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RANGE EXPANSION IN THE INVASIVE ROUND GOBY (*NEOGOBIUS MELANOSTOMUS*): BEHAVIOURAL AND GENE TRANSCRIPTIONAL COMPONENTS OF A SUCCESSFUL INVADER

By

Lida Nguyen-Dang

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2017

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DECLARATION OF CO-AUTHORSHIP

I. Co-Authorship Declaration

I certify that, with the above qualification, this thesis, and the research to which it refers, is the product of my own work. I am the sole author of Chapters 1 and 4 and am the primary author on all other chapters (Chapter 2 and 3). This thesis also incorporates the outcome of a joint research undertaken in collaboration under the supervision of Dr. Christina Semeniuk and Dr. Daniel Heath. The collaboration is covered in Chapter 2 and 3 of this thesis. In all cases, the key ideas, experimental designs, data analysis and interpretation, were performed by the author, and the contribution of my supervisors was solely in an advisory capacity.

I am aware of the University of Windsor Senate Policy on Authorship and I certify that I have properly acknowledged the contribution of other researchers to my thesis. I have obtained written permission from each of my supervisors to include the above material(s) in my thesis. I certify that, with the above qualification, this thesis, and the research to which it refers, is the product of my own work.

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ABSTRACT

Range expansion of an invasive species can be influenced by intrinsic mechanisms such as behaviours described as being highly flexible and/or of specific behavioural types that are associated with dispersal ability. In addition, related gene transcription can also be influential in invasion success, promoting acclimation to novel environments. My study species, the round goby (*Neogobius melanostomus*), is an invasive fish continuously expanding its range in the Laurentian Great Lakes and its tributaries. This thesis aims to examine: 1) the behavioural repertoire of the round goby 2) differential gene transcription for gobies "natural" and environmental captive "treatment" using brain candidate genes associated with behavioural traits specific to aggression, boldness, stress response, learning, and activity; and 3) how behaviour and gene transcription vary between residents and dispersers and detection time since North American invasion. I found that round goby possess an "invasion behavioural phenotype" consisting of boldness, exploration, sociality and predator habituation. In addition, I found juveniles were bolder, explored more, were social and habituated to predation more compared to adults, but more so at established sites than recently invaded ones, contrary to predictions. Adults did not show any overall invasion stage differences, possibly due to conspecific densities, habitat-feature differences, and/or time-since-first detection. I showed evidence that there could be a genetic mechanism driving these behaviours, genes expressed for the "natural" group (aggression, stress-response, learning). My natural gene transcription results support that detection time can result in differences most likely driven by density, but round gobies are most likely able to produce "alternative ontogenies" due to plasticity, where individuals acclimatize to novel stressors over time, resulting in shifts in phenotypes. By examining all the facets that could drive range expansion one can gain a deeper insight underlying "invasiveness".

DEDICATION

To my family and friends

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CHAPTER 1: GENERAL INTRODUCTION

Invasive species

With the rise of globalization, anthropogenic activities, and habitat degradation, species are continuously being transported outside their native range (Hulme 2009). Organisms that are found outside of their natural range are considered to be non-indigenous species (NIS) (IUCN 2000); and once introduced, a subset of organisms could potentially become invasive (Blackburn et al. 2011) when the initially small population persists in its new environment and impacts native species or causes economic or ecological harm (McNeely 2001; Perrings et al. 2005; Pimentel et al. 2005; Meyerson and Mooney 2007). The process of an accidental biological invasion consists of several stages that NIS must overcome to be successful invasive species. These stages include: 1) uptake (where the organism is taken up into a vessel), 2) transport (where the organism must evade detection and survive the voyage), 3) introduction (into its new habitat), 4) establishment (where the species is able to produce a sustaining population), and lastly, 5) spread (where the species actively disperses to another area) (Blackburn et al. 2011). Biological invasions are considered to be one the main threats to biodiversity, and their effects are often difficult to halt once the species has established (Thresher and Kuris 2004; Molnar et al. 2008).

For many years, invasion biologists have studied the factors that can influence invasion success in an attempt to understand mechanisms of invasiveness to potentially mitigate the impacts of invasive species. These factors have been broadly divided into three categories: 1) event-level, 2) location-level, and 3) species-level. “Propagule pressure”, an event-level factor, suggests that the number of individuals and introductions can be responsible for a successful invasive species (Lockwood et al. 2005). While there have been studies that strongly support propagule pressure as the main driver of invasion success (Skarpaas and Økland 2009), since high propagule pressure often means higher genetic diversity to overcome the stochastic events often associated with invasions, there are exceptions. A study by Sagata and Lester (2009) observed that invasive Argentine ants (*Linepithema humile)* were able to adjust their behaviour in regard to propagule size, where smaller-sized propagules were more likely to comprise individuals that tended to avoid conflict, and larger propagules possessed individuals more prone to aggression. These results suggested that propagule size would have lower predictive power of invasiveness for animals that display behavioural plasticity.

Other studies attribute invasion success to the "enemy-release hypothesis", a location-level factor, which states that the invasive species is free from parasites and predators in their new environment (Colautti et al. 2004). Contrary, an invasive species might thrive simply due to the new environment matching the native range of the NIS (Iacarella et al. 2015). Many studies also attribute invasion success to specific species-level factors such as habitat generalism (Tschinkel 1988; Gotelli and Arnett 2000), high reproductive rates (Corkum et al. 2004), parental care (Drake 2007), physiological tolerances (e.g., salinity and temperature) (Miranda et al. 2010), and high competitive abilities (i.e., outcompeting natives for resources) (Petren and Case 1996). It has become well-established that species-specific traits, along with propagule pressure and location-scale factors can explain the variability in the success of invasive species (Chapple et al. 2012). However, these traits lack an overarching mechanism that facilitates the successful transition from each stage of the invasion process.

Behavioural variation during the invasion process

More recently, the study of animal behaviour has been shown to play a critical role in invasion biology (Holway and Suarez 1999; Rehage and Sih 2004; Cote et al. 2010a). Behaviour mediates how an organism interacts with its environment (Clark and Ehlinger 1987; Sih et al. 2004). It can potentially explain how an animal can transition through all stages of the invasion process; for instance, what enables an animal to be taken up by a transport vessel, what innate behaviours permit a species to be transported and survive the transit, and how they find suitable habitats and evade novel predators (Chapple et al. 2012). As a result, the few individuals that surpass each selective barrier should not represent a random subset of individuals and this often results in distinct differences between non-indigenous individuals from their native range. These behavioural differences can be represented in behavioural syndromes, which are defined as a correlated suite of behaviours (i.e., boldness, activity levels, aggression, and exploration) that are exhibited across different contexts (mating, foraging, and parental care) and time (Sih et al. 2004). An invasive species could potentially possess within-species variation, where multiple behavioural types exist that could buffer against the perturbations often faced during the invasion process (Sih et al. 2004). However, the invasion process is a selective process, which means that there could be some traits that are advantageous throughout the entire process (Chapple et al. 2012). A term coined by Sih and colleagues (2004), called the “invasion syndrome”, has been defined as a suite of correlated behavioural traits that help an organism pass through several stages of the introduction process. An example of a species that demonstrates an invasion syndrome is the invasive mosquitofish (*Gambusia affinis*), where the mosquitofish is bolder and more explorative in its invaded habitat compared to its native range (Rehage and Sih 2004).

One theory presumes that behavioural flexibility can also be key for invasion success, which is defined as the ability to adjust one’s behaviour in response to a changing environment (Coppens et al. 2010). Behavioural flexibility falls under phenotypic plasticity (i.e., long term - the ability to produce multiple phenotypes in several situations) or adaptive flexibility (short-term responses) (Sih et al. 2004; Coppens et al. 2010), and is generally considered to be a trait that most invasive species possess that allows them to invade new areas because it allows the ability to respond to novel stressors (Wright et al. 2010). Invasion biologists have focused on plasticity as being forefront of what makes a successful invasive species (Sol and Price 2008), since the ability to switch and change certain behaviours in different contexts could in turn be advantageous for that animal's fitness (Wright et al. 2010). Plasticity is adaptive when it infers a fitness advantage or leads to smaller declines in fitness when exposed to a stressor, than if there were no plastic response (Conover and Schultz 1995; Duputie et al. 2015). However, plasticity can be irreversible or reversible (with time lags), but costly in a stochastic and variable environment or if the stressor subsides, as it then takes time to reverse the plastic phenotype (Tufto 2000). Therefore, it can be more beneficial to have limited, or short-term plasticity. While it may be extremely advantageous for organisms and invasive species to be adaptively flexible (akin to react-and-recover) in a variety of traits such as morphology, physiology, and behaviour, there are constraints to the evolution of flexibility. It can be energetically taxing on the individual to be able to constitutively express these traits due to the costs of maintaining sensory and regulatory mechanisms (Dewitt 1998; Murren et al. 2015). It can also be costly to process cues from the environment because it can expose individuals to predators and requires energy that can instead be used for foraging and parental care (Cohen 1967; Van Tienderen 1991; Sih 1992). Indeed, authors have posited that plasticity in certain behaviours coupled with other fixed syndrome-like traits (e.g., strong competitive advantage) may be the most advantageous in invading species (Matzek 2012). As such, multiple strategies (long-term plasticity, short-term flexibility and behavioural syndromes / behavioural types) are equally likely to play essential evolutionary adaptive roles in the invasion process (Sih et al. 2004; Wolf et al. 2007).

Dispersers and Residents

Animal movement is part of the life-history of several species, where this movement can be passive and active. Animals can migrate from patch to patch, which typically involves innate movement where individuals return to their natal patch (Clobert et al. 2009). Dispersal is defined as the active movement from one patch to another (Clobert et al. 2004) and is comprised of three stages, departure, transience, and lastly settlement (Clobert et al. 2009). Dispersal is a behavioural decision, which is a balance of benefits (e.g., food resources, shelter, avoiding competition) and costs (e.g., energy, risks, novel predators) (Bowler and Benton 2005; Ronce 2007; Clobert et al. 2009). In an invasion context, animals can disperse to expand their range and spread. Some benefits of range expansion can include increased prey, shelter and being free from predators that were present their natal habitat (Ronce 2007; Brown et al. 2013; Colautti et al. 2004). The decision to disperse, and the dispersers' ability to navigate and respond to novelty at the new introduction point are also key behavioural traits for invasion success (Martin and Fitzgerald 2005). The resident-disperser paradigm allows invasion biologists to explore the mechanisms that drive colonization of new areas from the initial introduction/ established point. Both invasion stages present different selection pressures that can drive morphological, behavioural and genetic differences between residents and dispersers (Clobert et al. 2009). The term "dispersal syndrome" has been defined as a suite of covarying physiological, morphological, and behavioural traits that are paired with dispersal (Clobert et al. 2009). This is a slight variant of the "invasion syndrome" that compares behaviours of species in their native vs. invaded habitats. Several studies have supported the occurrence of a dispersal syndrome at the invasion front and the persistent differences between the residents at the core/established region and dispersers at the invasion front (Belthoff and Dufty 1998; Phillips et al. 2006; Fraser et al. 2001; Dingemanse et al. 2003; Lielb and Martin 2012). It is predicted that individuals that disperse and those that reside should differ for several reasons: i) they are spatially separated and have limited gene flow, and thus more likely to differentiate due to spatial sorting and non-random mating (Phillips et al. 2008; Shine et al. 2011); ii) they deal with different selective pressures (e.g. habitat quality) (Clobert et al. 2009); and iii) dispersers possess traits (morphologically, physiologically, behaviorally) that more likely enable them to disperse and survive novel habitats compared to residents (Clobert et al. 2009; Wright et al. 2010; Chapple et al. 2012).

Specifically, a dispersal syndrome should be characterized by traits that facilitate spread, and therefore should encompass: boldness, exploration and activity levels (dispersal), asociality (cannot tolerate high densities), learning (habituating to predation), and stress coping (acclimatizing to novel environments). For a disperser, boldness, exploration and higher activity levels can facilitate individuals seeking out novel environments and exploring past their range (Rehage and Sih 2004). In addition, dispersal can be largely affected by social context, where asociality is often coupled with dispersal because asocial individuals typically prefer to be solitary and therefore disperse to colonize low-density habitats (Cote and Clobert 2007; Cote et al. 2010a). Habituation to predation risk can be a special form of plasticity that allows the animal to assess, learn, and remember the degree of risk and respond accordingly (Hemmi and Merkle 2009; Shettleworth 2010). In addition, coping styles, which look at behavioural correlations in regard to the physiological responses to stressful stimuli, where proactive individuals are risk-taking, aggressive and form rigid habits, while reactive individuals are shy, risk-adverse, and less aggressive (Koolhass et al. 1999). A disperser may be more proactive, in order to seek out novel environments and resources or possibly reactive if they are being forced out of ideal habitats.

Drivers of dispersal behaviours (ontogeny, sex, habitat quality and conspecific density)

Dispersal (especially from natal habitats) is often ontogenetically driven, and this could be the result of behavioural responses (behavioural types, range of flexibility) that can change as an animal develops (Groothuis and Trillmich 2011; Wuerz and Kruger 2015). Juveniles and adults are exposed to different challenges that could potentially influence how they respond behaviourally. For instance, juveniles should be focused on survival and growth, and finding an appropriate ecological niche, while an adult's main focus would be preparing for and carrying out reproduction (Groothuis and Trillmich 2011). These two contrasting goals should result in different movement rates. Differences in metabolism can also differ between adults and juveniles and drive behavioural differences, where bolder and exploratory individuals might require higher resting metabolic rate in order to compensate for these behaviours (Stamps 2007; Biro and Stamps 2010). This can ultimately result to differences in foraging activity, which can lead to behavioural differences (Biro and Stamps 2008). Furthermore, behavioural differences between sexes can result in skewed sex ratios at the invasion front (Miller and Inouye 2013). One sex might disperse more than others depending on the parental care and roles in sexual selection (Perrin and Mazalov 2000; Stiver et al. 2007). In addition, alternative reproductive tactics, though seldom studied within invasion biology, can play a role in which individuals disperse (Sunobe and Nakazono 1999; Manabe et al. 2009; Marentette et al. 2011).

Habitat characteristics of the environment can not only determine the range of an invading species (Baguette and Dyck 2007), but the type of dispersing individual, since movement and the decision to move can be influenced by habitat quality (e.g., hydrodynamics, temperature, turbidity), resource availability (food, shelter) (Holt 2003), and species assemblages (Baines et al. 2014). Abiotic features can be critical in dispersal, especially during the settlement stage (Clobert et al. 2009). For example, in round goby, Pennuto and Rupprecht (2016) observed that smaller round gobies were able to remain in place in a flume via passive pelvic adhesion power whereas larger gobies were incapable, suggesting that it might be possible for juvenile gobies to more efficiently surpass barriers that are submerged in water and therefore be more likely to expand the invasion front.

High quality patches containing shelter or food could create increased competition, where more aggressive individuals outcompete subordinate individuals to lower quality patches, from which they disperse (Ray and Corkum 2001). High conspecific densities can equally limit resource availability at a fast pace, resulting in individuals dispersing that are innately highly explorative, active and bold and therefore capable of sourcing other resources; or alternatively, individuals can be forced out of habitats and required to disperse due to lower competitive abilities and resource-holding potential. Species assemblage, more importantly predators, can affect behavioural variation where shyer individuals might opt for a hiding strategy - and hence residency; and bolder, more active individuals might disperse since they are more prone to be detected (Smith and Blumstein 2008). Predation risk has also been known to have an equalizing effect on behavioural types, and therefore residents and dispersers have the same suite of behaviours should they experience similar predation risk) (Cote et al. 2013). Taken together, the study of dispersal syndromes necessitates a comprehensive study of not only what behavioural phenotypes are involved but also the potential drivers that shape them.

Behavioural genomics of invasion

Investigating “invasive phenotypes” requires an integrative approach in order to understand the mechanisms that allow invasive species to persist, expand their range, in addition to acclimate to novel stressors. In particular, behaviour is important to incorporate in invasion biology because it is one of the first ways an animal can respond to a novel environment (Chapple et al. 2012). Moreover, the incorporation of genetics allows us to understand the possible underlying mechanism of behavioural variation (van Oers and Sinn 2011; van Oers and Sinn 2013). The study of both the behavioural and genomic aspects of disperser-residents in an invasion context can (i) unveil the genetic architecture underlying and differentiating these two phenotypes; (ii) assess whether there is geographic variation in the pattern of expression; and (iii) investigate how biotic and abiotic environmental features and challenges modulate expression (Canestrelli et al. 2016). Genomics can identify gene expression mechanisms for acclimatizing to novel habitats (Lee 2002), and does so by investigating differential gene transcription between individuals, populations, and/or species. The observable phenotype expressed can be mediated by gene transcription - the rate-limiting step for gene expression, and is the process of converting DNA code to mRNA that is later used in translation to generate functional protein (Platt 1986). Differential gene transcription can be in response to environmental cues (epigenetics) (Jaenisch and Bird 2003), variants in specific genes (Yan et al. 2002), or a gene by environment interaction (G × E) (Carrol et al. 2001). Differential gene transcription can also be due to heritable variation that selection acts upon, (i.e., individuals that have certain gene variants get selected on and subsequently fixed in the population based on selective events). It can further be due to environmental factors that induce a transcriptional response that facilitates acclimation to a stressor (Hogkins et al. 2013). Overall, gene transcription can play both an evolutionary and a transient role through acclimation in biological invasions and be an important mechanism in explaining how invasive species invade and persist.

The importance of understanding the intricate genetic mechanisms that drive invasive, and more specifically, disperser phenotypes is now made possible with the availability of massive parallel sequencing, microarrays and quantitative real-time polymerase chain reaction (qRT-PCR), whole genomes can now be sequenced and gene transcription profiles can be quantified much more efficiently (Gracey and Cossins 2003). Transcriptomes (set of RNA molecules being expressed) of certain tissues can reveal what genes are being downregulated or upregulated in response to a challenge (e.g. novel environment), or reveal resting gene expression – the constitutive transcription of genes. The invasion process provides the opportunity to understand genetic mechanisms that can enhance dispersal ability. Invasive species often have to deal with a multitude of stressors, novel predators, changing environmental conditions such as temperature and salinity, all of which can result in a transcriptional response (Sanogo et al. 2011; Lockwood et al. 2010; Clark et al. 2013) that helps the animal cope, behaviourally and physiologically.

Quantifying the genetic basis of these dispersal traits can be accomplished using a neural candidate gene list – for example genes related to behavioural traits such as boldness, aggression and sociality (Rehage and Sih 2004; Groen et al. 2012; Cote and Clobert 2007). Furthermore, genes can be targeted that are involved in stress-response and can therefore influence behavioural stress-coping styles (Koolhaas et al. 1999), important in dealing with novel stressors. Neuronal genes involved in synaptic plasticity and long term-potentiation can be important for learning and memory, imperative for a dispersing species to respond to their environment (Hazlett et al. 2002; Lefebvre and Sol 2008; Sol et al. 2008) and lastly, metabolic/activity genes can be important in maintaining the energy necessitated to disperse and respond to the environment. With this gene list, one may be able to assess the “invasiveness” potential – i.e., the ability to colonize new areas, by measuring which genes are differentially transcribed between dispersers and residents at rest and in response to novel environment challenges. This candidate gene list can therefore have the potential to aid in the screening process of future possible invasive- or high-risk species.

Round goby

The round goby, *Neogobius melanostomus* (1811 Pallas) is a prolific invader of the Laurentian Great Lakes and was first detected in North American waters in 1990 (Jude et al. 1992). It originated from the Ponto-Caspian and Black Sea in Eurasia, and has now made its way into all the Great Lakes, continuing to expand its range in various tributaries (Poos et al. 2010; Bronnenhuber et al. 2011). The round goby was initially found in St. Clair river (Sarnia ON, Canada) (Jude et al. 1992). This population has been identified to have the same six haplotypes as those found in the Dnieper river in Ukraine, originating from only one site and no other Eurasian sites (Dufour 2007; Brown and Stepien 2009). Therefore, serves as an appropriate source population for North America and a suitable baseline for comparison. The establishment success and transition of round gobies into freshwater systems has been attributed to their tolerance to temperature (Houston et al. 2013), multiple spawning events per season (Marentette et al. 2009), and parental care (Meunier et al. 2009).

While there have been multiple behavioural studies on the round goby invasion in North America (Groen et al. 2012; Capelle et al. 2015; Myles-Gonzales et al. 2015) and in Europe, (Thorlacius et at. 2015; Hirsch et al. 2016) there has yet to be a study that investigates dispersal and resident phenotypes on a scale that incorporates i) multiple established and invasion front paired rivers including the source, and ii) across ontogeny and sex, all which have been suggested to be important in dispersal (Groothius and Trillmich 2011; Sih et al. 2012). Furthermore, there have been limited studies examining transcription of genes that might have facilitated their invasion success either through genetic adaptation or acclimation (but see Vincelli 2016; Wellband and Heath 2017).

Overall, the round goby is an ideal study system because it is an invasive species that has had several negative repercussions for the Great Lakes: they predate on lake trout and mottled sculpin eggs (Chotkowski and Mardsen 1999), and small mouth bass in the Great Lakes (Steinhart et al. 2004). Round goby in Hamilton Harbour have been seen to display aggressive acts towards native logperch (*Percina caprodes*) and are “space competitors`` for habitats and food resources, which has resulted in dwindling numbers of logperch (Balshine et al. 2005; Bergstrom and Mensinger 2009). Round gobies have also been associated with being a vector for type E botulism mortality in birds (Yule et al. 2006). The expansion of round gobies into tributaries and rivers was not expected due to the physical constraints of upstream dispersal and swimming against the river current (Poos et al. 2010). Their continual spread in tributaries is concerning for rivers that already contain species at risk (SAR) (Poos et al. 2010); therefore, it is important to understand the mechanisms that have facilitate their further spread.

Thesis objectives

The overall goal of my thesis is to examine behavioural and genetic mechanisms of round goby spread during the invasion process, specifically looking at how residents and dispersers differ across the invasion stages (source, established, invasion) in the following traits: behaviour – boldness, exploration and activity levels, asociality, learning, and stress coping; and their transcriptional profile (neural genes involved in behaviour/stress-response, neuroplasticity, and activity (metabolism)) during range expansion, using “detection time since North American invasion”.

In chapter 2, I investigated whether round goby individuals exhibit consistent behavioural traits and predator responsiveness that would facilitate their successful invasion-spread in southern Ontario, Canada. Specifically, I sought to determine whether these behavioural traits: (1) differ across the source (S), establishment (E), and invasion stages (IS), (2) are consistent among sites and within invasion stage, (3) differ across life-stage (juvenile and adult), and within adults, and (4) differ between sex (including male alternative reproductive tactics).

In chapter 3, using a candidate approach, I investigated brain gene transcription in three gene ontologies- behavioural/stress response, neuronal, and metabolic/activity - of the invasive round goby with regards to detection time since North American invasion, to examine genetic mechanisms associated with dispersal vs resident phenotypes. Fish from multiple rivers ("natural") were sampled to examine whether innate differences in gene transcription occur (in the absence of a challenge) during range expansion, using "detection time since North American invasion". In addition, this study examined brain gene transcription response of round gobies following transfer to a captive environment - "treatment" group, to examine how these animals might cope with novel conditions, and comparing fish across "detection time since North American invasion"

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CHAPTER 2: BEHAVIOURAL VARIATION OF ROUND GOBY (*NEOGOBIUS MELANOSTOMUS*) DURING THE INVASION SPREAD ACROSS SOURCE, ESTABLISHED, AND INVASION-FRONT SITES

Introduction

Invasive species have long been considered a threat to biodiversity through competition with and predation of native species, and their effects on ecosystem dynamics (Mack et al. 2000; Didham et al. 2005; Poos et al. 2010; Pejchar and Mooney 2009). For a non-indigenous species to successfully establish in a new area (self-sustaining population), it must go through the invasion process, from transit, introduction, and establishment to spread - where once the non-indigenous species has expanded past its introduction point, it is considered 'invasive' should it cause ecological and/or economic harm (Blackburn et al. 2011). While it is difficult to completely eradicate a non-indigenous species once it has established, intervening when a nonindigenous species is localized to certain areas can at least prevent the species from expanding their range and colonizing new areas (Byers et al. 2002; Dextrase and Mandrak 2006). Over several years the spread stage (also known as the invasion front/stage/edge), has been studied to understand the drivers of dispersal, to recognize the range of an invasive species and the extent and magnitude of damage that they can incur; and to initiate appropriate management to mitigate further spread especially in already fragmented habitats (Sakai et al. 2001; Bowler and Benton 2005; Clobert et al. 2009; Juette et al. 2014). Dispersal is the active or passive attempt to move from a natal site to another site, and is the process that allows individuals to move from one patch to another (Clobert et al. 2009). The dispersal activity and continual spread of an invasive species can be explained by several theories such as i) natural selection - where individuals that have the capacity to disperse and survive in low density areas will reproduce and pass on genes to their offspring that allow them to be superior dispersers (Travis and Dytham 2002; Phillips et al. 2008), ii) spatial sorting - where individuals with greater dispersal tendencies will gather at the invasion front and promote further expansion via non-random mating (Phillips et al. 2006; Shine et al. 2011; Ashenden et al. 2017), and iii) behavioural variation - where dispersers that possess behavioural traits or behavioural responses that enhance colonization (i.e., behavioural syndromes, behavioural types, or varying degrees of flexibility) are more likely to disperse and survive novel habitats (Clobert et al. 2009; Wright et al. 2010; Chapple et al. 2012).

Because individuals at the leading edge often face different selection pressures and novel challenges compared to their established core populations, such as novel predators, unfamiliar food resources, fewer mating opportunities, and different abiotic habitat conditions, the spatial variability of a species' distribution should primarily and immediately be driven by behaviours that lead to enhanced survival under specific conditions (Cote et al. 2010a). While "invasion syndromes" have been extensively studied to contrast differences in a suite of correlated behavioural traits between native and non-native species that enable an individual to pass through the first stages of the introduction process (Sih et al. 2004; Rehage and Sih 2004; Bubb et al. 2006), more recently the concept of a "dispersal syndrome" has been introduced (Clobert et al. 2009). This idea describes the enhanced dispersal ability of invasive species individuals at the invasion edge in terms of covarying physiological, morphological, and behavioural traits, and typically compares these traits to the invasive species residents present at the core/established region (Belthoff and Dufty 1998; Phillips et al. 2006; Fraser et al. 2001; Dingemanse et al. 2003; Lielb and Martin 2012). For instance, highly capable dispersers that accumulate on the invasion front may possess traits that enable them to exploit new habitats and food sources. Being bold and explorative, and consequently gaining access to untapped resources, is one such strategy (Fraser et al. 2001; Pintor et al. 2008). Alternatively, a shy behavioural type, where individuals are less prone to take risks, could also potentially benefit a disperser, as they would be less likely to encounter novel predators or diseases that could potentially be present in a new habitat (Hudina et al. 2015). Dispersers could also be individuals that are more subordinate, having been competitively excluded by superior or larger conspecifics of established populations (reviewed by Bowler and Benton 2005; Guerra and Pollack 2010; Hudina et al. 2012; Hudina et al. 2015). In contrast, individuals at established sites could possibly possess a variety of behavioural types due to relaxed selection, but again, the optimal phenotype would be context specific. Behaviours at established sites could tend towards being less active and/or exploratory, and more social due to the familiarity with the conditions of the environment, the predator community, and other biotic and abiotic factors (Blossey and Notzold 1995). Should intraspecific competition be exceedingly high, however, established populations could also possibly be more active and aggressive due to these high densities, and dispersing individuals instead could experience relaxed selection and consequently exhibit behavioural variation at the invasion front due to density relief.

A dispersion syndrome implicitly describes a correlated suite of consistent behaviours across all contexts and time that also covaries with other phenotypic traits (Clobert et al. 2009). However, the ability to adjust one’s behaviour in response to a changing environment (Coppens et al. 2010) can also prove beneficial to a disperser. Behavioural flexibility (the ability to produce multiple phenotypes in several situations; Sih et al. 2004) is generally considered to be an important mechanism that allows invasive species to invade new areas (Martin and Fitzgerald

2005; Wright et al. 2010). For the past two decades, behavioural ecologists have focused on flexibility as being at the forefront of what makes a successful invasive species (Sol and Lefebvre 2000; Martin and Fitzgerald 2005; Webb et al. 2014). For example, it would be advantageous for a non-indigenous species to be highly explorative and bold in a situation such as foraging, but may not be advantageous for that organism to be bold at all times due to the risk of novel predation (Werner and Anhold 1993).

Behavioural responses can also change as an animal matures which in turn could affect dispersal potential (Groothuis and Trillmich 2011; Wuerz and Kruger 2015). Juveniles and adults face different challenges which could potentially influence how they respond behaviourally. For instance, a juvenile may prioritize growth and finding a suitable ecological niche which in turn could include dispersing. In contrast, an adult's main focus could be preparing for reproduction and settling rather than dispersing (Groothuis and Trillmich 2011). Therefore, behavioural syndromes might decouple (Matsumura et al.2017) or form as the individual matures (Bell and Stamps 2004), or an increase in flexibility might occur depending on experience (Groothuis and Trillmich 2011). These shifts in behaviour could potentially influence which individuals are more likely to be successful dispersers, and question whether dispersal is truly linked to specific behavioural traits or whether these behaviours are driven by ontogenetic-related dispersal behaviour. In addition to ontogenetic differences, males and females may differ behaviourally due to different selective pressures and life histories (Pruitt and Riechert 2009; Chapman et al. 2013; Michelangeli et al. 2016) that may in turn be further influenced by the invasion process favouring one sex and resulting in a skewed sex ratio (Gutowsky and Fox 2011; Hudina et al. 2012). As an extension, one sex may potentially be a better disperser than the other, depending on the mating system and parental care (Jones et al. 2003; Marentette et al 2010).

Lastly, while studies have investigated dispersion syndromes between established and invading sites, this has rarely been examined across multiple rivers. Certain environments might facilitate the expression of different adaptive behaviours that may be inconsistent across locations (Bowler and Benton 2005). For example, differences in species assemblage/predators present (Phillips and Shine 2006), flow rate (Crisp and Hurley 1991), and competitor density (Fronhofer et al. 2015) can each differentially favour one type of behavioural repertoire over another, which can lead to erroneous assumptions of what makes a successful 'spreader' when assuming one sole invasion site. In addition, the novelty of the new introduction point could also play a role in what shapes the behaviour of dispersers and how they behaviourally respond (habitat matching) (Edelaar et al. 2008), which can furthermore be shaped by the attributes of their natal environments (Bernard and McCauley 2008; Sih et al. 2011). To fully learn about the context behind dispersal behavioural phenotypes, one must include multiple habitats (paired established and edge), consider the times-since-invasion, and incorporate life-history traits to reveal the potential challenges that mediate any mitigation measures to control invasive spread.

Using the round goby (*Neogobius melanostomus*), a prolific invasive species of the Laurentian Great Lakes, we investigated whether round goby individuals exhibit consistent behavioural traits and predator responsiveness that would facilitate its successful invasion-spread in southern Ontario, Canada. Specifically, we sought to determine whether these behavioural traits: (1) differ across the source (S), establishment (E), and invasion stages (IS), (2) are consistent among rivers and within invasion stage, and (3) differ across life-stage (juvenile and adult), and sex (adults). The round goby (*Neogobius melanostomus*) (1811 Pallas), was first detected in 1990 in the St. Clair River near Sarnia, Ontario, Canada (Jude et al. 1992); they now currently occupy all five Great Lakes, and are still expanding their range into various tributaries and rivers (Poos et al. 2010). Previous studies on behavioural variation in the round goby in North America have examined aggression, boldness, and dispersal on the invasion front; however, these studies were conducted in one river only - the Trent-Severn waterway in Southeastern Ontario, and used only one behavioural assay at a time (i.e., boldness or aggression) to characterize the behavioural type (Groen et al. 2012; Myles-Gonzalez et al. 2015). These studies found no significant differences in aggression between the established site and the invasion front (Groen et al. 2012), but did find that round gobies on the invasion front dispersed further and were bolder compared to the core population in the Trent river (Myles-Gonzalez et al. 2015). Moreover, previous studies have found contrasting information about the effects of habitat quality on round goby invasion. For example, Hôrková and Kováč (2015) found that round goby females in disturbed habitats had smaller oocytes (generalized phenotype) compared to those at less disturbed habitats while controlling for time since invasion. This study highlighted the effect of habitat quality and differences in anthropogenic pressures on round goby life-history. In contrast, Cerwenka and colleagues (2017) found that environmental factors did not explain any differences in biological traits or invasion progress in the round goby. In terms of size, there has also been contrasting evidence between juveniles and adults regarding dispersal potential. In Europe, they found that larger round gobies at the invasion front, suggesting the invasion front allots for better conditioned round gobies possibly due to more prey ability and reduced competition (Bradner et al. 2013). However, it has been suggested that juveniles are more likely to disperse because they are forced to less ideal habitats (i.e. sandy habitats) from which they disperse from (Ray and Corkum 2001). It has also been suggested that since round gobies lack a swim bladder, juveniles might be better dispersers because it less physically constraining for them to disperse within rivers due to their smaller size. Interestingly, there has also been conflicting information about male and female dispersal, whereas Brownscombe and Fox (2012) found females were more prevalent on the invasion front, but males have been seen to be overall more active in a natural and laboratory setting (Marentette et al. 2011). Overall, the literature presents contrasting information about the round goby invasion in North America and Europe and the effects of life-stage, sex, habitat quality on round goby life-history which can ultimately affect round goby behaviours. This study aimed to holistically examine behaviours from the original established source population in North America, and between established and invasion front/stage populations and across different rivers, among juveniles and adults, and sex; and finally, how collectively these factors may potentially work in distinguishing residents from dispersers. We hypothesize that there would be distinct differences between invasion stages, where round gobies from the source to the invasion stage would have increasing scores for boldness, exploration, and asociality (S>E>IS) (Fraser et al. 2001; Cote and Clobert 2007; Cote et al. 2010a; Cote et al. 2010b). We hypothesize that behavioural traits will be consistent across rivers and that invasion stage will be context-independent of rivers. We also hypothesize that there will be a significant difference between adults and juveniles, where juveniles would exhibit a “dispersal phenotype” that would be consistent across all invasion stages, but that adults would have increasing scores of boldness, exploration and asociality from the source to the invasion stage. We predict that within each stage, adult males will be bolder, more explorative and asocial than females.

Materials and Methods

Sampling sites

Four sites were chosen in Southwestern Ontario: St. Clair River, Ausable River, Saugeen River, and Thames River. The St. Clair River in Sarnia is considered the source site where the round goby was initially found by anglers, and is assumed to be the first, longest-established site (Jude et al. 1992; Dufour 2007; Brown and Stepien 2009). The Ausable, Saugeen and Thames rivers were invaded at different times of the invasion process (from 0-25 years; Table 2.1) (USGS database; Poos et al. 2010; Bronnenhuber et al. 2011) and are currently still being invaded. Within each of these three rivers, both established and invasion-stage locations were used, where the established locations were located at the river mouth and the invasion-stage locations were further upstream. Because these rivers are presumed to have undergone natural invasion and dispersal (i.e., no multiple founding populations) and therefore are not genetically divergent, they may be considered comparable replicates (Dufour 2007).

Collection and Sampling

Surveys to locate current invasion fronts were conducted from August-September 2015 via seine nets, electrofishing, and minnow traps (Bronnenhuber et al. 2011). Behavioural sampling for round goby sampling occurred from August-October 2015. These gobies were captured by seine net (i.e., active sampling), rather than angling or minnow traps (i.e. passive sampling) at all sites to eliminate the potential selection of behavioural types and for capturing both juveniles and adults (Nett et al. 2012; Diaz Pauli et al. 2015). The established sites consisted of the river mouths, and gobies sampled at the river-paired invasion sites were considered to be of the ‘invasion-front/stage’ (Bronnenhuber et al. 2011) (Figure 2.1). Sampling occurred along the river moving upstream from the river mouth. When we reached an access point where gobies were no longer seined, we sampled an additional access point further upstream to confirm there were no gobies past the last access point where round gobies were captured. Between 30-40 gobies were collected from each site (7 populations total) consisting of juveniles, and male and female adults of all sizes were collected, transported to the Great Lakes for Environmental

Research (GLIER) aquatic facility at the University of Windsor, and were allowed to acclimate for 5-7 days before behavioural testing. All transport, holding, and experimental protocol followed the Canadian Council on Animal Care guidelines (AUPP #13-04).

Rearing and holding

The round gobies used for behavioural testing were kept in a recirculating-flow tank system, where each population was kept in separate tanks (151.4 L; 68.58 cm. x 96.52 cm. x 33.02 cm) under red-light conditions and approximately 22°C water temperature (Balshine et al. 2005) to reduce stress and facilitate acclimation. They were fed once per day in the morning *ad-lib* with sinking cichlid pellets (Omega One, United States). The housing tanks were monitored daily to check for mortalities. After behavioural assays were completed, round gobies were sexed and classified as adult (spawning capable, presence of eggs or testes/secondary reproductive traits) or juvenile (indistinguishable papillae, absences of eggs and testes and secondary reproductive traits) by methods from Marentette et al. (2009) and Young et al. 2010. Following experiments, gobies were humanely euthanized using tricaine methanesulfonate (MS-222) (CCAC 2005).

Behavioural assays

Behavioural assays were conducted in a 1.21m by 2.0 m experimental tank (Figure 2.2). The experimental tank was plumbed into the same recirculating system that housed the round gobies during the acclimation week. The tank was divided into two equal sections with neutral flow (Myles-Gonzalez et al. 2015). The behavioural assay was conducted on 30 individuals (15 juveniles and 15 adults) from each location (n_{total} = 211). The behavioural assay tested for specific behaviours on the boldness-to-shyness continuum scale, predator response to a repeated presentation of a predator silhouette, sociality (preference / avoidance of a mirror), dispersal ability (zone switches and distance travelled), and furthest zone reached in the maze - white PVC barriers (3” wide and 10” long), were situated along the edges of the maze. The individual was placed in a PVC-refuge box (15.3cm by 15.3cm by 7.8cm) and positioned at one end of the arena for 10 minutes (Meurnier et al. 2009). After this time, if the individual had not exited, the refuge box was tipped over to dislodge the goby, and removed. After release (if necessary), individuals were exposed to a predator stimulus every 10 minutes, and each trial consisted of five 10-minute intervals, for a total of 50 minutes; 1. Acclimation period 2. Post-Acclimation period 3. Post-predator #1 4. Post-predator #2 5. Post-predator #3.

Behavioural Analysis

All behavioural trials were video recorded from above the tanks with a monochrome GigE camera with a 4-8 mm F1.4 megapixels lens (Basler, Germany) attached to a laptop using Media Recorder (Noldus Inc.). Videos were then analysed using automated software, Noldus Ethovision (Version 10), to calculate distance moved and velocity. Manual scoring was done using Solomon coder (Version: beta 15.03.15) to determine time latencies to reach each zone and number of zone switches. Predator response was scored manually using Solomon coder on a rank-based system to each individual predator presentation (Table 2.2a), and then summarized across all three predator-stimulus presentations (Table 2.2b). Following each trial, test individuals were photographed for measurement of standard and total length, body depth and mass. Sex was determined for adults, distinguished by their urogenital papillae (Charlebois et al. 1997). Seven sneaker males were identified amongst our samples as they were small in size but contained large testes when dissected (Marentette et al. 2009). However, since we did not observe sneaking behaviour and did not quantitively measure gonadal somatic index (GSI) (just by visual inspection of large testes) we considered them “small parental males”. The data for these individuals were removed from the pool and analyzed separately. Juveniles were determined if they did not have distinct papillae, as well as if they were under ~60 mm in total length (Johnson et al. 2005; Walsh et al. 2007).

Statistical Analysis:

Principal Components Analysis

The data collected were assigned *a priori* to specific categories and were log-transformed as necessary: a) Acclimation- which encompassed all the variables collected during the first ten-minute period with the shelter; b) Sociality- which included all interactions with the mirror; c) Maze exploration- which included dispersal distance, velocity, and overall mobility; and d) and Predator response- which included velocity and distance moved after a predator stimulus. Principal components analysis (PCA) using varimax orthogonal rotation was conducted on each category, where components for each category that had an eigenvalue > 1, and variables with a loading >0.55 were retained, indicating a "good" significance level (Comrey and Lee 1992) (Table 2.3a-d). Analyses were conducted in JMP (Version 12).

For the Acclimation category, two components were identified that explained over 85.04% of the variance: the first was labeled "Acclimation Exploration and Activity", and individuals with high scores were those who left the shelter quickly, had a lower latency time to first explore, moved a greater total distance, and had a high velocity average. The second factor was named "Acclimation Shelter-seeking", with higher scores denoting individuals that frequently returned / visited the acclimation shelter.

For the Sociality category, two components were identified that explained over 93.54% of the variance. The first factor was named "Acclimation Sociality", which included more specific variables: latency to approach mirror, duration at mirror and mirror frequency, where high values indicate the individual quickly visited the mirror, and spent a long time at the mirror, and visited the mirror frequently during the acclimation period only. The second factor was labeled “Overall Sociality”, which included mirror frequency and mirror duration over the entire 50 minutes, where high values indicate individuals that visited the mirror frequently and with long durations over the course of the entire assay.

For the Maze exploration category, two components were identified that explained over 88.47% of the variance. The first component was named “Maze activity” which included velocity and distance moved, with high scores denoting individuals who moved a greater distance overall and at high velocities. The second component was termed “Maze Exploration” which included factors such as number of times swimming over PVC tubes, maze near frequency, maze far frequency. Individuals with high scores swam over (vs. around) the PVC tubes frequently and made more near-far zone changes.

Lastly, for the Predator response category, two components were identified that explained over 86.80 % of the variance. The first component was labeled “Delayed Activity to Predator Exposure”, and individuals with high scores were those that exhibited movement following the second and third presentation of the predator silhouette more so than after the first, exhibited higher velocity, and greater variance in velocity. The second factor was labeled “Early Activity to Predator Exposure” with high scores denoting individuals displaying greatest movement rates following the first predator presentation only.

A second-ordered PCA was then conducted on the scores from the eight principal components (PCs) to create a behavioural phenotype. Overall, three components were identified but were deemed less informative, where the second and third component explained only 18.5% and 13.0% respectively, so only the first component was considered since it explained the most variance. The first component, explaining over 37.8% of the behavioural variance, was retained and used as a response variable in subsequent analyses (summarized Table 2.4). This behavioural phenotype comprised the exploration and activity PC (during acclimation), sociality PC (during acclimation), exploration behaviours PC (overall) and post-predator activity PC (Table 2.5), where individuals with a high behavioural phenotype score were bolder and explorative, more social (acclimation only), and were highly active post-predator 2 and 3.

Linear mixed-modeling – behavioural phenotype

Statistical analyses were completed using R version 3.3.2 (R Core Team 2016). A linear mixed model was used to explore drivers of variation in goby behavioural phenotype. All models included days in captivity, trial ID, holding tank ID and experimental tank ID as random effects. Model assumptions of normality and homogeneity of variances for the behavioural-phenotype response variable were tested by visual inspection of residual versus fitted and quantile-quantile plots. Analyses were first run with invasion-stage only as a fixed effect to test for an overall invasion syndrome, with river ID added as an additional random effect. A second model was run to test for an overall ontogenetic effect on behavioral type, with life-stage as the sole fixed effect, with river ID and invasion-stage added as additional random effects. Data were then split by life-stage, and within each age category, the following fixed effects were simultaneously included: invasion stage (S, E, IS), river ID, stage × river ID, sex (for adults only, small parental males being excluded), and sex × stage and sex × river ID. Likelihood ratio tests were conducted to test for the significance of fixed interaction effects and to compare model fit. To test the significance of fixed effects the (anova) function was used (lmerTest; Kuznetsova et al. 2016). When categorical variables were significant, pairwise differences between groups were assessed using Tukey post hoc tests (lsmeans package; Lenth 2016).

Because the inclusion of the interaction effect between invasion stage and river would necessitate the exclusion of source gobies (since there is no E or IS in the St. Clair river), and to be able to compare location scores to the reference (i.e., source) level, the behavioural score for each fish was mean centered to St. Clair river scores. Specifically, scores from each individual fish from the six non-source locations (established and invasion edge) were subtracted from the mean behavioural phenotype score for either juveniles or adults (depending on the analysis) from the source site. Afterwards, for clearer interpretation this value multiplied by negative 1. Therefore, a positive value means that an individual had a higher behavioural phenotype score in relation to St. Clair, whereas a negative value denotes the individual's behavioural score was lower than St. Clair's average for that life-stage.

To examine behavioural phenotypes across alternative reproductive tactics due to our chance discovery of small parental males, data from all rivers and invasion stages were pooled due to the low sample size of these males (n =7), Parental males (n = 59) and females (n =55) were tested separately against small parental males for behavioural differences, with days in captivity, trial ID, holding tank ID and experimental tank ID, river ID and invasion stage as random effects. Behavioural scores were not mean centred to St. Clair river.

Ordinal regression analysis – overall predator responsiveness

The response of gobies to the predator stimuli (i.e., habituation, fleeing/freezing) was manually ranked and differed from the PC scores which focused only on activity after predator exposure. Due to the rank order nature of the manually scored predator-responses, ordinal regressions were conducted using the package "ordinal" (Christensen 2015). The cumulative link function (clmm) was used to specify a mixed model. Overall predator reaction scores were given a rank on a scale of -2 to +2 (see Table 2.3b). These scores were regressed against explanatory variables such as invasion stage, life-stage, river, population; with days in captivity and trial ID as random effects. The package "RVAideMemoire" was used to run Type II ANOVA tests to obtain the p-values for each fixed factor (Hervé 2017). One model was used to test for invasion-stage effects only, and a second model was run to examine differences between juveniles and adults.

For a more in-depth examination of variables driving life-stage differences, river ID and invasion stage were analyzed separately for juveniles and adults. To include St. Clair data in our models, we ran two separate models for each life-stage. For juveniles, we ran a model that included invasion stage as a fixed effect while controlling for site as an additional random effect, and a second model that included. river ID and controlling for invasion stage instead. For adults, we ran similar models, but included sex × invasion stage or sex × river ID as well. Analyses here were simplified since rank-values cannot be effectively mean centered.

Similar to the linear mixed models ran for small parental males (n =7), data across rivers and invasion stages were pooled, and the predator responsiveness of males and females were tested against small parental males, with days in captivity, trial ID, river ID and invasion stage as random effects.

Results

Behavioural phenotype

Overall, there was no significant difference in behavioural phenotype scores across invasion stages (F=0.70, df = 2, P=0.79) (Figure 2.3). When comparing life-stages, juveniles had a significantly higher behavioural phenotype score compared to adults (F=45.98, df=1, P<0.0001) (Figure 2.4). Due to this difference, juveniles and adults were analyzed separately in subsequent analyses.

There was a significant invasion-stage effect for juvenile gobies (*F*=4.14, df=1, P=0.045), where juveniles at established sites had a higher (i.e., less negative) behavioural phenotype score than at the invasion front (Figure 2.5; although both had lower scores compared to the source river). Overall, juvenile gobies exhibited the same invasion-stage behaviour patterns (i.e., no invasion stage × river interaction effect; P=0.50) (Figure 2.6). However, there was a significant river effect (*F*=10.6, df=2, P<0.0001), where juvenile gobies from the Ausable river had a higher positive behavioural phenotype score than the other rivers (Ausable > Saugeen > Thames), the latter two of which had negative behaviour scores to the source river gobies (Figure 2.7).

There was no significant invasion-stage effect for adults (*F*=0.043, df=1, P=0.84) (Figure 2.8), although this was due to gobies behaving differently between the established versus the invasion front, depending on their inhabited river (*F*=5.14, df=2, P=0.008) (Figure 2.9). Individuals at established locations at the Ausable and Thames rivers had higher behavioural phenotype scores compared to their counterparts at the invasion front whereas invading Saugeen-river gobies had the opposite (lsmean invasion: 0.510 ± 0.51 and lsmean established:-0.637 ± 0.44). For adult gobies, there was additionally a significant interaction effect between sex and site (*F*=4.08, df=2, P=0.02) (Figure 2.10), with females from the Ausable River having significantly higher (and positive) behavioural phenotype scores than females from the Thames River that had negative scores (P=0.042). Male behaviours (with mean negative scores) did not differ across sites (lsmeans: Ausable=-0.523 ± 0.55; Saugeen=-0.174 ± 0.46; Thames=-0.063 ± 0.44). For the adults, there was no overall river effect (*F*=0.88, df=2, P=0.42) (Figure 2.11).

Overall, smaller parental-males had significantly higher behavioural phenotype scores than parental males (*F*=4.88, df=1, P=0.031) and females (*F*=5.37, df=1, P=0.025) (Figure 2.12a-b).

Predator responsiveness

An invasion stage effect on overall predator responsiveness score was not observed when controlling for river ID (χ^2= 0.45, df=2, P=0.80) (Figure 2.13). However, there was a significant life-stage effect (χ^2= 3.91, df=1, P=0.048), where juveniles spent less time hiding as opposed to adults (Figure 2.14). Due to these differences, we analyzed juveniles and adults separately to look at within life-stage differences among invasion-stage, river- and sex effects (limited to adults only).

For juveniles, there was no significant invasion-stage effect (χ^2=1.80, df=2, P=0.41) nor significant river effect (χ^2=4.60, df=3, P=0.20). Similarly, there were no significant main effects within adults for invasion stage (χ^2=2.83, df=2, P=0.24), sex (χ^2= 0.00047, df=1, P=0.98) or their interaction (invasion stage x sex) (χ^2=2.38, df=2, P=0.30). We also did not observe any significant main effects for river ID (χ^2=3.82, df=3, P=0.28), sex (χ^2=0.0002, df=1, P=0.99) or their interaction (river ID x sex) (χ^2=4.74, df=3, P=0.19). Lastly, no significant differences in predator responsiveness were found between smaller parental-males and other males (χ^2=0.04, df=1, P=0.84) or females (χ^2=0.05, df=1, P=0.82).

Discussion

Our aggregate behavioural phenotype score revealed that exploration and activity, sociality, and predator habituation, explained the most variance across all the behavioural variables collected. Overall, we found no invasion stage effect, but a highly significant life-stage effect where juveniles had a higher behavioural phenotype score than adults. For the juveniles, we observed a significant invasion stage effect, where established juveniles were bolder, more explorative, social and had greater predator habituation. We also observed a significant river effect, where juveniles from Ausable River had a significantly higher behavioural phenotype score compared to Thames River gobies and marginally higher than Saugeen River. For the adults, we observed a significant invasion stage × river effect, and a significant sex × river effect, where Ausable River females had a significantly higher behavioural phenotype score than Thames River females. Lastly, small parental males had a significantly higher behavioural phenotype score compared to parental males and compared to females. Our predator response rankings were less informative, and revealed a life-stage effect only, where juveniles hid less in the presence of a predator compared to adults, nonetheless supporting our PC results, suggesting predator habituation upon repeated exposure.

When assessing the aggregate phenotype of round gobies overall, individuals did not display the purportedly characteristic "invasion behavioural phenotype" consisting of boldness, exploration and asociality. Instead, we found that gobies characterized as being bold, explorative, and risk-prone were equally quite social. Asociality is typically touted as being a critical driver of invasiveness because as density increases, asocial animals typically tend to be the first ones to disperse, expanding the range of an invasive species (Cote and Clobert 2007; Cote et al. 2010a). However, studies have shown that social gregarious invaders, like round gobies, can tolerate high conspecific density, actually preferring the presence of a conspecific, and instead are selectively aggressive to out-compete native species for resources (Capelle et al. 2015). Our results lend further support, and found it most evident in juveniles (and smaller parental males).

Despite the invasion-like behavioural phenotype exhibited by gobies overall in Southwestern Ontario rivers, there was no observable invasion-stage effect on behavioural phenotypes. However, upon closer inspection, strong life-stage effects were apparent instead, where juveniles had a higher behavioural-phenotype score compared to adults (Figure 2.4). These differences could be attributed to juveniles and adults facing different selective pressures in the wild, where juvenile round gobies are focused on survival and finding optimal habitats and adults are focused on reproduction, thus necessitating a different suite of behaviours (Biro and Stamps 2008, Groothuis and Trillmich 2011). For instance, juvenile gobies have different metabolic requirements than adults, as they must actively forage to find food such as insect larvae and fish eggs (French and Jude 2001). In contrast, adult round goby (>70 mm) will feed primarily on sessile zebra mussels (Ray and Corkum 1997). These different foraging strategies can explain the more active behaviours of juveniles. Round goby are also space competitors, where size plays a significant factor in who wins transgressive events (Stammler and Corkum 2005; Groen et al. 2012). Because their sheer size gives adults the competitive advantage, juvenile round goby may be bolder and more explorative to seek out areas of their own. Juveniles were also more likely to have a reduced response and habituate more quickly to the repeated presentation of the predator stimulus potentially due to their innate boldness and activity (Guillette et al. 2009; Sih and Del Giudice 2012; DePasquale et al. 2014). Overall, we found that juveniles have higher dispersal potential compared to adults, however Gutowsky and Fox (2011) and Bradner et al. (2013) and found that larger adults were most prevalent at the invasion front. Brownscombe and Fox (2012) found smaller individuals, were found at the invasion front. However, they utilized angling and which did not capture individuals <50 mm, while our study utilized seining to capture a variety of size classes, juveniles and adults.

Invasion stage effects among juveniles and adults

Within life-history stages, we did find an invasion stage-effect for juvenile gobies; most surprisingly however, behaviours were inconsistent to what was predicted. Juveniles at the invasion front were less bold and explorative but more asocial and predator sensitive than at their respective established populations. This was most evident in the Thames and Ausable River, whereas juveniles at the Saugeen River appear to have similar behavioural phenotype scores. This may have been due to the dam at the Saugeen River. Whereas we can say with confidence that Ausable and Thames invasion-front populations have not yet become "established" due to the low frequency of gobies captured (effort was less than 1 goby per seine on average), we captured many round gobies at the Saugeen invasion front in comparison. We also verified that the last-identified invasion front, according to Bronnenhuber et al. (2011) has not changed, as we were unable to find gobies further upstream due to the presence of a dam. As such, one may consider this population at the front to be "recently established" as opposed to an actively moving invasion front. Since there is no density relief, individuals at the "recently established" population are just as bold, explorative, social and predator habituated as those at the established Saugeen population. Additionally, juvenile behavioural scores at established and invasion-front sites were lower still than those from the source population, indicating individuals from the originator site were most social, explorative and bold, and predator-habituated. Sociality is important in achieving high densities and facilitating establishment (Holway et al. 1998); for example, Western bluebirds (*Sialia Mexicana*), employ their aggression to displace native mountain bluebirds (*Sialia currucoides*), however their aggression decreases after establishment (Duckworth and Badyaev 2007). However, in the dispersal process there are potentially roles for both asocial and social individuals, the first subset of dispersers may be more asocial seeking out new habitats, however the individuals that follow afterwards, “followers” are more social, following the asocial individuals to new habitats possessing the tolerances needed to achieve higher densities and establishing a new population (Fogarty et al. 2011).

In contrast, adults were consistent, exhibiting no differences in behaviour across invasion stages. Again, our results are somewhat unexpected, as we had anticipated for both adults and juveniles to have a dispersion syndrome at the invasion front. Despite this lack of support that has been (partially) found in other invasive goby work (e.g., Groen et al. 2012; Thorlacius et al. 2015; Myles-Gonzales et al. 2015), studies like ours that have also found little evidence for expected behavioural syndromes (e.g., Lopez et al. 2012; Ashenden et al. 2017) point to abiotic and biotic factors influencing the behavioural repertoires that would be most adaptive at an invasion front (e.g., Ashenden et al. 2017; Dubuc-Messier et al. 2017).

Specifically, we might have not observed a traditional invasion stage effect due to differences in river habitat characteristics, species assemblage, conspecific densities, or time since invasion (Belanger and Corkum 2003; Young et al. 2010). The Thames River presents a unique challenge for the round goby; it is turbid and difficult to navigate due to fast-flowing water, and is home to several large predators known to consume round goby (Thames River Ontario Fact Sheet 2014). The Saugeen River is very rocky with large rocks and boulders and contains vegetation and rapid flowing water in areas. It also hosts several sport fish known to eat round goby (Bence et al. 2004; Saugeen Conservation Fishing Map website: http://saugeenconservation.com/ downloads/fishingmap.pdf). The Ausable River substrate ranges from boulders to pebbles with slow-flowing water and sandy bottoms with fallen logs. This river is not known for sport fishing, but hosts several Cyprinidae species (Ausable River Fisheries Survey Report 2005). Lastly, St. Clair River has ideal goby habitat, with plenty of vegetation, a rocky substrate of cobbles, and clear and slow flowing water (Jude and Deboe 1996). Over 130 000 fish are released here each year (website: http://www.bluewateranglers. com/) including predators of round goby. Both habitat and species assemblage, in particular predators, are known to affect the development of different behavioural types (Bell and Sih 2007, Kobler et al. 2009, Cote et al. 2013). Although speculative, one would expect gobies from the Ausable river to be most distinct in its behaviours from the other rivers due to lower predation pressure, possible higher heterospecific competition, and favourable habitat substrate and flow. Our juvenile results lend support to this habitat-quality theory since individuals from the Ausable river, regardless if they were from the established or invasion-front site, were significantly bolder, explorative and more social compared to the Thames and Saugeen rivers respectively, and also had a greater positive behavioural score than even the source river, St. Clair (Figure 2.7).

Conspecific density could have also played a significant role in the behavioural variation observed (Cote and Clobert 2007). All of our established populations including the source population had round gobies in high densities compared to the invasion stage populations. Due to high densities, higher conspecific interactions and intra-specific aggression could allow the behavioural type we found at these locations to locate and secure suitable habitats, shelter (and mates) and remain (since dispersal costs would outweigh its benefits (Stamps 2001)). As a result, the ones forced to disperse could be the shyer, less competitively able individuals (reviewed in Cote et al. 2010b). Our findings of invasion-front juveniles being generally less bold and risk-taking than their established counterparts lends support to this hypothesis of reduced intraspecific competition, although not directly examined. For adult gobies, the increase in the behavioural phenotype score at the Saugeen River invasion front can also be explained by conspecific density. Since there is no density relief, adults exhibiting higher levels of boldness, exploration and sociality would be favoured, and we did indeed find adults with this phenotype at the invasion front.

While it is plausible that habitat context could be driving behavioural differences, time since invasion may also be a contributing factor. Each of these rivers were invaded at different time points, and upstream invasion started at different times as well (Table 2.1). As such, at the time of initial invasion, the novelty of the environment itself (regardless of environment characteristics) could have acted as a stressor on the invading population, but over time, populations “acclimatized”, resulting in observable shifts in plastic phenotypes along the invasion process (Kováč et al. 2009). In parallel studies of round gobies in the Baltic Sea and North America, researchers have found differences in reproductive parameters, condition, and age-at-maturity that are time dependent (Gutowsky and Fox 2012, Hôrková and Kováč 2015). In our North American system this theory of alternative ontogenies has yet to be tested in a behavioural-dispersal context, although our results suggest the temporal length of range expansion can possibly also mediate behaviours of juveniles (Figure 2.15).

Sex differences

Oftentimes only round goby males are used for behavioural assays because they generally are more active compared to females (Young et al. 2010; Marentette et al. 2011; but see Capelle et al. 2015). However, we collected gobies outside of their breeding season during late August to mid October 2015 (MacInnis and Corkum 2000) so that we could purposefully not largely overlap with the round goby reproductive season. None of the males tested had the typical reproductive signs such as puffy cheeks and black colouration (Corkum et al. 1998). Therefore, we could not attribute male parental care as the reason why the females generally had a higher behavioural score. The observed sex-specific behavioural trends, and how they differed among rivers rather than invasion stage remains to be further explored and underscores the need to investigate sex differences among round goby. In the literature there are contrasting results with regards to male and female biased invasion fronts. Gutowsky and Fox (2011) found the invading populations were mainly male biased, while Brownscombe and Fox (2012) found that the invasion front in the Trent river was mainly female biased. Our results reveal that male and female dispersal potential could be river or temporally specific and could change as invasion progresses. We also found significant differences between smaller parental males and adult males and females, although owing to the limited sample size, our results should be interpreted with caution. Nevertheless, the significantly higher behavioural phenotype score of small parental males could be attributable to metabolic differences due to the different sizes (Biro and Stamps 2008); sneaker/small parental males are vastly smaller than adult males that are approximately 34 % larger in total length (Marentette et al. 2009). These behavioural differences may also be due to the underlying reproductive tactics of sneaker males (if indeed they are sneaker males) that must seize opportunities for covert fertilization that carry inherent risks (i.e., should be bolder and explorative) (Bleeker et al. 2017). Sneaker-males might have to also exhibit social behaviours to exploit fertilization opportunities (i.e., posing as females) however this has yet to be further investigated (Marentette et al. 2009). Taken together, our findings stress how important it is to be inclusive of all individuals (size, sex, life-stage) when examining mechanisms of dispersal and spread in invasive species.

Conclusions

In summary, we found support for an “invasion behavioural phenotype” (overall behavioural phenotype) in line with what is already known for round gobies in Southern Ontario. This behavioural type was driven mainly by juveniles; however, those at the invasion front were overall less bold, less active and exploratory, were less likely to take-risks and were more asocial. This dispersion phenotype could potentially be explained, in part, by reduced intraspecific competition and habitat-feature differences. Other spatio-temporal factors such as time since establishment, time of upstream dispersal, and the temporal separation of the established and invading populations can also be contributing to the lack of a universal explanation, and requires further investigation, using rivers more spatio-temporally divergent than those chosen in this study. The behavioural variation observed in our study highlights the importance of an integrative and holistic approach that incorporates multiple abiotic and biotic factors to explain the invasion process. While many studies only exclusively study behavioural variation among adults, we argue that it is important to be inclusive of all life-stage and sexes when trying to understand mechanisms that promote invasiveness (Brown et al. 2005; Loftus and Borcherding 2016). By studying multiple rivers, we have revealed that the behaviour of an invasive species can depend on many factors, such as the habitat complexity and species-assemblage features of the invasion stage. We found there is not often just one optimal behavioural strategy for a non-indigenous species to be successful. In fact, our results, when taken together with others' studies of the invasive behaviours of the round goby, make a strong argument that round gobies may instead have flexible invasion behaviours that are context dependent (Wright et al. 2010; Clobert et al. 2009). By understanding all the facets that could be driving behavioural variation in invasive species we can better develop more effective management strategies and develop a stronger understanding of the drivers of range expansion.

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Tables

Table 2.1 Approximate year that the round goby was initially detected in the river mouth/upstream movement and the GPS coordinates that the round gobies were captured

Population	*Year detected/Upward movement*	*Detection time since North American Invasion*	*Location coordinates*	*Distance from the river mouth*	*Reference*
St. Clair	1990	0	42°58'56.438"N 82°24'40.392"W 42.982344,-82.41122000000001	-	Jude et al. 1992
Ausable-Established	1998	8	43°13'58.9"N 81°54'07.5"W 43.233032, -81.902095	-	EDDMaps
Ausable-Invasion	2007	25	43°09'05.7"N 81°48'36.6"W 43.151574, -81.810181	22.5 km	Poos et al. 2010
Thames-Established	1998	8	42°19'3.396"N 82°27'11.7"W 42.317609,-82.453250	-	USGS

Thames-Invasion	2003	25	42°36'27.0"N 81°50'00.2"W 42.607500, -81.833389	110.6 km	Poos et al. 2010
Saugeen-Established	2004	14	44°30'04.1"N 81°22'16.4"W 44.501133, -81.371220	-	Bronnenhuber et al. 2011
Saugeen-Invasion	2006	19	44°30'25.4"N 81°20'18.8"W 44.507055, -81.338554	4.4 km	Bronnenhuber et al. 2011

Table 2.2a Ranking system for individual fish responses to the presentation of the predator stimulus and the associated behavioural description.

Rank	**Individual Predator Response**
0	Not in view- individual was hiding when exposed to predator (no encounter)
1	i) Escape - individual sought out shelter when exposed to predator ii) Freezing- the individual was openly swimming and stopped its activity when exposed to the predator
2	i) Not responsive (Active)- individual was openly swimming during predator exposure, did not respond ii) ii) Not responsive (Inactive) individual was in the open during predator exposure but not moving during the predator exposure, and did not respond

Table 2.2b Overall ranking system of the response to all three predator stimuli with associated behavioural description.

Rank	Overall Predator Response
-2	Froze or fled each time the predator stimulus went over
-1	Hid when the predator stimulus went over/never encountered
0	Delayed response to predator stimulus (does not respond initially)
1	Habituated (Initially fled but by second and third time did not respond to the predator stimulus)
2	Consistently did not respond to the predator stimulus

Table 2.3 a-d. Final PCA loadings, eigenvalue and percent variance for each behavioural category a) Acclimation period b) Sociality c) Maze exploration d) Predator recovery. Bolded values (>0.55) indicate factor loadings that contribute significantly to the PC scores.

a)

Trait	**Acclimation Exploration and Activity**	**Acclimation Shelter-seeking**
Eigenvalue	3.19	1.06
% variance explained	63.76	21.28
Shelter visits	-0.01	**0.98**
Shelter latency	**-0.88**	0.18
Maze near latency	**-0.81**	0.21
Distance moved during Acclimation	**0.94**	0.13
Average velocity during Acclimation	**0.93**	0.11

b)

Trait	Acclimation Sociality	Overall Sociality
Eigenvalue	3.62	1.06
% variance explained	56.30	37.24
Latency to mirror (acclimation)	**-0.94**	-0.19
Duration at mirror (acclimation)	**0.93**	0.27
Mirror frequency (acclimation)	**0.93**	0.28
Mirror frequency overall	0.42	**0.86**
Mirror duration overall	0.14	**0.96**

c)

Trait	Maze Activity	Maze Exploration
Eigenvalue	2.93	2.37
% variance explained	48.89	39.58
Number of times over the PVC	0.14	**0.87**
Number of maze near visits	**0.40**	**0.79**
Number of maze far visits	0.31	**0.87**
Distance travelled post-acclimation	**0.93**	0.34
Total distance travelled	**0.95**	0.24
Average velocity post-acclimation	**0.94**	0.28

d)

Trait	**Delayed Activity to Predator Exposure**	**Early Activity to Predator Exposure**
Eigenvalue	6.79	1.03
% variance explained	51.67	35.13
Distance travelled post-predator 1	0.43	**0.87**
Distance travelled post-predator 2	**0.84**	0.38
Distance travelled post-predator 3	**0.86**	0.33
Velocity average post-predator 1	0.34	**0.93**
Velocity average post-predator 2	**0.82**	0.38
Velocity average post-predator 3	**0.86**	0.31
Velocity variance post-predator 1	0.37	**0.89**
Velocity variance post-predator 2	**0.81**	0.36
Velocity variance post-predator 3	**0.84**	0.35

Table 2.4 Summary of fixed-effect variables and levels within

Factor	Levels
Invasion stage	Established, Invasion, Source
River ID	Ausable, Thames, Saugeen, St. Clair
Life-stage	Adult, Juvenile
Sex	Female, Male, Sneaker-male

Table 2.5 Second-ordered unrotated principal component assay representing behavioural phenotype score (BPS), eigenvalue and percent variance for each behavioural category. Bolded values (>0.55) indicate factor loadings that contribute meaningfully to the PC scores.

Trait	Behavioural phenotype score
Eigenvalue	3.03
% variance explained	37.8
Acclimation Exploration, and Activity	**0.87**
Acclimation Shelter frequency	0.055
Maze Activity	0.47
Maze Exploration	**0.78**
Acclimation Sociality	**0.73**
Overall Sociality	0.52
Delayed Activity to Predator Exposure	**0.62**
Early Activity to Predator Exposure	0.51

Table 2.6 Summary of mean (±SE) behavioural-phenotype scores and body morphometrics of adult and juvenile round goby collected at each population (mass, total length, standard length and body depth). Est = established; Inv = invasion front.

Population	Juvenile						Adult					
	N	Behavioural phenotype score	Mass (g)	Total Length(mm)	Standard Length(mm)	Body Depth(mm)	N	Behavioural phenotype score	Mass (g)	Total Length(mm)	Standard Length(mm)	Body Depth(mm)
StClair-Source	15	1.31±0.19	0.96±0.11	42.20±1.22	35.14±1.41	7.50±0.28	15	-0.40±0.44	4.96±0.38	72.10±2.32	60.40±1.53	13.50±0.34
Ausable-Est	14	1.98±0.22	1.56±0.18	51.40±1.90	40.91±1.55	8.34±0.36	17	-0.38±38	6.68±1.05	76.77±4.20	62.15±3.47	13.32±0.79
Ausable-Inv	19	1.30±0.32	1.26±0.10	47.64±1.21	37.95±1.00	7.83±0.24	8	-0.65±037	13.21±3.72	96.78±7.82	78.01±6.37	17.54±1.58
Saugeen-Est	7	0.49±0.75	1.61±0.10	51.89±1.11	42.87±1.14	9.24±0.42	23	-1.32±0.30	5.20±0.46	76.70±2.28	63.57±1.82	13.41±0.49
Saugeen-Inv	12	0.51±0.51	1.87±0.10	54.14±0.88	43.48±0.75	9.33±0.30	14	-0.34±0.46	4.35±0.6	69.96±2.34	55.89±1.84	12.34±0.43
Thames-Est	15	0.30±0.42	1.76±0.14	53.53±1.59	44.24±1.37	9.46±0.32	22	-0.27±0.35	3.95±0.37	66.30±1.81	54.95±1.90	11.56±0.35
Thames-Inv	15	-0.62±0.41	1.43±0.13	50.09±1.42	41.26±1.35	8.21±0.25	15	-1.73±0.24	9.92±0.96	89.51±2.74	72.64±2.24	16.23±0.60

Table 2.7 Summary of linear mixed models examining the effect of 1) life-stage and 2) invasion stage on behavioural phenotype score (significant P-values are in bold). For model 1 river ID was included as a random effect, and for model 2 both river ID and invasion stage were included as random effects.

Behavioural phenotype score looking at main effects							
	Model	**Variable**	**Estimate**	**SE**	**F**	**df**	**P**
All individuals	1	Intercept	0.040	1.01	-	-	-
		Invasion stage (Invasion)	-0.27	0.70	0.26	2	0.79
		Invasion stage (Established))	-0.40	0.72			
	2	Intercept	-0.54	0.49			
		Life-stage (Juveniles)	1.39	0.20	45.98	1	**<0.0001**

Table 2.8 Summary of linear mixed models examining the effect of site, invasion stage and sex on adults' behavioural phenotype score (corrected to St. Clair) and the effect of site and invasion stage on juveniles' behavioural phenotype score (corrected to St. Clair) (significant P-values are in bold).

Behavioural phenotype score mean centered to source, St. Clair river

Adults	**Model**	**Variable**	**Estimate**	**SE**	**F**	**df**	**P**
	1	Intercept	0.68	0.54			
		Site (Saugeen)	-1.45	0.61	0.88	2	0.42
		Site (Thames)	-1.21	0.60			
		Invasion stage (Invasion)	0.18	0.64	0.043	1	0.84
		Sex (Male)	-0.80	0.60	0.54	1	0.46
		Site x Invasion stage (Saugeen * Invasion)	1.46	0.78	5.14	2	**0.008**
		Site x Invasion stage (Thames * Invasion)	-0.71	0.78			
		Site * Sex (Male * Saugeen)	1.07	0.73	4.39	2	**0.015**
		Site * Sex (Male * Thames)	2.15	0.73			
		Invasion stage * Sex (Invasion *Male)	-0.98	0.59	2.72	1	0.10

Behavioural phenotype score corrected to St. Clair

	Model	**Variable**	**Estimate**	**SE**	**F**	**df**	**P**
Juveniles	1	Intercept	0.97	0.49			
		Site (Saugeen)	-0.92	0.42	10.6	2	**<0.0001**
		Site (Thames)	1.70	0.37			
		Invasion stage (Invasion)	0.66	0.33	34.14	1	**0.045**

Table 2.9 Summary of linear mixed models comparing behavioural phenotype scores between sneaker-males and males and females (significant P-values are in bold). Invasion stage and river ID were included as random effects.

Behavioural phenotype score							
	Model	**Variable**	**Estimate**	**SE**	**F**	**df**	**P**
Males	1	Intercept	-0.55	0.39			
		Sex (Sneakermale)	1.35	0.65	4.88	1	**0.031**

Behavioural phenotype scores							
	Model	**Variable**	**Estimate**	**SE**	**F**	**df**	**P**
Females	1	Intercept	-0.71	0.39			
		Sex (Sneakermale)	1.45	0.63	5.37	1	**0.025**

Table 2.10 Summary of ordinal regression examining the effect of life-stage and invasion stage on overall predator response (significant P-values are in bold) of all individuals included. **River ID was included as a random effect for both models.**

Overall predator scores with gobies from source, St. Clair river, included							
	Model	**Variable**	**Estimate**	**SE**	χ^2	**df**	**P**
All individuals	1	Invasion stage (Invasion)	-0.19	0.29	0.45	2	0.80
		Invasion stage (Source)	-0.093	0.40			
	2	Life-stage	0.52	0.27	3.91	1	**0.048**

Table 2.11 Summary of ordinal regression examining the effect of sex and invasion stage (model 1) and site and sex (model 2) on adult overall predator response (significant P-values are in bold).

Overall predator scores with gobies from source, St. Clair river, included							
	Model	**Variable**	**Estimate**	**SE**	**χ^2**	**df**	**P**
Adults	1	Invasion stage (Established)	1.15	0.95	2.83	2	0.24
		Invasion stage (Invasion)	1.48	0.97			
		Sex (Male)	0.63	1.21	0.00047	1	0.98
		Invasion stage * Sex (Established*Male)	-0.28	1.33	2.38	2	0.30
		Invasion stage * Sex (Invasion* Male)	-1.56	1.42			
	2	Site (Ausable)	0.41	1.08	3.82	3	0.28
		Site (Saugeen	1.03	1.00			
		Site (Thames)	2.13	1.00			
		Sex (Male)	0.64	1.22	0.0002	1	0.99
		Site*Sex (Ausable*Male)	0.66	1.49	4.74	3	0.19
		Site*Sex (Saugeen*Male)	-0.59	1.41			
		Site*Sex (Thames*Male)	-1.63	1.40			

Table 2.12 Summary of ordinal regression examining the effect of invasion stage (model 1) and site on juvenile overall predator response (significant P-values are in bold). River ID and invasion stage were included as random effects for model 1 and 2, respectively.

Overall predator scores with St. Clair							
	Model	**Variable**	**Estimate**	**SE**	χ^2	**df**	**P**
Juveniles	1	Invasion stage (Established)	-0.42	0.55	1.80	2	0.41
		Invasion stage (Invasion)	-0.72	0.54			
	2	Site (Ausable)	-0.13	0.56	4.60	3	0.20
		Site (Saugeen	-0.83	0.62			
		Site (Thames)	-0.92	0.57			

Table 2.13 Summary of ordinal regression comparing overall predator response between sneaker-males and males and females (significant P-values are in bold).

Sneaker-male overall predator scores with St. Clair (controlling for site and invasion stage)							
	Model	**Variable**	**Estimate**	**SE**	**χ^2**	**df**	**P**
Male	1	Sex (Sneakermale)	-0.19	0.97	0.04	1	0.84
Female	2	Sex (Sneakermale)	-0.20	0.88	0.05	1	0.82

Figures

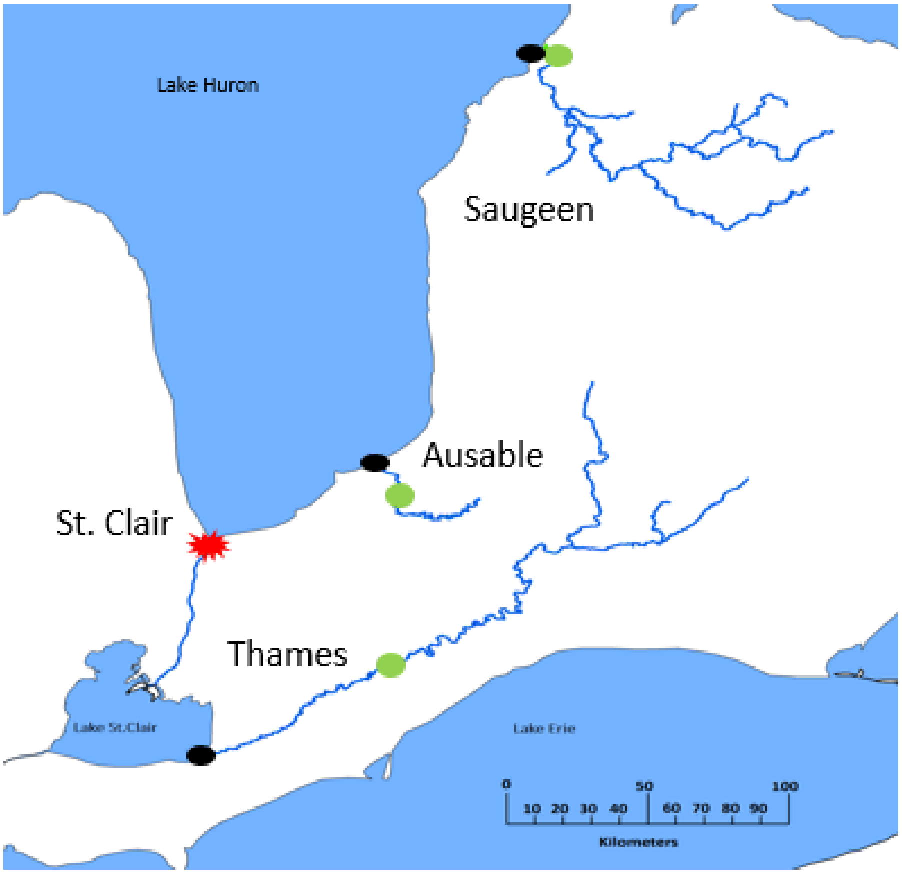

Figure 2.1 Map showing sampling sites (not drawn to scale), Ausable River, and Saugeen River, Thames River and St. Clair River. The black dots represent the established sites at the river mouth and the green dots represent the invasion stage population. The red dot represents the source site (St. Clair River).

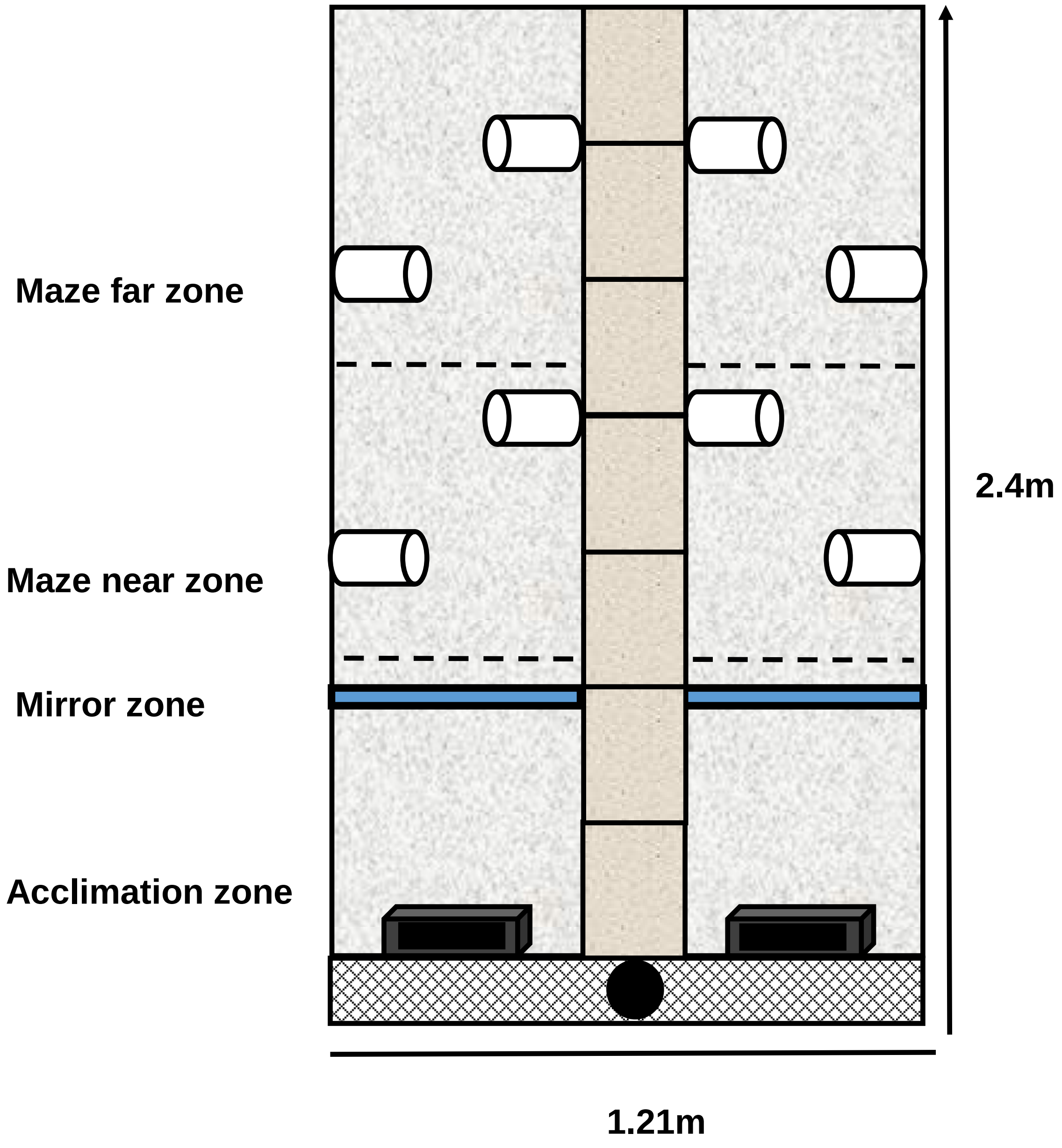

Figure 2.2 Schematic diagram of behavioural assay used to test for various behavioural traits (2.4 m by 1.21 m holding tank).

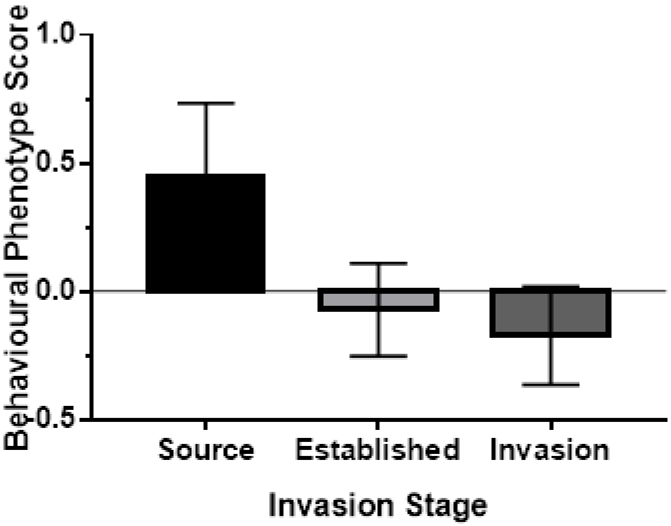

Figure 2.3 Mean (±SE) values comparing behavioural phenotype score between invasion stages, source (n=30), established (n=98), invasion front (83). No significant invasion stage effect.

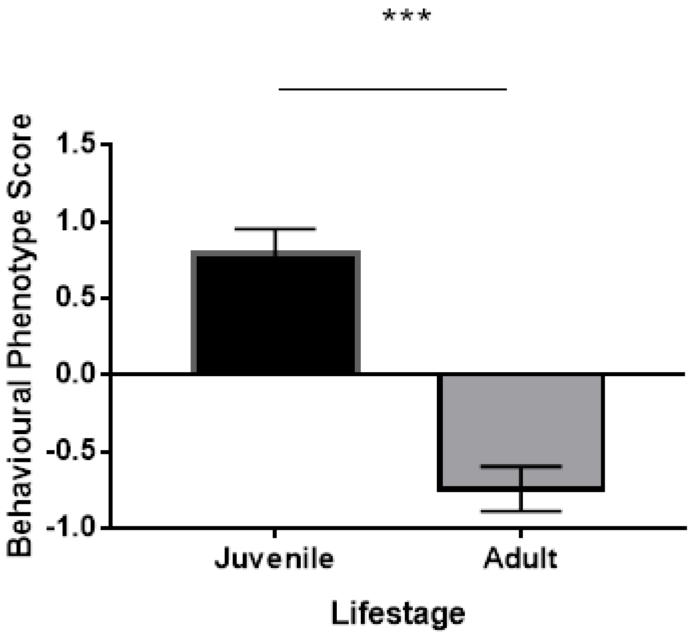

Figure 2.4 Mean (±SE) values comparing behavioural phenotype score between juveniles (n=97) and adults (n=114). *** represents P-values that are <0.001, respectively

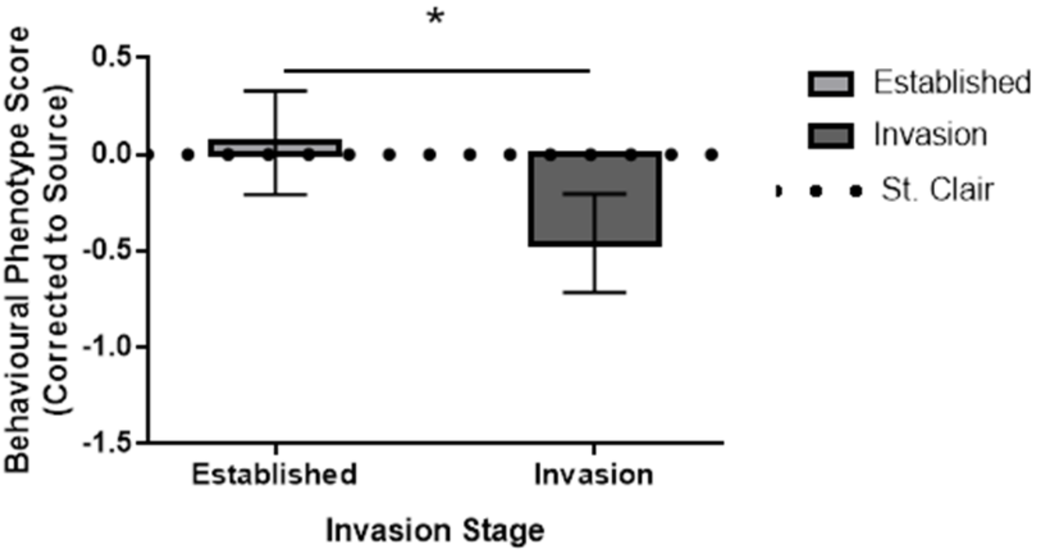

Figure 2.5 Mean (±SE) values comparing juvenile behavioural phenotype score (corrected to St. Clair – dashed line) between invasion stage, established (n=36), and invasion (n=46). NS, *, **, and *** represent P-values that were >0.05, <0.05, <0.01, and <0.001, respectively.

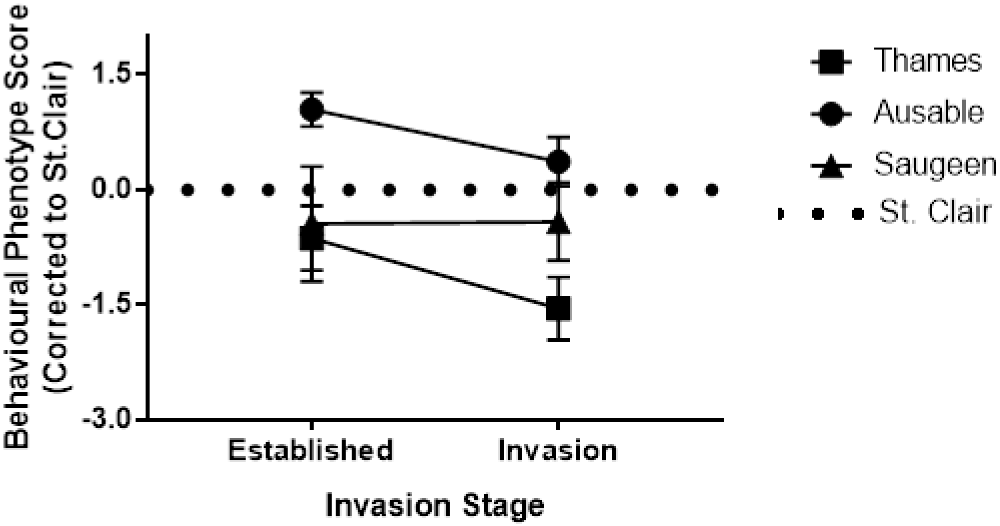

Figure 2.6 Mean (±SE) values comparing juvenile behavioural phenotype score (corrected to St. Clair – dashed line) between established and invasion stages within rivers.

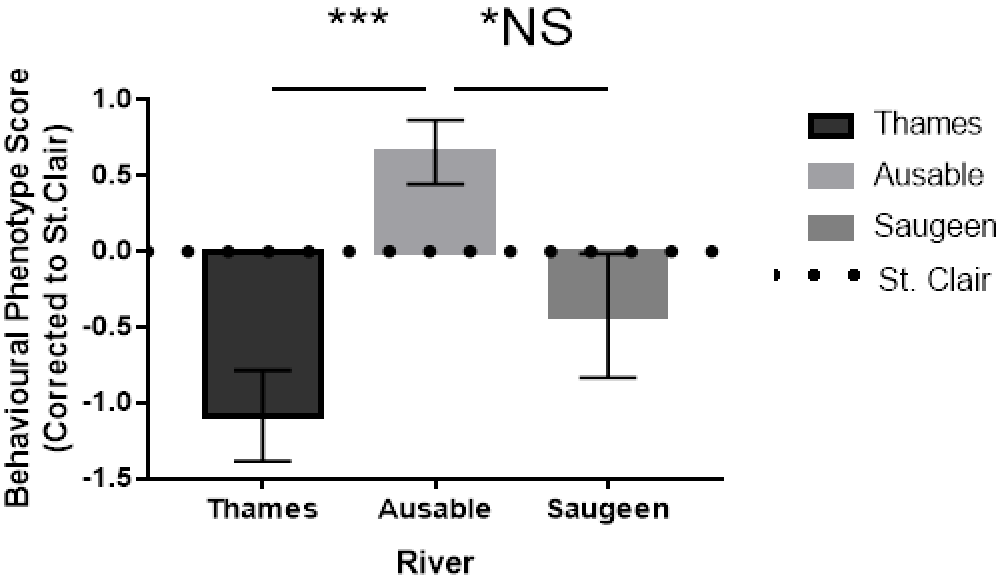

Figure 2.7 Mean (±SE) values juvenile behavioural phenotype score (corrected to St. Clair – dashed line) between rivers, Thames (n=30), Ausable (n=33), Saugeen (n=19). NS, *, **, and *** represent P-values that were >0.05, <0.05, <0.01, and <0.001, respectively. NS* indicates a marginal significant difference (P=0.082).

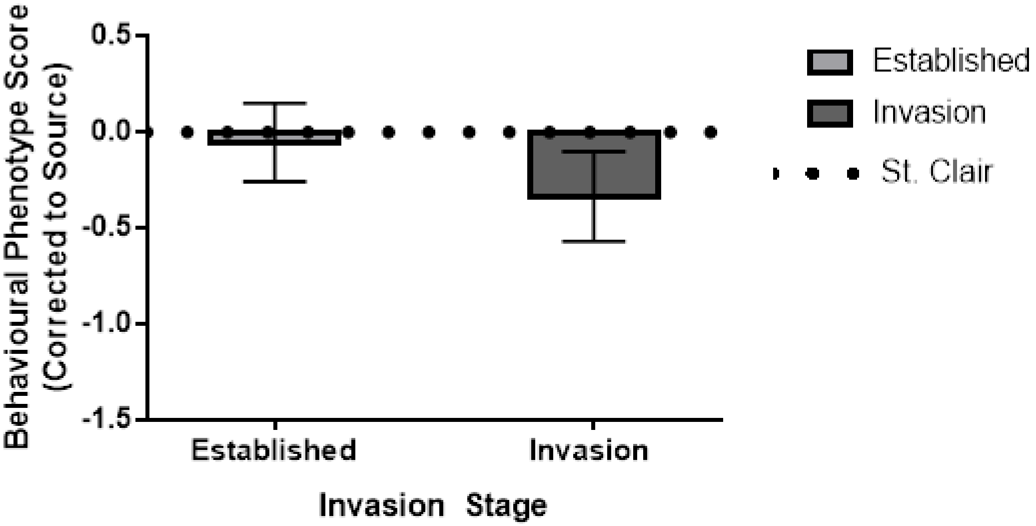

Figure 2.8 Mean (±SE) values comparing adult behavioural phenotype score (corrected to St. Clair – dashed line) between invasion stage, established (n=62), and invasion (n=37). No significant invasion stage effect.

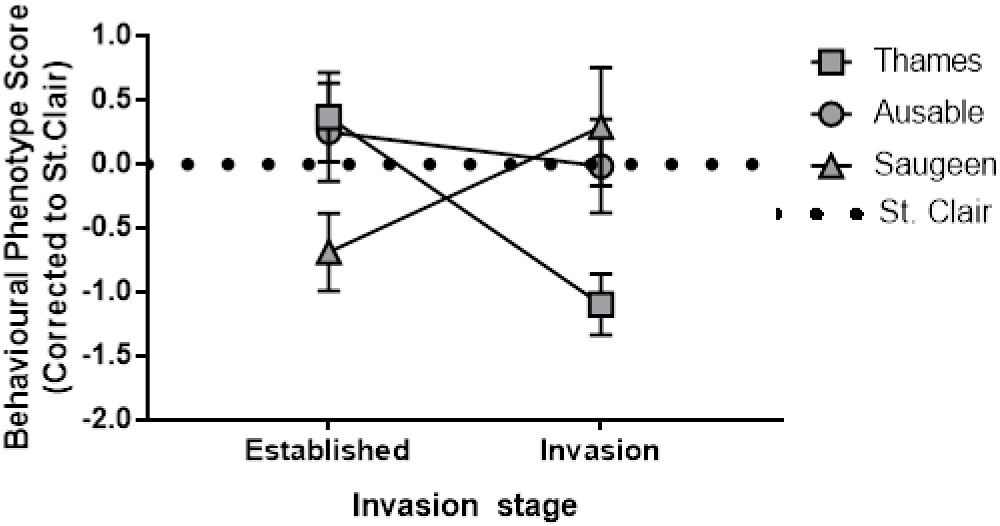

Figure 2.9 Mean (±SE) values comparing adult behavioural phenotype score (corrected to St. Clair – dashed line) between established and invasion stages within rivers. Overall significant river × invasion stage effect (P<0.05). Sample numbers shown in Table 2.7.

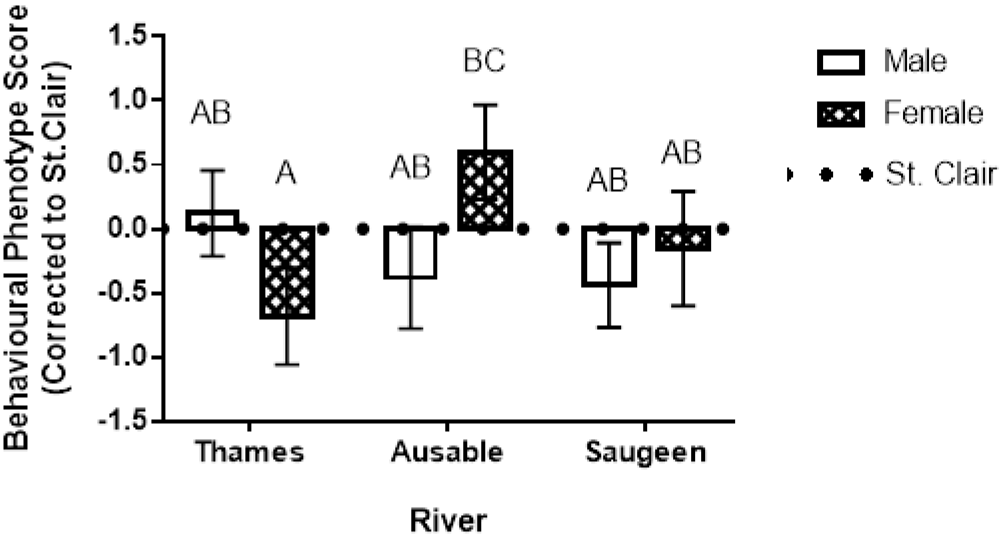

Figure 2.10 Mean (±SE) values comparing adult behavioural phenotype score (corrected to St. Clair – dashed line) between males and females within rivers, Thames (male n=21 female n=16), Ausable (male n=11 female n=14) and Saugeen (male n=21 female n=16). Bars that do not share the same letter are significantly different.

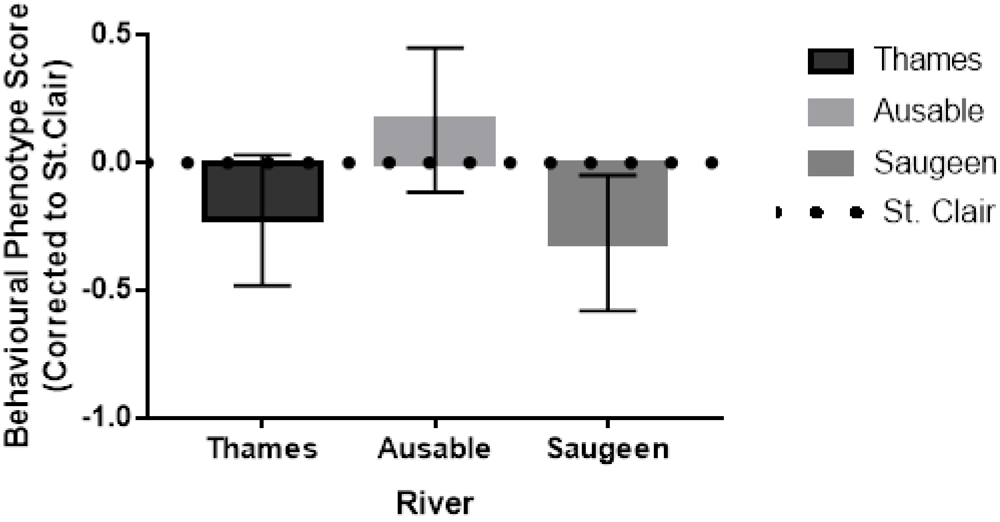

Figure 2.11 Mean (±SE) values adult behavioural phenotype score (corrected to St. Clair – dashed line) between rivers, Thames (n=37), Ausable (n=25), Saugeen (n=37). No significant river effect.

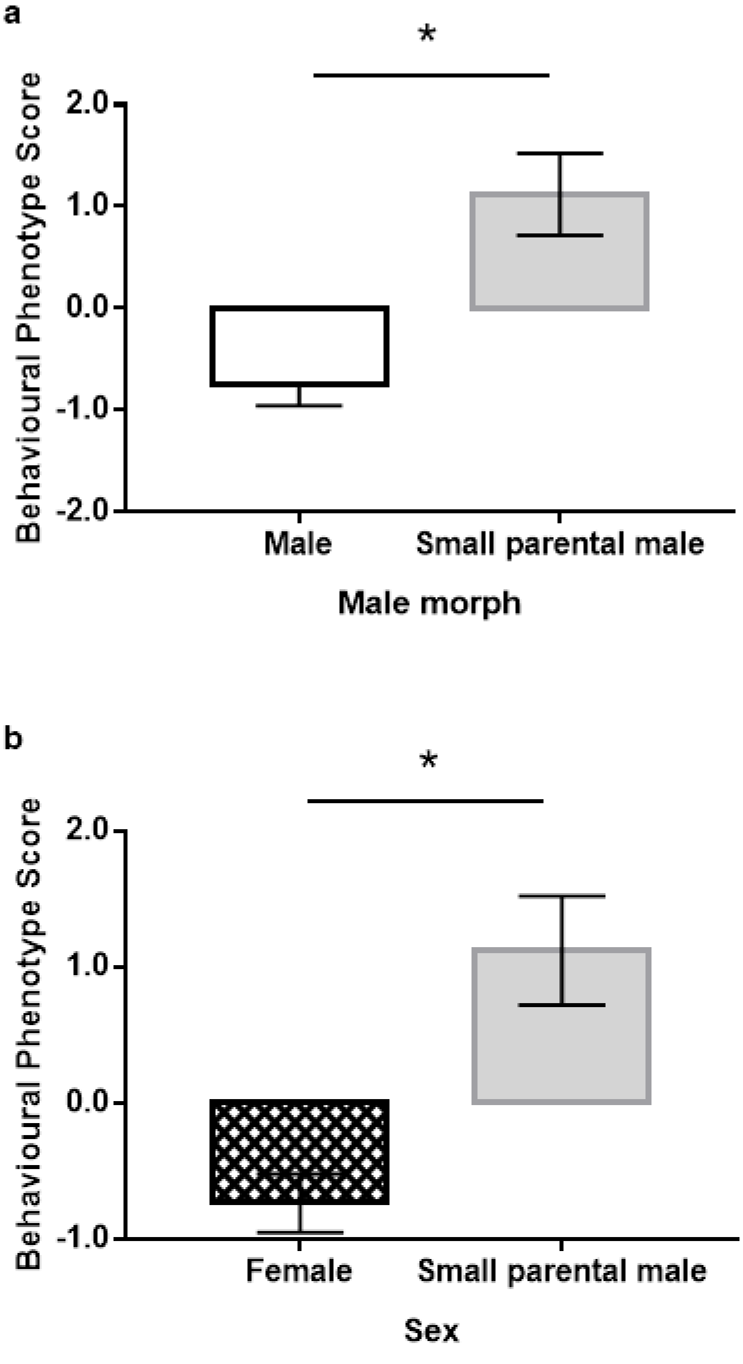

Figure 2.12 Mean (±SE) values comparing behavioural phenotype score (±SE) between males (n=59) and sneaker-males (n=7) (a) and females (n=55) and sneaker-males (n=7) (b). * represents a P-value <0.05.

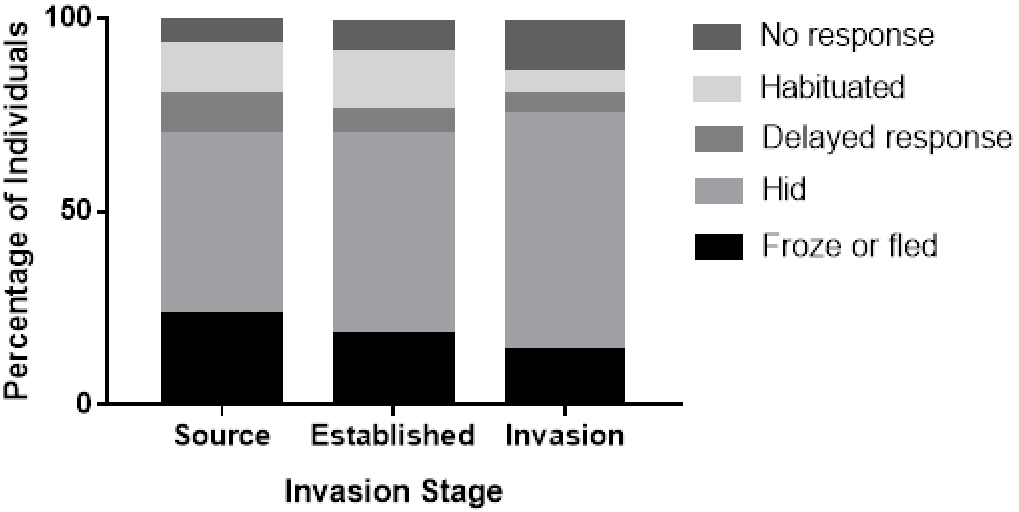

Figure 2.13 Distribution of predator response rankings between invasion stage (source, established and front). No significant invasion stage effect.

Figure 2.14 Distribution of predator response rankings between life-stage (juveniles and adults). * represents a P-value <0.05.

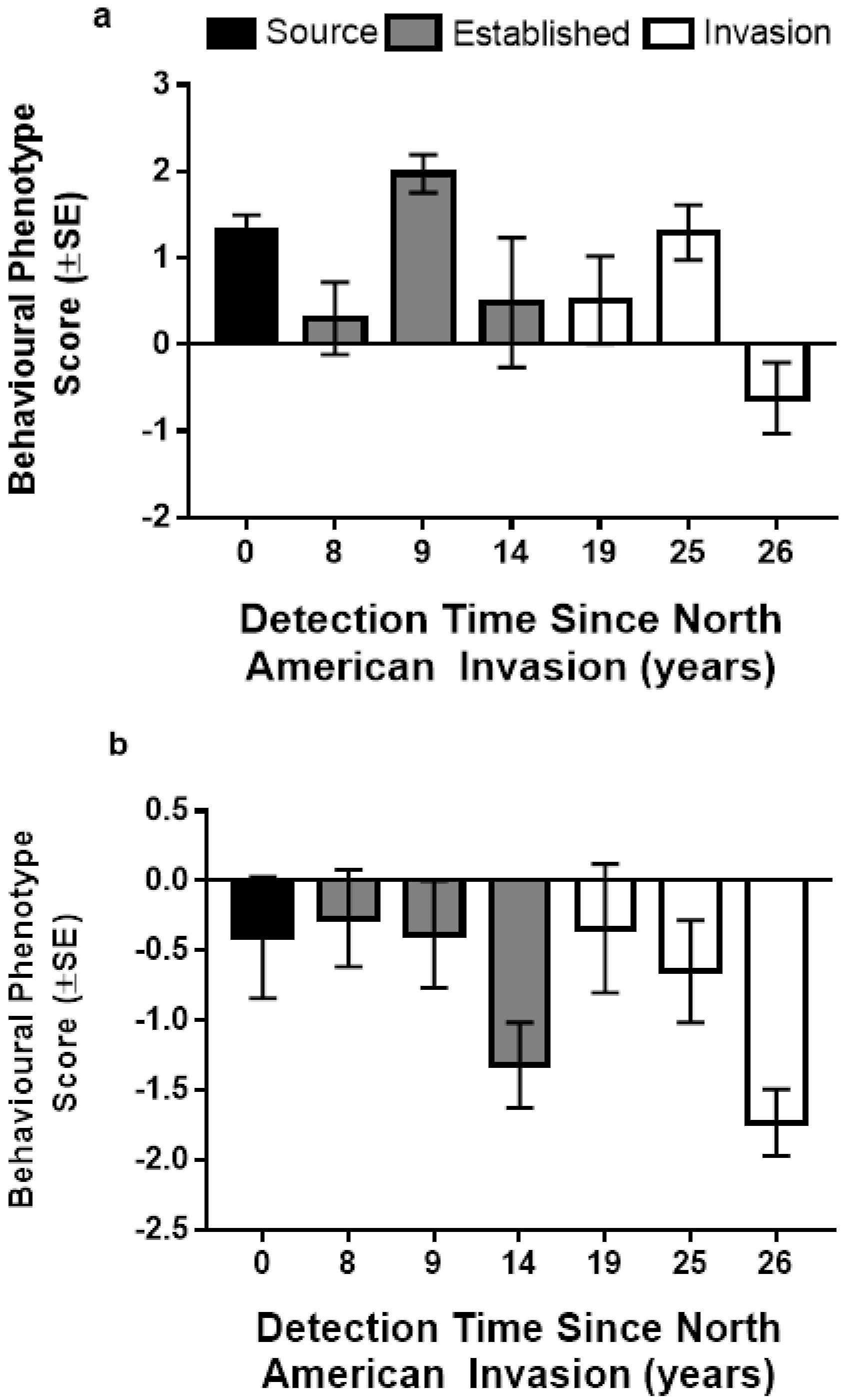

Figure 2.15 Mean (±SE) values comparing behavioural phenotype score across detection time since North American Invasion (Ausable-Est and Thames-Inv are offset by 1 year to show all seven populations) (0-25 years) juvenile (a) adults (b).

CHAPTER 3: TRANSCRIPTOMIC PROFILES OF ROUND GOBY (*NEOGOBIUS MELANOSTOMUS*) DURING RANGE EXPANSION

Introduction

For a non-indigenous species to be invasive they must pass through various stages to be successful: transport, introduction, establishment, and lastly, spread (Blackburn et al. 2011). At each stage, there are barriers which can selectively screen for individuals with certain traits, (Karatayev et al. 2009; Chapple et al. 2012). Invasion biologists have been most interested in the spread stage; specifically, how residents (individuals that remain in the same place) and dispersers (individuals that leave) differ phenotypically, and the various mechanisms that facilitate further range expansion (Ronce et al. 2001). Dispersal is the active or passive movement from a natal site to another site, and is the process that allows individuals to move from one area to another (Clobert et al. 2009). Since dispersal is more often considered a behavioural decision (Clobert et al. 2004; Bowler & Benton 2005), this can result in a variety of phenotypic differences between dispersers and residents (Clobert et al. 2009); for example, dispersing individuals are more asocial (Cote et al. 2010) and aggressive compared to residents (Duckworth and Badyaev 2007). In addition, dispersal has been associated with behaviours such as boldness, explorative tendency (to facilitate departure) (Pintor et al. 2008; Myles-Gonzalez et al. 2015), and higher cognitive functioning (to deal with stressors faced in a new environment; i.e., predators, novel food, conditions) (Sol and Lefebvre 2000; Amiel et al. 2011). Moreover, behaviours can be driven by metabolic processes, where individuals with high resting metabolism are generally more bold, explorative and have “fast” pace of life, and are more likely to disperse (Careau et al. 2008; Reale et al. 2010). Alternatively, less aggressive and shy individuals may be subordinate and forced out of ideal habitats and hence involuntarily disperse

(Bowler and Benton 2005; Guerra and Pollack 2010). Overall, behavioural differences can also be mediated by life-stage, as behaviour can shift ontogenetically as an animal incurs more experiences (Groothuis and Trillmich 2011; Wuerz and Kruger 2015); as well as by size differences, that influence territorial and dominance behaviours (Ray and Corkum 2001), all of which can ultimately affect the probability of dispersal.

Despite the intensive behavioural characterization of individuals occupying the "invasion front" (a low-density population, beyond which the density is zero) (Phillips 2016), the genetic component of dispersal behaviours has seldom been investigated in an invasion context (but see Mueller et al. 2014). Studies that do explore the genetic component of dispersal generally compare gene coding sequence such as single nucleotide polymorphisms (SNPs) rather than differences in gene expression. Transcriptional profiling allows the detection of multiple differentially expressed genes (Wellband and Heath 2017) and involves either characterising whole transcriptomes (all mRNA expression), or a more targeted approach (Wang et al. 2009) that examines specific candidate genes with putative known functions (Höglund, 2009; Primmer 2009). While behavioural traits are generally thought to be primarily flexible, underlying genetic components of different behavioural types have been reported in several natural and domesticated populations across taxa (e.g., Van Oortmerssen and Bakker 1981; Fidler et al. 2007; Suarez et al. 2008; Korsten et al. 2010; Van Oers et al. 2004; Sneddon et al. 2005; Norton et al. 2011; Rey et al. 2012; Thomson et al. 2011).

Specific to dispersal and invasive behaviours, Dingemanse et al. (2002) found a genetic association (by estimating heritability) with exploratory behaviour and natal distance in the great tit (*Parus major*) (Dingemanse et al. 2003), and Mueller and colleagues (2014) found the *DRD4* gene to be associated with neophilia and activity in the invasive yellow-crowned bishop

(*Euplectes afer*). Other studies have suggested that brain size in invasive species and their native counterparts could play a role in invasion success due to a relatively larger brain being capable of greater learning ability, and hence better invaders (Sol et al. 2005; Amiel et al. 2011). Larger brain size can be mediated through neurogenesis and synaptic connections that are often implicated in cognitive function (i.e. learning ability), and can be controlled through developmental gene transcription (Cao et al. 2004; Ebbesson and Braithwaite 2012; Johansen et al. 2012). An invasive species may need to cope with novel stressors (e.g. novel predators, limited food). Studies have shown invasive species are better able to cope with environmental change (Lopez-Maury et al. 2008) and the presence of specific receptors involved in neurotransmission has been shown to play a critical role in the response to novel stressors (Gunn et al. 2015). For example, the binding of glutamate and GAB_A (gamma-Aminobutyric acid) to their respective receptors have been implicated in synaptic plasticity and regulating synaptic transmission (Contractor and Heinemann 2002; Lüscher and Keller 2004). *GABA*$_A$ receptors also regulate the hypothalamic pituitary axis (HPA) and stress hormones via GABAergic inhibition (Decavel and van den Pol 1990; Herman et al. 2004). Additionally, glucose and energy regulation is important in the maintenance of all cellular processes and overall activity of individuals (Soengas and Aldegunde 2002) which in turn can affect movement, and hence dispersal, of an individual. Overall, the examination of multiple genes across several functional groups (i.e., transcriptional profiling) can help characterise the underlying mechanisms of behaviour-based dispersal differences and acclimation to novel environments during range expansion of invasive species.

Nonetheless, both the complexity and the genetic control of behaviour have been shown to be multifaceted due to pleiotropy, epistasis and plasticity (De Jong 1990; Falconer and

Mackay 1996; Van Oers et al. 2005). Taken together, the underpinnings of gene transcription and its role in behavioural variation, cognitive ability, stress-response, and activity should give rise to “invasive phenotypes”. If behaviours differ between dispersers and residents then there may also be an underlying genetic component allowing these phenotypic differences to exist as well, potentially leading to spatial assortment and non-random mating at the invasion front (Shine et al. 2011); or equally, phenotypes developing due to rapid evolution (Phillips 2009; Perkins et al. 2013).

One alternative to heritable variation driving the evolution of invasive behaviours is that invasive individuals may exhibit elevated phenotypic flexibility via plasticity in gene transcription. Phenotypic plasticity is defined as one genotype that can produce multiple phenotypes depending on the nature of the environment (Pigliucci 2001; West-Eberhard 2003); and plasticity in gene transcription can be key in facilitating both short and long-term acclimation to novel environments and stressors (Lopez-Maury et al. 2009). For instance, factors acting at the pre-transcriptional stage (e.g. epigenetic modifications, enhancers, repressors, etc.) can ultimately affect gene transcription levels in response to an unpredictable environment (Pigliucci 2001; Schneider and Grosschedl 2007; Flavell and Greenburg 2008), which in turn can lead to an altered behavioural response (e.g. response to a novel predator is due to increased synaptic plasticity which is due to calmodulin protein kinase transcription). The ability for synapses to strengthen or weaken their connections is a form of plasticity, and has been cited as a mechanism by which behaviours can be flexible (Bell and Aubin-Horth 2010). Plasticity in the transcription of genes related to cognition is another important factor mediating how animals respond to their environment via consolidating learning and memory: cognition studies have revealed the upregulated expression of immediate-early genes (IEG) after a learning task, thus providing a genetic basis for learning (Guzowski et al. 2005; Rajan et al. 2011). Consequently, individuals at the invasion front might be selected to be more transcriptionally plastic, and therefore responsive to a challenge or novel environment, compared to their established counterparts, due to the novel stressors they are likely to encounter as a result of dispersing.

This study investigates brain gene transcription profiles - behavioural/stress response, neuronal, and metabolic/activity - of the invasive round goby in various stages of invasion in Southwestern Ontario, Canada. The goal is to characterise genetic mechanisms associated with dispersal vs resident phenotypes, and therefore range expansion of an invasive species. We sampled fish from multiple rivers ("natural"), to examine whether there are innate differences in gene transcription (in the absence of a standardised challenge) between round gobies from the invaded source, established and invasion stage populations. Specifically, we predicted that round gobies on the invasion front should have lower transcription of genes related to inhibition of aggression or boldness, higher transcription of stress response genes, higher transcription in genes related to neuronal response and synaptic plasticity, and overall higher transcription of metabolic/activity genes to govern these neuronal and behavioural processes. Using a candidate gene approach, while utilizing gene category transcription profiles, allows us to examine the transcriptional differences between resident and dispersers across multiple tributaries to provide insight into whether there is a genetic basis driving phenotypic differences between residents and dispersers. This study furthermore examines brain gene transcription response of round gobies following a transfer to a novel captive environment, "treatment" group, to examine how these animals might respond to novelty in a new (and presumably stressful) environment, and compares these differences across fish from the source, established and invasion stage populations. We predict round gobies from established populations will be less responsive and therefore have lower transcription of genes related to behavioural/stress response, neuronal and metabolic/activity, due them being settled for a long period of time, allotting them time to adjust and acclimate to their environment and face few challenges. The invasion-front gobies would have higher transcription levels in response to captivity because they are constantly being exposed to novel challenges in their wild habitats (e.g., limited resources, novel predators) and therefore should acclimate via gene transcription (especially genes related to stress response) in the novel environments such as captivity (Sol et al. 2007).

By examining gene transcription across multiple gene ontologies in the brain we can gain insight across multiple biological processes that could underlay "invasiveness" and the organism's ability to colonize new habitats. Quantifying the genetic basis of behavioural variation, cognitive ability, stress-response, and activity traits by using a candidate gene list can aid in the screening process of future potential invasive or high-risk species by examining which genes typically are differentially transcribed between dispersers and residents both at baseline (natural) and during a captive environmental treatment.

Methodology

Study species

The round goby is an ideal study system to examine gene transcription differences associated with invasion because it is undergoing active invasion in various tributaries across Ontario (Poos et al. 2010; Bronnenhuber et al. 2011;). Their establishment success and transition into freshwater systems has been attributed a variety of traits, ranging from their tolerance to temperature and salinity extremes (Houston et al. 2013; Karsiotis et al. 2012), multiple spawning events per season (Marentette et al. 2009), parental care (Meunier et al. 2009) to competitive ability for resources (Bergstrom and Mensinger 2009). Several studies have focused on behavioural traits at established and invasion-front sites in round gobies (Groen et al. 2012; Myles-Gonzalez et al. 2015), some suggesting spatial sorting (Phillips et al. 2008). More recently, there have been gene transcription studies characterising thermal stress and acclimation in the round goby between recently invaded and established populations (Vincelli 2016) and the comparison of round goby and tubenose goby and gene transcription plasticity to a thermal stressor (Wellband and Heath 2017). However, no study has quantified the behavioural and genetic correlates of the round goby that facilitate range expansion. From chapter 2, we know that overall round goby displays an invasion phenotype consisting of boldness, exploration, sociality, and being predator habituated, that varies across life-stage and within life-stage but among invasion stages. Due to the characterized behavioural repertoire of the round goby and their on-going range expansion, the round goby makes an excellent study species to examine genetic mechanisms that facilitate range expansion, and their ability to respond to novel stressors.

Tissue Collection

In August and September 2015, round gobies were caught via seine netting, at seven sites (populations), St. Clair (invaded source) and established (river mouth) and invasion front (upstream) in three other rivers, Thames, Ausable and Saugeen. A subset of round gobies were humanely euthanized using MS-222 (CCAC 2015) and twenty whole brains (heads) were collected by exposing the skull cap to let RNAlater sufficiently saturate the brain tissue, in under 5 minutes, in the field, for “natural” gene transcription (Figure 3.1). Samples were transported in coolers on ice and subsequently kept frozen at -20ºC in RNAlater until whole brain tissue including the brain stem was dissected for RNA extractions. A total of n=134 wild-caught gobies were used for gene transcription analysis (Supplementary Table S2A).

From August to October 2015 a separate set of round gobies - considered the "treatment" group – were caught via seine net and transferred from each site to the aquatic facility at the Great Lakes Institute for Environmental Research (GLIER) at the University of Windsor and held for 5-7 days to acclimate to the laboratory conditions. These fish underwent a 50-min behavioural challenge (Chapter 2), were then euthanized, decapitated and their brains were exposed to let RNAlater saturate the tissue, stored in RNAlater and immediately placed in the -20^{o}C freezer until tissue dissection and RNA extraction. A total of 194 individuals were used in the gene transcription analysis of the treatment fish (Supplementary Table S2B).

Candidate gene selection

Candidate genes were selected after extensive literature review. Genes were selected based on their function/association with behavioural invasive traits and comprised three ontologies: genes related to aggression and stress responsiveness, to neuronal activity of learning and memory, and to metabolism and thus activity-exploration behaviours (Table 3.1).

Behavioural/stress response genes

Genes that have been associated with specific behaviours observed in invading species are related to aggression, stress response, and plasticity. Selected genes within this category include, *5HT3A, AVT, FGFR1A, GABAAR, HBA2, MAO-A, POMC, STRA6. 5HT3A* receptor gene is one of the only serotonin receptors to be a ligand ion channel. It binds the neurotransmitter serotonin and has been implicated in modulating aggression (Grimes and Melloni 2005; Miczek et al. 1989; Ricci et al. 2005), and learning and memory in mice (Pitsikas et al. 1994; Harrell and Allan 2003). Arginine vascotocin (*AVT*) is known to control social behaviour in many fish species (Goodson and Bass 2000; Greenwood et al. 2008). *AVT* has also been shown to be upregulated in courting aggressive fish (Godwin et al. 2000; Aubin-Horth et al.

2007). Differential *AVT* expression has also been linked to stress response, where there was an increase in *AVT* transcription in response to an acute stressor (Gilchriest et al. 2000). Fibroblast growth factor 1-A (*FGFR1A*) has been seen to modulate histamine signalling, and zebrafish mutants that had reduced Fgf signalling resulted in highly aggressive, bold and explorative fish (Norton et al. 2011). The $GABA_A$ receptor gene ($GABA_A$), and the GABA system in general has multiple functions and has been linked with fearfulness (Caldji et al. 2000) and aggression (Miczek et al. 2003). High expression has also been linked with proactive stress coping (Thomson et al. 2011; Gunn et al. 2015). Hemoglobin alpha-2 (*HBA2*) is involved in oxygen transport and is important in stress response (Stankiewicz et al. 2014); elevated levels of hemoglobin have been seen in more subordinate fish which has been proposed to help them cope with increased demand for oxygen when escaping in response to attacks (Ferraz and Gomes 2009). The *MAO-A* gene encodes an enzyme that degrades serotonin, dopamine and noradrenaline, neurotransmitters critically involved in the regulation of aggression (Shih and Thompson 1999); and the inhibition of *MAOA* has been shown to result in reduced aggression in isolated male mice (Florvall et al. 1978). Pro-opiomelanocortin (*POMC*) encodes for a precursor polypeptide that is cleaved into various other peptides, such as adrenocorticotropic hormone (ACTH), also involved in the stress response (Winberg and Lepage 1998). Stress response is important in a behavioural aspect via coping styles (Koolhaas et al. 1999; Ducrest et al. 2008). *STRA6* gene is the high-affinity receptor for plasma retinol-binding protein (RBP) and mediates cellular vitamin A uptake (Kawaguchi et al. 2007). Retinoic acid and vitamin A uptake have been shown to be important in immune and stress response, as well as hippocampal-dependent memory (McCaffery et al. 2006). Retinoic acid may also be crucial for homeostatic synaptic plasticity, which is the mechanism where neurons adapt the strength of their synaptic networks (synaptic scaling) in response to external stimuli (Pozo and Goda 2010).

Neuronal genes

Neuronal genes are associated with behavioural plasticity, which in turn is often suggested in the literature as one of the mechanisms that allows a non-indigenous species to be a successful invader (Hazlett et al. 2002). Neuronal genes associated with synaptogenesis and cellular response (excitatory or inhibitory via neurotransmitter receptor binding) may be important in the response to novel stressors since the transcription of these genes can aid in long-term potentiation and learning. In addition, genes that promote long-term potentiation may be critical for learning and memory. The selected genes in the neuronal category include, *CAMK2G*, *CAMK2N2*, *C-FOS*, *DLG2*, *GLRK*, KCNN2, and *NRG2*. *CAMK2G* is involved in calcium signalling which is involved in long-term potentiation (Lisman et al. 2012), which is important for learning (Zhou et al. 2007) and memory (Lisman et al. 2002; Lucchesi et al. 2011). The *CAMK2N2* gene encodes for an inhibitor of calmodulin kinase, and plays a role in regulating long-term potentiation and possibly memory maintenance (Vigil et al. 2014). The *DLG2* gene codes for a protein part of the membrane-associated guanylate kinase (MAGUK) family; and this encoded protein is involved in N-methyl-D-aspartate (NMDA) signalling which is important for synaptic transmission and synaptic plasticity (Li and Tsien 2009). *GLRK* codes for glutamate receptor-U1, which binds L-glutamate, an excitatory neurotransmitter (Meldrum 2000), thus facilitating the majority of the excitatory transmission in the brain (Dingledine et al. 1999), important for learning and memory (Riedel et al. 2003). *KCNN2* encodes for an integral membrane protein and is involved in regulating neuronal excitability (Lin et al. 2008) and modulating memory and synaptic plasticity in the hippocampus (Stackman et al. 2002). *NRG2* codes for neuregulin-2, important in dendrite morphology, and regulates neurotransmitter receptor function (Garcia et al. 2000; Huang et al. 2000; Longart et al. 2004); it has also been implicated in plasticity (Yan et al. 2017).

Metabolic genes

Metabolic and activity-regulating genes are important in generating ATP for energy and maintaining individual performance, regulating circadian rhythms, and overall fitness, especially in dispersing individuals. In addition, metabolic activity in the brain is vital to cope in stressful environments. The selected genes in this category include, *CRY1, CRY2, FAM50A, G6PD, GALC, GYG1, HPRT1, MID1IP1, MSRA, PFKFB1,* and *PRVB. CRY1* and *CRY2* are transcriptional repressors that form a core component of the circadian clock, but each play distinct roles differentially regulating per (period) proteins (Vitaterna et al. 1999). *CRY1* and *CRY2* regulate various physiological processes and rhythms in metabolism and behaviour (Lowrey and Takahashi 2011) which in turn can affect their dispersal ability and potential for range expansion. *FAM50A* codes for a DNA-binding protein that acts as a transcription factor (Mazzarella et al. 1997) and is thought to be a circadian clock regulator. *G6PD* encodes the enzyme glucose-6-phosphate dehydrogenase that is involved in the normal processing of carbohydrates, and is also involved in the prevention of oxidative stress (Pandolfi, et al. 1995). *GALC* codes for the enzyme galactosylcerimidase, and is responsible for the breakdown of fats in the brain and kidney (Luzi et al. 1995), and an important component in myelin that conducts the rapid transmission of nerve impulses (Norton and Cammer 1984). *GYG1* codes for an enzyme involved in the synthesis of glycogenin, a glucose polymer used as a reserve when glucose levels fall (Dringen et al. 1993), and is consumed during a sensory stimulus (Dienel and McKenna 2014) and exercise (Matsui et al. 2011) which can be important for a dispersing species. The *HPRT1* is involved in the recycling of purines - building blocks of DNA (Craig and Eakin 2000); and higher transcription of this gene is more efficient and requires less energy than generating more purines (Craig and Eakin 2000). *MIDI1P1* gene plays a role in regulating lipogenesis where transcription has been seen to be reduced in fasted states (Tsatsos et al. 2008). *MSRA* codes for the enzyme methionine sulfoxide reductase A and functions as a repair enzyme for proteins that have been inactivated by oxidation (Moskovitz et al. 1996). *MSRA* is involved in mitochondrial ATP synthesis (Dun et al. 2013). *PFKFB1* encodes an enzyme that catalyzes the synthesis and degradation of fructose-2,6-biphosphate, an activator of the glycolysis pathway and an inhibitor of the gluconeogenesis pathway. Consequently, regulating fructose-2,6-biphosphate levels through the activity of this enzyme is thought to regulate glucose homeostasis (Minchenko et al. 2003). The *PRVB* gene codes for one Parvalbumin (PV) which are a class of intracellular calcium-binding proteins that regulate calcium homeostasis (Celio 1990); and expression levels in the brain are linked with high metabolic and firing rates (Baimbridge et al. 1992), and altered locomotor activity (Farré-Castany et al. 2007).

Primer Optimization

Five primer pairs were designed using Geneious software v6.1.6. and Genbank sequences. Genbank sequence data from closely related teleost species were aligned to identify conserved regions for primer design. The resultant PCR product was sequenced to obtain the round goby gene sequence using Sanger sequencing at the Quebec Genome facility (Montreal, QC, Canada). The round goby sequence was used to develop round goby-specific primers and probes for those five genes. Twenty primer pairs were designed using annotated gene sequence from a round goby liver transcriptome (Wellband and Heath 2017). Primers and probes were designed using an online program, PrimerQuest® (amplicon length ranging from 60-250 bp).

Primers for the two endogenous control genes *β-actin* and *EF1a* and *G6DP* gene were obtained from (Vincelli 2016). The optimal annealing temperature for each primer set was determined using gradient PCR (annealing temperature range: 55-65ºC) and visualized using gel electrophoresis. In addition, primer specificity was verified using SYBR™ Green PCR Master Mix on the QuantStudio™ 12K Flex Real-Time PCR by melt-curve analysis. All primers were designed specifically for this study.

RNA extraction

RNAlater was decanted from the preserved brain tissue and total RNA was extracted from the whole brain by mechanical homogenization using glass beads followed by Isol-RNA lysis solutions, TRIzol™ (ThermoFisher Scientific) following the manufacturer's protocol. The extracted RNA was washed in 75% ethanol and stored at -80ºC until reconstituted. At that time, ethanol was removed and the RNA pellet was air-dried to remove excess ethanol and resuspended in ddH_2O. RNA quality was assessed by running samples on a 2% agarose gel; and the purity of RNA was assessed using the NanoVue spectrophotometer and the $A_{260/260}$ ratio, acceptable samples had values from 1.8-2.1. The concentration of RNA was also quantified, and acceptable samples contained at least 60 ng/µL of RNA. Afterwards, the RNA samples were stored at -80 ºC until DNase treatment and cDNA synthesis.

DNase Treatment and cDNA synthesis

A DNase treatment using RQ1 RNase-Free DNase (Promega Corporation, Madison WI, USA) following the manufacturer's protocol was applied to all RNA samples to eliminate genomic DNA contamination. Samples were diluted to ensure there was a concentration of 500ng/µL per 10 µL reaction. Subsequently, complementary DNA (cDNA) was synthesized using the High Capacity cDNA Reverse Transcription (RT) kit (Applied Biosystems, Burlington, ON, Canada) following manufacturer's protocol. cDNA was stored at -80 ºC until qRT-PCR quantification.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Quantification of gene transcription was by quantitative real-time polymerase chain reaction (qRT-PCR) using TaqMan® OpenArray ® chips (Applied Biosystems, Burlington, ON, Canada) following the manufacturer's protocol. OpenArray ® chips were run on the QuantStudio™12K Flex Real-Time PCR System (Life Technologies Inc., Carlsbad, CA, USA). For each cDNA sample, a 5.0 µL reaction was prepared using 2.5 µL Taqman® OpenArray® Real-Time PCR Master mix and 2.5 µL of diluted cDNA (4.8 µL cDNA stock and 5.2 µL ddH_2O) in 384-well plates. The 5.0 µL reactions were then loaded onto the OpenArray® chips using the OpenArray® Accufill® System to reduce inter-assay variation. Each OpenArray® chip contained 48 subarrays, and each subarray contained 64 through-holes, which allowed 28 genes to be analyzed simultaneously (26 candidate genes and 2 endogenous controls) in duplicate per cDNA sample. Each qRT-PCR reaction on the OpenArray® was conducted in a 33 nL volume.

Selection of endogenous control

Two endogenous control genes were used, beta-actin (*β-actin*) and elongation factor 1a (*EF1a*). We tested transcriptional stability of raw Ct values with the NormqPCR package using the GeNorm method in R (Perkins et al. 2012). *EF1a* and β-actin were both ranked high in stability (consistent across samples) We also ran linear mixed models to test for invasion stage, life-stage, and river effects for each endogenous control gene, and the average of both. *β-actin* showed site effects and *EF1a* showed significant life-stage stage effects. The average of *β-actin* and *EF1a* showed no significant invasion stage, life-stage or site effects, and was chosen for ΔC_T normalization for both the natural and treatment. For seven individuals out of a total of 328 individuals *EF1a* did not show amplification, so only *β-Actin* was used for ΔC_T normalization in these cases.

Transcriptional Analysis

All transcriptional analyses were completed using ExpressionSuite software v1.1 (Applied Biosystems, Burlington, ON, Canada). Reactions that showed no amplification or had raw C_T (critical threshold) values that were over 32 cycles were eliminated from further analyses. The *MAO* and *AVT* gene assays were excluded from the analyses because they did not show sufficient amplification, this may be due to very low transcription levels or primer/probe efficiency. The C_T mean was calculated and between the technical replicates for every gene for each sample. In some incidences, only one technical replicate was used, because the other technical replicate was deemed not reliable. Δ C_T values were calculated by subtracting the C_T mean for the endogenous controls from the candidate gene C_T mean. A lower Δ C_T value indicates higher transcription of that gene.

Statistical analysis

Comparisons between natural and treatment gene transcription

For each gene, the mean Δ C_T for each population was graphed for the natural and treatment individuals to visually inspect if populations (St. Clair (source) and established and invasion fronts of Thames, Ausable, Saugeen rivers) transcriptionally responded to the captive environment/behavioural assay. A least-squared means fit model JMP (SAS Inc. v.13) was done to identify genes that responded to the environmental treatment (captivity+ behavioural assay) (Supplementary: Table S3). This model tested for i) treatment (natural versus treatment) ii) population and iii) the interaction of treatment and population effects. This procedure was done instead of calculating a ΔΔ C_T: i) due to other factors that could have affected the behaviour and gene transcription of the individuals (e.g. transport to captivity), and ii) to justify against using pooled data (should differences exist). These results were not included in subsequent analyses.

Principal coordinates analysis (PCoA)

A principal coordinates analysis was conducted using the package “vegan” (Oksanen et al. 2017) in R version 3.3.2 (R Core Team 2016) using Bray-Curtis distances to examine clustering of genes (behavioural/stress response, neuronal and metabolic/activity). This was done for all 24 genes and all individuals for both i) natural gene transcription and ii) treatment gene transcription. This was done for visual purposes only.

Candidate approach

Transcriptional data were analyzed using a candidate gene approach, where each gene was tested individually against explanatory variables in linear-mixed model. All statistical analyses were completed using R version 3.3.2 (R Core Team 2016). Model assumptions of normality and homogeneity of variances for the relative gene transcription (ΔC_T) variable were tested by visual inspection of residual versus fitted and quantile-quantile plots.

Linear mixed-modelling

A global linear mixed model using the package “lme4” (Bates et al. 2015) was conducted to examine the effects of i) detection time since North American invasion (0 – 25 years), and ii) life-stage, mass, and their interaction, for all 24 genes. Each analysis was conducted separately for the natural group (controlling for seine effort as a random effect) and treatment gene transcription (with days in captivity, trial ID, holding tank ID and experimental tank ID as random effects). If life-stage, mass, life-stage × mass or the random effects were not significant, they were dropped from the model to focus on the effect of “detection time since North American invasion”. Separate Bonferroni corrections were conducted within each functional gene category (behavioural/stress response, neuronal, and metabolic/activity) for natural and treatment gene transcription. This resulted in adjusted alpha levels for the behavioural/stress response category p-values <0.008, for the neuronal category p-values <0.007, and for the metabolic/activity category p-values <0.0045.

Results

Principal coordinates analysis (PCoA)

For the natural gene transcription, principal coordinates analysis revealed that $GABA_A$ and *CAMK2N2* genes visually clustered. *POMC* and *HBA2* were distinctly different from the other genes There was no obvious pattern of gene function clustering of the behavioural/stress response, neuronal or metabolic/activity gene transcription (Figure 3.2a).

For the treatment gene transcription, principal coordinates analysis revealed similar clustering patterns as natural gene transcription PCoA, indicative of conserved gene transcription patterns. $GABA_A$ and *CAMK2N2* genes clustered together. *POMC* and *HBA2* also were distinct in transcription and separate from all other genes. There was no distinct clustering of behavioural/stress response, neuronal or metabolic/activity genes (Figure 3.2b).

Transcriptional response to captivity

For the behavioural/stress response category we found a significant treatment effect, for *5HT3A, FGFR1A,* $GABA_A$ and *HBA2*. There was a significant treatment × population and population effect for *HBA2* (Supplementary Table S3; Figure S1)

For the neuronal gene category there was a significant treatment effect for *CAMK2G2*, CAMK2N2. There was a significant treatment × population effect for *CAMK2N2* and *KCNN2*. There was also a significant population effect for *CAMK2N2* and *DLG2* (Supplementary Table S3; Figure S2).

For the metabolic/activity category there was a significant treatment effect for *GALC* and *MID1IP1*. There was also a significant treatment by population effect for *CRY2*. Lastly, there was a significant population effect for *CRY1, CRY2, GALC, MSRA,* and *PRVB* (Supplementary Table S3; Figure S3).

Natural-gene analysis

For the natural gene transcription analyses, there were two genes in the behavioural/stress response gene category that showed a significant "detection time since North American invasion" effect: *5HT3A* and *HBA2*, where invading populations had lower transcription of both genes relative to more established populations (Table 3.3; Figure 3.3)

For the neuronal gene category, one gene, *KCNN2*, had a significant "detection time since North American invasion" effect, where invading populations also had lower transcription compared to more established populations (Table 3.3; Figure 3.4).

Lastly, no genes from the metabolic/activity category showed a significant detection time since North American invasion" effect (Table 3.3; Figure 3.5)

Treatment-gene analysis

For the all three gene categories, there were no significant "detection time since North American invasion" effects (Table 3.4; Figure 3.6-3.8). *MID1IP1,* a metabolic gene, had a significant mass and life-stage × mass interaction effect.

Discussion

The genes in this study were specifically selected because of their known or suspected role in behavioural variation (e.g. aggression and boldness), ability to cope with stress, long-term potentiation and learning/memory, and synaptic plasticity that would allow individuals to make optimal decisions and learn and process cues from the environment (e.g. predator response), and metabolism/activity (dispersal and exploration). These attributes have been identified to be important for invasion success and range expansion across a range of populations and species (Dingemanse et al. 2003; Rehage and Sih 2004; Bubb et al. 2006; Duckworth and Badyaev 2007; Lande 2015). For a species to expand its range, dispersers must be able to cope with novelty and environmental stressors not present at the core population site (Novak 2007). If the most capable dispersers are individuals actively leaving established populations, then one would expect dispersers to have a specific phenotype (Clobert et al. 2004) and possibly an underlying genotype that provide adaptive advantages in the novel environment (Pasinelli et al. 2004). Alternatively, it is possible that dispersers simply exhibit greater plasticity to produce new phenotypes that are better adapted to deal with novelty and stress (Pigliucci 2001; West-Eberhard 2003). Hence, it is reasonable to predict that there are genetic components or a combination of genetic and environmental factors mediating phenotypic differences between dispersers and residents (Lee 2002).

PCoA natural and treatment gene transcription profiles

We observed very similar patterns of visual clustering for both “natural” and “treatment” gene transcription profiles. This suggests that these genes are highly conserved in transcription regardless of context (rest and challenge). Interestingly, we saw the *GABA*$_A$ and *CAMK2N2* genes cluster together. GABA$_A$ receptors which are involved in inhibitory neurotransmission and *CAMK2N2*, which is an inhibitor of calmodulin protein kinase which involved in calcium signalling cascades. Calmodulin protein kinase has been known to localize on GABA$_A$ synapses to further increase inhibitory neurotransmission (Marsden et al. 2013). This could possibly explain why they were clustered together. *HBA2* and *POMC* separated from all the other genes possibly due to their distinct and independent roles in stress responsiveness.

Natural gene transcription

Overall, we found two genes in the behavioural/stress response category with a significant “detection time since North American invasion” effect: *5HT3A* and *HBA2*. For both genes, we observed significantly lower transcription at invading populations (higher detection time) compared to established populations (lower detection time). *5HT3A* is involved in the inhibition of aggression, and so it would reasonable to assume individuals at established sites have higher transcription of *5HT3A* since they have to be able to withstand high conspecific densities. Capelle et al. (2015) found that round gobies overall are socially gregarious, which in turn helps them establish and tolerate high-density populations. If lower transcription of *5HT3A* is associated with generally higher aggression, then round gobies at the invasion front would be more aggressive or possibly asocial, which has been suggested previously (Groen et al. 2012). We initially predicted that there would be higher transcription of *HBA2* (oxygen transport and stress response) at the invasion front to cope with the novelty and stressors in an unknown habitat. However, we found the opposite, where individuals at the invasion front have lower transcription of this gene. This might mean that leaving established areas also means leaving “enemies” which can include both conspecifics and predators, thus experiencing less stress and supporting the enemy release hypothesis (Colautti et al. 2004). In addition, there could be more opportunity for resources (e.g., food and shelter) at the invasion front (Brown et al. 2013). Both these genes support that density and social context may play a role lower transcription of these genes in the invading populations, where asocial individuals might experience density relief and lowered stress.

For the neuronal gene category, we found one gene to have a significant “detection time since North American invasion” effect; *KCNN2,* which codes for a potassium-calcium channel and is involved in regulating neuronal excitability which has been implicated in modulating memory and synaptic plasticity (Lin et al. 2008; Stackman et al. 2002; Vick et al. 2010). Overexpression of this gene has been shown to impair memory in transgenic rats (Hammond et al. 2006; Stackman et al. 2008). Furthermore, protein expression of *KCNN2* decreased after learning in rats (Brosh et al. 2008). We expected that individuals in the invasion front would face stressors and novel environments that would require them to be better learners (e.g., recognizing unfamiliar predator cues). For this neuronal gene, we expected that individuals would have lower transcription on the invasion front to facilitate neuronal excitability (learning and memory) in response to the novelty of the invasion front. Our results agree with our prediction and supports other studies that have examined learning in invasive species. For example, invasive crayfish showed longer retention of a learned association (goldfish odour to conspecifc alarm cue) compared to native crayfish (Hazlett et al. 2002). Higher learning ability at the invasion fronts may aid in exploiting novel resources or navigating in unknown territory (Roudez et al. 2008).

Environmental behavioural treatment gene transcription

Overall, we did not observe any “detection time since North American” effects on genes in our environmental behavioural challenge. This may have been due to several reasons: overall the round goby is phenotypically plastic and may thus acclimate well under novel conditions, such as our captive environment. We saw several genes responding to our captive environment challenge (Supplementary Table S3; Figure S1-3), but overall, populations responded in the same way (Supplementary Table S4), suggesting that when brought into a novel and captive environment round gobies, despite their origin, respond similarly. We observed on average the variance for the genes in the treatment group was lower than the natural group (Supplementary Table S4). Alternatively, different ecologically relevant challenges (e.g. predator cue, or conspecific alarm cue) may need to be used with the round goby to drive differences in gene transcription responses between newly invaded populations and established ones.

To summarize, we did find some evidence for a transcriptomic "invasion phenotype" based on the detection time since North American invasion. We found gene transcription differences at three genes among the populations within the resting gene transcription group, and these genes were related to behaviour-related genes such as aggression, boldness, and stress responsiveness, as well as neuronal excitability. We did not find any effect of detection time since North American invasion for any of the 24 genes in our treatment group. This potentially suggests that overall, the round goby is phenotypically plastic which may explain why they have become such a well-established invasive species (Pettit-Wade et al. 2015; Vincelli 2016; Wellband and Heath 2017). Indeed, the round goby and other invasive species have been shown to be plastic in their response to stressors (Lockwood et al. 2010; Wellband and Heath 2017).

We had expected for transcriptional profiles at the invasion front populations to be most distinct from the source population. In the literature, we often see several phenotypic traits showing divergence based on time since invasion or spatial distance (Philips et al. 2008). Since the source is the longest established, relaxed selection is expected to have occurred, allowing for more genetic variation to emerge and different phenotypes to persist. We might have not seen more genes differentially expressed in the natural gene transcription group due to not enough time for evolution and selection to act upon these genes to transcriptionally distinguish them; or perhaps round gobies exhibit plasticity where they can transcriptionally respond to environmental stimuli, but this response may be transient and not long-lasting (West-Eberhard 2003).

Conclusions

This study is one of few to have examined both a baseline and treatment transcriptional response to a novel environment in a species across the invasion stage and multiple rivers (Mueller et al. 2014). Our findings suggest that variation in gene transcription in targeted functional categories is diverse and may be due to a multitude of underlying factors – life-stage, plasticity, dispersal strategy (deliberate vs. not), and environmental heterogeneity. Nevertheless, gene transcription can be a useful tool in providing insight into an invasive species’ response to novel environments and how well they will cope and acclimate to these conditions.

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Tables

Table 3.1 Twenty-eight genes biologically relevant to invasive behaviours used in transcriptional profiling in round goby, divided into three gene ontologies, behavioural/stress response, neuronal and metabolism/activity and endogenous controls

Functional Gene Category	Symbol	Gene	Reference
Behavioural/stress response	5HT3A	5-hydroxytryptamine 3A receptor	Loveland et al 2014
	AVT	Arginine vasotocin	Sneddon et al. 2005 Thomson et al 2011
	FGFR1A	Fibroblast growth factor receptor 1a	Norton et al. 2011
	$GABA_A$	γ-Aminobutyric acid A receptor	Sneddon et al. 2005 Thomson et al 2011
	HBA2	Haemoglobin α2 subunit	Sneddon et al. 2005 Thomson et al. 2011
	MAOA	Monoamine oxidase	Shih et al. 1999
	POMC	Proopiomelanocortin	Sneddon et al. 2005 Thomson et al. 2011
	STRA6	Stimulated-retinoic acid 6	Sneddon et al. 2005 Thomson et al. 2011

Neuronal	CAMK2G	Calcium/Calmodulin Dependent Protein Kinase II Gamma	Sneddon et al. 2005 Thomson et al. 2011
	CAMK2N2	Calcium/calmodulin-dependent protein kinase II inhibitor 2	Rey et al. 2013
	C-FOS	c-fos	Okuyama et al. 2011
	DLG2	Disks large homolog 2	Rey et al. 2013
	GLRK	Glutamate receptor U1	Rey et al. 2013
	KCNN2	Potassium Calcium-Activated Channel Subfamily N Member 2	Rey et al. 2013
	NRG2	Neuregulin 2	Rey et al. 2013
Metabolism/Activity	CRY1	Cryptochrome 1	Rey et al. 2013
	CRY2	Cryptochrome 2	Rey et al. 2013
	FAM50A	Family With Sequence Similarity 50 Member A	Rey et al. 2013
	G6PD	Glucose -6-phosphatase dehydrogenase	Pandolfi et al. 1995
	GALC	Glactosylcermidase	Rey et al. 2013
	GYG1	Glycogenin 1	Rey et al. 2013
	HPRT1	Hypoxanthine phosphoribosyltransferase 1	Rey et al. 2013
	MID1IP1	MID1 interacting protein 1	Rey et al. 2013
	MSRA	Methionine sulfoxide reductase A	Rey et al. 2013

	PFKFB1	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4	Rey et al. 2013
	PRVB	Parvalbumin beta protein	Derome et al. 2006
Endogenous control	β-actin	Beta-actin	Zheng and Sun 2011
	EF1A	Elongation factor 1A	Olsvik et al. 2005

Table 3.2 Approximate year that the round goby was initially detected in the river mouth/upstream movement and the GPS coordinates that the round gobies were captured

Population	*Year detected/Upward movement*	*Time since North American Detection*	*Location coordinates*	*Distance from the river mouth*	*Reference*
St. Clair	1990	0	42°58'56.438"N 82°24'40.392"W 42.982344,-82.41122000000001	-	Jude et al. 1992
Ausable-Established	1998	8	43°13'58.9"N 81°54'07.5"W 43.233032, -81.902095	-	EDDMaps
Ausable-Invasion	2007	25	43°09'05.7"N 81°48'36.6"W 43.151574, -81.810181	22.5 km	Poos et al. 2010
Thames-Established	1998	8	42°19'3.396"N 82°27'11.7"W 42.317609,-82.453250	-	USGS
Thames-Invasion	2003	25	42°36'27.0"N 81°50'00.2"W 42.607500, -81.833389	110.6 km	Poos et al. 2010

Saugeen-Established	2004	14	44°30'04.1"N 81°22'16.4"W 44.501133, -81.371220	-	Bronnenhuber et al. 2011
Saugeen-Invasion	2006	19	44°30'25.4"N 81°20'18.8"W 44.507055, -81.338554	4.4 km	Bronnenhuber et al. 2011

Table 3.3 Linear mixed model summary of all 24 genes for natural gene transcription. Global model included detection time, life-stage, mass and life-stage × mass as fixed effects and seine effort a random effect. Dash (–) represent non-significant terms. **Bolded *** indicates significant P-values after Bonferroni correction.

Gene			**Estimate**	**SE**	**F**	**P**	**df**
5HT3A	Fixed	(Intercept)	8.14	0.25			
		Detection Time	0.05	0.02	9.43	**0.003***	1
		Life-stage (Juvenile)	-		-	-	1
		Mass	-	-	-	-	1
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-	1
	Random	Seine				-	
FGFR1A	Fixed	(Intercept)	5.1	0.19			
		Detection Time	0.001	0.01	0.0006	0.91	1
		Life-stage (Juvenile)	-	-	-	-	1
		Mass	-	-	-	-	1
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-	1
	Random	Seine				-	
GABAA	Fixed	(Intercept)	-0.26	0.16			
		Detection Time	0.03	0.01	5.97	0.02	1
		Life-stage (Juvenile)	-	-	-	-	1
		Mass	-	-	-	-	1
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-	1
	Random	Seine				-	
HBA2		(Intercept)	-3.32	0.33			

	Fixed	Detection Time	0.07	0.02	10.02	**0.002***	1
		Life-stage (Juvenile)	-	-	-	-	1
		Mass	-	-	-	-	1
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-	1
	Random	Seine				-	
POMC		(Intercept)	1.06	0.78			
	Fixed	Detection Time	0.06	0.05	1.26	0.26	1
		Life-stage (Juvenile)	-	-	-	-	1
		Mass	-	-	-	-	1
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-	1
	Random	Seine				-	
STRA6		(Intercept)	6.91	0.18			
	Fixed	Detection Time	0.02	0.01	1.92	0.17	1
		Life-stage (Juvenile)	-	-	-	-	1
		Mass	-	-	-	-	1
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-	1
	Random	Seine				-	
CAMK2G		(Intercept)	5.07	0.17			
	Fixed	Detection Time	0.01	0.011	1.4	0.24	1
		Life-stage (Juvenile)	-	-	-	-	1
		Mass	-	-	-	-	1
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-	1
	Random	Seine				-	
CAMK2N2		(Intercept)	0.07	0.45			

	Fixed	Detection Time	0.01	0.02	0.53	0.47	1
		Life-stage (Juvenile)	-	-	-	-	1
		Mass	-	-	-	-	1
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-	1
	Random	Seine				-	
CFOS		(Intercept)	7.86	0.33			
	Fixed	Detection Time	-0.01	0.02	0.27	0.6	1
		Life-stage (Juvenile)	-	-	-	-	1
		Mass	-	-	-	-	1
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-	1
	Random	Seine				-	
DLG2		(Intercept)	6.01	0.21			
	Fixed	Detection Time	0.02	0.01	2.81	0.1	1
		Life-stage (Juvenile)	-	-	-	-	1
		Mass	-	-	-	-	1
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-	1
	Random	Seine				-	
GLRK		(Intercept)	8.51	0.48			
	Fixed	Detection Time	0.02	0.03	0.45	0.5	1
		Life-stage (Juvenile)	-	-	-	-	1
		Mass	-	-	-	-	1
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-	1
	Random	Seine				-	
KCNN2		(Intercept)	8.17	0.2			

	Fixed	Detection Time	0.04	0.01	7.67	**0.007***	1
		Life-stage (Juvenile)	-	-	-	-	1
		Mass	-	-	-	-	1
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-	1
	Random	Seine				-	
NRG2		(Intercept)	7.94	0.23			
	Fixed	Detection Time	-	-	-	-	1
		Life-stage (Juvenile)	-	-	-	-	1
		Mass	-	-	-	-	1
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-	1
	Random	Seine					
CRY1		(Intercept)	3.92	0.18			
	Fixed	Detection Time	0.03	0.012	8.17	0.005	1
		Life-stage (Juvenile)	-	-	-	-	1
		Mass	-	-	-	-	1
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-	1
	Random	Seine				-	
CRY2		(Intercept)	4.87	0.25			
	Fixed	Detection Time	0.02	0.02	1.75	0.19	1
		Life-stage (Juvenile)	-	-	-	-	1
		Mass	-	-	-	-	1
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-	1
	Random	Seine				-	
FAM50A		(Intercept)	4.31	0.28			

	Fixed	Detection Time	0.00	0.02	0.00	0.95	1
		Life-stage (Juvenile)	-	-	-	-	1
		Mass	-	-	-	-	1
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-	1
	Random	Seine				-	
G6PD		(Intercept)	8.44	0.25			
	Fixed	Detection Time	0.02	0.01	0.97	0.33	1
		Life-stage (Juvenile)	-	-	-	-	1
		Mass	-	-	-	-	1
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-	1
	Random	Seine				-	
GALC		(Intercept)	6.56	0.19			
	Fixed	Detection Time	0.01	0.01	0.59	0.44	1
		Life-stage (Juvenile)	-	-	-	-	1
		Mass	-	-	-	-	1
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-	1
	Random	Seine				-	
GYG1		(Intercept)	5.21	0.21			
	Fixed	Detection Time	0.02	0.01	1.27	0.26	1
		Life-stage (Juvenile)	-	-	-	-	1
		Mass	-	-	-	-	1
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-	1
	Random	Seine				-	
HPRT1		(Intercept)	8.75	0.3			

	Fixed	Detection Time	-0.004	0.02	0.06	0.81	1
		Life-stage (Juvenile)	-	-	-	-	1
		Mass	-	-	-	-	1
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-	1
	Random	Seine				-	
MID1IP1	Fixed	(Intercept)	5.55	0.40			
		Detection Time	-0.02	0.02	0.59	0.44	1
		Life-stage (Juvenile)	-	-	-	-	1
		Mass	-	-	-	-	1
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-	1
	Random	Seine				-	
MSRA	Fixed	(Intercept)	4.6	0.18			
		Detection Time	-0.005	0.01	0.21	0.65	1
		Life-stage (Juvenile)	-	-	-	-	1
		Mass	-	-	-	-	1
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-	1
	Random	Seine				-	
PFKFB1	Fixed	(Intercept)	7.48	0.27			
		Detection Time	0.029	0.02	2.49	0.12	1
		Life-stage (Juvenile)	-	-	-	-	1
		Mass	-	-	-	-	1
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-	1
	Random	Seine				-	
PRVB		(Intercept)	7.41	0.46			

Fixed	Detection Time	-0.02	0.03	0.63	0.43	1
	Life-stage (Juvenile)	-	-	-	-	1
	Mass	-	-	-	-	1
	Life-stage x Mass (Juvenile:Mass)	-	-	-	-	1
Random	Seine				-	

Table 3.4 Linear mixed model summary of all 24 genes for treatment gene transcription. Global model included detection time, life-stage, mass and life-stage × mass as fixed effects and days in captivity, trial ID, holding tank ID and experimental tank ID as random effects. Dash (–) represent non-significant terms. **Bolded *** indicates significant P-values after Bonferroni correction.

Gene			**Estimate**	**SE**	**F**	**P**
5HT3A	Fixed	(Intercept)	8.39	0.23		
		Detection Time	0.004	0.01	0.09	0.76
		Life-stage (Juvenile)	-	-	-	-
		Mass	-	-	-	-
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-
	Random	Trial ID	Holding Tank ID	Experimental Tank ID	Days in Captivity	
	P	-	-	-	-	
FGFR1A	Fixed	(Intercept)	4.55	0.11		
		Detection Time	-0.007	0.006	1.23	0.27
		Life-stage (Juvenile)	-	-	-	-
		Mass	-	-	-	-
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-
	Random	Trial ID	Holding Tank ID	Experimental Tank ID	Days in Captivity	
	P	-	-	-	-	
GABAA	Fixed	(Intercept)	-0.31	0.11		
		Detection Time	-0.007	0.007	0.96	0.33

		Life-stage (Juvenile)	-	-	-	-
		Mass	-	-	-	-
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-
	Random	Trial ID	Holding Tank ID	Experimental Tank ID	Days in Captivity	
	P	-	-	-	-	
HBA2		(Intercept)	-2.04	0.17		
	Fixed	Detection Time	0.005	0.01	0.23	0.63
		Life-stage (Juvenile)	-	-	-	-
		Mass	-	-	-	-
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-
	Random	Trial ID	Holding Tank ID	Experimental Tank ID	Days in Captivity	
	P	-	-	-	-	
POMC		(Intercept)	2.08	0.65		
	Fixed	Detection Time	-0.03	0.04	0.71	0.4
		Life-stage (Juvenile)	-	-	-	-
		Mass	-	-	-	-
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-
	Random	Trial ID	Holding Tank ID	Experimental Tank ID	Days in Captivity	
	P	-	-	-	-	
STRA6		(Intercept)	7.2	0.14		
	Fixed	Detection Time	0.007	0.008	0.82	0.37
		Life-stage (Juvenile)	-	-	-	-
		Mass	-	-	-	-

		Life-stage x Mass (Juvenile:Mass)	-	-	-	-
	Random	Trial ID	Holding Tank ID	Experimental Tank ID	Days in Captivity	
	P	-	-	-	-	
CAMK2G	Fixed	(Intercept)	4.65	0.26		
		Detection Time	0.002	0.01	0.02	0.9
		Life-stage (Juvenile)	-	-	-	-
		Mass	-	-	-	-
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-
	Random	Trial ID	Holding Tank ID	Experimental Tank ID	Days in Captivity	
	P	-	-	-	-	
CAMK2N2	Fixed	(Intercept)	-0.32	0.12		
		Detection Time	-0.01	0.008	2.7	0.1
		Life-stage (Juvenile)	-	-	-	-
		Mass	-	-	-	-
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-
	Random	Trial ID	Holding Tank ID	Experimental Tank ID	Days in Captivity	
	P	-	-	-	-	
CFOS	Fixed	(Intercept)	7.18	0.28		
		Detection Time	0.01	0.02	0.61	0.43
		Life-stage (Juvenile)	-	-	-	-
		Mass	-	-	-	-
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-

	Random	Trial ID	Holding Tank ID	Experimental Tank ID	Days in Captivity	
	P	-	-	-	-	
DLG2		(Intercept)	6.2	0.37		
	Fixed	Detection Time	0.01	0.02	0.24	0.63
		Life-stage (Juvenile)	-	-	-	-
		Mass	-	-	-	-
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-
	Random	Trial ID	Holding Tank ID	Experimental Tank ID	Days in Captivity	
		-	-	-	-	
GLRK		(Intercept)	9.04	0.27		
	Fixed	Detection Time	-0.001	0.02	0.01	0.91
		Life-stage (Juvenile)	-	-	-	-
		Mass	-	-	-	-
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-
	Random	Trial ID	Holding Tank ID	Experimental Tank ID	Days in Captivity	
	P	-	-	-	-	
KCNN2		(Intercept)	8.53	0.17		
	Fixed	Detection Time	-0.01	0.01	0.91	0.34
		Life-stage (Juvenile)	-	-	-	-
		Mass	-	-	-	-
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-
	Random	Trial ID	Holding Tank ID	Experimental Tank ID	Days in Captivity	
	P	-	-	-	-	

		(Intercept)	7.72	0.23		
	Fixed	Detection Time	-0.01	0.01	0.26	0.61
		Life-stage (Juvenile)	-	-	-	-
NRG2		Mass	-	-	-	-
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-
	Random	Trial ID	Holding Tank ID	Experimental Tank ID	Days in Captivity	
	P	-	-	-	-	
		(Intercept)	3.96	0.13		
	Fixed	Detection Time	-0.02	0.008	6.78	0.01
		Life-stage (Juvenile)	-	-	-	-
CRY1		Mass	-	-	-	-
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-
	Random	Trial ID	Holding Tank ID	Experimental Tank ID	Days in Captivity	
	P	-	-	-	-	
		(Intercept)	5.13	0.32		
	Fixed	Detection Time	-0.02	0.01	1.15	0.12
		Life-stage (Juvenile)	1.37	0.53	6.71	0.01
CRY2		Mass	0.04	0.03	4.64	0.03
		Life-stage x Mass (Juvenile:Mass)	-0.72	0.3	5.99	0.02
	Random	Trial ID	Holding Tank ID	Experimental Tank ID	Days in Captivity	
	P	-	-	-	-	
FAM50A		(Intercept)	4.57	0.42		
	Fixed	Detection Time	-0.01	0.02	0.08	0.77

		Life-stage (Juvenile)	-	-	-	-
		Mass	-	-	-	-
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-
	Random	Trial ID	Holding Tank ID	Experimental Tank ID	Days in Captivity	
	P	-	-	-	-	
G6PD		(Intercept)	8.71	0.17		
	Fixed	Detection Time	-0.02	0.01	2.4	0.12
		Life-stage (Juvenile)	-	-	-	-
		Mass	-	-	-	-
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-
	Random	Trial ID	Holding Tank ID	Experimental Tank ID	Days in Captivity	
	P	-	-	-	-	
GALC		(Intercept)	6.39	0.15		
	Fixed	Detection Time	-0.003	0.009	0.09	0.76
		Life-stage (Juvenile)	-	-	-	-
		Mass	-	-	-	-
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-
	Random	Trial ID	Holding Tank ID	Experimental Tank ID	Days in Captivity	
	P	-	-	-	-	
GYG1		(Intercept)	5.22	0.18		
	Fixed	Detection Time	-0.008	0.011	0.59	0.44
		Life-stage (Juvenile)	-	-	-	-
		Mass	-	-	-	-

		Life-stage x Mass (Juvenile:Mass)	-	-	-	-
	Random	Trial ID	Holding Tank ID	Experimental Tank ID	Days in Captivity	
	P	-	-	-	-	
HPRT1	Fixed	(Intercept)	9	0.24		
		Detection Time	-0.01	0.01	0.88	0.35
		Life-stage (Juvenile)	-	-	-	-
		Mass	-	-	-	-
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-
	Random	Trial ID	Holding Tank ID	Experimental Tank ID	Days in Captivity	
	P	-	-	-	-	
MID1IP1	Fixed	(Intercept)	4.52	0.35		
		Detection Time	-0.01	0.02	0.28	0.6
		Life-stage (Juvenile)	2.15	0.71	9.07	-
		Mass	0	0.04	12.64	0.001
		Life-stage x Mass (Juvenile:Mass)	-1.42	0.4	12.71	0.0005
	Random	Trial ID	Holding Tank ID	Experimental Tank ID	Days in Captivity	
	P	-	-	-	-	
MSRA	Fixed	(Intercept)	4.34	0.13		
		Detection Time	0.003	0.008	0.17	0.68
		Life-stage (Juvenile)	-	-	-	-
		Mass	-	-	-	-
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-

	Random	Trial ID	Holding Tank ID	Experimental Tank ID	Days in Captivity	
	P	-	-	-	-	
PFKFB1		(Intercept)	7.82	0.27		
	Fixed	Detection Time	-0.02	0.02	1.56	0.21
		Life-stage (Juvenile)	-	-	-	-
		Mass	-	-	-	-
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-
	Random	Trial ID	Holding Tank ID	Experimental Tank ID	Days in Captivity	
	P	-	-	-	-	
PRVB		(Intercept)	7.83	0.31		
	Fixed	Detection Time	-0.02	0.02	1.52	0.22
		Life-stage (Juvenile)	-	-	-	-
		Mass	-	-	-	-
		Life-stage x Mass (Juvenile:Mass)	-	-	-	-
	Random	Trial ID	Holding Tank ID	Experimental Tank ID	Days in Captivity	
	P	-	-	-	-	

Figures

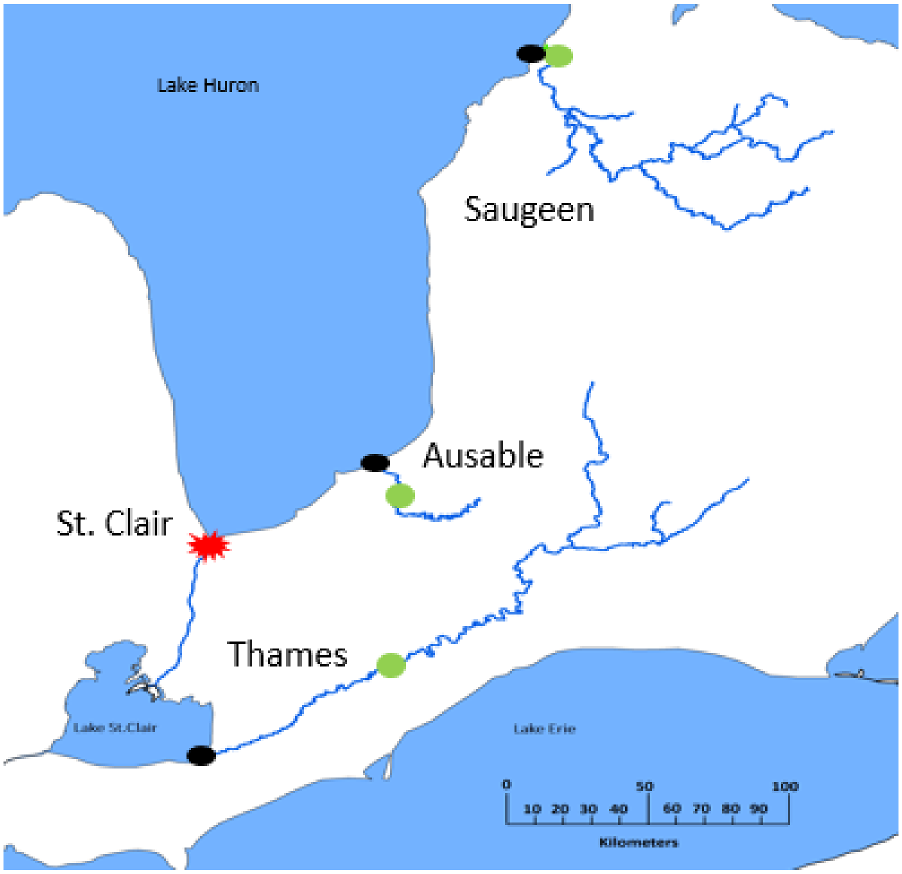
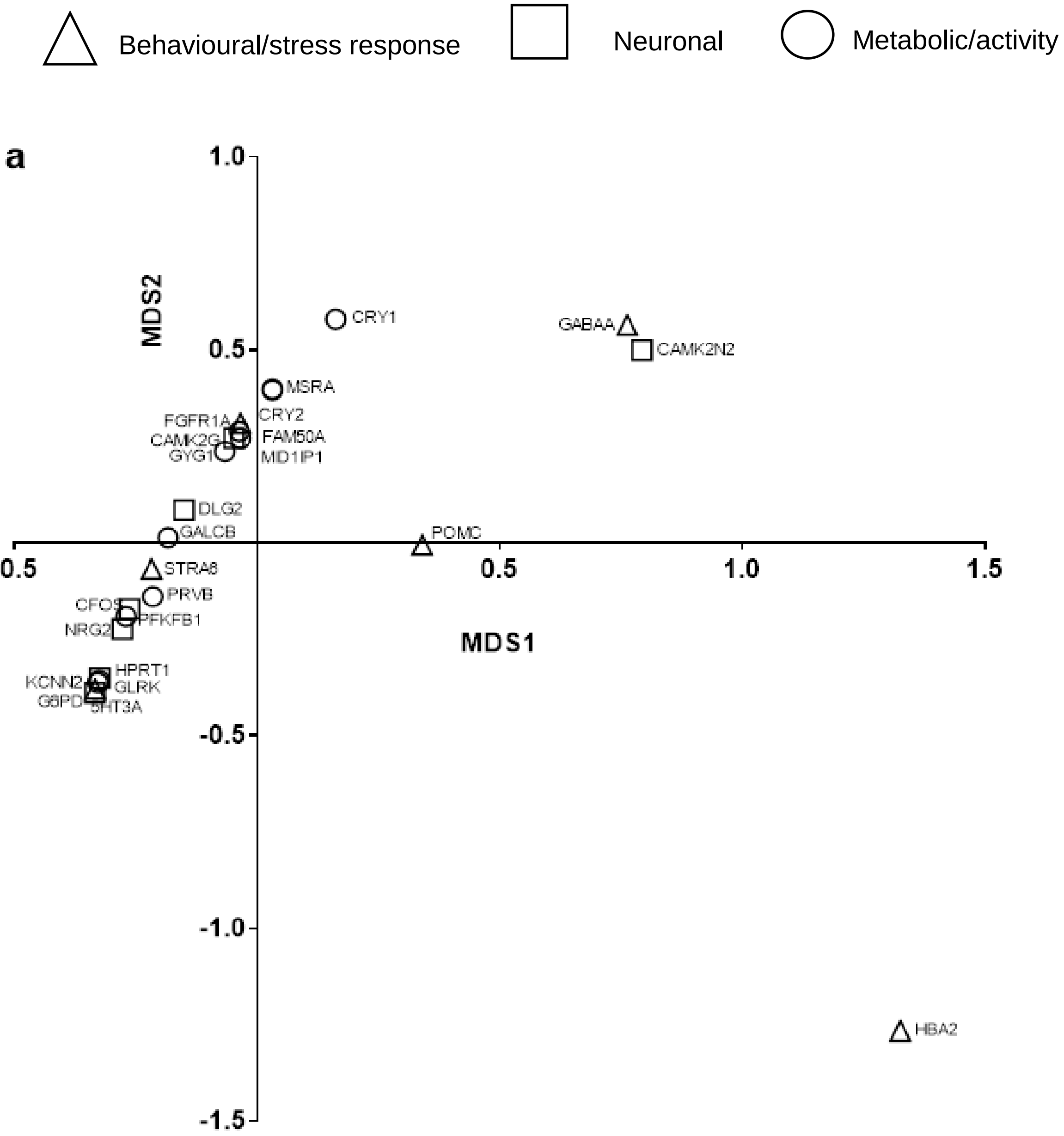


Figure 3.1 Map of sampling locations in Ontario, St. Clair (source), Thames-Est, Thames-Inv, Ausable-Est, Ausable-Inv, Saugeen-Est, and Saugeen-Inv. Est=Established, Inv=Invasion. Black represent the established sites at the river mouth and the green represent the invasion stage populations. The red represents the source population (St. Clair River).



Behavioural/stress response
Neuronal
Metabolic/activity
a
MDS2
MDS1
1.0
0.5
-0.5
-1.0
-1.5
-0.5
0.5
1.0
1.5
CRY1
GABAA
CAMK2N2
MSRA
FGFR1A
CRY2
CAMK2G
FAM50A
GYG1
MD1P1
DLG2
GALCB
POMC
STRA8
PRVB
CFOS
PFKFB1
NRG2
HPRT1
KCNN2
GLRK
G6PD
5HT3A
HBA2

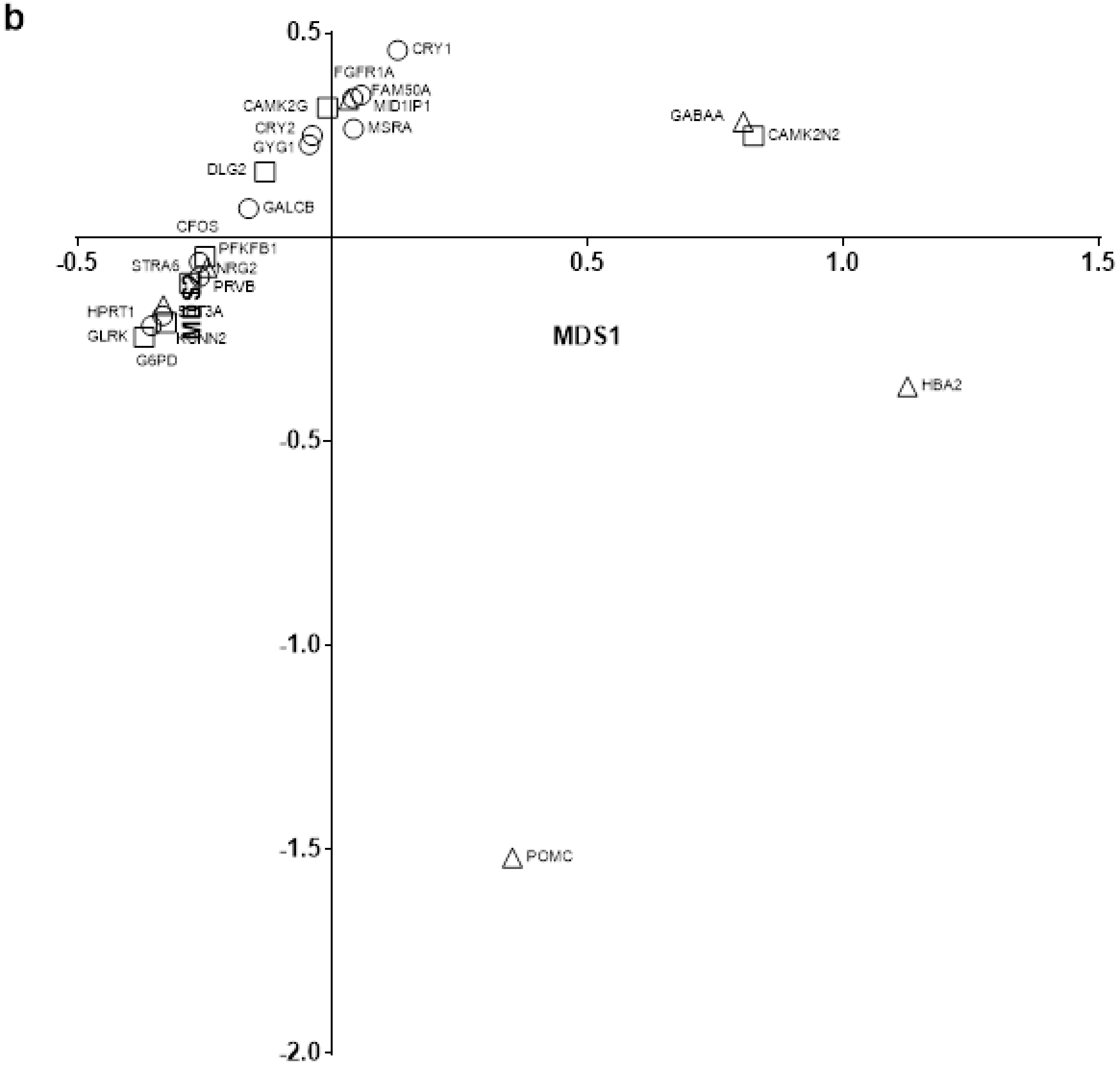


Figure 3.2 (a-b) Principal coordinate scatter plot of all ΔC_T of all 24 genes to examine functional clustering (a) natural populations (b) environmental behavioural treated populations

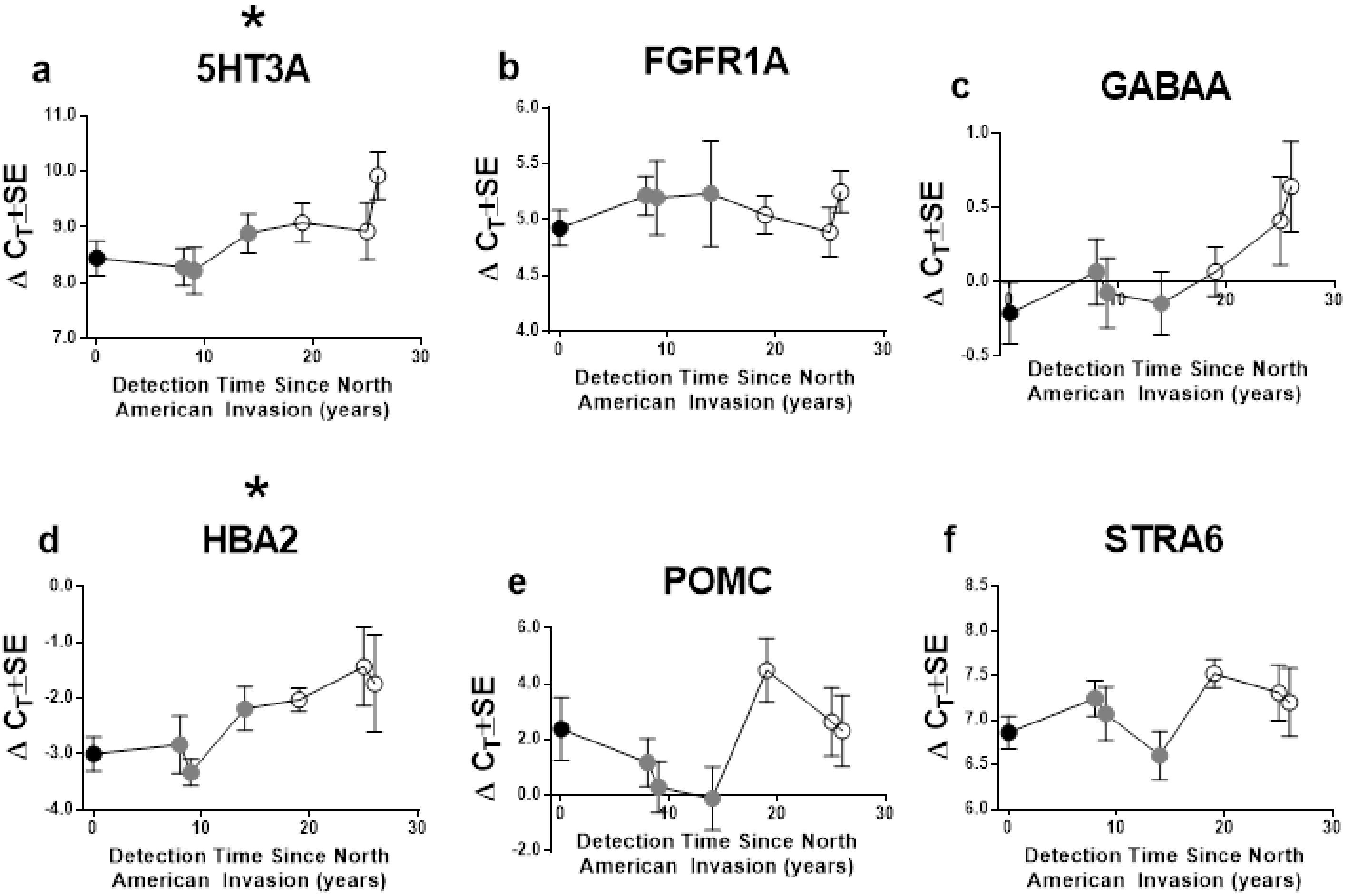


Figure 3.3 (a-f) Scatterplots of natural gene transcription for behavioural/stress response genes mean ΔC_T (±SE) examining the effect detection time since North American invasion

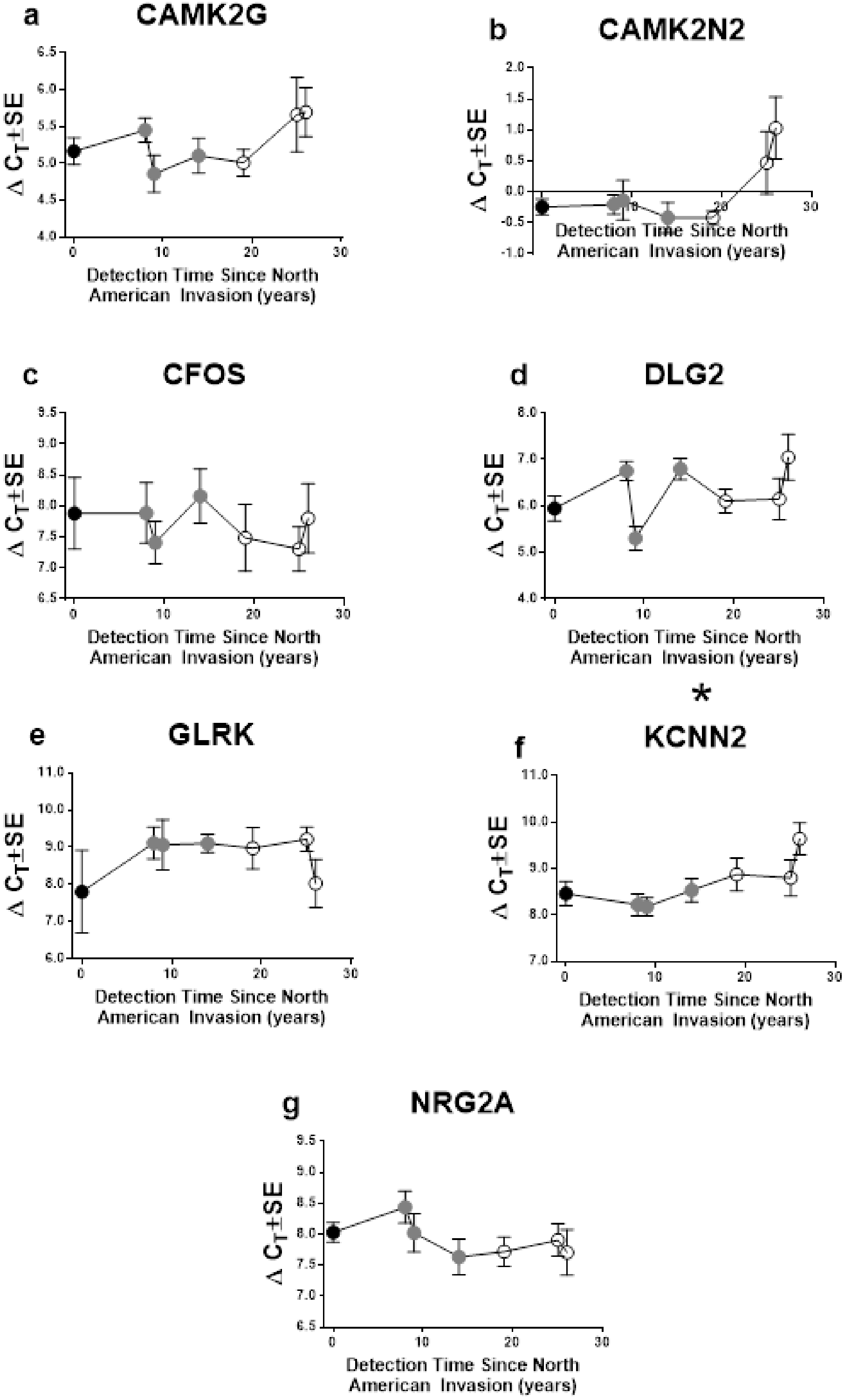
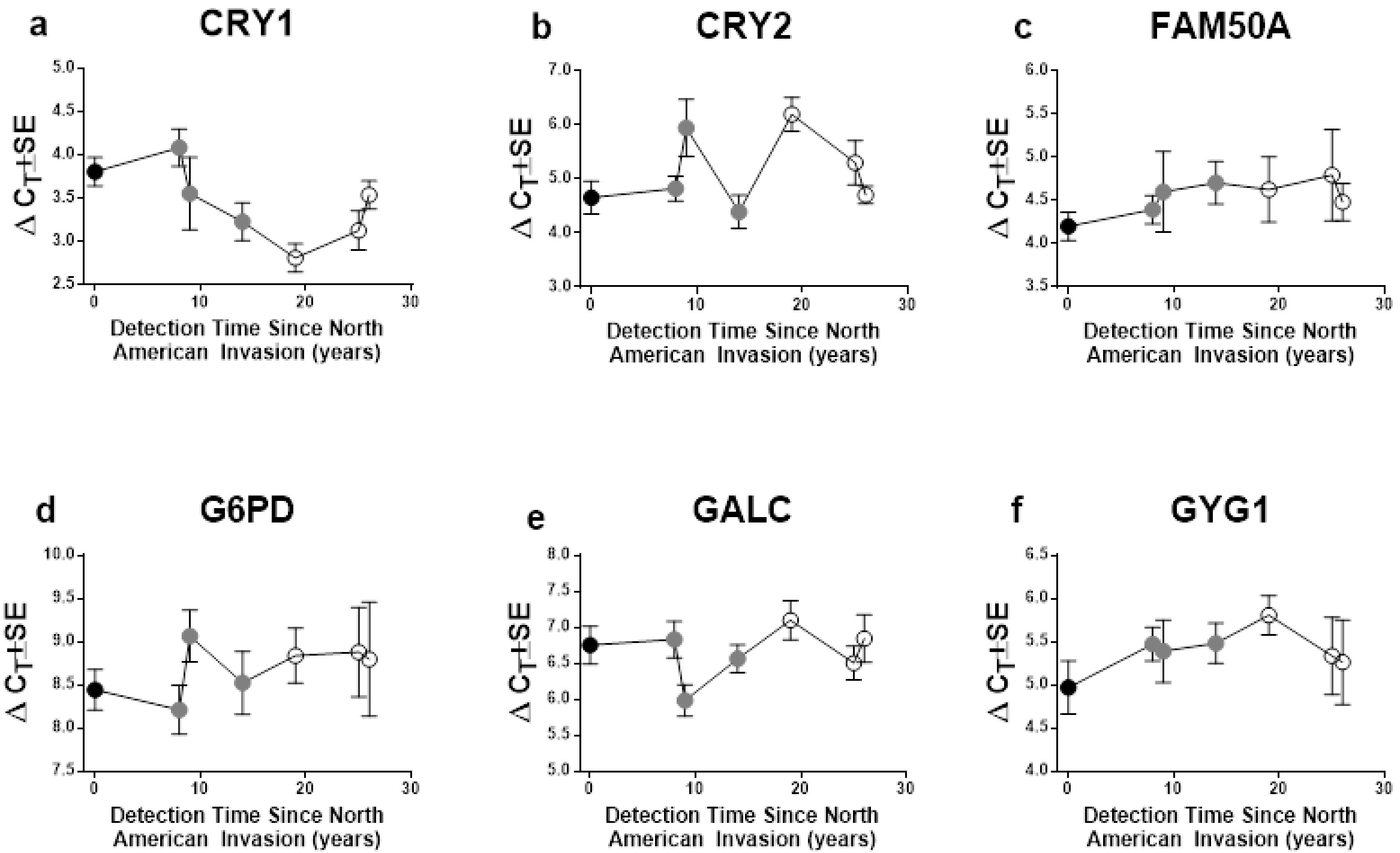


Figure 3.4 (a-g) Scatterplots of natural gene transcription for neuronal genes mean ΔC_T (±SE) examining the effect detection time since North American invasion


a
CRY1
5.0
4.5
4.0
3.5
3.0
2.5
Δ C_T±SE
0
10
20
30
Detection Time Since North American Invasion (years)
b
CRY2
7.0
6.0
5.0
4.0
3.0
Δ C_T±SE
0
10
20
30
Detection Time Since North American Invasion (years)
c
FAM50A
6.0
5.5
5.0
4.5
4.0
3.5
Δ C_T±SE
0
10
20
30
Detection Time Since North American Invasion (years)
d
G6PD
10.0
9.5
9.0
8.5
8.0
7.5
Δ C_T±SE
0
10
20
30
Detection Time Since North American Invasion (years)
e
GALC
8.0
7.5
7.0
6.5
6.0
5.5
5.0
Δ C_T±SE
0
10
20
30
Detection Time Since North American Invasion (years)
f
GYG1
6.5
6.0
5.5
5.0
4.5
4.0
Δ C_T±SE
0
10
20
30
Detection Time Since North American Invasion (years)

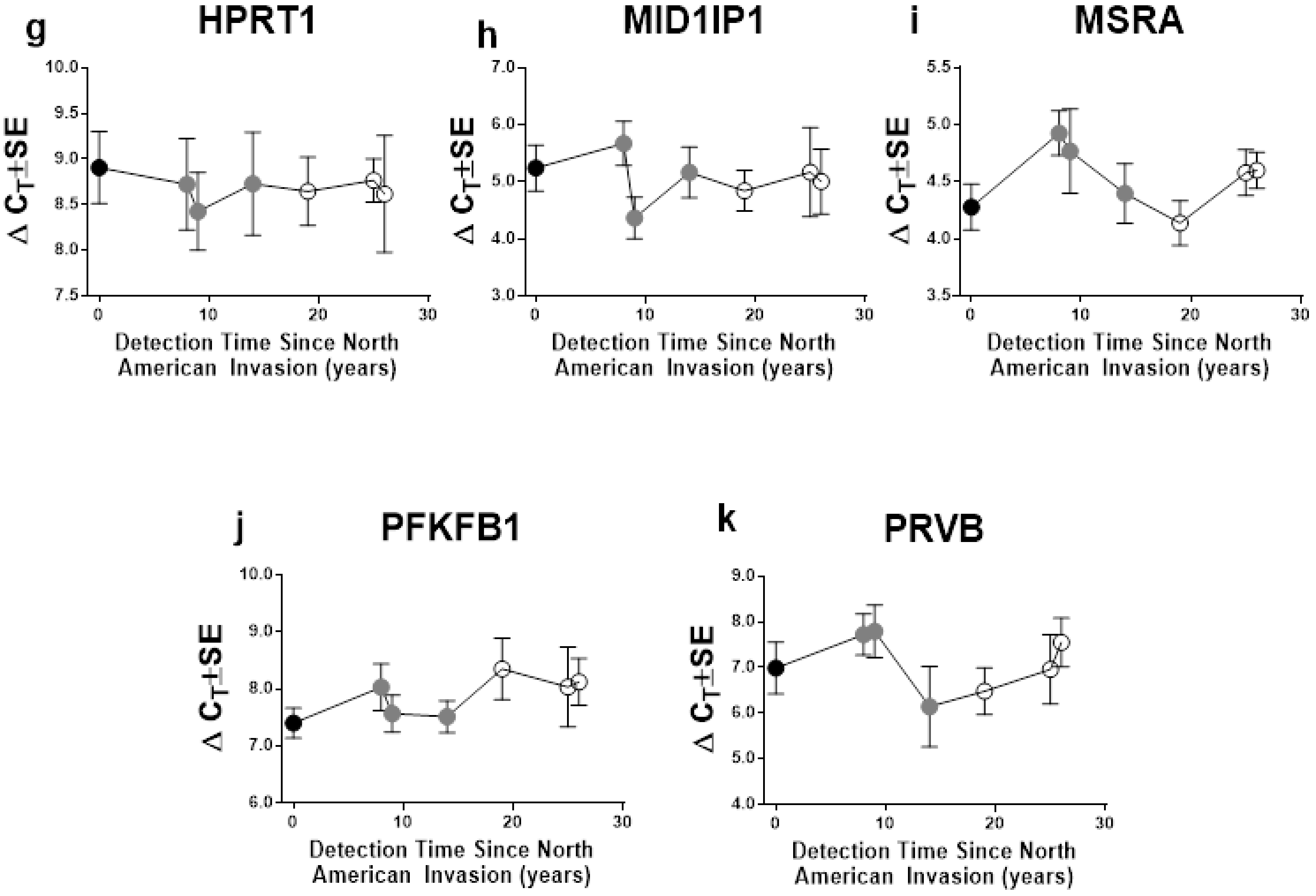


Figure 3.5 (a-k) Scatterplots of natural gene transcription for metabolic/activity genes mean ΔC_T (±SE) examining the effect detection time since North American invasion

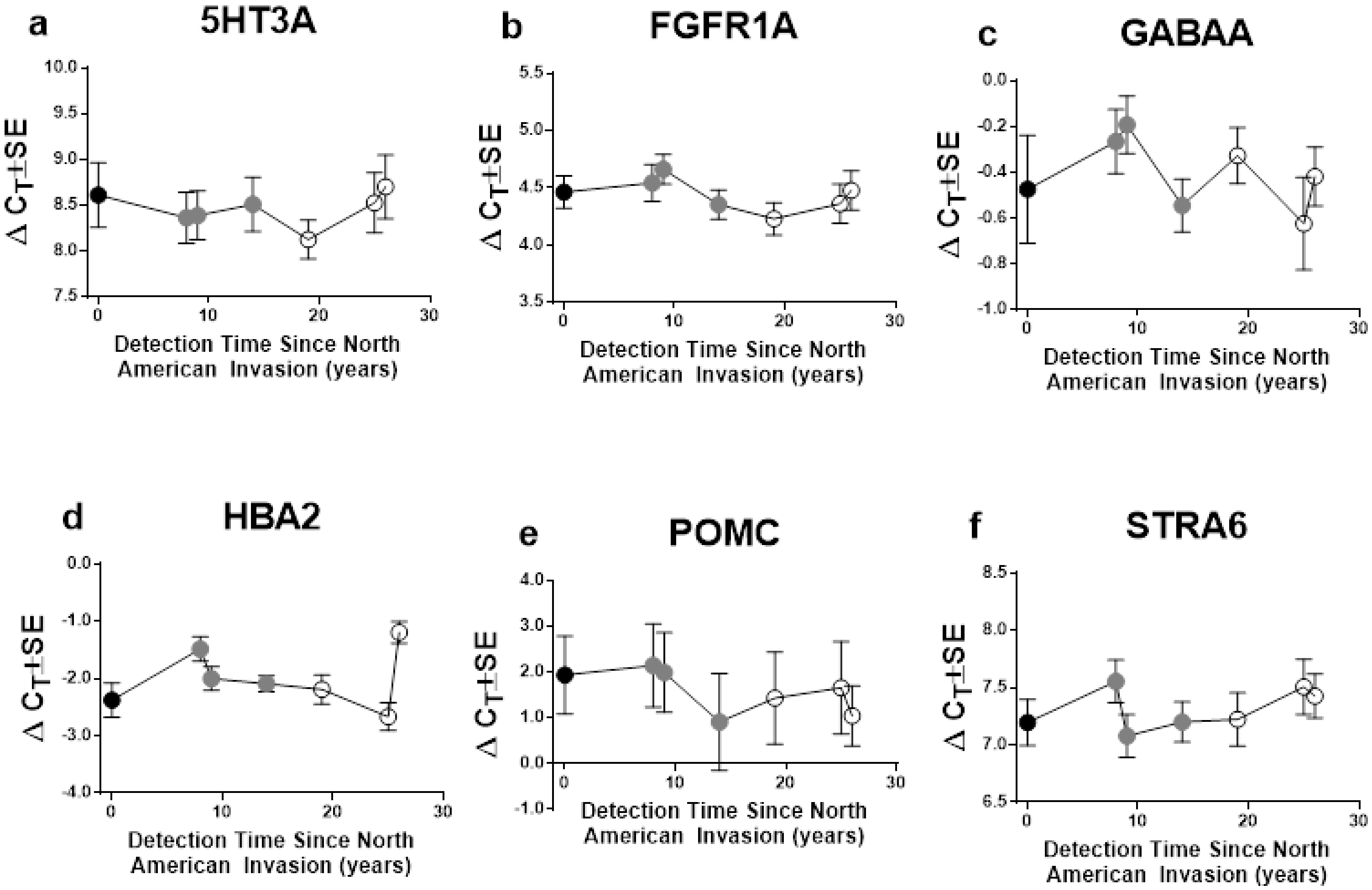


Figure 3.6 (a-f) Scatterplots of treatment gene transcription for behavioural/stress response genes mean ΔC_T (±SE) examining the effect detection time since North American invasion

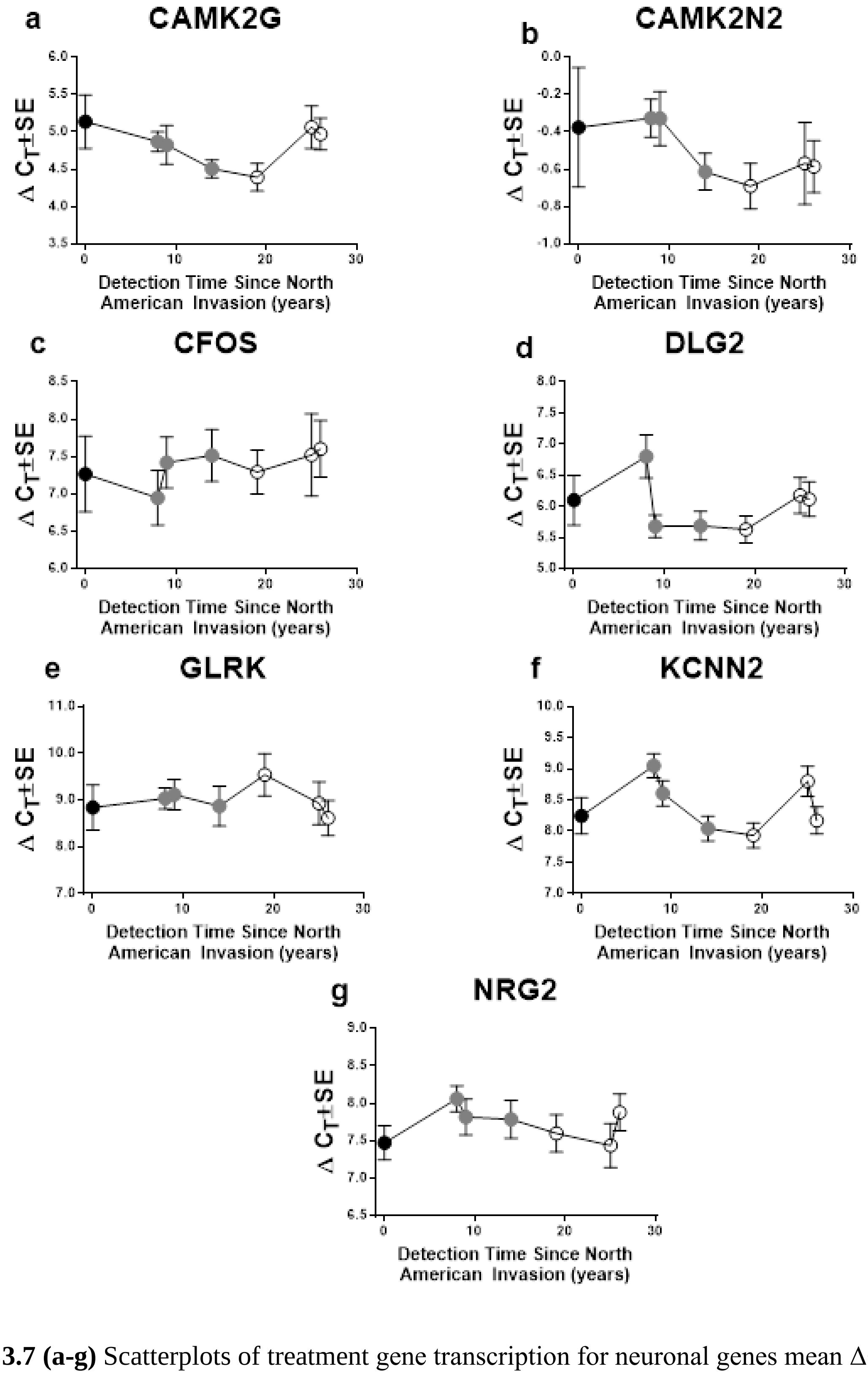
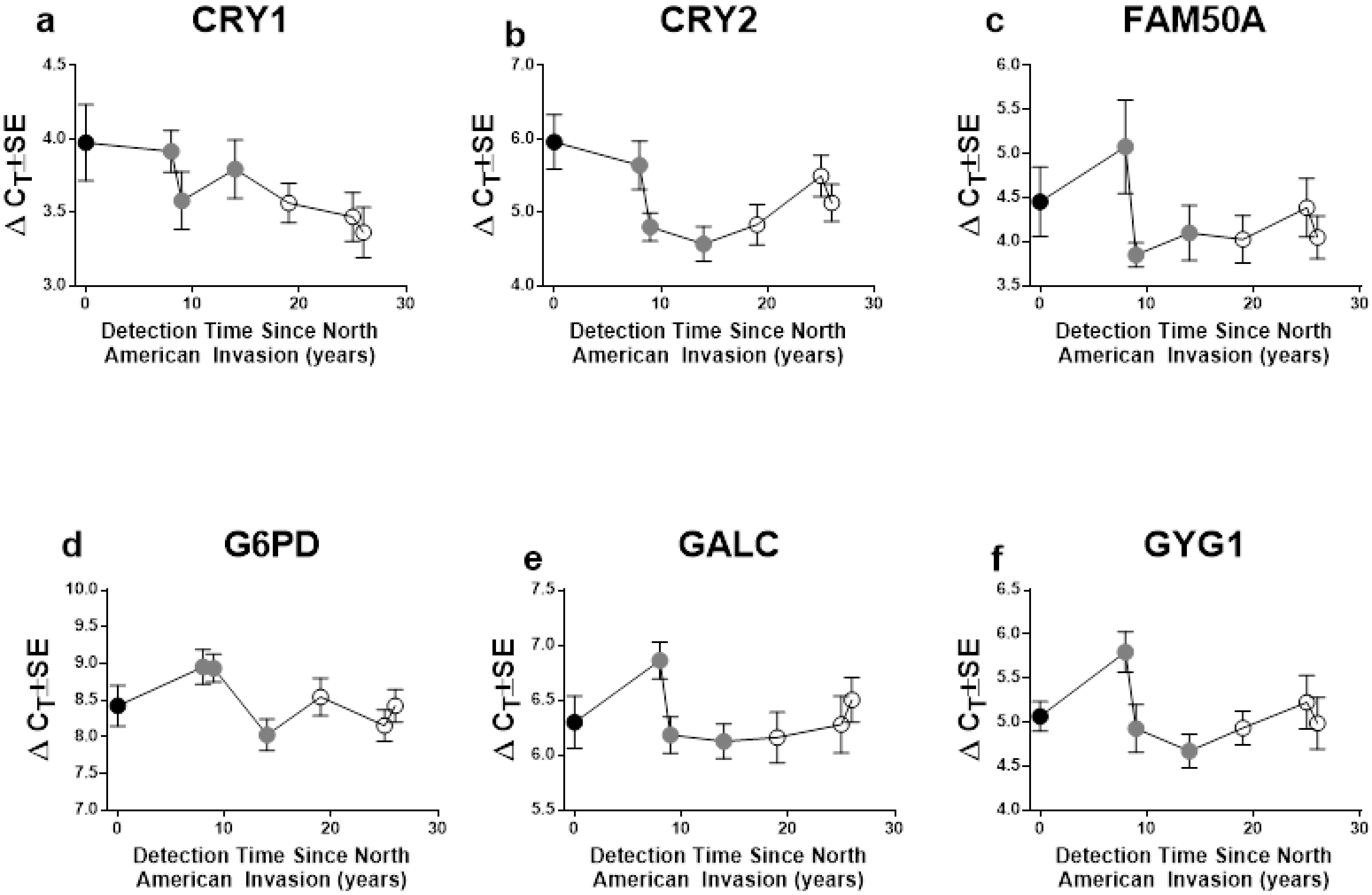


Figure 3.7 (a-g) Scatterplots of treatment gene transcription for neuronal genes mean ΔC_T (±SE) examining the effect detection time since North American invasion


a
CRY1
4.5
4.0
3.5
3.0
0
10
20
30
Δ C_T±SE
Detection Time Since North American Invasion (years)
b
CRY2
7.0
6.0
5.0
4.0
0
10
20
30
Δ C_T±SE
Detection Time Since North American Invasion (years)
c
FAM50A
6.0
5.5
5.0
4.5
4.0
3.5
0
10
20
30
Δ C_T±SE
Detection Time Since North American Invasion (years)
d
G6PD
10.0
9.5
9.0
8.5
8.0
7.5
7.0
0
10
20
30
Δ C_T±SE
Detection Time Since North American Invasion (years)
e
GALC
7.5
7.0
6.5
6.0
5.5
0
10
20
30
Δ C_T±SE
Detection Time Since North American Invasion (years)
f
GYG1
6.5
6.0
5.5
5.0
4.5
4.0
0
10
20
30
Δ C_T±SE
Detection Time Since North American Invasion (years)

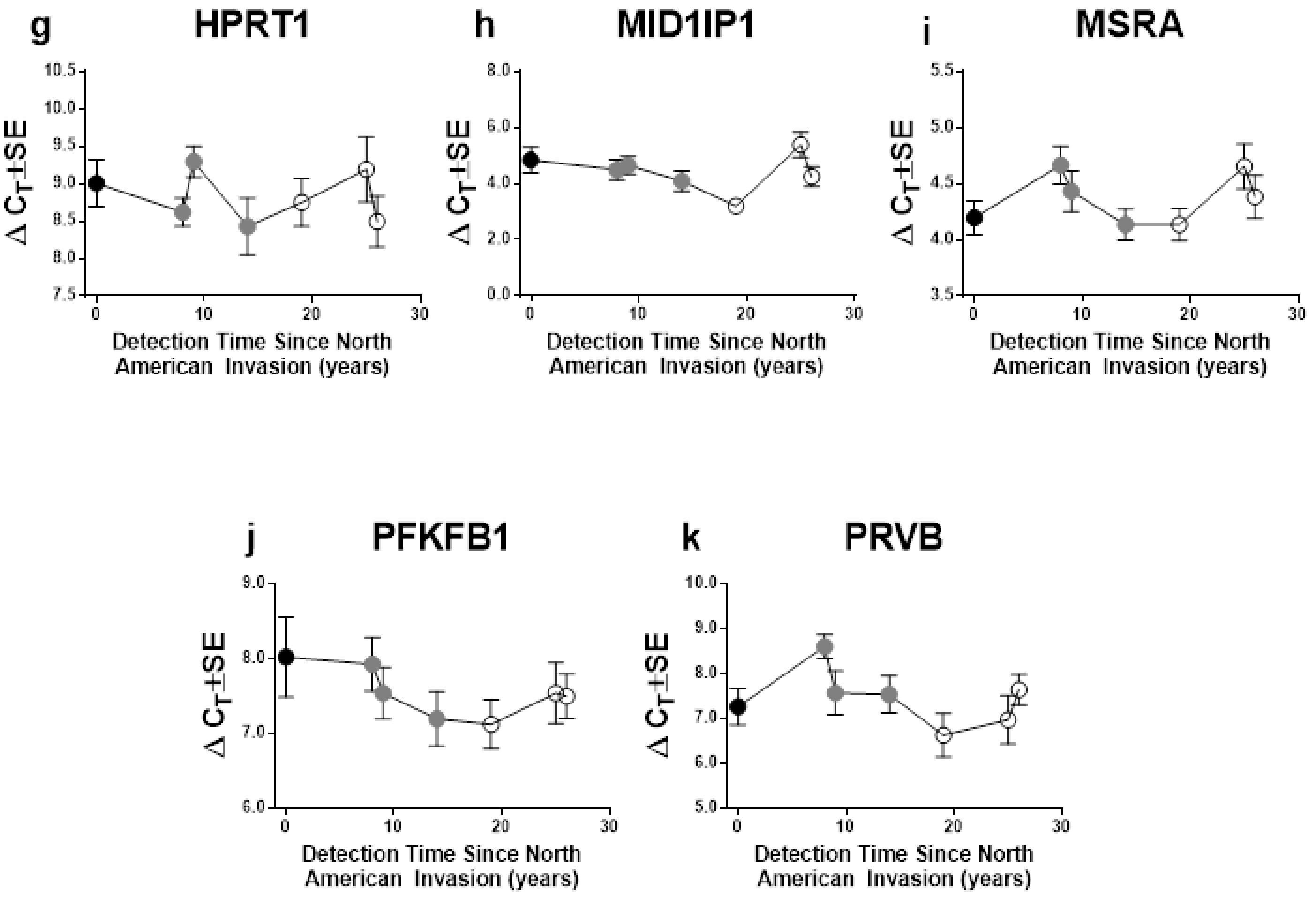


Figure 3.8 (a-k) Scatterplots of treatment gene transcription for metabolic/activity genes mean ΔC_T (±SE) examining the effect detection time since North American invasion

Supplementary information

Table S1 Forward and reverse primer, and probe sequences for 28 genes designed for quantitative real-time PCR for Taqman OpenArray. Amplicon size 60-250 bp

Gene	*Forward (5´ – 3´)*	*Reverse (5´ – 3´)*	*Probe*	*Size (bp)*
HBA2	GTTCGCATGCTACCCTCAGACCAAG	TCAAGCAGCCCTTTGTTCATGTCGT	TGGACTGATGTCTCTTACG	153
GABAA	GCTACCATGGGATTGTTGTACC	ACACTCTCATCACTGTAGGCAATG	ACAGGAGCATCATGAAGA	151
AVT	AAAGGGAGAGTAACGATGCAG	TGGAAGCGAACATGATGAAGA	TGATGATGACCAACGACCA	97
STRA6	TTTTCTACAATGTGGTGATGGGAAT	GATCATTCCAACCCATGTACGATAA	ATCAGCTTCATGTTGGGAA	158
POMC	TGTGGAAGTGTCTCCCTCTGA	CTTCTTCTTCTGCTGCGGGA	TGAGACGCGCCTTGT	127
FGFR1A	ACGGAACAGAAATAGTACGGCTTTT	GTCTGTCCTTTTATCCCAACTCGTT	ACAATCCGCACGTTCT	166
5HT3A	CTGTTCATATCAGTCAGCTTCATCA	TTCACAGCACAATTAACATTTCCAC	CCGGGCTTCATGAGTT	237
MAOA	GACTGTTCCTGCTATTATGGGATT	TCTCCTCATAGTGCACAGGATG	AGGCTGAAGAGGATCT	152
CAMK2G	GATCAGTGACTTCGGTCTCTCCAAA	CGGTCTCTTCGTAGAAAGGAGGGTA	CGTGGACTGTTGGTCCA	188
CAMK2N2	AATAGATGAGGTACTGAAGGGGATG	CTTCAAATCGGTGATCAAAATTAAA	AAACCTGACAGATGCC	101
CFOS	CCACAAACTCTAATGCTTCCTTCTA	TGA CTA TCG CTG CAA ATA CAA TAC A	AGCCCTGTGATCTGC	249

DLG2	GGACTGTACATAGCAAACAGAGAAA	CTTCTCATAGTCAAATAAGGCCCTC	TGCGGGAACAGATGATGA	223
GLRK	TTTTAATACCCCAGCAGATTTGTCA	ATCATGATTATTACCTGCCCAAGAC	TGCCGGACACTGACTT	247
KCNN2	GACAGATTTCTACTCATCTCAGCAG	CAT CCC ACA GAC TAA AGC ATA ATC A	TGCAGCTTCTGGAGGAC	223
NRG2	GCTTTGAAAGTTTACAGGATTGCTT	TTCAGCTCAGGTCTAACACACTATA	TTTCTGGAAACAACAGTGCC	152
CRY1	GTCCTGTCCACCTTAACTTGTTAAT	TTATGAGCTGTTGAACTACTTTGCT	TCCACACGATATCATG	167
CRY2	AAATGTGTGAGGACCAGTTAGTTAC	CATTGACTGACAGGAACATTTATGC	TGCACAAAGAGCTGGAA	231
FAM50A	CCAATATTGACAAGAAATTCTCGGC	GCAATTTTCCTTTTCTGCTCTTCTT	AGCGGGAGAAGCA	226
G6PD	TGGTGCAGCAGCTCCTTAAA	CGGACCTGCATGTCTGTTGT	AGGAGGATGTTCTTTC	58
GALC	CTGGTTAACTACGATGAGCCTTATC	TTCCTCTTCTTTGCTTCTTTCATGA	TTCCGTGGCTATGAGTG	212
GYG1	TTTGTTTAGGATTTTAACACGCCAC	AATGAGCCACAAGAATACTGAAGAA	ACCCACTGAAGACATAAG	192
HBA2	GTTCGCATGCTACCCTCAGACCAAG	TCAAGCAGCCCTTTGTTCATGTCGT	TGGACTGATGTCTCTTACG	153
HPRT1	AAAGGCAGTGTTTCTCAGCTATTTA	TTTGTAATCCAGGAATAGACGCAAA	AGTAAAGCCAAACTAAAGAC	153
MID1IP1	ACCACTCTGACATTTGTAATTGAGT	AAAAGGACAGACTAACAAGCATTCA	TGACGCCTGCATGTT	234
MSRA	CACTTGGTCAACACTCGGATG	GAAACAGCCCATACCGAACATG	AGCATCAAAGTCAGCG	229
PFKFB1	CAATCAACCAGTGCTATATCCATGA	AGTCCTCTCATTAGAAGTCAGAGAC	CATGAGGCTGCACAAC	186

PRVB	CTGCAAGATACAGTAATCATCAGCT	ATGTACAATTTGCACGTGTAATTCC	TGCCTGCGGACTGT	154
BACTIN	GGAGCGTGGCTACTCCTTCAC	TCCTTGATGTCACGGACAATTT	ACCACAGCCGAGAGG	60
EF1A	AACCCCAAAGCCCTGAAGTC	TTTCCCGGGACCATGGT	AGACGCCGCCATC	62

Table S2 (a-b) Summary of mean (±SE) body morphometrics of round goby collected at each population for **A**-natural gene transcription **B**-treatment gene transcription (mass, total length, standard length and body depth). Est = established; Inv = invasion front

a

Population	**N**	**Mass (g)**	**Total Length(mm)**	**Standard Length(mm)**	**Body Depth(mm)**
St. Clair-Source	20	4.07±0.35	66.55±2.05	53.23±1.58	12.04±0.33
Thames-Est	26	3.23±0.31	62.82±1.74	49.89±1.43	11.51±0.36
Thames-Inv	15	5.72±1.55	65.45±7.34	52.71±6.05	12.41±1.47
Ausable-Est	20	3.93±0.74	64.23±3.87	50.88±3.06	12.34±0.85
Ausable-Inv	12	7.45±2.61	72.04±9.12	57.08±7.23	13.34±1.85
Saugeen-Est	19	5.47±0.82	73.66±3.40	58.82±2.78	16.37±3.41
Saugeen-Inv	22	3.14±0.44	61.90±2.74	48.80±2.19	11.01±0.51

b

Population	**N**	**Mass (g)**	**Total Length(mm)**	**Standard Length(mm)**	**Body Depth(mm)**
St. Clair-Source	27	3.18±0.45	58.87±3.23	48.78±2.74	10.79±0.64
Thames-Est	32	3.06±0.31	61.47±1.81	50.22±1.46	10.68±0.32
Thames-Inv	27	5.90±0.99	70.79±4.22	57.82±3.37	12.41±0.85
Ausable-Est	31	4.37±0.74	65.31±3.35	52.56±2.78	11.07±0.64
Ausable-Inv	25	5.08±1.61	63.43±5.30	50.82±4.32	10.95±1.05
Saugeen-Est	28	4.24±0.46	70.32±2.71	58.28±2.20	12.36±0.52
Saugeen-Inv	24	3.34±0.37	63.49±2.12	50.82±1.67	11.16±0.40

Table S3 Summary of results (p-values) from standard least squares model to examine treatment (natural and treatment gene transcription), population and treatment × population effects. Bolded *, **, and *** represent P-values that were <0.05, <0.01, and <0.001 respectively

	Gene	**Treatment**	**Treatment × Population**	**Population**
Behavioural/stress response	5HT3A	**0.048***	0.08	0.29
	FGFR1A	**<0.0001*****	0.94	0.64
	GABA$_{A}$	**<0.0001*****	0.12	0.26
	HBA2	**0.038***	**0.002****	**0.008****
	POMC	0.63	0.26	0.25
	STRA6	0.09	0.47	0.16
Neuronal	CAMK2G	**0.00082*****	0.51	0.036*
	CAMK2N2	**<0.0001*****	**0.003****	**0.01***
	C-FOS	0.19	0.88	0.90
	DLG2	0.10	0.09	**0.0003****
	GLRK	0.43	0.87	0.46
	KCNN2	0.06	**0.0003*****	0.15
	NRG2	0.18	0.80	0.24
Metabolic/activity	CRY1	0.05	0.23	**0.0005*****
	CRY2	0.74	**<0.0001*****	**0.016***
	FAM50A	0.15	0.27	0.74
	G6PD	0.24	0.20	0.29
	GALC	**0.02***	0.22	**0.021***
	GYG1	0.06	0.17	0.24
	HPRT1	0.57	0.77	0.93
	MID1IP1	**0.006****	0.20	0.06
	MSRA	0.19	0.95	**0.008****
	PFKFB1	0.15	0.45	0.79
	PRVB	0.15	0.64	**0.02***

Table S4 ΔC_T average variances for all 24 genes for natural and treatment gene transcription

	Gene	**Natural**	**Treatment**
Behavioural/stress response	5HT3A	1.47	1.47
	FGFR1A	1.36	0.62
	$GABA_A$	1.03	0.67
	HBA2	4.36	1.55
	POMC	20.88	21.21
	STRA6	1.06	1.03
Neuronal	CAMK2G	1.03	1.43
	CAMK2N2	1.54	0.82
	CFOS	2.22	2.59
	DLG2	1.42	1.91
	GLRK	2.61	1.19
	KCNN2	1.19	1.09
	NRG2	0.91	1.31
Metabolic/Activity	CRY1	1.30	0.95
	CRY2	2.10	1.97
	FAM50A	1.53	2.95
	G6PD	1.94	1.24
	GALC	1.13	1.03
	GYG1	1.47	1.63
	HPRT1	1.74	1.69
	MID1IP1	2.88	3.36
	MSRA	1.15	0.82
	PFKFB1	2.01	2.84
	PRVB	5.96	4.45

Figure S1. Graphical representations of all behavioural/stress response genes comparing mean ΔC_T (±SE) natural and treatment gene transcription across all seven populations

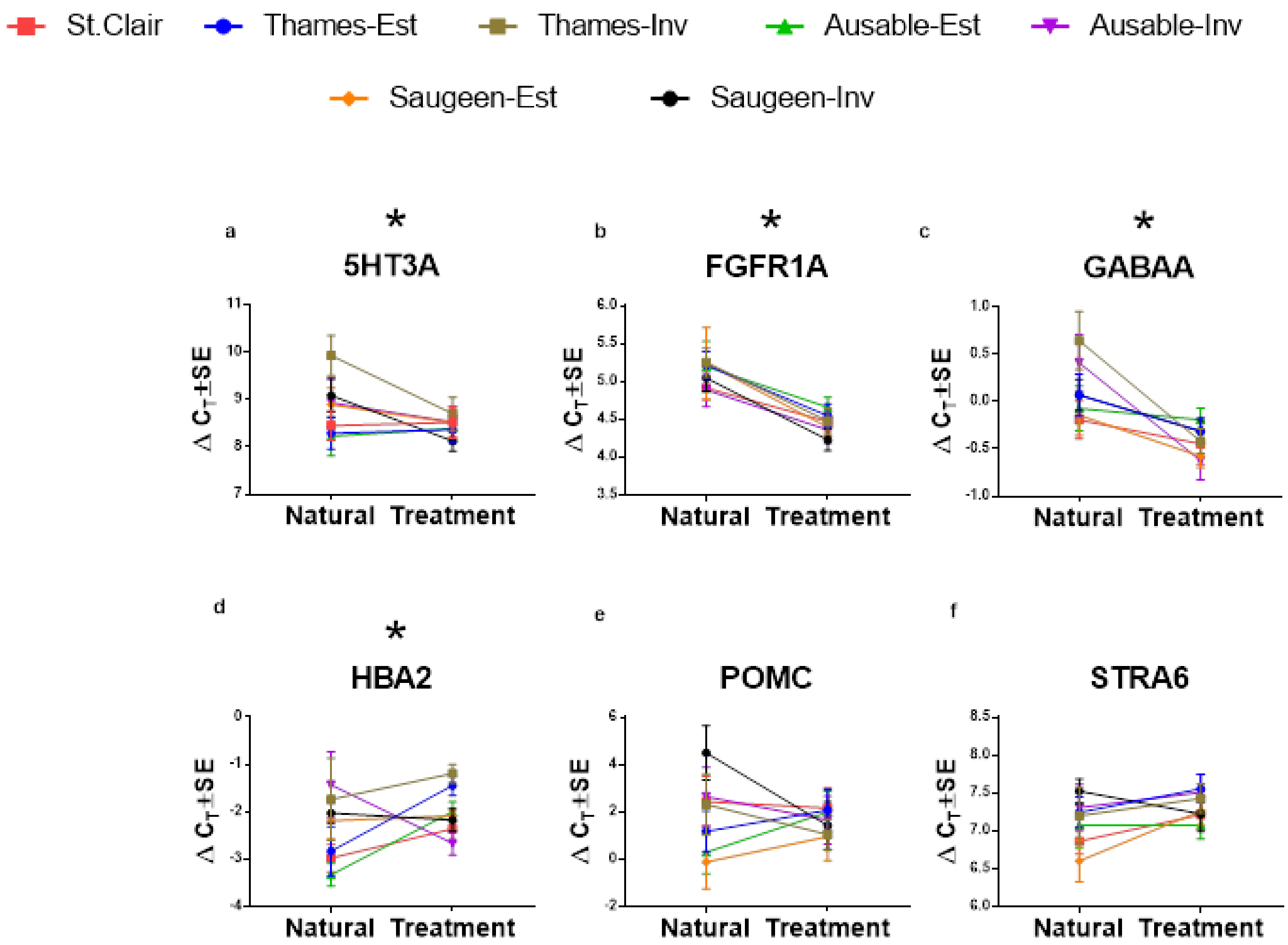


Figure S2. Graphical representations of all neuronal response genes comparing mean ΔC_T (±SE) natural and treatment gene transcription across all seven populations

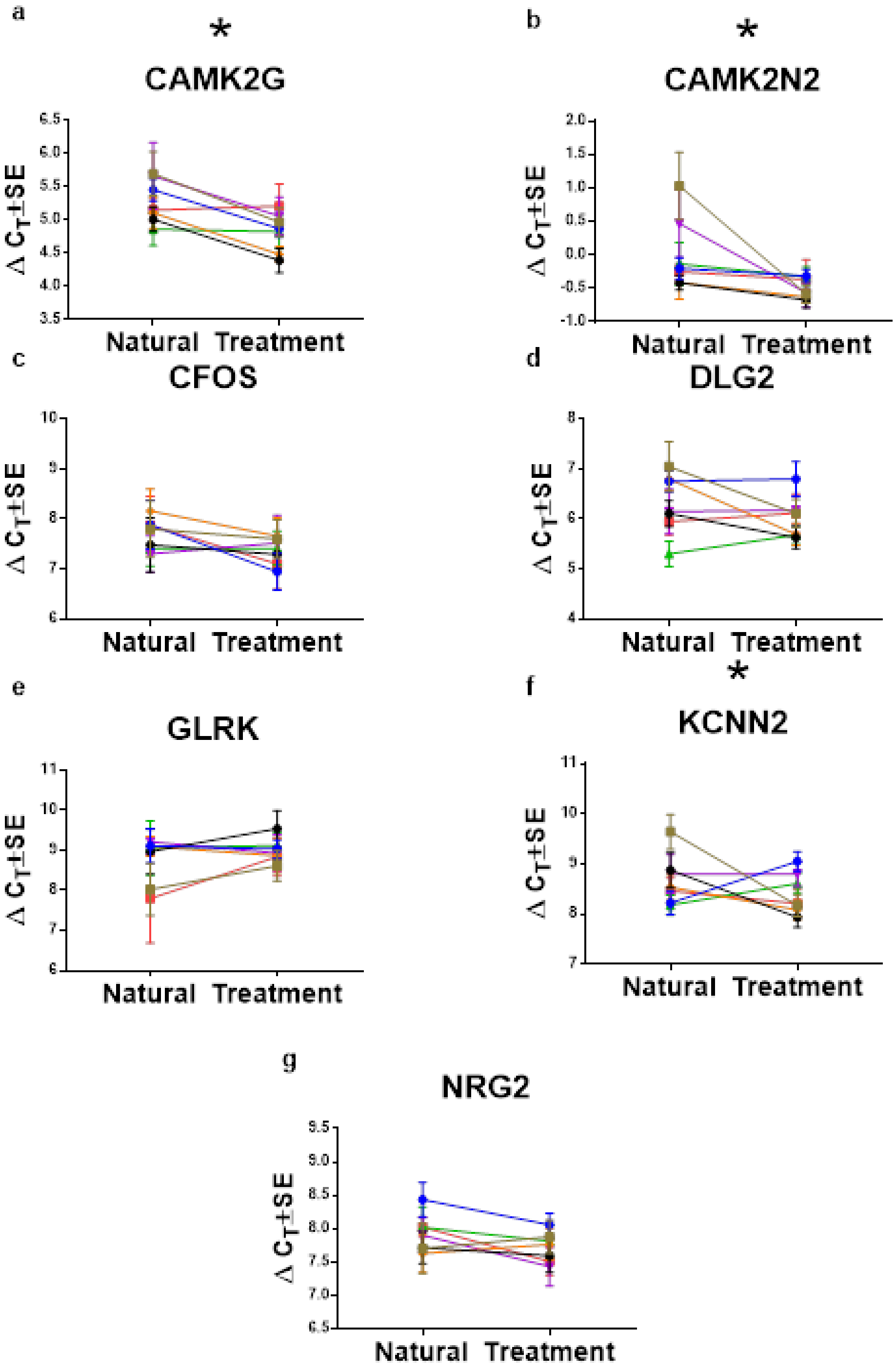
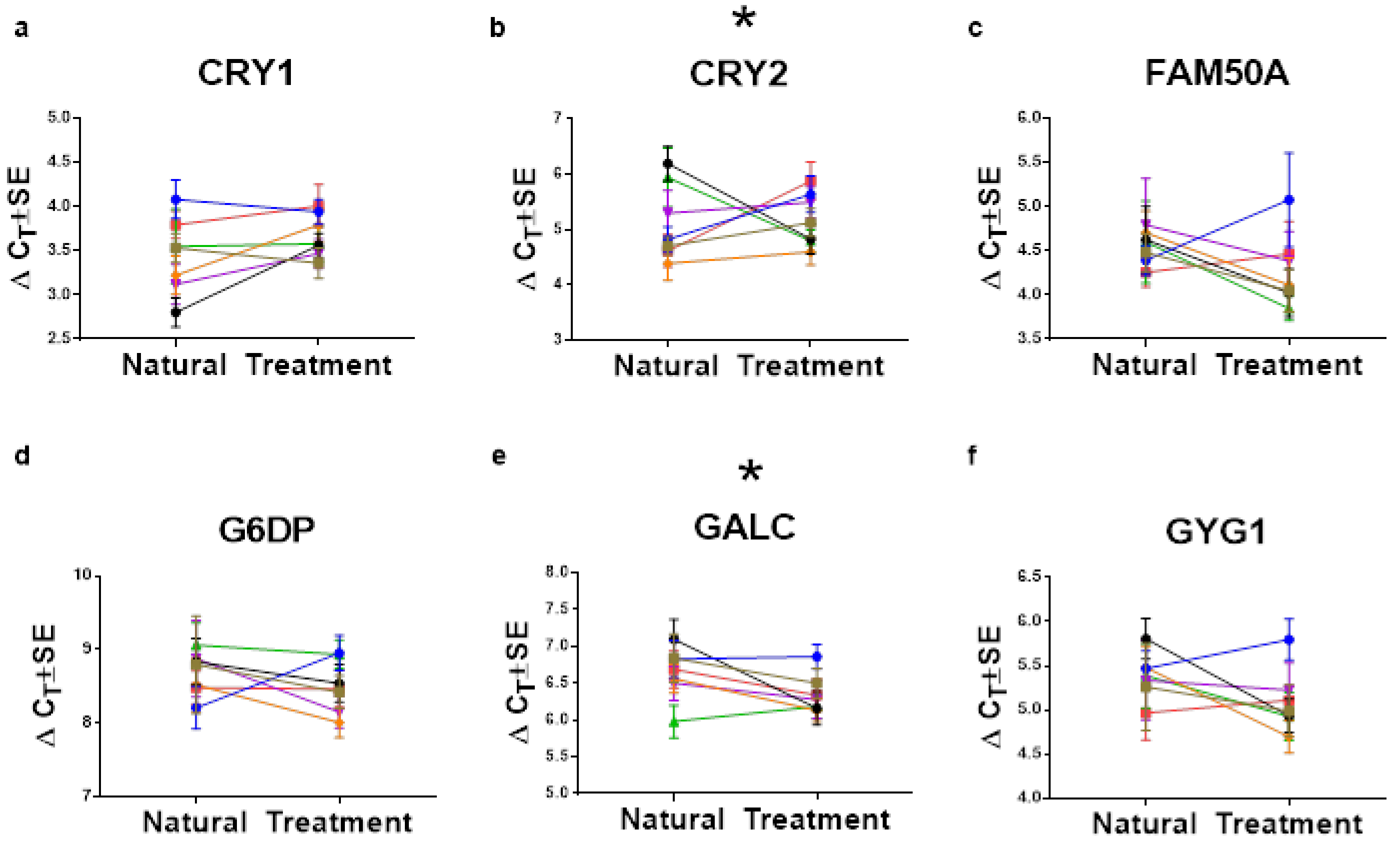


Figure S3. Graphical representations of all metabolic/activity genes comparing mean ΔC_T (±SE) natural and treatment gene transcription across all seven populations



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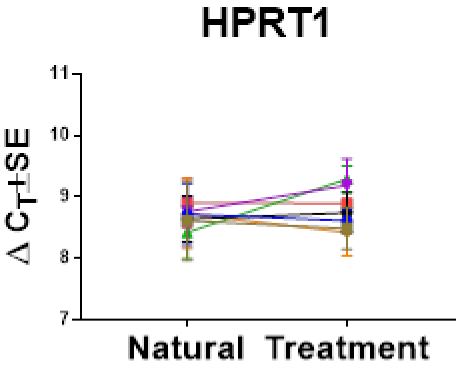
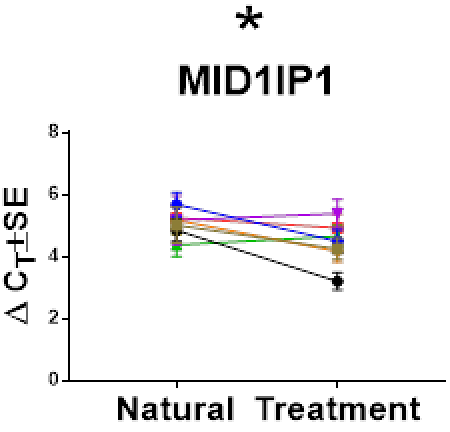

HPRT1
Δ C_T±SE
11
10
9
8
7
Natural
Treatment

h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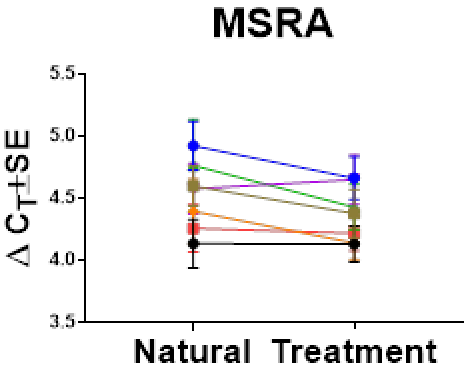
i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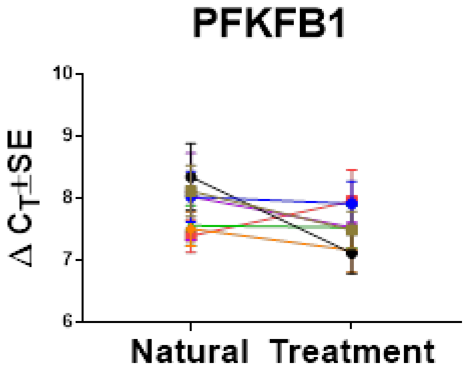
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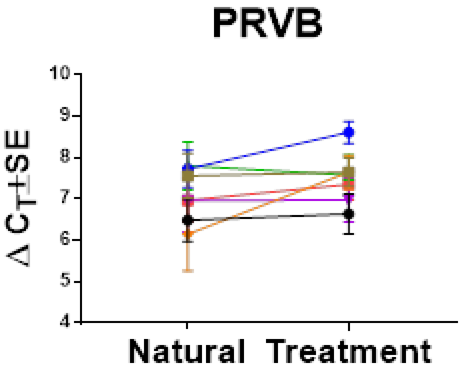
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CHAPTER 4: GENERAL DISCUSSION AND CONCLUSIONS

My thesis research found support for an "invasion behavioural phenotype" (overall behavioural phenotype), in line with what is already known for round gobies in Southern Ontario. In **Chapter 2** we found this behavioural type was driven mainly by juveniles; juveniles at the invasion front were overall less bold, and exploratory, more asocial and tended towards lower predator habituation. Adults, in contrast, did not differ across the invasion stage, and differences that we did find were river-specific. We suggest that this dispersion phenotype pattern could be explained, in part, by reduced intraspecific competition and habitat-feature differences; and that the round goby likely exhibits flexible behaviours that are context-dependent (Wright et al. 2010; Clobert et al. 2009). The behavioural variation observed in our study highlights the importance of an integrative approach that incorporates multiple abiotic and biotic factors to explain the invasion process. While many studies only exclusively study behavioural variation among adults and/or single invaded locations, we argue that it is critically important to be inclusive of all life-stage and sexes in a multi-site context when trying to understand mechanisms that promote invasiveness (Brown et al. 2005; Loftus and Borcherding 2016). In **Chapter 3** we showed evidence that there could be a genetic mechanism driving these behaviours in our "natural" group. We exhibited that our environmental behavioural challenge did exhibit a transcriptional response where animals acclimated to captivity; and our gene transcription results support that detection time since North American invasion can result in differences most likely driven by density, and possibly "alternative ontogenies" where individuals can display plastic responses in response to time since invasion/detection time, where the novel environment (i.e. invasion front) acts as a stressor, where animals initially acclimate and shift their phenotypes in response to time since invasion (Hôrková and Kováč 2015). While we only examined intrinsic mechanisms that differ between residents and dispersers, we discussed the possible extrinsic factors that could also have played a role in the behavioural and gene transcription results we observed. Overall, individuals that disperse are most likely due to both intrinsic (behavioural type, genotype) and the extrinsic (habitat, species assemblage) and how they interact, which is why we stress it is important to consider these mechanisms when studying invasive/dispersal phenotypes (Clobert et al. 2009). By understanding all the facets that could be driving behavioural variation and the mechanisms that in turn regulate this variation in an invasive species (via gene transcription studies), we can understand the underlying "invasiveness" and invasion success of a species; and we can better develop more effective management strategies and develop a stronger understanding of the drivers of range expansion.

Why study the invasion process across multiple contexts (ontogeny, sex, river systems)?

The invasion process is a highly selective and dynamic process that requires a multifaceted approach to truly understand the drivers of invasion success. More recently, the dispersal syndrome, which is defined as a suite of traits (behavioural, physiological, and genetic) that covary, is being applied to distinguish individuals with enhanced dispersal ability (i.e. dispersers) from residents (individuals that reside). However, phenotypes expressed can vary across life-stage, between sexes, and among habitats (Quinn et al. 2009; Marentette et al. 2010), and the unpredictable nature of the invasion process - in particular establishment and spread, can be highly selective of these phenotypic differences.

Invasion phenotype

We sought to characterize the overall behavioural repertoire of the round goby, and found contradictory results with respect to sociality. While this is contrary to what is seen in the literature for invasion syndromes (Fraser et al. 2001; Dingemanse et al. 2003; Cote et al. 2010), we know sociality is important in establishing high density populations; in addition, being social for the round goby permits them to withstand being in high densities allowing them to establish. While we did not look at aggression specifically, other studies have shown they employ their aggression towards (native) heterospecifics instead (Pintor et al. 2009), similar to other invasive species (Groen et al. 2012; Pintor et al. 2008; Duckworth and Baydrev 2007). Boldness, exploration and predator habituation were also part of the round goby's behavioural repertoire, which supports studies that examined boldness and exploration in invasive species compared to natives (Rehage and Sih 2004; Cote et al. 2010a; Cote et al. 2010b). For example, signal crayfish (*Pacifastacus leniusculus*) from introduced populations are bolder, compared to natives and non-native mosquitofish were found to be bolder than native populations (Pintor et al. 2008; Rehage and Sih 2004). Overall, boldness and exploration can be advantageous in seeking new habitats and exploiting resources, in addition to facilitating dispersal (Cote et al. 2011). Furthermore, predator habituation can be also paired with boldness, exploration and sociality, where individuals, that are bolder, explorative and social are more likely to encounter predators and habituate to their presence (Rodriguez-Prieto et al. 2011). Overall, my study supports what is found in the literature and contributes to the invasive literature on behavioural phenotypes.

Ontogenetic driven dispersal?

The majority of studies opt to include a single life-stage when investigating behavioural mechanisms for dispersal. However, if behaviour can be largely dependent on size, metabolic requirements, and different selective pressures then there should be distinct behavioural differences between juveniles and adults (Biro and Stamps 2008; Biro and Stamps 2010; Groothuis and Trillmich 2011). Wuerz and Kruger (2015) also posit that behavioural syndromes are not always consistent across ontogeny and should be more considered when studying behaviour. Christian (1970) first suggested that subordinate individuals might be forced to disperse (i.e. juveniles). Moreover, Cote et al. 2010c, suggested that resident and dispersal phenotypes might be affected by ontogenetic shifts. However, there is a lack of studies that examine both adult and juvenile dispersal (in an invasive species) when studying behaviour-dependent dispersal. Supporting studies on the round goby do suggest adults and juveniles have different foraging techniques, where juveniles are required to actively swim to obtain insect larvae since they cannot consume zebra mussels until they reach a certain size unlike adults (Ray and Corkum 1997) and can therefore be the life-stage that facilitates range expansion (Ray and Corkum 2001). Our results provide supporting evidence for juvenile gobies being the primary "invaders". If there is evidence that one life-stage is more prone to dispersing compared to another one, then one might consider targeting the dispersing life-stage for control and eradication (Buhle et al. 2005). For example, one might consider targeting the weaker and easily targetable individuals or the ones that cause the most ecological impact, or have high dispersal ability (e.g. juveniles).

Importance of "Context"

Overall, context-dependent dispersal is highly important in the consideration of species-range expansion because it can be the motivator for dispersal (Bowler and Benton 2005; Clobert et al. 2009). Environmental heterogeneity and habitat quality differences can furthermore affect which behavioural types develop (Bell 2005; Quinn et al. 2009; Dubic-Messier et al. 2016), and in turn affect who disperses or remains (Bowler and Benton 2005; Clobert et al. 2009). However, while the importance of environmental context has been suggested in the invasion literature (Bowler and Benton 2005; Clobert et al. 2009; Cote et al. 2010), habitat-focused studies evaluating behaviour-dependent dispersal has yet to be empirically tested (Clobert et al.

2009). Our study did not explicitly examine habitat-dependent behaviour, but nonetheless found behaviour to vary across rivers, suggesting that round goby is behaviourally flexible and able to adjust its behaviour based on its environment, perhaps facilitating their invasion success in the Great Lakes. One example of behavioural flexibility is response to a novel food source, and a study on invading house sparrows (*Passer domesticus)*, demonstrated these birds had a shorter latency to approach novel food compared to an established population (Martin and Fitzgerald 2005). Although the objective of this thesis was not to examine behavioural differences based on habitat/temporal variables, we consider that there maybe be complex temporal (e.g., time since detection, time since invading upstream) and habitat quality differences driving contrasting behavioural trends between established and invasion-front sites (for adults). However, to empirically test these suppositions, studies need to be done across multiple rivers, either controlling for time since first detection or habitat similarities (by using a common garden experimental design) and test dispersal behaviours.

Density

Density can play a large role in dispersal behaviour, due to limiting resources and space. Cote and Clobert (2007) found that social personalities (as assessed by repulsion or attraction to a conspecific odour) affected common lizard (*Lacerta vivipara*) propensity to disperse to high and low-density areas. Those repulsed (asocial) were more likely to leave high-density areas, while those attracted (social) were more likely to settle in high-density areas. In addition, Cote and colleagues (2011) found that dispersal ability increased with asociality, suggesting that dispersers cannot tolerate conspecifics. For the round goby, the St. Clair (source), despite being the longest established population and presumably probably one of the densest populations, was one of those most social, as well as boldest, most explorative and flexible. These findings suggest gobies at the source are capable of outcompeting individuals that are either smaller, or with a contrasting behavioural phenotype.

Gene transcription profiles across North American range expansion

The genes in this study were specifically targeted because of their role in: i) behavioural variation (e.g. aggression and boldness); ii) ability to cope with stressors; iii) long-term potentiation, learning/memory, and synaptic plasticity that would allow individuals to make optimal decisions, learn and process cues from the environment (e.g. predator response); and iv) metabolism/activity that would support dispersal and energy for the brain that allows animals to respond. For the natural group, we found that *5HT3A* (involved in the suppression of aggression) was more highly transcribed at established populations than invading populations, which supports the idea residents must be able to withstand high conspecific densities and be social rather than aggressive, in order to tolerate high-density populations (Capelle et al. 2015). Similarly, sociality can be beneficial to an invasive species such as the invasive argentine ant, which exhibited a loss of aggression over time towards other conspecifics, which resulted in lowered mortality among conspecifics and an increase in resources shared (Holway et al. 1998).

An increased stress response can allow animals to deal with the novel stressors found at the invasion front (Jessop et al. 2013). In the invasive house sparrow (*Passer domesticus*), authors Lielb and Martin (2012) observed a hyper-stress response at the invasion front compared to the introduced established population during the breeding season, in addition to higher exploratory behaviour, which they suggested aided the birds in exploiting novel resources (Lielb and Martin 2012). In contrast, we observed a significant detection time since North American invasion, with higher transcription of stress response genes *HBA2* at longer established populations compared to invading populations, suggesting that individuals dispersing might be leaving stress environments. *KCNN2* (involved in regulating synaptic plasticity and implicated in learning), where lowered transcription has been seen after learning in rats (Brosh et al. 2008), was found to have lower transcription at invading populations. Learning might be important for an invading species to process cues from the environment, for example, invasive crayfish have a higher propensity to learn, where they were able to locate a hidden food source faster than the native range (Roudez et al. 2008). Overall, learning on the invasion front might be important for range expansion in order to locate food or recognize novel predator cues (Hazlett et al. 2002).

Behavioural and Genomic Integration of Invasiveness

Overall, this thesis highlights both the behavioural and genetic correlates of range expansion. We found support that round gobies found at the invasion front are likely forced out of ideal habitats as the trigger for dispersal (Ray and Corkum 2001). These individuals are most likely juveniles that are small, less bold and explorative, and asocial. Juveniles that remain have the capabilities (and phenotype) to secure the resources necessary without leaving. As the invasion-front population becomes more established, juveniles and adults exhibit bolder, explorative, predator habituation and social phenotypes due to higher densities, and these phenotypes can be further mediated by habitat abiotic and biotic features. Our gene transcription results for our "natural" individuals appear to support our behavioural findings suggesting that these behavioural types may be regulated by a genetic mechanism (gene transcription). This mechanism is most likely the result of plasticity (since potentially not enough generations have passed for genetic assimilation or genetic adaptation), where individuals can reversibly adjust transcription level in response to their environment (Pigliucci 2001; West-Eberhard 2003). Our environmental behaviourally-treated group provides support for the plasticity argument because we did not observe consistent gene transcription in captivity compared to the "natural", where a majority of the genes respond the same in captivity irrespective of detection time since North American invasion, which suggests that round gobies are capable of acclimating to their captive environment regardless of what population they originated from; which could explain how they have widely spread across the Great Lakes. We argue that it is likely both behavioural and transcriptional plasticity that permits the round goby to acclimate to change and novel stressors and facilitate colonization success across the Great Lakes (Vincelli 2016; Wellband and Heath 2017).

Future directions

Range expansion can be a behavioural decision, and those dispersing and those that reside inherently have specific and even differentially transcribed genes that allow for phenotypic differences. The goal of this thesis was to characterize the behavioural repertoire of the round goby and whether there were genetic mechanisms (i.e. gene transcription) underlying these behaviourally distinct phenotypes across invasion stages. In the future, other studies can be conducted to investigate various forms of learning in round goby, across life-stage and environments. In addition, we can also perform social behavioural assays, to examine whether phenotypes that disperse are affected by group composition. While my study only looked at captivity as a stressor (novel environment), other stressors can be used to assay behavioural differences (such as flow, temperature, substrate type), given our suggested habitat-mediating factors. Transplantation experiments can also be done to investigate whether populations have locally adapted behaviourally or genetically to the habitats (Urszán et al 2015). Epigenetic studies looking at methylation or acetylation which allow gene transcription levels to change irrespective of gene sequence can also be conducted to examine the regulators of gene transcription, which has already been considered in behavioural studies (Robinson et al. 2008;

Bell and Robinson 2011; Lester et al. 2011). This can be especially important for an invading species, where there typically is low genetic diversity at the invasion front due to limited individuals, and epigenetic modifications could help individuals respond to environmental changes (Prentis et al. 2008; Bossdorf et al. 2008). Lastly, my study is only able to correlatively look at the link of behaviour and specific genes; however, i) since we have transcription and behavioural data on the same individual, we can perform more causal analyses; and ii) one can conduct knock-out or mutation-induced experiments, where the gene is removed or mutated can be done to examine the causal role of a gene on a certain behaviour (Norton et al. 2011). For the receptor genes, one could do *in-situ* hybridization to understand and localize receptor mRNA to specific tissues (Gall and Pardue 1969) or immunohistochemistry to ensure that transcription is correlated to receptor abundance (Shukla et al. 2014).

Invasive species management

Invasion biology is an integrative science that reaches across several disciplines (Leung et al. 2002). Interestingly, we found support that invasive phenotypes (behaviour) can be regulated via transcription at least at a population level and that round goby do transcriptionally respond to a captive novel environment. By understanding the mechanism that drives range expansion, we can use better and more effective control and eradication methods. For example, if juveniles are driving range expansion, and we could potentially introduce sterile males to limit reproduction. However, if juveniles or subordinate individuals are dispersing due to high densities, then we might suggest targeting established populations to lower their population densities. Conversely, the invasion front may be better to target since populations are small. We found that juveniles on the invasion fronts to be overall less social, bold, explorative and predator sensitive which might make trapping them difficult if they do not have the tendency to explore. A chemical pesticide could potentially be developed to eliminate small populations, similar to lampricides used on the invasive sea lamprey. We demonstrated that it is likely that plasticity in behaviours and gene transcription facilitate the invasion success of the round goby, which has also been observed in other round goby studies with respect to thermal stressors (Vincelli 2016; Wellband and Heath 2017). The behavioural profile of the round goby can be used for as an outline for risk-assessment of other potential invasive fish species that have similar life-history traits (Lennox et al. 2015). In particular, managers can conduct behavioural tests to assess the risk of an introduced species and utilize more specific techniques or develop a more strategic approach to attract them and eradicate them before they colonize a new area (Juette et al. 2014; Côté et al. 2014).

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