1251.9	49.3
	Population	5	-1203.6	97.6
	null	0	-1151.9	149.3
	Location + population + interaction	11	-1457.9	0
Body shape	Location	2	-1452.1	5.8
	Population	5	-1449.7	8.1
	null	0	-1447.4	10.5

Table 4-4. Standardized selection differentials on body size (length, mm), head shape (Relative Warp 1), body shape (Relative Warp 4) of four groups (origin) of brown trout released into three rearing locations in Newfoundland. The wild origin fish represent individuals raised in their rearing location, while other groups are F_1 offspring of each population origin reared in the laboratory until release.

			i (linear)			*j* : Quadratic (-stabilizing/+disruptive)		
Release Location	Origin	N (Alive:Dead)	Body size	Head shape	Body shape	Body size	Head shape	Body shape
Parkers	Parkers	30:71	0.0222 (0.0231)*	−0.0222 (0.0227)*	0.0493 (0.0528)*	−0.342 (0.173)††	0.308(0.133)††	0.365 (0.160)††
	Rennies	16:84	0.0293 (0.0611)*	−0.0605(0.128)**	−0.0142 (0.0290)	−0.624 (0.283)†††	0.539(0.189)†††	−0.255 (0.228)††
	Waterford	22:78	0.0463 (0.0681)*	−0.0564(0.0871)**	−0.0642 (0.103)**	−0.786 (0.261)†††	−0.227(0.195)††	−0.408 (0.269)††
	Wild	22:43	−0.134 (0.208)***	0.0932(0.120)**	−0.0844 (0.116)**	−0.0732 (0.378)†	−0.432(0.289)††	−0.984 (0.365)†††
	Pooled	90:276	−0.0101 (0.00699)	−0.0311(0.0206)*	−0.0168 (0.0113)	−0.200 (0.0803)††	−0.135(0.0825)†	−0.203 (0.0978)††
Rennies	Parkers	9:91	−0.0103 (0.0506)	0.000824 (0.00351)	0.0503 (0.267)**	−2.68 (0.957)††††	0.141(0.254)†	−0.0302 (0.251)
	Rennies	11:89	−0.0384 (0.130)*	0.0383 (0.127)*	−0.0297 (0.0976)*	−1.31 (0.489)†††	−0.242(0.223)††	−0.515 (0.307)†††
	Waterford	14:88	−0.123 (0.487)**	0.107(0.367)**	0.0519 (0.130)**	−0.827 (0.633)†††	0.453(0.231)††	0.255 (0.248)††
	Wild	25:74	0.0143 (0.0171)	0.00395(0.00485)	0.0453 (0.0585)*	0.146 (0.109)†	−0.253(0.199)††	0.167 (0.141)†
	Pooled	59:342	0.00 (0.00)	−0.0129(0.0143)	0.0304 (0.0349) *	0.154 (0.0434)†	0.436(0.0930)††	0.244 (0.0879)††
Waterford	Parkers	17:84	0.00269 (0.005)	0.00549(0.0105)	−0.0182 (0.00347)	0.0953 (0.161)†	0.531(0.179)†††	−0.137 (0.213)†
	Rennies	12:86	−0.0241 (0.0736)*	−0.0441(0.125)*	−0.0479 (0.155)*	−0.0444 (0.194)†	−0.127(0.205)†	0.181 (0.238)††
	Waterford	18:82	0.0349 (0.0637)*	−0.0583(0.110)**	0.0166 (0.00222)	0.476 (0.268)††	−0.386(0.266)††	0.381 (0.164)††
	Wild	28:72	−0.0275 (0.0312)*	0.0215(0.0238)*	0.0344 (0.0389)**	−1.02 (0.268)†††	−0.143(0.215)†	−0.305 (0.186)††
	Pooled	75:324	−0.00860 (0.00726)	−0.0370(0.0306)*	0.00456 (0.00381)	−0.358 (0.112)††	−0.0645(0.104)†	0.0732 (0.0887)†

* > 10th percentile ** > 25th percentile *** > 50th percentile

† > 25th percentile †† > 50 th percentile ††† > 80 th percentile †††† > 95th percentile

Table 4-5. Standardized selection gradients on body size (length, mm), head shape (Relative Warp 1), body shape (Relative Warp 4). of four groups (origin) of brown trout released into three rearing locations in Newfoundland. The wild origin fish represent individuals raised in their rearing location, while other groups are F_1 offspring of each population origin reared in the laboratory until release.

			β (linear)			γ: Quadratic (-stabilizing/+disruptive)		
Release Location	Origin	N (Alive:Dead)	Body size	Head shape	Body shape	Length^2	Head shape^2	Body shape^2
Parkers	Parkers	30:71	0.0159 (0.0194)	−0.0049 (0.00611)	0.0469 (0.0513)*	−0.384 (0.197)††	0.398 (0.139)††	0.340 (0.165)††
	Rennies	16:84	0.00255 (0.00627)	−0.0593 (0.139)**	−0.0143 (0.0301)	−0.860 (0.321)†††	0.811 (0.247)†††	−0.165 (0.225)†
	Waterford	22:78	0.00753 (0.0154)	−0.0454 (0.0946)*	−0.0597 (0.0999)**	−0.891 (0.294)†††	0.0538 (0.212)†	−0.490 (0.272)††
	Wild	22:43	−0.175(0.463)***	−0.0337 (0.0800)*	−0.0952 (0.152)**	0.746 (0.469)†††	−0.262(0.327)††	−1.61 (0.442)††††
	Pooled	90:276	−0.0616 (0.0634)**	−0.0717 (0.0706)**	−0.0188 (0.013)	−0.149 (0.0978)†	0.121 (0.104)†	−0.197 (0.0968)††
Rennies	Parkers	9:91	0.00347 (0.0231)	0.000636 (0.00387)	0.0512 (0.278)*	−3.56 (1.07)††††	0.568 (0.323)††	0.283 (0.260)††
	Rennies	11:89	−0.0182 (0.0735)	0.0331 (0.132)*	−0.0343 (0.123)*	−1.45 (0.504)††††	0.154 (0.242)†	−0.649 (0.339)†††
	Waterford	14:88	−0.0804 (0.386)**	0.0658 (0.260)**	0.0152 (0.0461)	−0.955 (0.667)†††	0.589 (0.228)††	0.177 (0.271)††
	Wild	25:74	0.0418 (0.0670)*	0.0287 (0.0474)*	0.0524 (0.0688)*	0.117 (0.122)†	−0.343 (0.211)††	0.170 (0.144)††
	Pooled	59:342	−0.00941 (0.0151)	−0.0215 (0.0334)	0.0302 (0.0355)*	0.123 (0.0477)†	0.377 (0.100)††	0.231 (0.0894)††
Waterford	Parkers	17:84	0.00226 (0.00495)	0.00316 (0.0454)	−0.0173 (0.0342)	0.0742 (0.175)†	0.574 (0.185)††	−0.223 (0.237)††
	Rennies	12:86	−0.0494 (0.169)*	−0.0632 (0.218)**	−0.0544 (0.199)**	−0.235 (0.226)††	0.240 (0.281)††	0.409 (0.250)††
	Waterford	18:82	0.00145 (0.0034)	−0.0567 (0.132)**	0.0172 (0.0324)	−0.891 (0.361)†††	−0.659 (0.344)†††	0.575 (0.287)†
	Wild	28:72	−0.0257 (0.0376)*	0.00596 (0.0084)	0.0359 (0.00677)*	−1.12 (0.294)†††	0.164 (0.236)†	−0.317 (0.189)††
	Pooled	75:324	−0.0307 (0.0289)*	−0.0499 (0.0461)*	−0.00100 (0.000877)	−0.398 (0.115)††	−0.00685 (0.109)	0.122 (0.0895)†

* > 10th percentile ** > 25th percentile *** > 50th percentile

† > 25th percentile †† > 50 th percentile ††† > 80 th percentile †††† > 95th percentile

Table 4-6. Percentage of released fish recaptured (a proxy for survival) in the fall of 2009 and summer 2010 of four groups (origin) of brown trout released into three rearing locations in Newfoundland. The wild origin fish represent individuals raised in their rearing location, while other groups are F_1 offspring of each population origin reared in the laboratory until release.

Rearing Location	Origin	n	survival (%)	
			fall	over-winter
Parkers	Parkers	101	30	12
	Rennies	100	16	4
	Waterford	100	22	8
	Wild	65	34	4
Rennies	Parkers	100	9	2
	Rennies	100	11	1
	Waterford	102	14	2
	Wild	99	25	1
Waterford	Parkers	101	17	2
	Rennies	98	12	3
	Waterford	100	18	3
	Wild	100	28	5

Chapter Four Figures

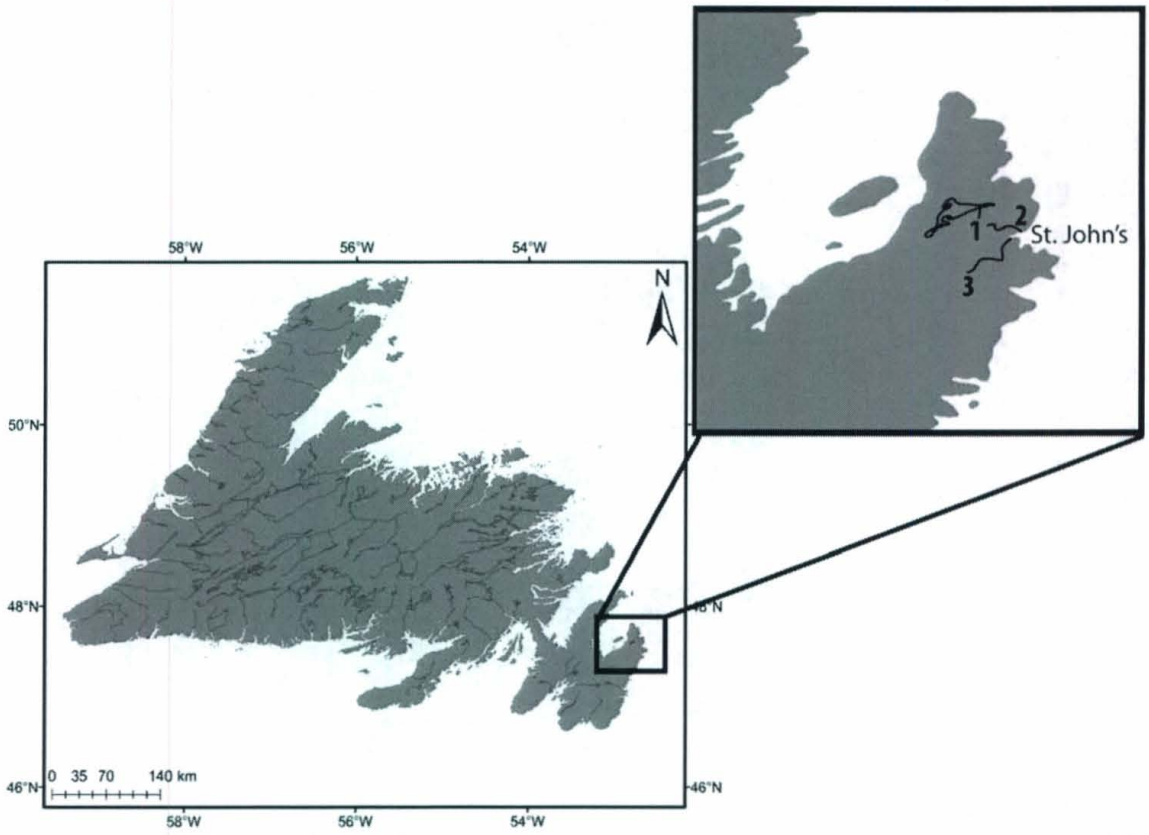


Fig. 4-1. Island of Newfoundland, Canada, showing the approximate locations of the three study rivers, Parker's Pond Brook (1), Rennie's River (2), and Waterford River (3). See Table 2 for specific coordinates of release locations within the rivers and habitat characteristics.

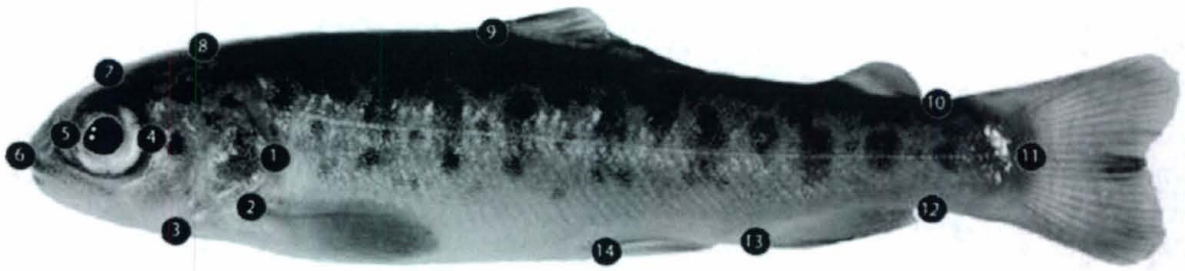


Fig. 4-2. Homologous landmarks used in geometric morphometric analyses, shown on a representative 51 mm F_1 individual from the Parker's Pond Brook population grown in common laboratory conditions. Landmarks represent: 1) most posterior point of the operculum, 2) insertion of the pectoral fin, 3) intersection of the pre-opercle and opercle plates, 4) posterior insertion of the orbit, 5) anterior insertion of the orbit, 6)tip of the snout, 7) point directly above the middle of the eye, 8) insertion of the skull, 9) insertion of the dorsal fin, 10) narrowest point of the caudal peduncle, 11) insertion of the caudal fin to the hyperual at the lateral line, 12) same as 10, 13) anterior insertion of the anal fin, 14) anterior insertion of the pelvic fin.

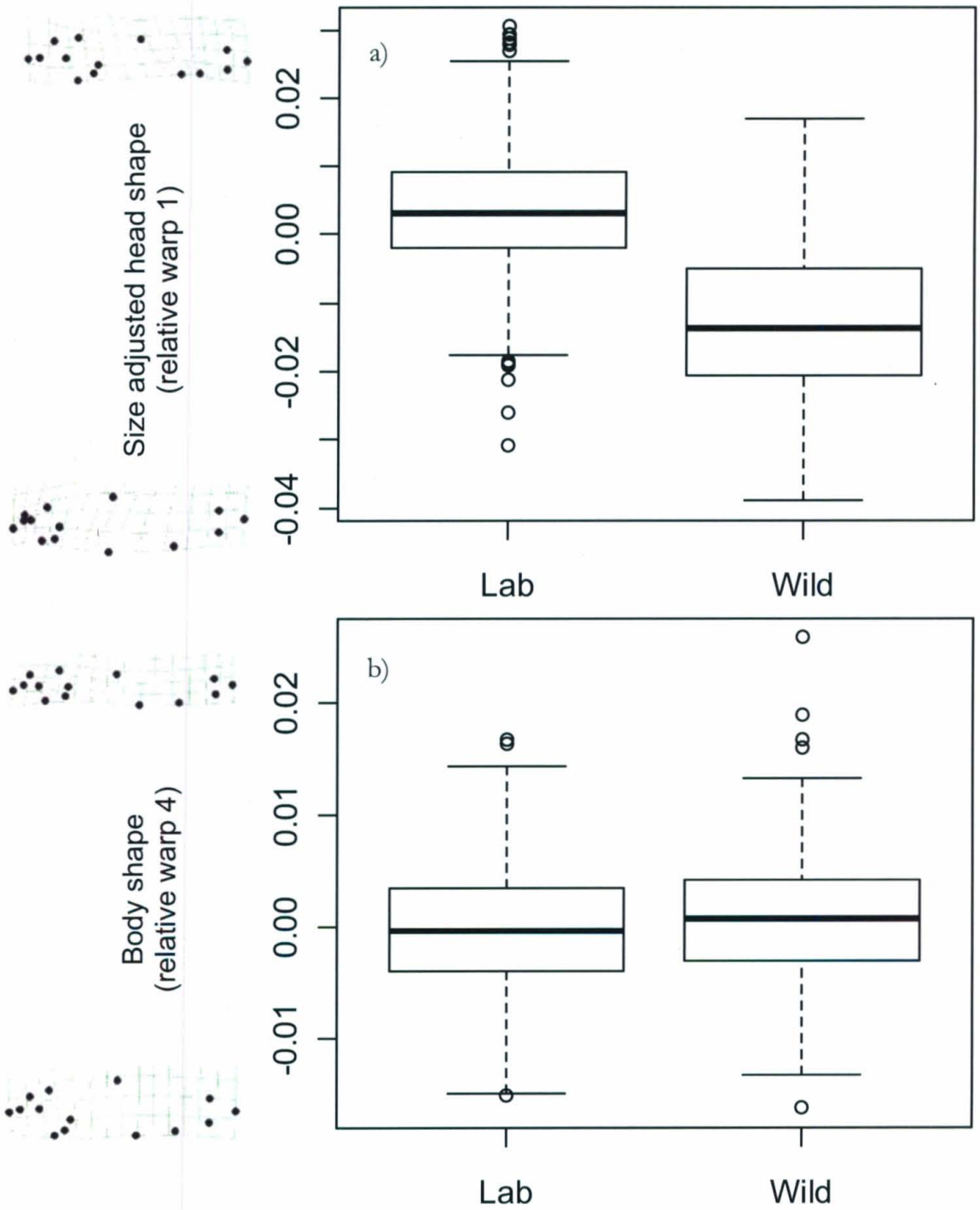


Fig. 4-3. Head shape (relative warp 1, a) and body shape (relative warp 4, b) of brown trout raised in laboratory versus the wild. Note that increasing values of the first relative warp correspond to increasing head size and eye size, whereas increasing values of relative warp four correspond to increased streamling (shallow-bodies and caudal peduncles). Deformation grids of maximum and minimum observed warps are exaggerated by 3 x times to facilitate interpretation

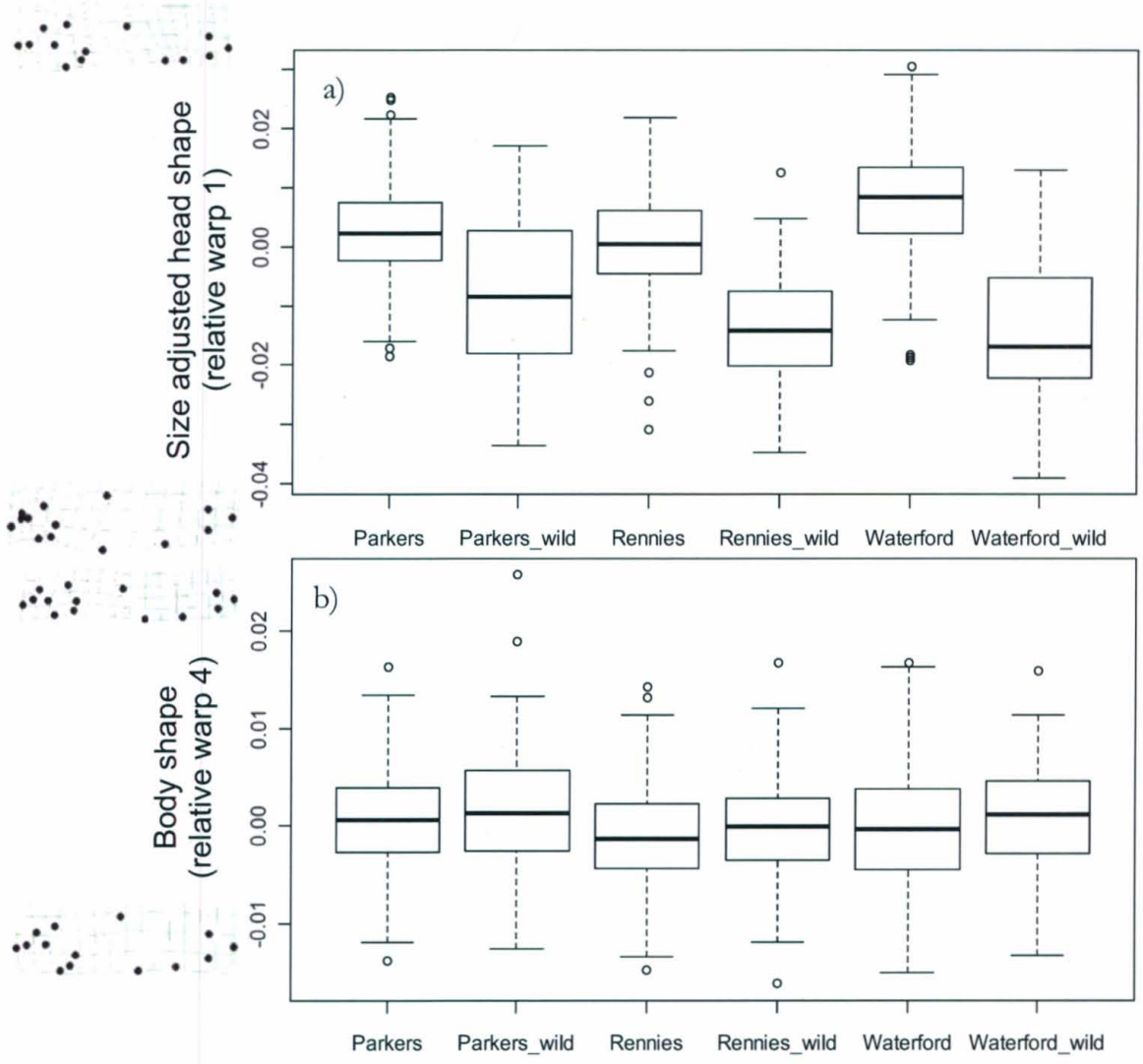


Fig. 4-4. Head shape (relative warp 1, a) and body shape (relative warp 4, b) among populations of brown trout. Note that increasing values of the first relative warp correspond to increasing head size and eye size, whereas increasing values of relative warp four correspond to increased streamling (shallow-bodies and caudal peduncles). Deformation grids of maximum and minimum observed warps are exaggerated by 3 x times to facilitate interpretation. The wild origin fish represent individuals raised in their rearing location, while other groups are F_1 offspring of each population origin reared in the laboratory until release

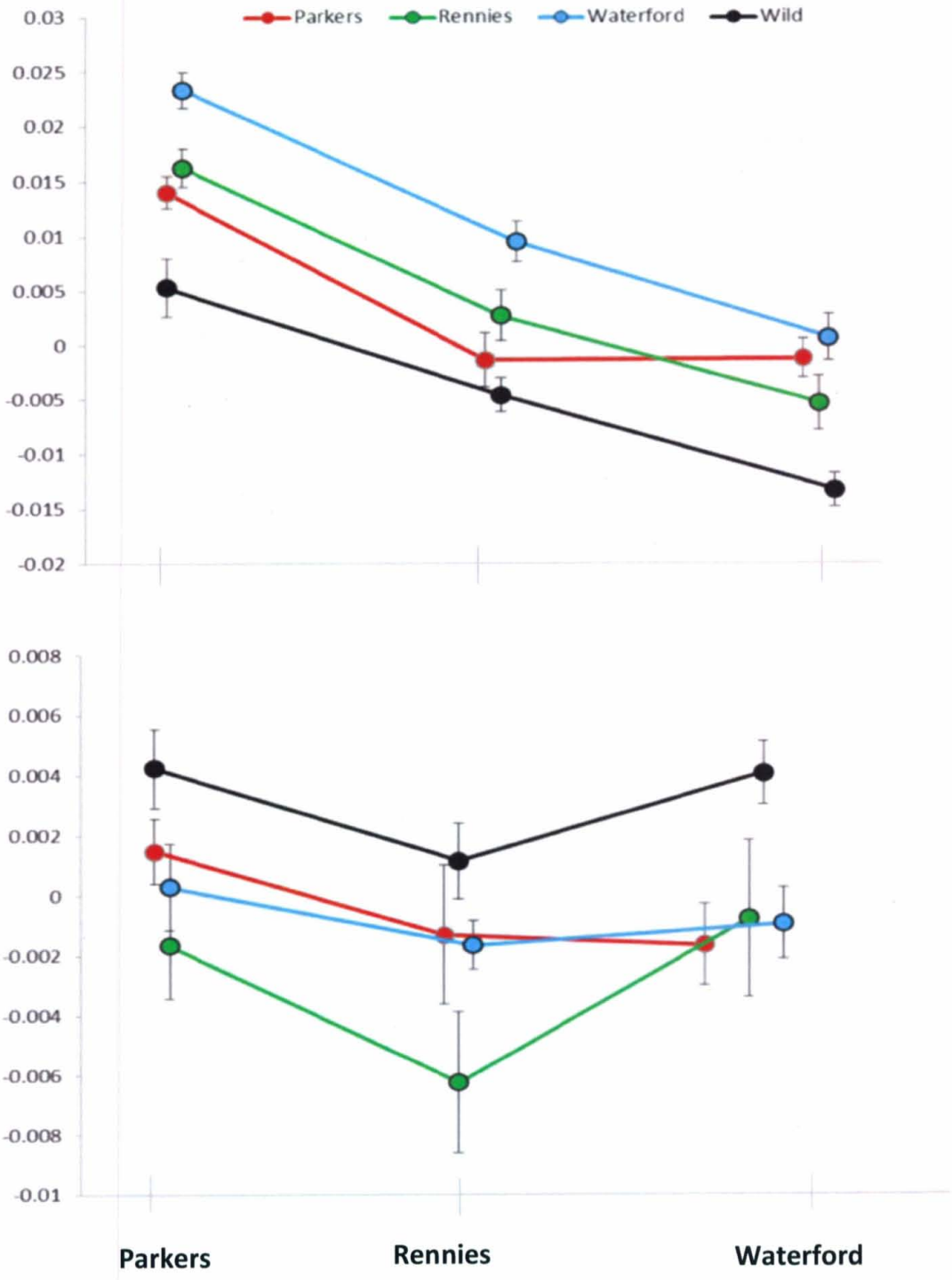


Fig. 4-5. Reaction norms of head shape (relative warp 1, a) and body shape (relative warp 4, b) among populations of brown trout reared in three wild environments. Each point represents average head shape (top panel) and body shape (bottom panel) of Parkers (red symbols), Rennies (green), Waterford (blue), and Wild (black) origin fish when reared in Parkers, Rennies, or Waterford environments. Error bars are ±1 SE.

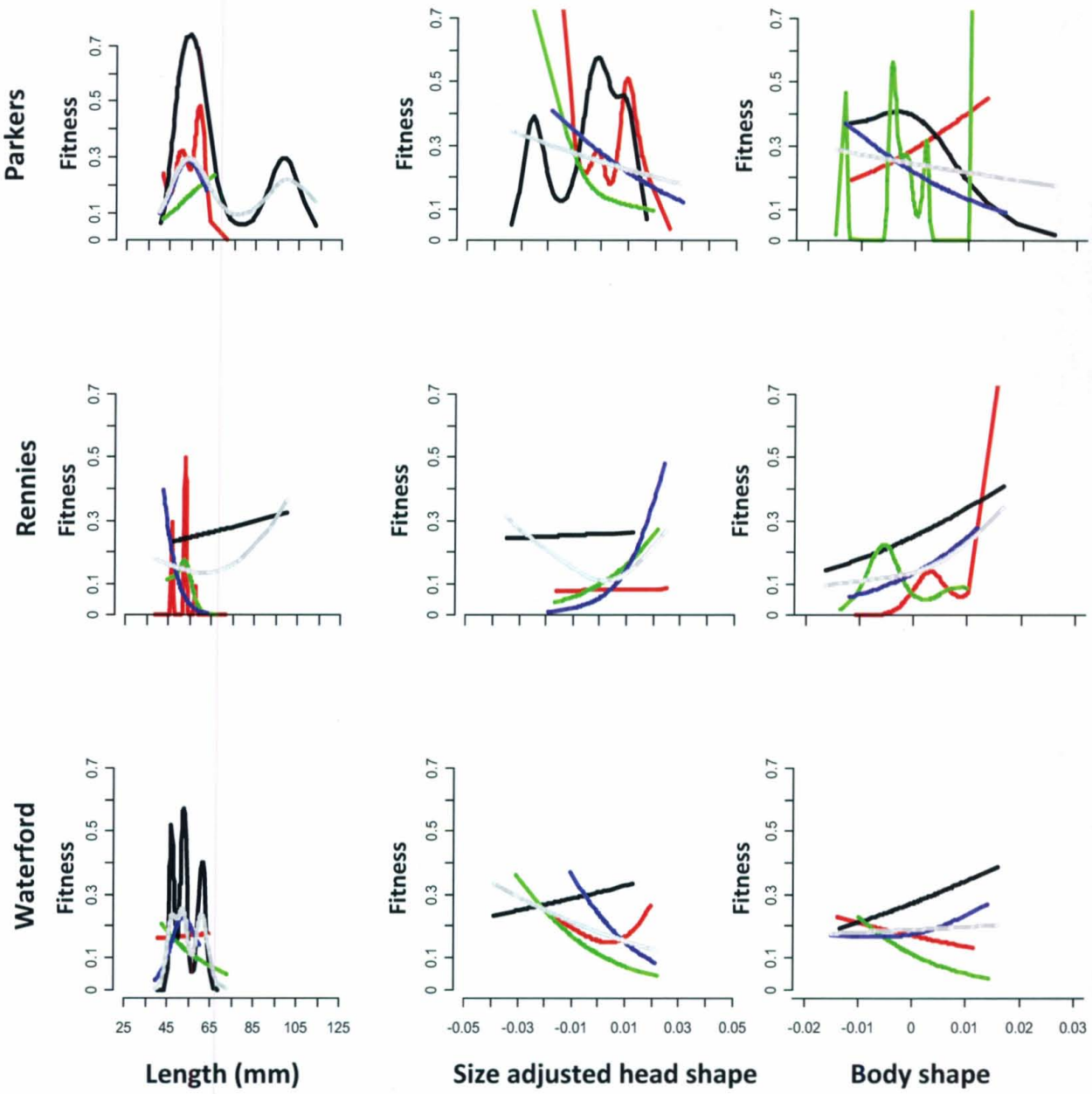


Fig. 4-6. Cubic splines to visualize fitness as a function of body size (length), head shape, and body shape, for Parkers (red lines), Rennies (green lines), Waterford (blue lines), and Wild (black lines) origin fish reared in the Parkers (top rows), Rennies (middle rows), and Waterford (bottom rows) environments. Increasing values of head shape are interpreted an increasing relative size of the head and eye, whereas increasing values of body shape are interpreted as increased streamlining (decreasing dorsal ventral compression)

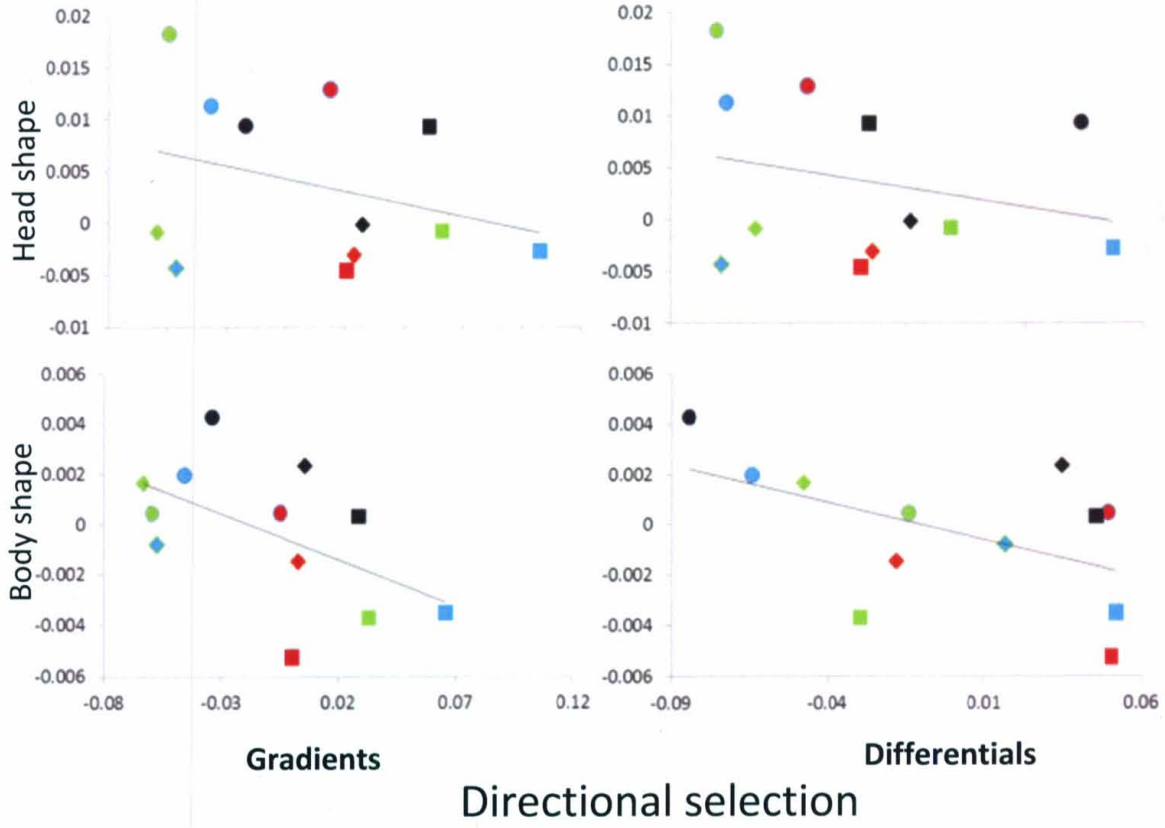


Fig. 4-7. Relationship between directional selection and phenotypic plasticity (shape at recapture – shape at release) in head shape and body shape. Each point represents the average plasticity of Parkers (red symbols), Rennies (green), Waterford (blue), and Wild (black) origin fish when reared in Parkers (circles), Rennies (diamonds), or Waterford (squares) environments.

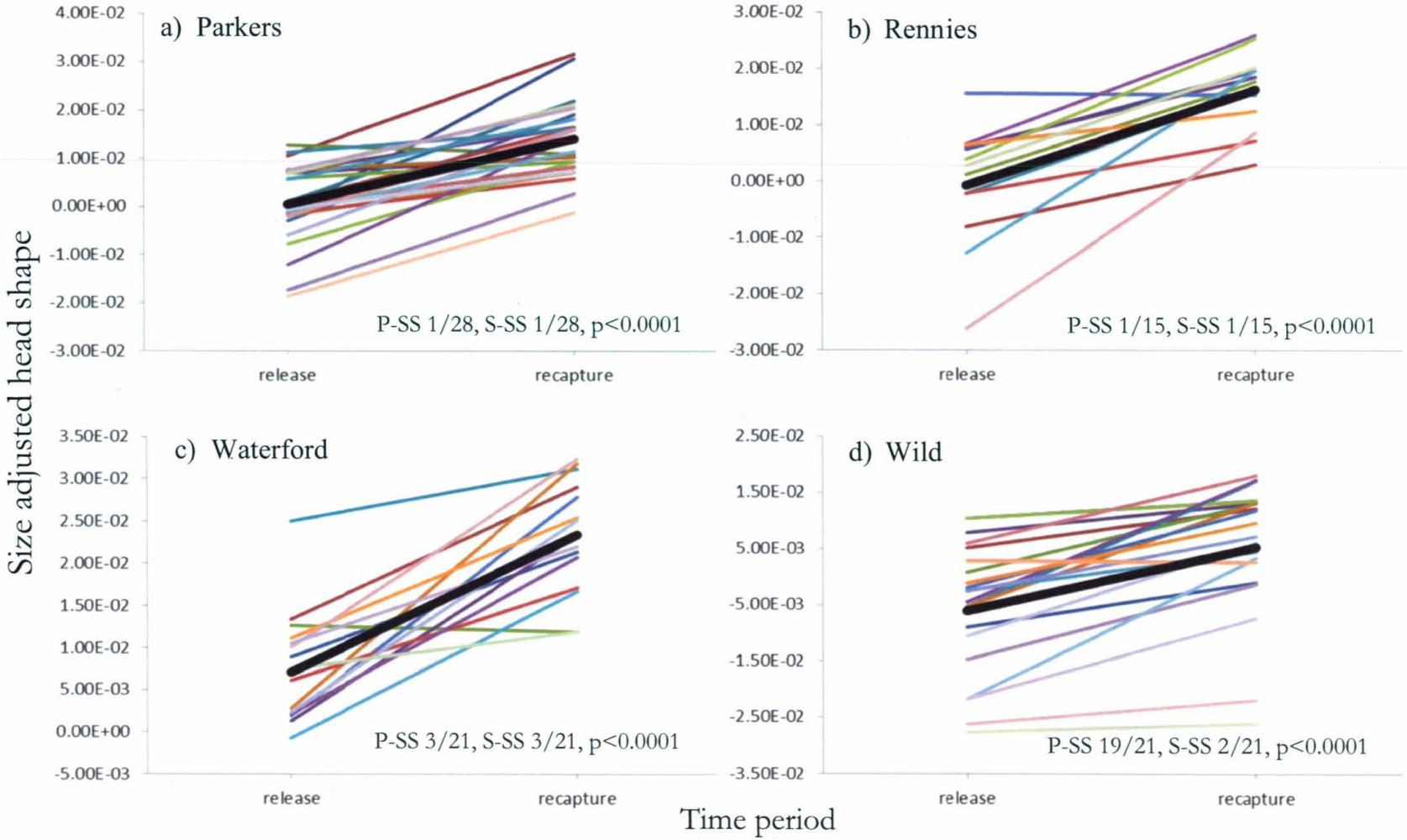


Fig. 4-8. Individual reaction norms of Parkers (a), Rennies (b), Waterford (c), and Wild (d) origin fish reared in Parkers Pond Brook. Each coloured line represents an individual's change in size adjusted head shape after ca. 70 days post-release. The thick black line is the population-average response. The number of individuals responding in the direction predicted based on population-specific selection (P-SS) or site-specific selection (S-SS), based on differentials are shown as a fraction of the total individuals sampled and compared to a binomial process.

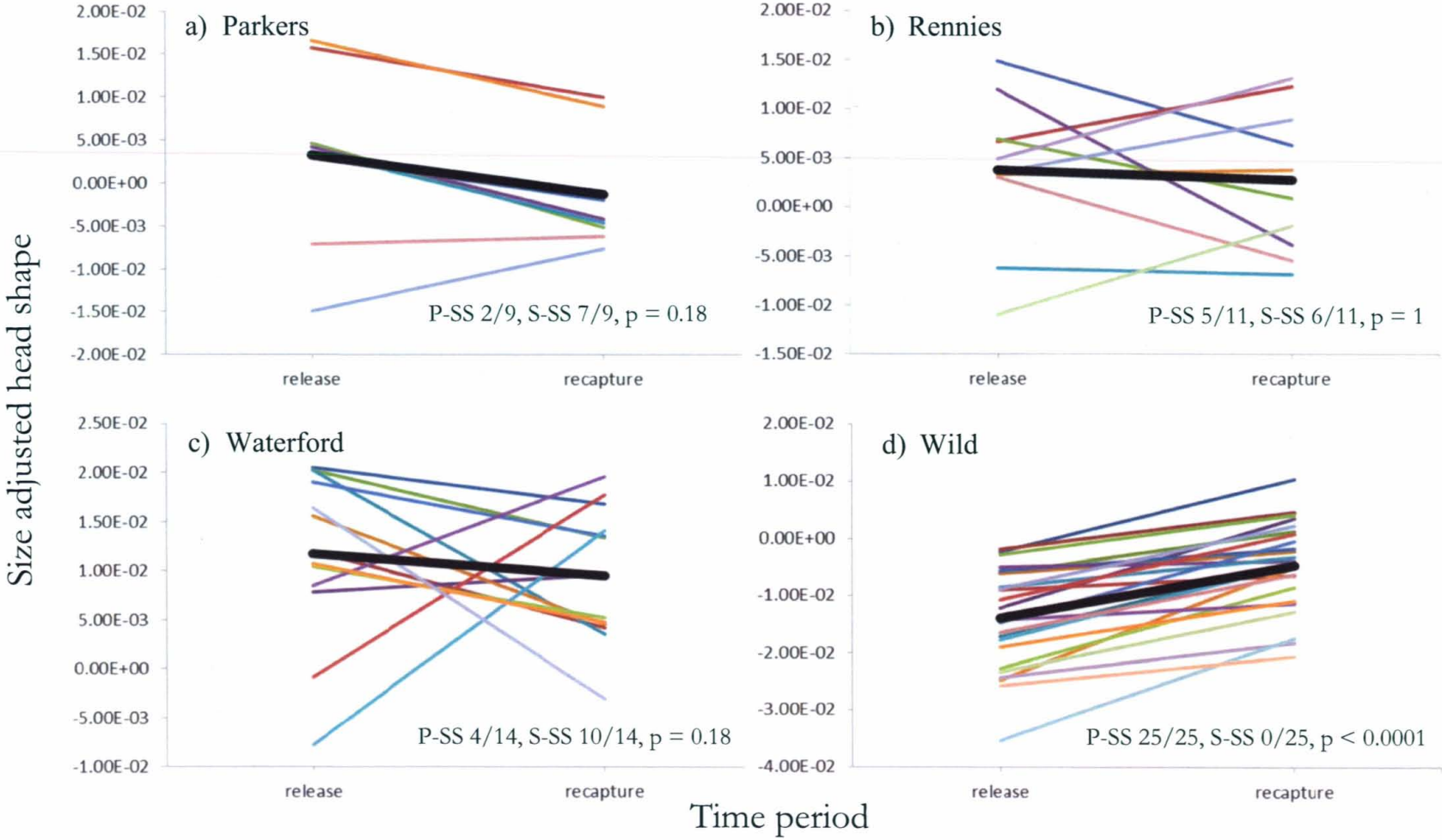


Fig. 4-9. Individual reaction norms of Parkers (a), Rennies (b), Waterford (c), and Wild (d) origin fish reared in the Rennies River. Each coloured line represents an individual's change in size adjusted head shape after ca. 70 days post-release. The thick black line is the population-average response. The number of individuals responding in the direction predicted based on population-specific selection (P-SS) or site-specific selection (S-SS), based on differentials are shown as a fraction of the total individuals sampled and compared to a binomial process.

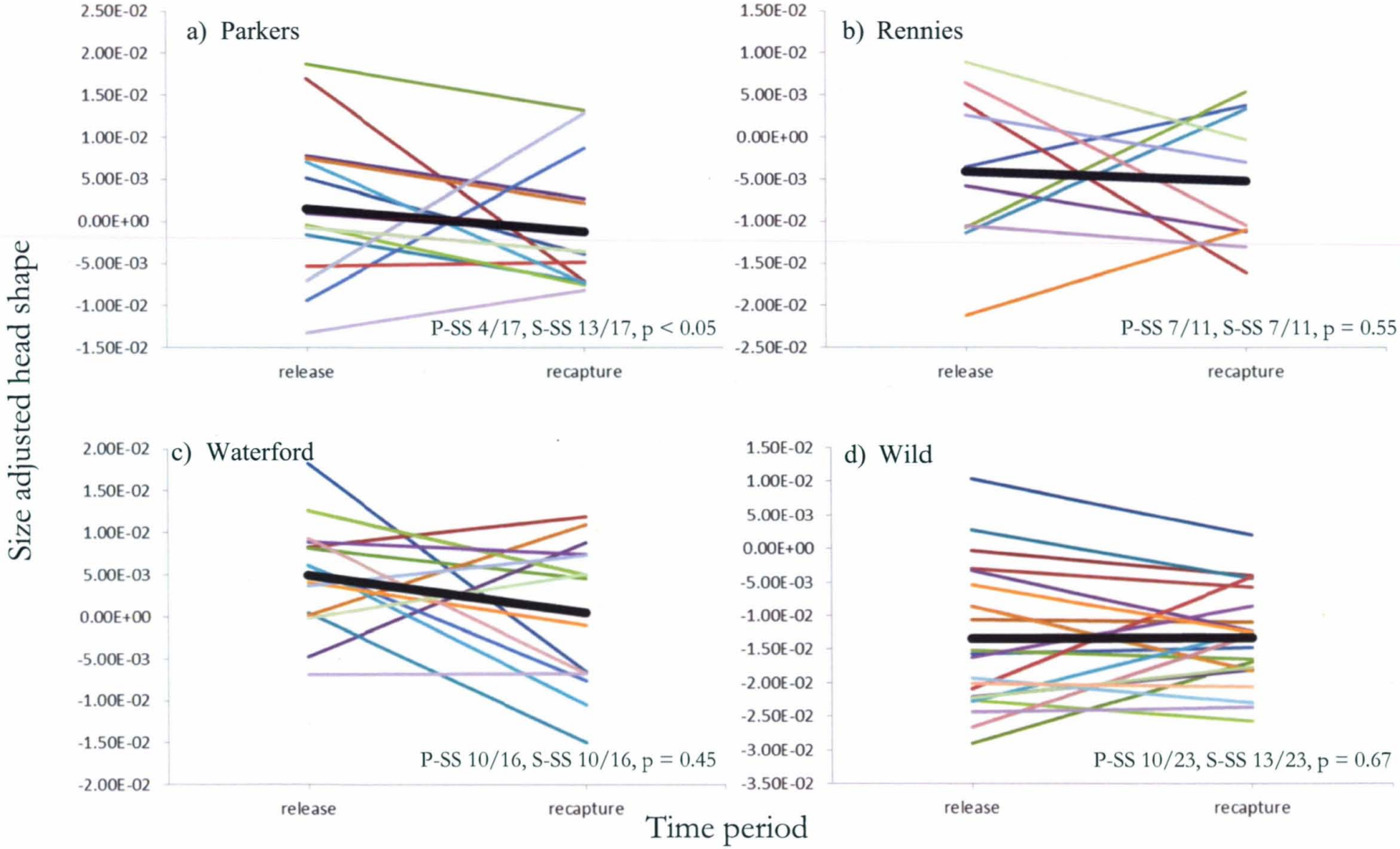


Fig. 4-10. Individual reaction norms of Parkers (a), Rennies (b), Waterford (c), and Wild (d) origin fish reared in the Waterford River. Each coloured line represents an individual's change in size adjusted head shape after ca. 70 days post-release. The thick black line is the population-average response. The number of individuals responding in the direction predicted based on population-specific selection (P-SS) or site-specific selection (S-SS), based on differentials are shown as a fraction of the total individuals sampled and compared to a binomial process.

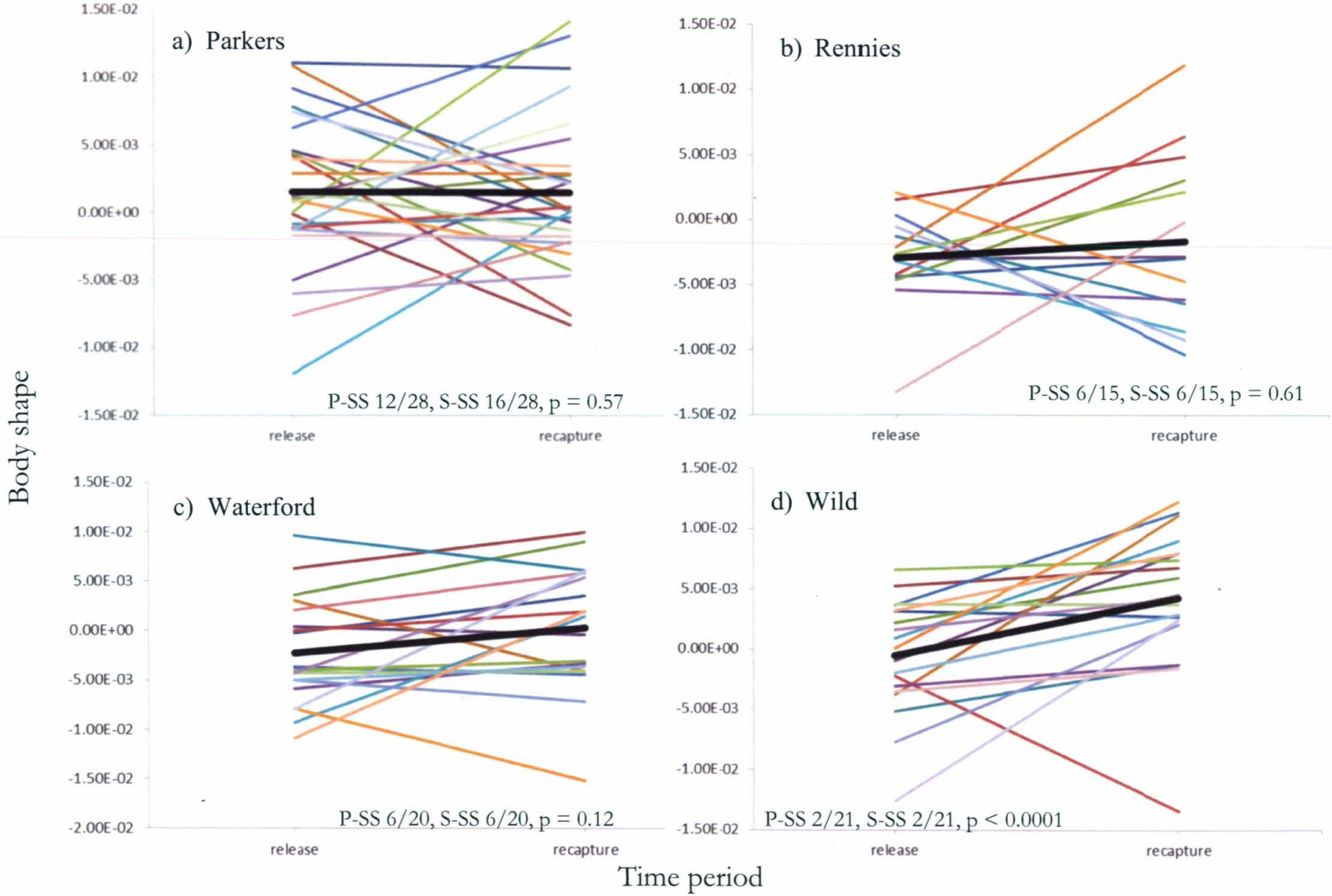


Fig. 4-11. Individual reaction norms of Parkers (a), Rennies (b), Waterford (c), and Wild (d) origin fish reared in Parkers Pond Brook. Each coloured line represents an individual's change in body shape after ca. 70 days post-release. The thick black line is the population-average response. The number of individuals responding in the direction predicted based on population-specific selection (P-SS) or site-specific selection (S-SS), based on differentials are shown as a fraction of the total individuals sampled and compared to a binomial process.

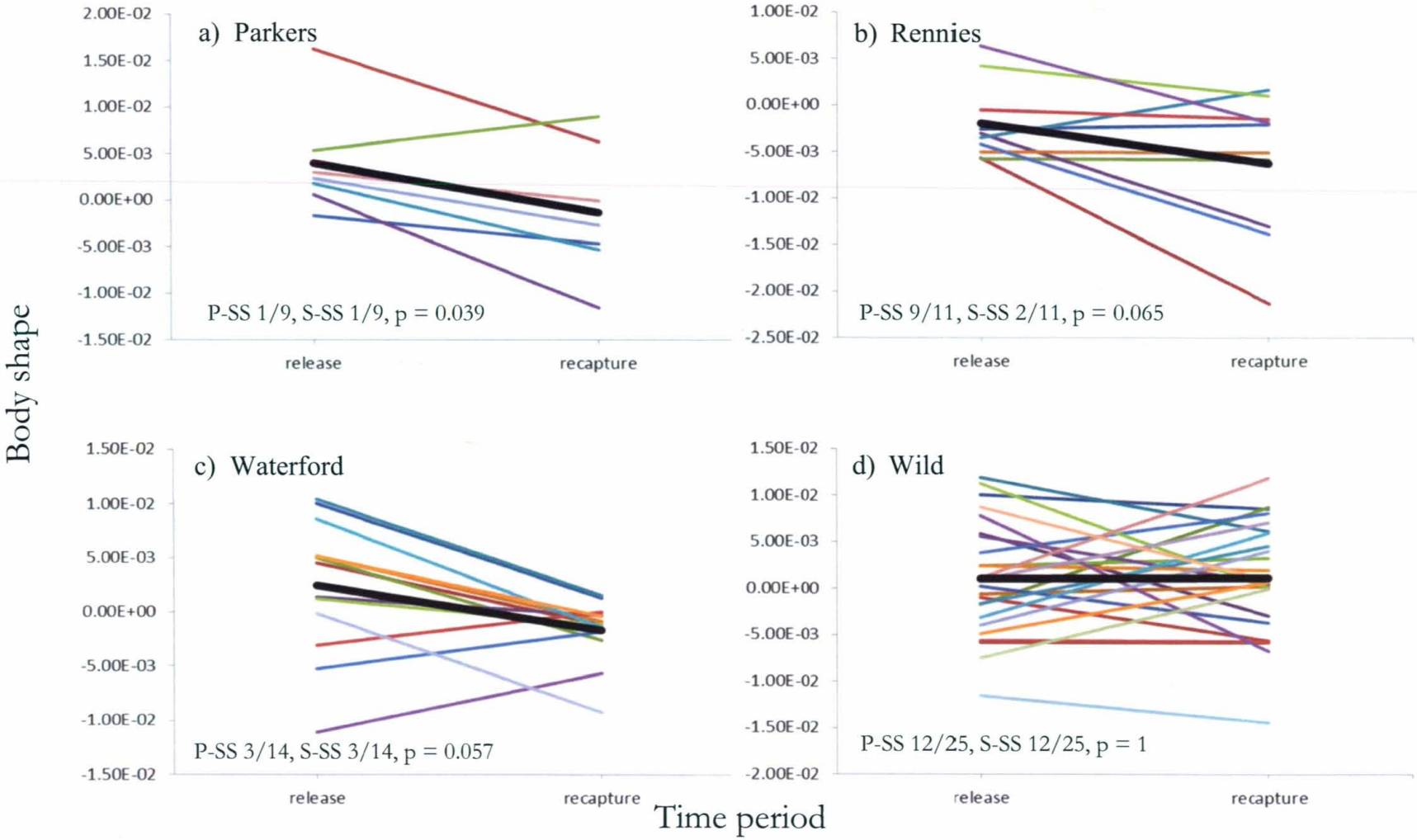


Fig. 4-12. Individual reaction norms of Parkers (a), Rennies (b), Waterford (c), and Wild (d) origin fish reared in the Rennies River. Each coloured line represents an individual's change in size adjusted head shape after ca. 70 days post-release. The thick black line is the population-average response. The number of individuals responding in the direction predicted based on population-specific selection (P-SS) or site-specific selection (S-SS), based on differentials are shown as a fraction of the total individuals sampled and compared to a binomial process.

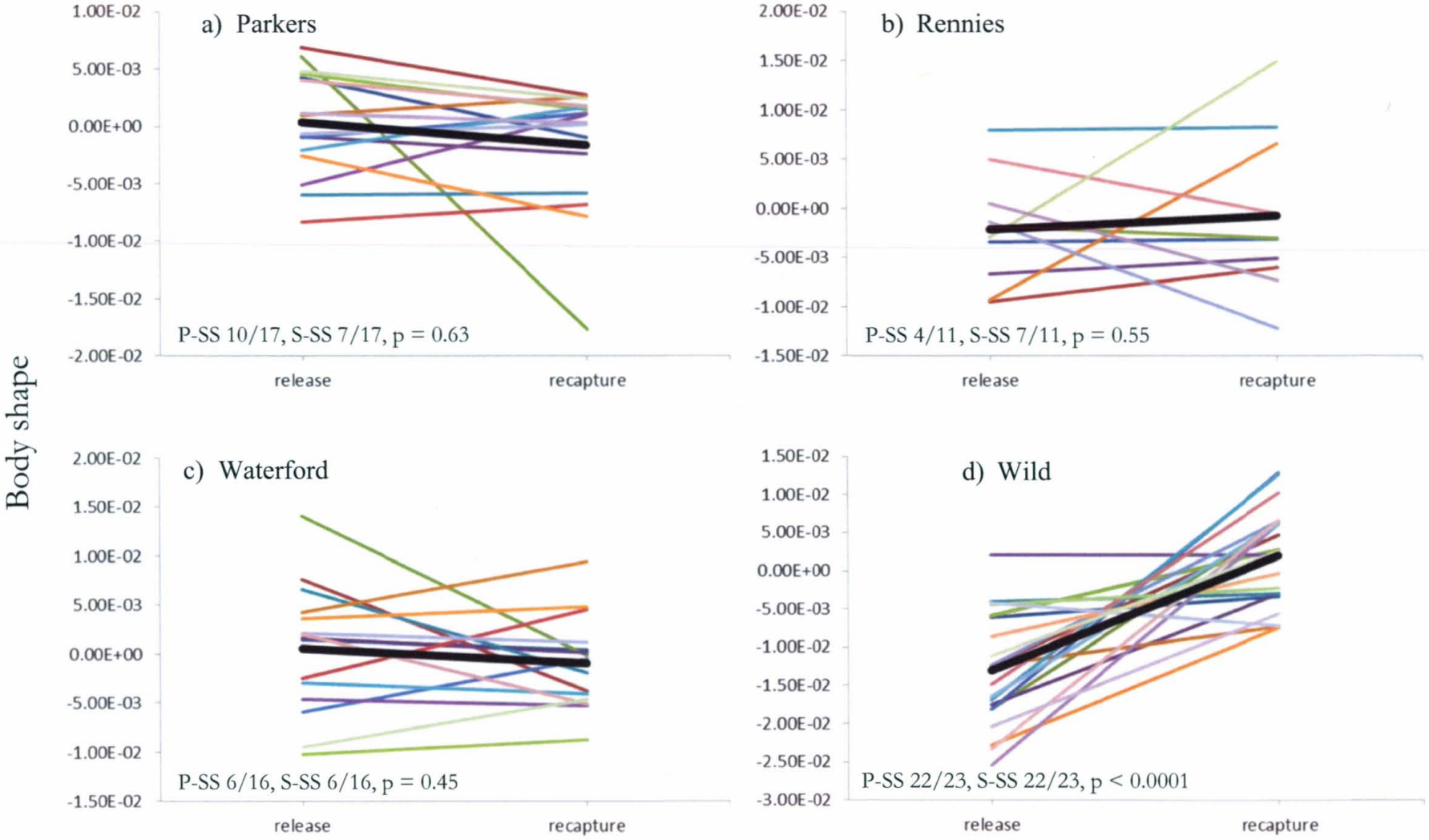


Fig. 4-13. Individual reaction norms of Parkers (a), Rennies (b), Waterford (c), and Wild (d) origin fish reared in the Waterford River. Each coloured line represents an individual's change in size adjusted head shape after ca. 70 days post-release. The thick black line is the population-average response. The number of individuals responding in the direction predicted based on population-specific selection (P-SS) or site-specific selection (S-SS), based on differentials are shown as a fraction of the total individuals sampled and compared to a binomial process.

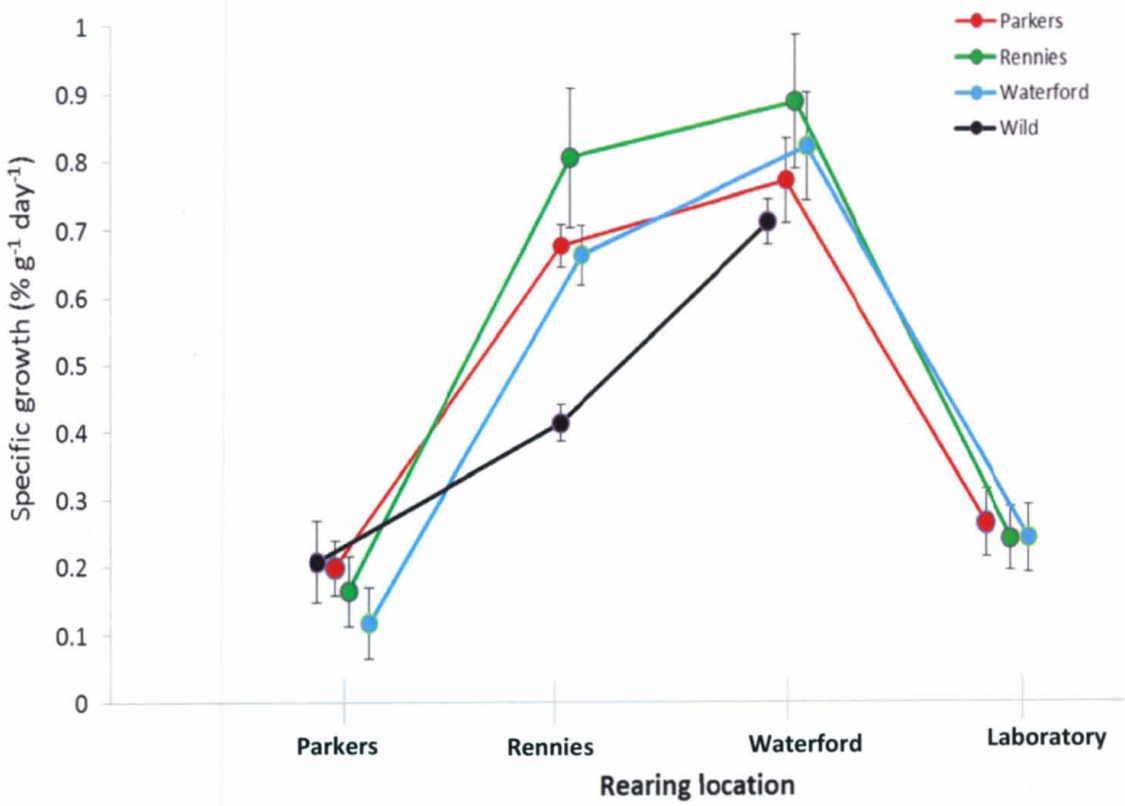


Fig. 4-14. Average growth (± SE) of Parkers (red), Rennies (green), Waterford (blue), and Wild (black) origin brown trout reared in four environments.

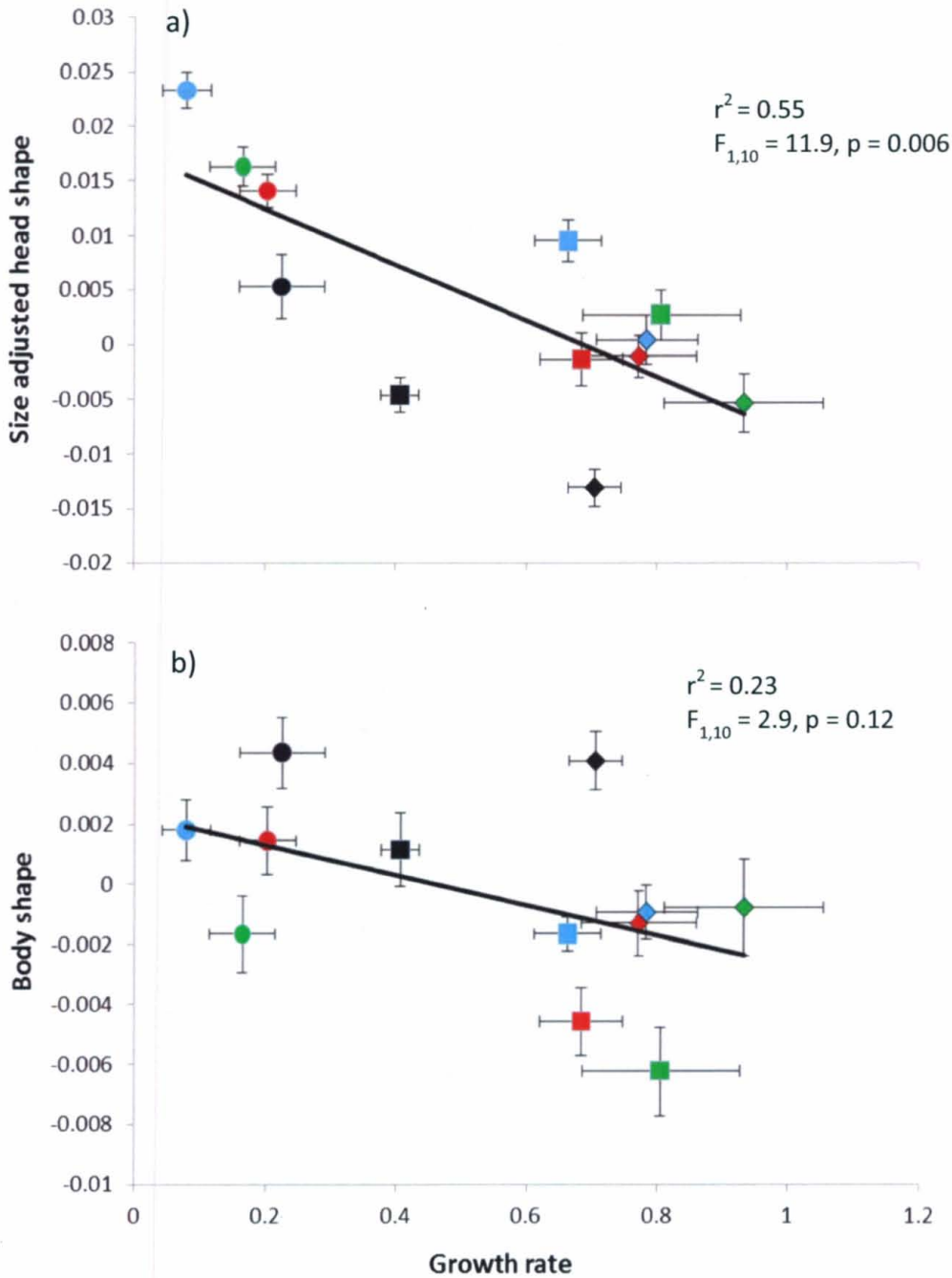


Fig. 4-15. Relationship between head shape (a), body shape (b), and growth. Each point represents the average growth of Parkers (red symbols), Rennies (green), Waterford (blue), and Wild (black) origin fish when reared in Parkers (circles), Rennies (diamonds), or Waterford (squares) environments. Error bars represent ± 1 SE.

General discussion and summary

The benefits of transporting species around the globe (e.g., to establish new agricultural crops and increase food supplies and recreation) notwithstanding, there is little doubt that the biological invasions that sometimes ensue can cause lasting ecological and economic damage (e.g., Townsend, 1996). Indeed, the last global ecosystem assessment highlighted the detrimental impact of non-native invasive species not only on native species, but also on the long-term maintenance of ecosystem health and biodiversity in all its forms (Hassan et al., 2005). This thesis was an attempt to highlight what we have and can learn about evolution and ecology through the examination of one biological invasion – brown trout in Newfoundland. My motivation to do so was inspired by the call to consider biological invasions as unplanned, large-scale, replicated experiments in nature to investigate fundamental questions (Sax et al., 2005). Though researchers are increasingly thinking of invasions as fortuitous research opportunities (Sax et al., 2007), the sentiment in the literature is that the opportunities remain woefully under-utilized (see comments in Reed et al., 2010a, Ghalambor et al., 2007). In this discussion I review the key points of each chapter and highlight, where appropriate, the next research steps to be taken.

Differential rates of phenotypic evolution and plasticity in native versus invasive species

In Chapter One, I confirm in a quantitative sense the sentiments of Reznick & Ghalambor (2001) and Kinnison & Hairston (2007), who suggest that many of the examples of contemporary evolution emerge from contexts of invasion and colonization. Perhaps most importantly, the results revealed that invasive species and native species are generally evolving along similar trajectories and both are influenced by the environment and thus

phenotypic plasticity. That is, our interpretation of the rate and form of how populations evolve is not biased by the frequent use of invasive species as subjects. The lack of difference between native species and invasive species is somewhat surprising as natural selection is predicted to be strong during the first stages of colonization or invasion, which seems likely to drive dramatic phenotypic change during biological invasions. An analogous rationale was used by Darimont et al. (2009) to explain the observed differences in trait change of species subject to human predation in the form of hunting and commercial fishing versus other anthropogenic disturbance and natural conditions. Specifically, they concluded that the markedly greater trait change observed in harvested populations was the result of intense human-mediated 'unnatural' selection.

Assuming that natural selection, even in small populations, is the primary driver of trait change the lack of difference between native species and invasive species suggests generally similar selection pressures in contexts of invasion vs. natural conditions. One potential, mentioned in the discussion of Chapter One, is that the conditions identified as 'natural' may be in fact periods of exceptional selection pressure. For example, changes in beak size and shape in Galapagos finches were quantified during periods of abrupt and intense, natural environmental change (i.e. El Nino climatic events). Alternatively, selection during invasion and colonization may not be as intense as previously thought. In Chapter Four, I quantified natural selection on swimming morphology during the first stage of invasion and detected *weak* directional selection and *strong* non-linear selection.

These results, coupled with the patterns observed in Chapter One highlight a knowledge gap and fodder for a review. How does the strength and shape of natural selection vary as a function of time since colonization? Is selection higher in contexts of

anthropogenic disturbance, including invasion, compared to natural settings? To my knowledge, no one has tested the hypothesis that natural selection is comparably strong during the early stages of population establishment or that selection varies among contexts of natural vs. anthropogenic disturbance (as suggested by Darimont et al., 2009). This is somewhat surprising as the raw material for such as synthesis, namely the Kingsolver selection database, is freely available. Moreover, the realization that selection varies through time has become prominent (Siepielski et al., 2009, Siepielski et al., 2011, Bell, 2010) and contentious in the literature (Morrissey & Hadfield, 2011). Ultimately, it is unclear whether we have a false sense of how selection operates in nature if indeed many of the estimates of selection are based on species and systems in the context of invasion or other anthropogenic disturbance.

The approach and results of Chapter One should be considered a starting rather than an end point. This is not meant to minimize the utility and importance of the findings, but rather to suggest there are far too few species included in the database, with a strong bias towards vertebrates. I also suggest caution in comparing multiple traits among multiple taxa to infer fundamental differences between invasive and native species. Increasingly, there is a trend to test for differences *within species* that exist in a native and introduced range, especially in plants. This approach, represents a more robust experimental design to control for phylogenetic differences in species responses (see empirical example by Davidson et al., 2011). However, this approach has the obvious short-coming in only allowing comparisons among species that are, in fact, invasive (i.e. have populations established out of the native range).

Role of the landscape and biological interactions in shaping invasions

Chapter Two marks the initial steps to understand the brown trout invasion of Newfoundland within the context of: Who, where and how many, why, and so-what? Specifically, this chapter addresses the first three of these questions and sets the stage for investigating the consequences of the invasion in the remaining chapters. The most important aspects of this second chapter were that: 1) brown trout are established non-randomly across the landscape in watersheds that are relatively large and productive, 2) the invasion is occurring slowly relative to other salmonid invasions elsewhere (e.g., Chinook in Patagonia).

The first finding is in general agreement with theoretical predictions of biogeography. Namely, larger areas are capable of supporting a larger number of species compared to relatively smaller areas. In addition to having more resources to support rich flora and fauna, large areas – watersheds in this case – may be easier to find or encounter based on chance. By way of a non-fish analogy, a wandering bird is more likely to find Japan (227,000 km^2) than Java (127,000 km^2) based on landmass alone. Moreover, productive environments may facilitate establishment by relaxing intra- or inter-specific competition for resources (see chapter 6 in Lockwood et al., 2007). Results from Chapter Four support this; outcomes of foreign vs. local population performance were associated with habitat productivity (inferred by growth rates and conductivity). Specifically, local fish performed better in relatively unproductive habitats and on par with foreign groups in productive environments.

The second general finding of relatively slow population expansion is curious. The discussion of Chapter One highlights some of the possibilities of why this may be occurring, with specific regard to abiotic factors. The ability of a community to resist invasion has

traditionally been thought to vary inversely with native species richness (i.e. communities with large numbers of native species should be less invasible and thus have fewer invasive species). Support for this hypothesis, first articulated by Elton (1958) and revisited by Levine & D'Antonio (1999) apparently differs among spatial scales of examination (Shea & Chesson, 2002). Biological resistance has been indicated in studies conducted at small spatial scales (Levine, 2000), but not larger spatial scales (Stohlgren et al., 2003, Marchetti et al., 2004).

Watersheds in Newfoundland are depauperate and contain only a handful of fish species (De Jong et al., 2005). Brook trout is the most likely competitor with brown trout, as the other species (e.g. Atlantic salmon, eels, and sticklebacks) occur in sympatry with brown trout in its native range. An emerging hypothesis is that habitat-mediated interactions between brook trout and brown trout may shape the distribution of brown trout in Newfoundland and elsewhere (Korsu et al., 2007). Briefly I outline the evidence leading to this hypothesis:

1. The spatial distribution of brown trout *among* watersheds mirrors distribution *within* watersheds. In both the native and introduced range, brown trout tend to occupy the lower elevation and gradient, warmer and more productive areas of watersheds whereas brook trout tend to occupy the cooler, higher elevation and gradient, headwater sections of watersheds (Budy et al., 2008, Dunham et al., 2002, Korsu et al., 2007). Within watershed distributions of brook trout and brown trout in Newfoundland have not been published, but personal observations formed during the data collection for chapters two and three of this thesis support the pattern described above. Moreover, extensive sampling of the

Renews River watershed, from the estuary to the upper headwaters, provides quantitative support (Lucas Warner, personal communication 2010).

2. Distributions reflect habitat-specific performance and fitness. Brook trout appear to outcompete brown trout in oligotrophic environments whereas brown trout are competitively superior in more productive habitats (Korsu et al., 2007). This competition appears to occur even to the point of population extirpation in some systems. For example in northern Sweden, introduced brook trout greatly increase the probability of population extinction in brown trout, but only in the highest altitude lakes (Spens et al., 2007). The proximate mechanisms underlying the differential performance are not entirely clear but may relate, at least in part, to spawning site requirements. Specifically, brook trout exclusively use groundwater for spawning whereas brown trout may or may not spawn in upwelling areas (Witzel & MacCrimmon, 1983). Survival of brook trout embryos is much higher in areas of upwelling suggesting local adaptation to these environments (Guillemette et al., 2010).

Given these patterns and evidence, the distribution of brown trout in Newfoundland may likely be shaped by competition with brook trout that is context and habitat specific. To test this species-interaction hypothesis one could compare a suite of performance metrics between the species in either relatively productive or unproductive environments. This could be accomplished by direct manipulation and stocking of fish in each environment (e.g. move fish from upstream environments to downstream and vice versa), or again utilize the fortuitous opportunities afford by invasions. An assessment of this hypothesis is timely and

has consequences for predicting the response of brown trout and brook trout to global changes in temperature and freshwater productivity.

Environmental shaping of adaptive within - species diversity

It is increasingly clear that *within species* diversity –analogous to a diverse financial portfolio – buffers against perturbations and promotes long-term persistence in heterogeneous environments (Schindler et al., 2010, Hilborn et al., 2003, Reed et al., 2011). What is less clear is how quickly this diversity arises and the role of the environment in its shaping. Great phenotypic diversity is observed among trout populations established for no more than 130 years and that much of this variation is predictable based on aspects of the physical environment. Moreover, the relationship between aspects of the phenotype, such as swimming morphology and body colouration, and the environment were in the direction often assumed to be adaptive. For example, body shape of small fish in high gradient rivers was more streamlined and fusiform, presumably an adaptive response to living in fast water flow (see logic in Pakkasmaa & Piironen, 2000). Additionally, colouration patterns in populations inhabiting darker-more overgrown environments were drabber than populations inhabiting brighter and relatively open environments, which I interpret as a likely adaptive response for crypsis (but see the discussion in Chapter Three for other explanations).

Salmon and trout colouration has plastic (Donnelly & Dill, 1984) and genetic (Blanc et al., 1994) components, but it is unclear the extent to which the observed colour 'matching' is environmentally vs. genetically controlled. In an attempt to address this question, all fish collected in the wild and raised in the laboratory were photographed in standardized positions with colour vignettes to allow for correction and robust comparisons. Future analyses will investigate how colour change influences fitness (e.g. survival and growth) in

the wild as well as assess the underlying genetic control by comparing colouration of four populations maintained in common-laboratory conditions. Additionally, an experiment was conducted in the winter of 2009 to examine population differences in colour reaction norms in response to colour of rearing substrate. This experiment compliments the recent findings that colonizing freshwater sculpins in southeast Alaska plastically modify their colouration patterns to match novel stream conditions (Whiteley et al., 2009). In addition to quantifying the extent of plasticity, the experiment undertaken with brown trout will illuminate the potential for reaction norms to respond to natural selection in nature.

The results of Chapter Three support the now unequivocal evidence that adaptive variation can arise in contemporary time (Carroll et al., 2007, Reznick et al., 1997, Losos, 2009, Hendry & Kinnison, 1999). Since Darwin, it has been accepted that ecology can influence the patterns of evolution but only recently has it emerged that evolution may happen rapidly enough as to shape ecological patterns (Hairston et al., 2005). Like in Chapter Two, the role of the biological community and the influence of inter-specific competition represent an unfortunate knowledge gap in Chapter Three. The potential for competition with Atlantic salmon and/or brook trout to influence morphology and perhaps lead to character displacement in colonizing brown trout (or the native species) is intriguing and worthy of future investigation. Here again, patterns of the invasion across the landscape lend themselves to natural experiments; brown trout are found in systems comprised entirely of brown trout (e.g. the Waterford River) or in sympatry (e.g. Raymond's Brook) with the other species. However, brown trout occur more often in sympatry with Atlantic salmon than brook trout, presumably due to intense competition with the latter.

Local adaptation, phenotypic plasticity, and the Baldwin effect

The propensity of salmon and trout to return to natal locations for reproduction, coupled with varying patterns of natural selection among locations, can lead to reproductive isolation (Hendry et al., 2000) and local adaptation of populations (Carlson et al., 2009, Quinn et al., 2001a, Hendry & Stearns, 2004). Without question, salmon home to tributaries within large rivers and evidence suggests the capacity to return to microhabitats within small streams (Quinn et al., 2006). This leads to the intriguing question of the spatial scale at which local adaptation may arise. In a recent meta-analysis, Fraser *et al.* (2011) address the magnitude and spatial scale of local adaptation in salmonid fishes and conclude that 1) local adaptation is common, and 2) the magnitude of local adaptation increases with spatial scale, such that populations inhabiting environments further separated in space are more likely to show greater adaptation. However, they also reveal great variation in adaptation at small spatial scales. To assess the extent and scale of adaptation observed in Chapter Four I plotted results from the reciprocal transplant experiments against the data presented in Fraser *et al.* (2011). The outcome of this comparison is shown in Figure 3. Of the 15 comparisons, all but one suggested that local groups performed better (based on recapture probability, a proxy for survival) than foreign groups. Additionally, the estimates of effect size fall within expectations given the spatial proximity among populations. Moreover, the largest effect sizes were the result of comparisons between local wild groups and laboratory raised foreign populations. Taken as a whole, and viewed within this larger context, the results of Chapter Four provide strong support that populations of brown trout have evolved local adaptations to environmental conditions within 130 years of establishment.

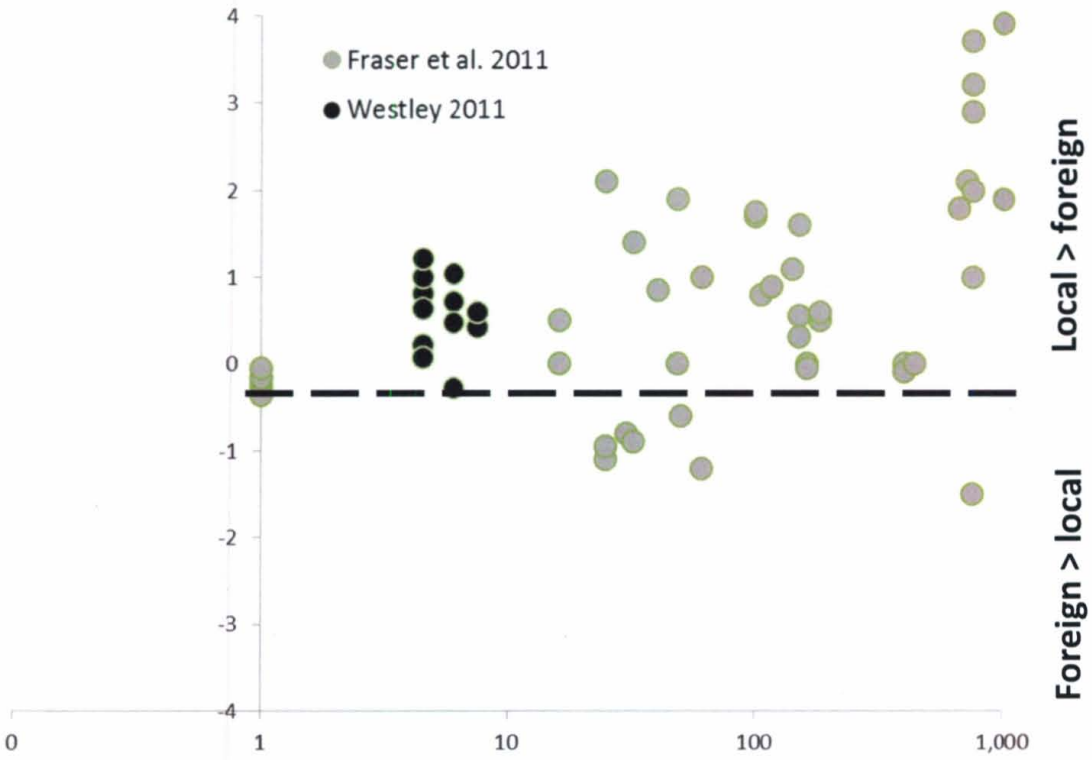


Fig. 3. Effect size (log odds ratio effect size) as a function of distance (km). Effect sizes greater than zero suggest local advantage whereas effects sizes less than zero suggest foreign advantage over local groups. Grey points are taken from Fig. 1a and Supplemental Table 1 in Fraser et al. (2011), and black points are data from Chapter Four of this thesis. Effect sizes were calculated with data from Table 4-6 following the equation on page 406 in Fraser et al. (2011).

As with most empirical studies of local adaptation, the mechanisms leading to the observed local advantage over foreign groups are not clear. Predation is an important force in driving and maintaining ecological divergence in some systems (Nosil & Crespi, 2006, Carlson et al., 2009), and may be the proximate mechanism of mortality in foreign groups here. Though we scanned all potential predators for ingested PIT tags (i.e. tags they may have had in their stomachs by preying upon tagged trout), we only found definitive evidence of one predation event. Interestingly, the predator (a 128 mm brown trout) was not

recovered in the Rennies or Waterford where survival was low (implying high predation). Rather, we found evidence of predation in Parkers, where the relatively large trout consumed a Waterford fish released at 49 mm in length. Piscivorous coho salmon (*O. kisutch*) of approximately equal size to the brown trout predator we observed are capable of completely digesting sockeye salmon fry (*O. nerka*, approximately the same size as the brown trout fry we released) within 12 hours at 13°C (Ruggerone, 1989). Assuming generally similar metabolic rates between coho and brown trout, and given the markedly warmer temperature during the time of our experiment, digestion and passing of tags would have been even more rapid. Thus, it is not surprising that we saw so little evidence of direct predation even if it were occurring frequently. Future work, especially in the relatively small and secluded Parkers location, could be conducted to assess the mechanistic role of predation to drive and maintain local adaptation. All predators could be experimentally removed from sections and performance between groups compared. Moreover, transplanted predators could be used to artificially inflate predation pressures elsewhere in stream sections to examine the effects of an increasing gradient of predation risk.

Functional morphology, namely the shape of the head and depth of the body and caudal peduncle, displayed marked plasticity among environments and to a limited extent, among populations. The population differences is phenotypic response suggest underlying genetic variation that, if additive, could respond to selection in subsequent generations. Previous work suggests that evolutionary responses to selection are likely as morphology in salmonids is heritable and has underlying additive genetic variance (Kinnison et al., 2003, Hard et al., 1999). The potential for anthropogenic sources of selection, such as through hatchery practice, to shape population norms of reactions is increasingly emerging (e.g.

Morris et al., 2010). In a recent paper, Morris et al. (2010) reveal that domestication appears to affect the height (i.e. the y-intercept) of the growth reaction norm in Atlantic salmon, but not the slope. Indeed, the researchers in this paper report generally parallel reaction norms, similar to the overall pattern observed in Chapter Four.

Similar to the findings above, parallel reaction norms maintained population differences in morphology among environments countering predictions that morphology would converge towards presumed site-specific optima. This is counter to the findings resulting from reciprocal transplants of barnacles (Neufeld & Palmer, 2008) and fountaingrass (Williams et al., 1995), both of which report nearly complete phenotypic convergence, resulting from plasticity, of groups reared in common conditions. The feeding morphology of benthic and limnetic ecotypes of sticklebacks (*Gasterosteaus aculeatus*) becomes more similar when reared on the other's diet, but differences remain (Day et al., 1994). This finding is more congruent with the results of Chapter Four: plasticity resulted in more similar morphology among populations in different environments than if plasticity was absent, and yet significant population differences persist. This result suggests underlying constraints on plasticity that are not clear, and may reflect past selection pressures (Ghalambor et al., 2007, Cook & Johnson, 1968, Valladares et al., 2007).

Theory predicts that plasticity should increase the probability of persistence when directional selection acts on extreme phenotypes in novel environments (Price et al., 2003, Ghalambor et al., 2007, Chevin et al., 2010). In contrast to this prediction, we detected weak (relative to estimates of directional selection in the Kingsolver database) directional selection acting on body size, and two aspects of shape. This finding is curious given theoretical predictions and published empirical results (Anderson et al., 2010, Kingsolver & Diamond,

2011, Kingsolver et al., 2001, Charmantier et al., 2008). Moreover, the direction of the phenotypic response was not predictable given the observed patterns of directional selection, counter to expectations arising from the early stages of the Baldwin effect (Reed et al., 2011, Badyaev, 2009, West-Eberhard, 2003). I conclude this discussion with several thoughts of how to understand these contradictions.

First, a publication bias may exist towards studies reporting strong directional selection and plastic responses in the direction of selection. Moreover, many attempts to quantify selection in nature are done so during periods of abrupt change when the conditions are presumably conducive for detecting strong selection. To my knowledge a formal test for a publication biases in studies of selection has not been undertaken. While the potential for such a bias to exist, I consider it unlikely that it would sufficiently explain the outcome here. For example, in a recent review by Fraser et al. (2011) showed no bias in publications reporting local adaptation in salmonids.

Second, directional selection may have been weak as phenotypic differences among populations were perhaps sufficiently close to the local optima that non-linear selection could prevail (Ghalambor et al., 2007). It is possible that we would have seen more of an effect if we had transplanted populations further in space (e.g. transplants from the origin of the invasion to areas at the edge of the range) or if we had selected populations based on extreme phenotypic values. Indeed, the populations of trout reciprocally planted differed less from one another than classic models of ecological species like sticklebacks (Schluter, 1993) and certainly differed less than the well-known morphs of brown trout found in some lakes (Ferguson, 1989). While comparisons between more divergent populations may have led to different interpretations, the design of our experiments may actually have better represented

the true dynamics of invasions. If invasions proceed as stepping-stones rather than long dispersal leaps, then colonizing individuals are likely not to be so dissimilar from source populations.

Finally, the disparity between predictions and reality may have arisen from my attempts to actually quantify selection, rather than *assuming* plasticity was adaptive. Indeed to my knowledge no other study has simultaneously quantified swimming morphology and selection in juvenile salmonids (see Fleming & Gross, 1994 for an example of selection and sex traits in adult salmon), though assumptions about adaptive significance of morphology is common (see logic in Pavey et al., 2010, Pakkasmaa & Piironen, 2001, Franssen, 2011). Though it is true that repeated patterns in morphology consistent with ecology strongly implicates adaptation driven by natural selection, it is equally true that there is still considerable progress to be made towards holistically understanding and predicting how organisms will respond to a rapidly changing world.

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Appendices

Appendix 1-1 Evolutionary Indices: haldanes and darwins

John 'Jack' B.S. Haldane proposed two quantitative methods, now referred to as units of darwins and haldanes, for estimating the rate of evolution in nature (Haldane 1949).

$$darwin = \frac{\ln X_2 - \ln X_1}{y}$$

where X_2 and X_1 are mean trait values measured at time period 2 and 1 in allochronic studies or mean trait values between populations 2 and 1 in synchronic studies and y is time in years.

$$haldane = \frac{(\frac{X_2}{S_p}) - (\frac{X_1}{S_p})}{g}$$

where X_2 and X_1 are mean trait values measured at time period 2 and 1 in allochronic studies or mean trait values between populations 2 and 1 in synchronic studies, S_p is the pooled standard deviation, and g is number of generations (time of divergence divided by generation length).

Obvious fundamental differences between the darwin and haldane exist. First, the darwin assumes an exponential rate of change over time (linear rate between logarithims is equivalent to exponential rate between untransformed values), while the haldane has no such constraint. Both units are susceptible to self-correlation if plotted against time interval and thus should be avoided. The darwin has been employed more widely than the haldane, perhaps due to its charismatic name or for the simplicity of application; however, the haldane has a better grounding in the evolutionary process.

Appendix table 3-1. Average ± SD values for growth rate and size adjusted morphological and meristic traits. Averages are based on n=17, n=20, and n=15 fish per population in the <60 mm, 60-150 mm, and > 150 mm size groups, respectively. Units: Growth represents the specific growth rate in mm, weight (g), body depth (mm), caudal depth (mm), pectoral length (mm), caudal length (mm), and head surface (mm^2), eye surface (mm^2), is the first principal component axes scores of extracted red, green, and blue colour values, and % red is the percentage of pixels in standardized fish photographs that were determined to fall in the 'red' spectrum, spots are counts of pigmentation spots on the left sides of fish. See text for more information.

Size category	Population	Growth	Weight	Body depth	Caudal depth	Pectoral length	Caudal length	Head surface	Eye surface	Color	% red	Spots
<60 mm	Avondale	0.0084 ± 0.0010	1.0 ± 0.10	8.19 ± 0.50	3.72 ± 0.21	7.42 ± 0.32	6.63 ± 0.28	55.77 ± 4.64	5.93 ± 0.66	-0.40 ± 0.72	5.5 ± 2.3	8 ± 6
	Chance	0.0058 ± 0.0008	1.0 ± 0.14	8.36 ± 0.27	4.01 ± 0.12	8.10 ± 0.30	7.45 ± 0.35	60.41 ± 3.94	7.93 ± 0.52	-1.49 ± 1.00	19.8 ± 4.9	15 ± 6
	Chapel	0.0073 ± 0.0015	1.0 ± 0.11	8.43 ± 0.30	3.89 ± 0.15	7.05 ± 0.49	6.74 ± 0.31	55.07 ± 2.88	6.56 ± 0.65	-0.94 ± 0.90	1.8 ± 1.9	10 ± 5
	Parker's	0.0065 ± 0.0012	0.9 ± 0.12	8.56 ± 0.34	3.96 ± 0.16	7.77 ± 0.57	6.83 ± 0.33	63.19 ± 2.44	8.14 ± 0.78	-0.89 ± 0.86	7.0 ± 3.8	8 ± 4
	Raymond's	0.0092 ± 0.0018	1.2 ± 0.17	8.50 ± 0.63	3.79 ± 0.20	7.90 ± 0.44	6.72 ± 0.39	61.46 ± 4.86	6.57 ± 0.91	-2.40 ± 0.58	3.9 ± 3.2	7 ± 3
	Renews	0.0052 ± 0.0008	1.0 ± 0.15	8.29 ± 0.29	3.93 ± 0.19	8.17 ± 0.50	7.30 ± 0.33	61.38 ± 2.93	8.50 ± 0.61	-1.04 ± 0.58	17.9 ± 3.5	25 ± 8
	Rennie's	0.0079 ± 0.0015	1.1 ± 0.29	8.58 ± 0.30	3.88 ± 0.12	7.18 ± 0.36	6.60 ± 0.36	59.13 ± 4.67	7.07 ± 0.89	0.01 ± 1.05	2.4 ± 1.6	9 ± 10
	Rexton	0.0078 ± 0.0011	1.2 ± 0.29	8.28 ± 0.56	3.81 ± 0.14	7.72 ± 0.66	6.33 ± 0.36	53.55 ± 4.19	5.56 ± 0.75	-2.60 ± 0.99	0.4 ± 0.7	7 ± 2
	Salmon	0.0074 ± 0.0013	0.9 ± 0.15	8.29 ± 0.38	3.87 ± 0.20	7.62 ± 0.31	7.15 ± 0.46	60.50 ± 3.44	7.82 ± 0.73	2.22 ± 1.07	3.7 ± 2.0	9 ± 4
	Savage	0.0075 ± 0.0013	1.2 ± 0.32	8.54 ± 0.34	3.94 ± 0.13	7.24 ± 0.59	6.56 ± 0.43	55.40 ± 2.58	5.38 ± 0.71	-0.88 ± 0.49	5.2 ± 3.4	8 ± 4
	SE Placentia	0.0086 ± 0.0010	1.0 ± 0.11	8.54 ± 0.23	3.81 ± 0.13	7.54 ± 0.25	6.62 ± 0.34	58.05 ± 3.36	6.44 ± 0.63	0.40 ± 0.97	14.3 ± 7.0	9 ± 3
	Topsail	0.0074 ± 0.0012	1.0 ± 0.21	8.79 ± 0.40	4.10 ± 0.19	7.90 ± 0.41	6.92 ± 0.34	57.54 ± 3.86	6.80 ± 0.58	0.54 ± 1.38	9.1 ± 3.6	8 ± 7
	Witless	0.0080 ± 0.0021	1.1 ± 0.28	8.82 ± 0.42	3.87 ± 0.15	7.87 ± 0.42	7.19 ± 0.42	59.14 ± 3.57	6.33 ± 0.62	-1.02 ± 0.75	9.7 ± 2.8	7 ± 4
60-150mm	Avondale	0.0029 ± 0.0003	12.1 ± 0.71	19.54 ± 1	8.51 ± 0.40	16.62 ± 0.77	13.31 ± 0.96	263.49 ± 16.42	26.49 ± 3.83	0.34 ± 0.97	5.1 ± 4.6	45 ± 12
	Chance	0.0022 ± 0.0004	11.4 ± 1.12	19.67 ± 0.6	8.76 ± 0.29	17.88 ± 0.92	15.12 ± 0.84	270.32 ± 12.63	31.61 ± 3.24	-1.69 ± 0.81	16.0 ± 7.4	50 ± 10
	Chapel	0.0029 ± 0.0005	12.1 ± 1.4	19.86 ± 0.5	9.00 ± 0.27	17.76 ± 0.81	14.65 ± 0.72	257.63 ± 12.88	27.96 ± 2.35	-0.14 ± 1.23	4.2 ± 5.8	35 ± 8
	Parker's	0.0026 ± 0.0003	12.0 ± 0.62	21.02 ± 0.8	9.42 ± 0.30	17.68 ± 0.75	14.54 ± 0.93	282.60 ± 17.68	30.85 ± 3.96	-1.19 ± 1.17	4.8 ± 3.7	32 ± 10
	Raymond's	0.0032 ± 0.0004	13.4 ± 1.06	20.09 ± 1	8.75 ± 0.47	16.47 ± 0.72	14.22 ± 0.57	258.84 ± 25.34	24.12 ± 2.62	-1.02 ± 1.27	4.9 ± 5.6	38 ± 8
	Renews	0.0028 ± 0.0015	12.5 ± 2.58	20.05 ± 0.8	8.80 ± 0.36	17.98 ± 0.98	15.07 ± 1.28	277.83 ± 16.59	32.68 ± 3.06	-0.86 ± 1.06	14.6 ± 9.3	38 ± 12
	Rennie's	0.0037 ± 0.0018	11.7 ± 1.83	20.6 ± 0.7	8.75 ± 0.40	16.69 ± 0.69	14.40 ± 0.84	267.74 ± 11.72	27.62 ± 2.27	1.94 ± 1.40	3.9 ± 4.4	32 ± 10
	Rexton	0.0033 ± 0.0003	11.5 ± 1.23	19.97 ± 0.9	8.70 ± 0.48	15.89 ± 0.69	13.27 ± 0.83	249.33 ± 23.65	23.87 ± 2.00	0.48 ± 1.14	6.3 ± 5.7	40 ± 10
	Salmon	0.0066 ± 0.0039	11.8 ± 1.81	20.23 ± 1.1	8.82 ± 0.39	16.26 ± 1.36	14.63 ± 1.28	243.45 ± 22.95	25.44 ± 4.37	0.92 ± 2.25	5.0 ± 3.3	36 ± 12
	Savage	0.0034 ± 0.0003	11.5 ± 1.83	20.88 ± 0.8	9.47 ± 0.41	16.26 ± 0.96	14.26 ± 0.81	260.25 ± 14.87	24.11 ± 2.41	0.41 ± 0.90	8.7 ± 4.7	37 ± 10
	SE Placentia	0.0080 ± 0.0038	12.8 ± 0.66	21.36 ± 0.8	8.96 ± 0.49	16.34 ± 0.72	14.36 ± 0.71	257.00 ± 19.49	23.42 ± 4.45	0.67 ± 1.23	17.2 ± 8.4	34 ± 10
	Topsail	0.0032 ± 0.0005	12.1 ± 0.61	20.87 ± 0.7	9.05 ± 0.35	16.81 ± 0.60	14.56 ± 0.55	253.62 ± 12.93	26.44 ± 2.09	2.27 ± 0.90	8.7 ± 5.4	39 ± 11
	Torbay	0.0036 ± 0.0003	12.6 ± 2.28	21.23 ± 0.9	9.10 ± 0.43	16.78 ± 0.71	14.15 ± 1.11	251.54 ± 15.40	24.26 ± 1.96	-0.82 ± 1.07	5.7 ± 4.3	40 ± 12
	Virginia	0.0031 ± 0.0006	11.9 ± 1.05	20.96 ± 0.7	8.82 ± 0.31	15.93 ± 0.81	13.56 ± 0.97	258.61 ± 16.11	25.80 ± 3.87	0.63 ± 1.19	10.6 ± 8.5	38 ± 9
	Waterford	0.0035 ± 0.0005	11.4 ± 1.72	20.24 ± 0.9	8.78 ± 0.41	15.99 ± 0.90	14.23 ± 0.92	257.73 ± 24.86	27.10 ± 2.90	0.46 ± 1.62	3.4 ± 2.5	41 ± 13
	Witless	0.0031 ± 0.0003	12.1 ± 1.3	20.6 ± 0.9	8.99 ± 0.29	17.60 ± 0.62	15.28 ± 0.52	269.85 ± 15.73	29.15 ± 2.84	-0.34 ± 1.36	10.6 ± 7.7	46 ± 15
>150mm	Rennie's	0.002135 ± 0.0004	89.9 ± 7.95	42.07 ± 1.6	17.55 ± 0.81	32.51 ± 1.68	26.22 ± 1.79	955.94 ± 88.29	76.12 ± 6.72	2.46 ± 1.90	8.3 ± 6.4	57 ± 20
	Salmon	0.002907 ± 0.0009	92.4 ± 8.5	42.41 ± 1.7	17.93 ± 0.46	31.74 ± 1.73	25.72 ± 1.41	936.37 ± 106.44	67.46 ± 6.45	1.37 ± 1.06	6.0 ± 4.5	73 ± 21
	Savage	0.002421 ± 0.0002	84.4 ± 6.34	40.83 ± 1.9	17.93 ± 0.68	32.55 ± 1.55	26.55 ± 1.15	908.62 ± 75.64	66.51 ± 7.76	-0.10 ± 0.76	10.8 ± 7.8	52 ± 15
	Topsail	0.001939 ± 0.0004	83.6 ± 14.5	40.94 ± 2.8	17.14 ± 1.15	30.59 ± 2.45	25.03 ± 1.94	907.20 ± 87.36	66.71 ± 7.79	1.89 ± 2.00	9.5 ± 15.1	49 ± 20
	Torbay	0.002735 ± 0.0008	89.6 ± 12.9	43.09 ± 1.7	17.42 ± 0.69	31.59 ± 2.33	24.78 ± 2.13	995.70 ± 101.98	71.26 ± 7.91	-1.15 ± 1.04	4.3 ± 3.4	60 ± 19
	Virginia	0.002125 ± 0.0004	86.0 ± 15.2	41.61 ± 2.9	17.13 ± 1.05	32.69 ± 1.62	24.75 ± 1.31	1001.43 ± 103.74	82.17 ± 10.17	1.04 ± 1.28	18.1 ± 11.3	59 ± 16
	Waterford	0.002302 ± 0.0005	76.4 ± 17.4	41.68 ± 3.3	17.26 ± 0.73	33.20 ± 2.47	26.53 ± 1.18	1000.77 ± 109.10	80.07 ± 11.11	0.52 ± 2.03	4.3 ± 3.0	79 ± 23

Appendix table 3-2. Average physical habitat features at watersheds associated with brown trout populations in Newfoundland. Distances (km) calculated as a fish would swim the putative original source to each watershed, where negative values are south of the source and positive values are to the north of the source, riparian cover categorized between 1 and 4, stream wetted –width (cm) and depth (cm), ratio of wetted-width to depth, gradient % change in elevation (m) over the length of the sample reach (m), conductivity ($\mu S\ cm^{-1}$) , and water clarity (cm).

Location	Distance	Cover	Width	Depth	Width/Depth	Gradient	Conductivity	Clarity
Avondale	110	1.33	16.1	35.3	0.46	1.1	47.1	120
Chance Cove	-140	1	10.4	23.3	0.45	0.8	33.1	110.7
Chapel Arm	250	1.67	13.3	28.4	0.47	0.7	38.5	102.8
Parker's	4.5	3.33	1.7	16.6	0.10	4.4	43.6	110.5
Raymond's	-18	1.67	10.6	32.6	0.33	0.6	43.2	37.8
Renews	-116	1.33	16.9	19.4	0.87	0.9	34.5	92.5
Rennie's	0	1.67	7.4	29.8	0.25	1.5	246.3	38.5
Rexton	155	1	3.4	12	0.28	6.3	48.4	102.2
Salmon Cove	92	1	11.9	30.9	0.39	0.4	48.1	120
Savage	12	1.33	5.5	23.1	0.24	3.9	178.9	95.5
SE Placentia	-421	1	13.5	20.6	0.66	0.5	28.3	75.8
Topsail	92	2.33	5.9	36.4	0.16	3.9	200.2	84.5
Torbay	16	1	4.4	31.4	0.14	4.8	74	101.3
Virginia	0	1.33	4	25.7	0.16	2.2	278.3	49.5
Waterford	-6	1	5.6	38.1	0.15	1.5	299.3	98.8
Witless	-55	3	8.3	19.1	0.43	2.9	77.6	86.7

Appendix 4-1. AICc values from ANOVA models fit to head shape and body shape variables of surviving individuals reared in the Parkers, Rennies, and Waterford environments. Two models (one with a population term, and another with the population term set to zero, that is, a null model) were fit at time of release and time of recapture to test the hypothesis of phenotypic convergence following rearing in common environments.

		Time period			
		Release		Recapture	
Rearing location	Shape variable	Population	Null	Population	Null
Parkers	head	-514.9	-501.7	-502.9	-486.3
	body	-614.1	-610.32	-585.8	-582.7
Rennies	head	-357.8	-311.1	-378.5	-357.3
	body	-404.4	-404.3	-402.3	-397.7
Waterford	head	-402.9	-373.5	-427.7	-403.2
	body	-473.4	-474.4	-467.8	-461.7

Digitization of *Mouseion*

The journal has had three names in its history, and three numberings as a result. In its most current form, the journal is titled *Mouseion*, whose series runs from 2001 (volume 1) to the present (we're currently at 2010, volume 10 issue 1), with three issues per volume. Immediately before this, the journal was titled *Echos du Monde Classique/Classical Views*, which ran from 1982 (volume 1) to 2000 (volume19), again with three issues per volume. The original series was titled *Echos du Monde Classique/Classical News and Views*, running from 1957 (volume 1) to 1981 (volume 25), with varying numbers of issues per year (typically two or three).

In terms of naming the files for the sake of clarity, I would suggest listing the third series as, e.g., **Mouseion2007.v7.1**, the second as, e.g., ClassicalViews2000.19.2, and the first as ClassicalNewsandViews1981.25.3.

Missing Volumes/Issues (to be delivered later)

Third Series: *Mouseion*:	Complete
Second Series: *Classical Views*:	7.3 (1988)
First Series: *Classical News and Views*:	13.1 (1969) 2.3 (1968) 10.1-2 (1966) 1-8 [all issues] (1957[?]-1964)