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# Ancestral Variation and the Evolution of Sneaking Behavior in the Adaptive Radiation of Threespine Stickleback (*Gasterosteus aculeatus* L.)

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Behavior, a highly plastic and responsive group of phenotypic traits, has been characterized as having the potential to both drive and inhibit evolutionary change within populations. The Threespine Stickleback radiation (*Gasterosteus aculeatus* L.) is a model system that demonstrates the vital role environment plays in behavioral evolution – with multiple colonizations of variable freshwater habitats by ancestral oceanic metapopulations producing the behaviorally diverse populations observable today. The goal of this dissertation is to characterize both inter- and intra-population variation in sneaking behavior – an inconspicuous mating tactic used by threespine males to gain fertilizations as an alternative to courtship behavior.

I describe the pattern of geographic variation in sneak fertilization tactic use across populations in the Pacific Northwest, presenting evidence for regional and population-level variation in sneaking frequencies under natural conditions – a pattern that has been difficult to discern due to the large number of published accounts focused on aspects of sneaking behavior in single populations.

I used common garden laboratory techniques to determine the extent to which the difference in sneaking propensities observed under field conditions are genetically versus environmentally influenced and found no evidence of trait loss in populations not observed to express sneaking behavior under natural conditions. This study also provides evidence of individual variation in sneaking propensity, behavioral consistency, and tactic flexibility/plasticity.

Lastly, I investigate the extent to which body size, condition, or coloration and patterning often associated with the sneaker tactic is specifically characteristic of this behavioral tactic in an Alaskan freshwater population and find no evidence of tactic specific physical traits.

Overall, this collection of studies provides exciting results, as the flexibility in tactic expression and body coloration observed suggests that complex decision-making rules underlie reproductive tactic use in stickleback. At the population-level, a variety of options exist for males in terms of gaining reproductive success beyond those we have generally associated with species-typical tactics and have typically focused on measuring to connect morphological and behavioral traits in a functional way with reproductive success. In addition, the number of options utilized by males may greatly depend on population-specific environmental and social characteristics.

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Adaptive Radiation of Threespine Stickleback (*Gasterosteus aculeatus* L.)

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B.A., Clark University, Worcester MA, 2004

M.A., Clark University, Worcester MA, 2005

A Dissertation

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at the  
University of Connecticut

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Katherine A. Shaw

2014

APPROVAL PAGE

Doctor of Philosophy Dissertation

Ancestral Variation and the Evolution of Sneaking Behavior in the  
Adaptive Radiation of Threespine Stickleback (*Gasterosteus aculeatus* L.)

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*For my parents, who have provided the fundamental GxE interactions that have made me me.*

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## **CHAPTER 1**

### **SNEAKING BEHAVIOR AS A FLEXIBLE ALTERNATIVE REPRODUCTIVE TACTIC IN**

### **MALE THREESPINE STICKLEBACK (*GASTEROSTEUS ACULEATUS* L.):**

### **INTRODUCTION & LITERATURE REVIEW**

#### **1.1 ABSTRACT**

Behavior, a highly plastic and responsive group of phenotypic traits, has been characterized as having the potential to both drive and inhibit evolutionary change within populations. The Threespine Stickleback radiation (*Gasterosteus aculeatus* L.) is a model system that demonstrates the vital role environment plays in evolutionary change. Multiple colonizations of variable freshwater habitats by morphologically and behaviorally ancestral oceanic populations have produced the phenotypically diverse populations observable today. A growing number of studies demonstrating morphological plasticity and behavioral plasticity in threespine populations suggest that flexible aspects of the ancestral oceanic phenotype likely played a significant role in colonization. Given that behavioral plasticity can affect the evolutionary trajectory of populations, the goal of this dissertation is to characterize both inter- and intra-population variation in sneaking behavior – a flexible, inconspicuous mating tactic used by threespine males as an alternative to courtship to gain fertilizations. In this introductory chapter I will provide background necessary to frame the subsequent dissertation chapters. This background includes a review of the behavioral components that comprise the sneaker repertoire, a review of the geographic distribution of sneaking observations and the environmental and social characteristics associated with the propensity of individuals to exhibit this behavior, a review of flexible alternative reproductive tactics

to categorize stickleback sneak fertilization behavior, and an outline of the significance sneak fertilization behavior may have with respect to population dynamics and selection.

**KEYWORDS:** alternative reproductive tactics, sneaking, behavioral plasticity, behavioral flexibility, Threespine Stickleback, *Gasterosteus aculeatus*

## 1.2 INTRODUCTION

Maintenance of genetic variation within populations is the substrate necessary for natural selection to occur across generations and, consequently, an important prerequisite for evolutionary change. Sexually selected traits are often produced by strong, directional selection imposed by male-male competition or female choice for males of high genetic quality, high dominance status, or high resource-holding capacity (Andersson 1994). Sexual selection should erode genetic variation for male traits over time, although males of many species continue to show variation in both morphological and behavioral traits that are subject to sexual selection. Many mechanisms may be operating within populations to maintain genetic variation despite strong selection, including the presence of alternative reproductive tactics. Males may use alternative reproductive tactics, such as female mimicry and inconspicuous sneaking behavior, to steal fertilizations and copulations. By effectively bypassing species-typical tactics that require traits associated with male-male competition and female choice, use of such tactics increases genetic variation within the pool of reproductively successful males (Shuster & Wade 2003; Oliveira *et al.* 2008). Alternative reproductive tactics are quite common across the animal kingdom but the extent to which such tactics are influenced by underlying genetic differences and environmental cues can vary extensively (e.g. specialized morphs vs opportunistic behavioral tactics; Shuster & Wade 2003; Oliveira *et al.* 2008). Population dynamics and the evolutionary trajectories of populations may be affected differently depending on the extent to which alternative tactics are plastically expressed (Shuster &

Wade 2003, Fawcett *et al.* 2008). To better understand how the presence of alternative tactics helps maintain variation and influences evolutionary dynamics within populations, the complex interplay of genetics and environmental determination underlying mating tactics should be investigated within and among populations. Species with many populations spread across a range of environments are well-suited for investigating the genetic and environmental contributions underlying morphological and behavioral trait expression (Carroll & Corneli 1999). In this respect, the Threespine Stickleback (*Gasterosteus aculeatus* L.) is a useful model organism. Males exhibit variation in sexually-selected behavioral and morphological traits within and across populations despite seemingly strong female choice and male-male competition. Populations also vary in the extent to which males exhibit sneak fertilization behavior but the contribution of genetic and environmental determination to the expression of this alternative reproductive tactic is unknown. A major goal of my dissertation research is to describe geographic variation in alternative mating tactic use and the extent of environmental influence on mating tactic expression (i.e. behavioral plasticity) within and between populations.

The Threespine Stickleback is a small fish with a broad holarctic distribution in both marine and freshwater habitats. The geographic structure and genetic relationships among stickleback populations may be described as regional metapopulations, in which bi-directional gene flow has occurred between oceanic populations and many of the rapidly adapting freshwater populations derived from these oceanic source populations (Schluter & Conte 2009; Hohenlohe *et al.* 2012; Foster 2013b). The most recent colonization of hundreds of thousands of freshwater habitats followed the last glacial recession between 20,000 and 10,000 years ago (reviewed in Bell & Foster 1994). Offshoots of ancestral marine populations are greatly influenced by the habitats they colonized – whether lake or stream, deep oligotrophic or shallow eutrophic, predator-rich or predator-poor environments. New populations have been observed to exhibit similar phenotypic characteristics when colonizing similar environments while retaining marks of historical contingency (Hendry *et al.* 2009). The Threespine Stickleback has become

an important and unique system in studies of evolutionary biology, ecology, and genetics over the last few decades for several reasons: (1) the signature ability to compare ancestral and derived populations, enabling inference of mechanisms underlying evolutionary change, (2) the availability of hundreds of natural replicates for *in situ* comparisons or laboratory manipulations, and (3) the tractability of this species to lab-rearing and manipulations that are necessary for inferring the genetic and environmental components of phenotypic traits (Bell & Foster 1994; Ostlund-Nilsson *et al.* 2006; Foster 2013a). For all these reasons, the stickleback is also fast becoming an indispensable model organism in the “omics” fields (e.g. Cresko *et al.* 2004; Kingsley *et al.* 2004; Shapiro *et al.* 2004; Colosimo *et al.* 2005; Barrett *et al.* 2009; Kitano *et al.* 2009; Leder *et al.* 2009; Hohenlohe *et al.* 2010; Jones *et al.* 2012).

Comparative investigations have focused on anti-predator morphology and behavior, trophic morphology, physiology, and mating behavior – including male courtship behavior, parental behavior, and female preferences (reviewed in Bell & Foster 1994; Foster *et al.* 1996; Ostlund-Nilsson *et al.* 2006; Foster 2013b). Studies are increasingly demonstrating not only major trends in evolution of these phenotypic traits but also the intricate interactions between genetics, environments and stochasticity – such as the diverse patterns of relatedness among sympatric benthic and limnetic pairs (Taylor & McPhail 2000; Hendry *et al.* 2009) and the ecotype-specific relationship between genetic predisposition and learning in antipredator behavior (Huntingford *et al.* 1994; Kozak & Boughman 2012).

A growing area of research with regards to the Threespine Stickleback model system is the presence of gene x environment interactions or plasticity -- morphological plasticity (e.g. Day *et al.* 1994; Wund *et al.* 2008; Garduno-Paz *et al.* 2010; Frommen *et al.* 2011; Svanback & Schluter 2012; Wund *et al.* 2012; Ramler 2014), physiological plasticity (e.g. McCairns & Bernatchez 2010), and behavioral plasticity (e.g. Huntingford *et al.* 1994; Day & McPhail 1996; Odling-Smee & Braithwaite 2003; Zbinden *et al.* 2003; Bell & Sih 2007; Scotti & Foster 2007; Shaw *et al.* 2007; Webster *et al.* 2007; Delcourt *et al.* 2008; Dingemanse *et al.* 2009, 2012; Harcourt *et al.* 2009; Dzieweczynski & Forrette 2011; Kozak &

Boughman 2012; Stein & Bell 2012). The significant potential for behavioral plasticity to be a driver or inhibitor of evolutionary change is increasingly recognized (Plotkin 1988; Huey *et al.* 2003; West-Eberhard 2003; Duckworth 2009; Foster 2013a; Zuk *et al.* 2014). As a driver of evolution, changes in behavior can expose organisms to novel selection pressures and result in rapid evolution of morphological, life history, and physiological traits (Wcislo 1989; Huey *et al.* 2003; Losos *et al.* 2004; Sol *et al.* 2005). A high level of behavioral plasticity also has the potential to work as an inhibitor of evolution, by shielding organisms from strong directional selection (e.g. Wright 1931; Losos *et al.* 2004; Sol *et al.* 2005) or by allowing organisms to move to less stressful environments (e.g. Bogert 1949; Huey *et al.* 2003; Badyaev 2005).

Although behavior is considered to be highly plastic, a number of studies now demonstrate that it is not always optimally flexible. Behavioral traits are not able to change to conform adaptively in every scenario or context, especially considering the number of social and environmental stimuli individuals may experience across spatial and temporal scales is very large, if not infinitely large (Thompson 1999; McNamara & Houston 2009). Generally, the greater the number of environments in which a trait is expressed and subject to natural selection, the higher the probability genetic constraints on the evolution of that trait will form (Falconer 1952; Via & Lande 1985; Gomulkiewicz & Kirkpatrick 1992; Thompson 1999). Differences in personality (i.e. consistent temperament: Dingemanse *et al.* 2010; Herczeg & Garamszegi 2012) and behavioral syndromes (i.e. cross-context behavioral correlations: Sih *et al.* 2004; Ghalambor *et al.* 2010; Brommer 2014) have been observed within and among stickleback populations (reviewed in Bell, Foster & Wund 2013). In addition, individuals within populations may differ in their degree of behavioral plasticity (Wolf *et al.* 2008; Dingemanse *et al.* 2010; Reale & Dingemanse 2010) and behavioral consistency (Dingemanse *et al.* 2010; Reale & Dingemanse 2010; Stamps & Groothuis 2010a,b; Stamps *et al.* 2012). Intra-individual consistency and inconsistency in behavioral traits have been observed in stickleback (e.g. Bell & Stamps 2004; Bell & Sih 2007;



Dzieweczynski & Forrette 2011; Stein & Bell 2012). Understanding the complex interactions between environment, behavior and morphology will provide a better understanding of how trophic, anti-predator and sexually-selected traits evolved in the stickleback. It would be useful to investigate whether certain behavioral traits have precluded the evolution of morphological adaptations or whether they evolve in concert, providing positive feedback for each other (West-Eberhard 2003; Foster 2013a).

Expression of alternative mating tactics is a component of Threespine Stickleback behavior that has the potential to contribute to the maintenance of variation and evolutionary potential within and among populations but has yet to be a subject of comprehensive investigation. In threespines, this refers to a behavior performed by males in which they inconspicuously sneak to the nests of courting males in an attempt to steal fertilizations by depositing sperm over spawned eggs. Why is it important to know about the prevalence and potential variation in this behavior at the population-level? The frequency with which alternative mating tactics occur in a population can have effects on expression and maintenance of male sexually-selected traits, strength of female mate preferences, and estimates of effective population size (reviewed in Le Comber 2003 and in detail below). To better understand the evolutionary consequences of alternative reproductive tactics, the patterns of discontinuity in reproductive tactics and the threshold and developmental switch mechanisms responsible for producing alternative behavioral phenotypes should be described (Brockmann *et al.* 2008; Oliveira *et al.* 2008; Bergmuller & Taborsky 2010). A number of studies have investigated diverse aspects of this behavior in stickleback (reviewed below) but few have attempted a broad geographic comparison of the prevalence and the conditions under which this behavior occurs (Foster *et al.* 1996; Foster 2013b). The goal of this chapter (**Chapter 1**) is to provide a comprehensive and updated review of sneaking behavior in *G. aculeatus*.

### 1.3 REVIEW OF THE STICKLEBACK SNEAKING LITERATURE

Several authors have provided reviews of sneaking behavior in Threespine Stickleback. A review by von Hippel (2010) offers a thorough account of the seminal works originally characterizing sneaking behavior, along with nest cannibalism and egg-stealing behavior. Foster (1994) and Ostlund-Nilsson (2006) have published descriptions of sneaking behavior and reviewed more recent studies primarily focused on several variables linked to the expression of sneaking behavior. Here I aim to provide a comprehensive review of the stickleback literature with respect to: **(I)** describing the sneaker repertoire and distinguishing sneak fertilization from nest-raiding behavior; **(II)** describing the geographic distribution and ancestral character state of sneaking behavior; and **(III)** categorizing the variables investigated in relation to sneaking propensity. I then **(IV)** review stickleback sneak fertilization behavior with respect to the general classifications used in the alternative reproductive tactic literature. Lastly, I **(V)** discuss the potential influences of sneak fertilization tactic use on selection and trait evolution within populations.

#### 1.3.1 Methods

I conducted a literature search to determine the holarctic distribution of populations expressing the sneaker repertoire and to estimate the potential ancestral character state for the sneaker repertoire (i.e. present or absent) within *G. aculeatus*. Search terms included words related to sneaking behavior in Threespine Stickleback (e.g. sneak, steal/stolen fert\*, nest raiding/raid, paternity and stickleback or *Gasterosteus*/*Gasteroste*\*; ISI Web of Science database (Thomson Reuter, New York, NY, USA) and Google Scholar (Google, Mountain View, CA, USA)). Nest-raiding behavior (stealing eggs or nest material) was included in the literature search and noted in the compiled data but not considered stringent enough criteria to indicate the occurrence of sneak fertilizations in a study population unless authors explicitly noted observations of stolen fertilizations along with nest-raiding behavior (see '*Function of*

*the sneaker repertoire: Why separate stolen fertilizations from “nest-raiding”?*). Location, type of study (laboratory or field), and type(s) of "sneaking" behavior (sneak fertilizations, egg-stealing, or stealing of nest material) observed was determined for each population described in the literature (Table 1.1, Figure 1.1).

### 1.3.2 Results

#### **(I) Description of the sneaker repertoire in Threespine Stickleback**

##### **(a) Stealing Fertilizations**

The sneaker repertoire in the Threespine Stickleback was originally described in detail by Morris (1952) and van den Assem (1967). Both authors described sneaking as occurring in response to a male directing courtship towards a female, although Van den Assem (1967) provides a more detailed description of the motor patterns utilized during a sneak attempt. Sneaker males are described as moving along the substrate towards the courting male's nest and freezing intermittently. A very interesting and distinguishing feature of the sneaker repertoire described by van den Assem (1967) is the reduced pectoral fin movement observed during the substrate creeping behavior. This part of the repertoire has been reported in many sneaking observations in both the field and lab (K Shaw, pers. obs) and is a motor pattern specific to nest-directed sneaking, as it is not included in other stereotyped behaviors. Beyond substrate creeping, sneaking males take advantage of any aquatic vegetation nearby the courting male's nest. Similar to the vegetation freezing observed in *Pungitius pungitius* sneakers (Morris 1952), threespine sneakers utilize the concealment of vegetation while exhibiting behaviors similar to substrate creeping, in some cases freezing high above a courting male in vegetation surrounding the nest (K Shaw, pers. obs.).

The final approach of a sneaker towards a nest typically involves a quick dash from the substrate or vegetation to the nest: (a) while the courting male is showing the female the nest entrance, (b) while

the female is in the nest and the courting male is pushing on the female's abdomen (i.e. quivering) to help release her clutch, or (c) after the female exits the nest and the courting male has entered the nest or is about to enter the nest to fertilize the eggs. In some instances, the courting male is focused enough on spawning behavior that a sneaker is able to position himself at the nest entrance without eliciting a reaction. During a successful sneaking attempt, the sneaker enters the nest following the female, following the nest owner, or simultaneously with either the female or nest owner (van den Assem 1967; Goldschmidt & Bakker 1990; Goldschmidt *et al.* 1992; K Shaw, pers. obs.). In some cases the sneaker arrives at the nest prematurely in the sequence and enters the nest before the female enters. Several scenarios can follow this premature arrival: (a) the nest owner may chase both sneaker and female away, (b) the sneaker may enter and exit the nest and the female may follow and enter the nest followed by a spawning attempt, or (c) the sneaker may enter and exit the nest and the female may choose to abort the spawning with the nest owner and swim away (K Shaw, pers. obs.). Most sneaking attempts typically result in the sneaker male being spotted before entering the nest and being chased away by the courting nest owner (K. Shaw, pers. obs.; Chapter 2). The probability of successful sneak fertilizations, at least directed towards a single nest owner, likely declines with increasing attempts and increasing nest owner vigilance.

Paternity analyses performed both in the field and lab have found evidence of sneak fertilization success. Within-clutch estimates of stolen paternity range from 10.5-77% (Bakker *et al.* 2006) and within-population estimates of stolen paternity from pooled nests range from 8-21% (Rico *et al.* 1992; Largiader *et al.* 2001; Blais *et al.* 2004; Krobbach 2006; Eizaguirre *et al.* 2009; Candolin & Vlieger 2003). Stolen paternity can be substantial in some populations, highlighting the fact that successful use of alternative tactics has the potential to influence evolutionary outcomes in populations and the fact that the strength of this potential influence varies among populations.

Following most successful sneak fertilizations, the sneaker attempts to grab eggs and return to his own territory (van den Assem 1967; Goldschmidt & Bakker 1990; Goldschmidt *et al.* 1992; Jamieson & Colgan 1992; K. Shaw pers. obs). The behavior typically involves the sneaker exiting the nest and swimming back around to the nest entrance to retrieve eggs by digging in the nest. Sneakers unsuccessful at performing a sneak fertilization also attempt to grab eggs, but are often chased away before successfully stealing eggs. In either case, sneakers who successfully grab eggs – in the form of a partial clutch or whole clutch – often return to their own nest and deposit them. Males may still perform egg-stealing behavior if they do not have a nest and either eat the stolen eggs (van den Assem 1967; Kynard 1978; K. Shaw, pers. obs.) or, on occasion, begin building a nest around the stolen clutch (K. Shaw, pers. obs.). Whether males rear and hatch stolen eggs likely depends on probability of future courtship opportunities for the sneaker male (Rohwer 1978), sneaker male condition (van den Assem 1967), and the number of eggs a sneaker is able to steal (a threshold number of eggs or the act of egg care can trigger hormonal changes related to parental care, i.e. ‘priming effect’, Goldschmidt & Bakker 1990). However, the frequency with which sneakers successfully rear stolen clutches (either sneaker- or nest owner-fertilized) may be fairly low (van den Assem 1967; Sargent & Gebler 1980; Jamieson & Colgan 1992).

In published descriptions of sneaker motor patterns, sneakers are typically described as being drab in coloration, often displaying disruptive body patterning (e.g. mottled or striped), which is believed to contribute to the inconspicuous nature of the sneaker repertoire (van den Assem 1967; Jamieson & Colgan 1992; de Fraipont *et al.* 1993). The extent to which certain aspects and patterns of cryptic body coloration are characteristic of sneak fertilization behavior, the extent to which interpopulation variation in sneaker body coloration is observed, and the extent to which individual body coloration is plastic are topics discussed in further detail in Chapter 2 and 4.

***(b) Function of the sneaker repertoire: Why separate stolen fertilizations from “nest-raiding”?***

The focus of the literature search was to locate studies describing the sneaker repertoire, specifically observations of sneak fertilizations as a form of alternative reproductive tactic. In the stickleback literature, it is difficult to parse out specific behaviors observed when nest-directed behaviors are simply described as ‘nest-raiding’ without further description/clarification. ‘Nest-raiding’ is a term used to describe various nest-directed behaviors by males including sneak fertilizations and behaviors described as interference competition (i.e. nest destruction, stealing of nest material, cannibalism of eggs, and courtship disruption: van den Assem 1967; Black 1971; Wootton 1971, 1976; Li & Owings 1978a,b; Kynard 1978; Mori 1995; Ostlund-Nilsson 2006; Raeymaekers *et al.* 2010). It is possible that only a subset of these nest-directed behaviors are expressed in any given population (Wootton 1971, Kynard 1978, Foster *et al.* 1996; Raeymaekers *et al.* 2010; Chapter 2) so it should not be assumed that observation of one type of nest-directed behavior suggests that the other nest-directed behaviors are also exhibited by individuals of a given population.

Sneaking fertilizations is considered a parasitic reproductive tactic (Taborsky 1998). Sneaking fertilizations should be considered separately from other nest-raiding behaviors because it results in different consequences than competitive interference behaviors. While cuckoldry does put the cuckolded male at a disadvantage, it also directly benefits the parasitic male by increasing his reproductive fitness (Figure 1.2). In contrast, behaviors associated with interference competition may put a rival male at a disadvantage but do not directly increase the reproductive fitness of the egg-stealer, nest-destroyer, or courtship interrupter. For instance, nest destruction by male intruders can result in victimized males re-building lower quality nests, potentially due to increased aggression and decreased sexual motivation, which may also influence the victimized male’s future courtship success (Li & Owings 1978b).

Instead of using parasitic tactics to acquire mates or fertilizations, males can use alternative methods to gain resources required for mating or brood care for their offspring from males performing species-typical tactics, usually by force (van den Berghe 1988; Sargent 1989; Sinervo & Lively 1996; Mboko & Kohda 1999; Ota & Kohda 2006; reviewed in Taborsky 1998, 2008). Forceful acquisition of useful resources is an advantage of some of the nest-raiding behaviors performed by male stickleback (Figure 1.2). Beyond serving to disrupt the breeding cycle of a rival male (e.g. pure interference competition), stealing nesting material saves a male the time and energy to collect this resource. In some populations, females prefer nests with bright decorations and males preferentially add these materials to their nests (Ostlund-Nilsson & Holmlund 2003). Stealing these materials from other nests, much like the behavior observed in bowerbirds (Marshall 1954), saves considerable searching time and effort for the nest-raider. In fact, Ostlund-Nilsson & Holmlund (2003) argue that nest decorations serve as a signal of male vigilance to females in populations in which stealing nest material is common. A similar argument for honest signaling has been made for bower defense in bowerbirds (Wojcieszek *et al.* 2007; Doerr 2010). Stealing eggs to cannibalize may also serve to save time and energy in acquiring food sources (Kynard 1978). Generally, it is argued that stealing eggs and nesting material more likely represent interference competition rather than competition for scarce resources – considering, in many populations, neither food nor nesting material are limiting resources (van den Assem 1967; Li & Owings 1978b). Nest raiding behaviors can serve simultaneously as interference competition and easy access to required resources. Noncannibalistic egg-stealing is another nest-raiding behavior that serves as an alternative method to gain mates. Female stickleback prefer nests with eggs present, a potential trigger for mate choice copying (Kraak 1996; Rohwer 1978; Ridley & Rechten 1981; Goldschmidt & Bakker 1990). Use of stolen eggs may at least work initially to gain mates – this preference for eggs seems to reach an optimum between one to several clutches (Belles-Isles *et al.* 1990; Goldschmidt *et al.* 1993).

As shown in Table 1.1, the literature search resulted in a total of 30 research articles related to nest-raiding and/or sneak fertilization behavior, with a total of 31 populations in which nest-raiding behavior (specifically stealing eggs or nest material) and/or the sneaker repertoire was described, as well as populations in which the presence of sneak fertilization behavior was specifically investigated but not observed ( $n = 7$ ). The sneaker repertoire in relation to sneak fertilizations (denoted SF, or SFNR when nest-raiding was also present) was observed in 13 of the populations, described across a total of 16 studies (several studies utilized the same populations to investigate variables associated with sneak fertilization behavior, e.g. Li & Owings 1978a,b; Mori 1995, 1998; de Fraipont et al. 1993; Cubillos & Guderley 2000).

A number of studies specify whether the sneaker repertoire was present or not when describing egg-stealing or stealing of nest material (Black & Wootton 1970; Wootton 1971, 1972; Kynard 1978; Li & Owings 1978a,b; Jamieson & Colgan 1992; Raeymaekers *et al.* 2010). A handful of these studies used the term “nest-raiding” to describe use of the sneaker repertoire leading to nest destruction and egg-stealing but not fertilization attempts (e.g. Black & Wootton 1970; Wootton 1971; Kynard 1978). During 80 hrs of observation in several populations, Wootton (1971) did not observe any sneaking attempts on males performing courtship but did observe sneaking males stealing eggs and nesting material. In the case of the now-extinct Wapato Lake population of Washington, many specific accounts of sneaker behavior were described in the context of egg-stealing and cannibalism but not in the context of fertilization attempts despite the large number of courtships encountered (Kynard 1978).

On the other hand, there are some published accounts of attacks on nests during courtships and egg-stealing that do not provide enough descriptive information to confidently determine if nest-raiding males used the sneaker repertoire (e.g. Black 1971; Borg 1985; Mori 1995; Cubillos & Guderley 2000). There are also a number of studies that focus on “nest-raiding” in the context of conspecific or group cannibalism with no reference to the use of the sneaker repertoire, which further confounds behaviors



related to mating tactics, interference competition or feeding (e.g. Black 1971; Whoriskey & FitzGerald 1985; FitzGerald & van Havre 1987; Ridgway & McPhail 1987, 1988; Vickery *et al.* 1988; Hyatt & Ringler 1989; Belles-Isles *et al.* 1990; Whoriskey 1991; FitzGerald *et al.* 1992; Cubillos & Gurderley 2000). Studies in which nest-raiding was more indicative of group cannibalism were not included in Table 1.1.

The use of quite conspicuous behavior, rather than the sneaker repertoire, prior to instances of directed nest attacks, egg-stealing, or nest material stealing attempts can be fairly common depending on the population (Li & Owings 1978a; K Shaw, pers. obs.). Evidence suggests that interference tactics (stealing eggs and nesting material) in other stickleback species are typically not preceded by inconspicuous behavior similar to the sneaker repertoire (*P. pungitius*; McKenzie & Keenleyside 1970; Wootton 1976). However, use of the inconspicuous sneaker repertoire prior to interference behaviors may be effective in decreasing or postponing nest-holder aggression directed towards the intruder during a destructive act.

What can be deduced from the overall patterns of sneaker repertoire descriptions, observations of sneak fertilization, and observations of interference competition (e.g. stealing eggs/nest material, nest destruction, etc.) is that sneak fertilizations are overwhelmingly prefaced by performance of the sneaker repertoire but interference competition behaviors are not. Because of these discrepancies, these functional categories are treated separately throughout the studies presented in this dissertation. Specifically, 'sneaker repertoire' is used to describe instances in which males used inconspicuous behaviors, and often times cryptic coloration, to gain access to a competitor's nest. From that point, resulting behavior can involve attempts to enter the nest entrance, particularly during courtship behavior. This functional category is referred to as sneak fertilizations (or sneak fertilization attempts). Attempts to destroy a competitor's nest, steal nesting material, or steal eggs (without prior sneak fertilization) fall under the functional category 'nest-raiding behaviors'. Depending on the

circumstances, either the inconspicuous sneaking repertoire or conspicuous/disruptive behaviors may be performed prior to 'nest-raiding behaviors' (Figure 1.2).

There are two Alaskan populations included in a 1996 review by Foster *et al.* (Lynne Lake, Willow Lake) in which no observations of the sneaker repertoire were reported. Follow-up observations demonstrate the presence of sneaking behavior at low frequencies in both these populations and these data are included in the Chapter 2 interpopulation comparison. Thus, there is a discrepancy in sneaking behavior observations for these two populations between Foster *et al.* (1996) and Table 2.1 of Chapter 2. Exclusion of these observations for this literature review does not affect the general geographic patterns of sneak fertilization prevalence discussed here, since several other Alaskan populations were observed to express sneak fertilization behavior.

Besides these two Alaskan populations, there are a number of populations in the Pacific Northwest in which sneaking behavior was specifically investigated but not observed (i.e. Foster *et al.* 1996), there have been no attempts to simply catalog the frequency with which this behavior is present across populations, especially under natural conditions, in other regions of the threespine range. As will become apparent in Chapter 3, populations in which use of the sneaker repertoire is readily observable under laboratory conditions (yellow symbols, Figure 1.1) may express significantly lower frequencies of this behavior under natural, field conditions. Populations documented in the literature review that either demonstrated absence of sneak fertilizations or absence of nest-raiding behaviors deserve further investigation to elucidate the environmental and social dynamics that might be unique to these systems.

Overall, the collected literature serves as a conservative estimate of the prevalence of sneak fertilizations across the threespine Holarctic distribution. It is likely that the majority of populations in which genetic studies suggest mixed clutch paternity do indeed utilize the sneaker repertoire to obtain those stolen fertilizations but without observational evidence of such, these studies are categorized as suggestive but unconfirmed instances of the sneaker repertoire (Rico *et al.* 1992; Largiader *et al.* 2001;

Le Comber 2003; Blais *et al.* 2004; Krobbach 2006; Eizaguirre *et al.* 2009; Table 1.1, Figure 1.1). Although it seems laborious to regroup the nest-directed behaviors performed by males, it is essential considering the goal is to estimate alternative reproductive tactic prevalence across the Holarctic region based on the published literature. This is especially important knowing that some populations do not readily exhibit sneak fertilization behavior in the field, but do readily exhibit nest-raiding behaviors – with or without use of the sneaker repertoire (e.g. Kynard 1978; Foster *et al.* 1996; Chapter 2). Failure to specify the nest-directed behaviors observed in each study would otherwise lead to inflation of sneak fertilization prevalence across populations.

## **(II) Estimating ancestral character state for the sneak repertoire in *G. aculeatus***

Based on observations of sneak fertilization behavior in a handful of oceanic and freshwater populations of the Pacific Northwest, Foster *et al.* (1996) proposed the presence of this alternative reproductive tactic to be the ancestral condition in the threespine post-glacial radiation of this particular region. In addition, the distribution of sneak fertilization observations collected from the literature, coupled with information regarding the phylogenetic relationships among disjunct biogeographic regions of threespines and phylogenetic relationships among stickleback genera (Family: Gasterosteidae) provide strong evidence that the presence of sneak fertilization behavior is the ancestral condition across the entire threespine Holarctic distribution.

Of the 13 populations exhibiting sneak fertilization behavior, observations for 5 of 13 (38%) were conducted under natural field conditions. For the other 8 populations, it is assumed in these studies that the behavior observed under laboratory conditions (typically pool or aquaria observations with multiple males interacting, e.g. van den Assem 1967; Li & Owings 1978a,b; Sargent & Gebler 1980; Bakker *et al.* 2006; Candolin & Vlieger 2013; or paired male trials, e.g. Vlieger & Candolin 2009) is indicative of what

would be observed under natural conditions. Although the frequency of sneaking behavior can increase greatly under lab conditions (Chapter 3).

Figure 1.1 shows the Holarctic distribution of populations expressing sneak fertilization behavior (i.e. all arrow and circle symbols). This distribution demonstrates that sneak fertilization behavior is present throughout the range of the Threespine Stickleback and includes populations that are marine, anadromous and freshwater. Molecular evidence indicates that oceanic populations are very large, with regional breeding assemblages interconnected by high levels of gene flow (Withler & McPhail, 1985; Taylor & McPhail 1999, 2000; Cresko, 2000; Hohenlohe *et al.* 2010). Oceanic populations in the Cook Inlet region of Alaska exhibit such high genetic diversity at microsatellite loci that there is greater than a 90% probability that two alleles drawn at random will differ (Cresko, 2000). These data, in combination with the relative stability of oceanic environments, suggest that oceanic populations are unlikely to have experienced significant genetic change, due to either genetic drift or natural selection since giving rise to the recent freshwater radiation and, thus, trait expression in the oceanic form can be used to infer the ancestral condition. However, local gene flow between rapidly adapting post-glacial freshwater populations and oceanic populations can contribute to regional genetic differentiation within and across oceanic basins (Schluter & Conte 2009; Hohenlohe *et al.* 2012; Jones *et al.* 2012). Morphological traits vary little over the modern Holarctic range of oceanic threespines (Walker & Bell, 2000), and the fossil record demonstrates morphological stasis over at least the last 10 million years (Bell 1994). Indirect evidence indicates that the red nuptial coloration and some elements of behavior are ancestral too, as these traits can be expressed by oceanic fish throughout their range (Rowland 1994).

The most inclusive molecular phylogeny to date is a study by Orti *et al.* (1994) based on a single gene, *cytochrome b*. The best prediction of the relationships, based on the mtDNA haplotypes sampled, supports the hypothesis that one of two Pacific clades is basal to the Atlantic clade, possibly caused by a bottleneck in the Atlantic followed by a reinvasion of these waters by Pacific haplotypes before gene

flow through the Bering seaway declined (Orti *et al.* 1994). More recent genetic comparisons of oceanic and derived freshwater populations suggest strong bidirectional gene flow and caution that oceanic populations may be complex metapopulations complicating wide-sweeping generalizations across regions (Schulter & Conte 2009; Hohenlohe *et al.* 2012; reviewed in Foster 2013b). However, other phylogenetic analyses of mtDNA corroborate an ancient divergence between a group of Japanese populations and a group of Western and Eastern populations in the Pacific clade that are more closely related to the Atlantic clade (Haglund *et al.* 1992; O'Reilly *et al.* 1993; Johnson & Taylor 2004). Observations of sneak fertilization behavior in oceanic and freshwater populations from both the east and west Pacific (including Japan; Figure 1.1) demonstrate that the presence of the sneaker repertoire is a fairly ancient character state within *G. aculeatus*.

While the presence of the most basic form of inconspicuous sneaker repertoire behavior across the Holarctic threespine distribution indicates an ancestral origin, it is likely that this origin is even deeper within the Gasterosteid family. There have been multiple attempts to reconstruct the Gasterosteidae phylogeny using various combinations of morphological, behavior, and genetic data. Generally, the studies agree on the basal placement of *Spinachia spinachia* (fifteenspine stickleback) and *Apeltes quadracus* (fourspine stickleback) but differ in the relationships among *Culaea inconstans* (brook stickleback), *Pungitius pungitius* (ninespine stickleback), and *Gasterosteus spp.* (*aculeatus* and *wheatlandi*) (reviewed in Mattern 2006). However, the most recent phylogeny, only based on a molecular dataset but including whole mitochondrial genomes and a small set of nuclear genes, places the *Gasterosteus* species basal to the rest of the taxa and *S. spinachia* sister to all other extant Gasterosteid *spp* (Kawahara 2009). Either way, a shared origin of the sneaker repertoire is the most parsimonious explanation if multiple taxa within the Gasterosteid family demonstrate behaviors similar to the threespine sneaker repertoire, regardless of whether *G. aculeatus* is derived or basal. A literature search on behavioral repertoires of other Gasterosteid genera revealed documentation of behaviors

similar or identical to the sneaker repertoire and sneak fertilization behavior, as well as nest-raiding behaviors. In other cases, genetic analyses support the existence of alternative reproductive tactics in groups besides *G. aculeatus*.

Under laboratory conditions, nest-less male ninespine stickleback (*Pungitius pungitius*) have been observed to use sneaking behavior during courtship (Morris 1952). The behavior was originally described by Morris (1952) as 'homosexual' or 'pseudofemale' behavior because the males assumed the coloration and patterning of females and attempted to enter the nest during the time of courtship a female normally would enter. The described repertoire is similar to the threespine sneak fertilization repertoire also observed and described by Morris (1952), with a few exceptions related to post-repertoire behavior. Egg cannibalism, as opposed to egg-stealing, was often observed in the ninespine following sneaking occurrences in the laboratory. Egg cannibalism was also reported as a common occurrence in the field (Morris 1952). Without further data to support mixed paternity as an outcome of this behavior in *P. pungitius*, the use of the sneaking repertoire to steal fertilizations has yet to be determined in this species.

Genetic analysis of progeny in a natural population of fifteenspine stickleback (*Spinachia spinachia*) demonstrates the presence of mixed paternity in this species (Jones *et al.* 1998). The authors estimated that 18% of the nests contained progeny from sneak fertilizations and 17% contained progeny due to egg-stealing. Stolen fertilizations have also been observed in the lab (Ostlund-Nilsson 2002). However, there has been no published description of the behavioral repertoire used by fifteenspine cuckolders so the extent to which it is similar in context and motor patterns to the sneaker repertoire of threespines is unknown.

Sneak fertilization behavior in fourspine stickleback (*Apeltes quadracus*) originally was described under in the laboratory by Rowland (1979). However, there were no accounts of stealing nest material or egg-stealing. Willmott & Foster (1995) also described fourspine sneaking behavior in the laboratory.

They suggested that males use female mimicry of both coloration and behavior to sneak, considering nuptial coloration is typically restricted to pelvic spines and can be easily hidden. Many sneaking males assumed the head-up posture similar to receptive females, which resulted in low aggression towards sneaker males by nest-holders and fewer interruptions during courtship in the presence of sneakers (Willmott & Foster 1995). Threespine sneakers assume a similar head-up posture under both field and lab conditions. This behavior usually is observed if a nest-holder spots a sneaker and assumes a head-down posture. The sneaker then rises from the substrate in the head-up position before fleeing the territory (K. Shaw per. obs.). In this respect, the head-up postures also seems to function to decrease territorial male aggression.

No reports of sneak fertilization behavior have been published for either the blackspotted stickleback (*Gasterosteus wheatlandi*) or the brook stickleback (*Culea inconstans*). Egg-stealing has been observed in *C. inconstans*, particularly at high nesting densities – although most stealing attempts were followed by agonistic interactions that resulted in abandonment of the stolen eggs (McKenzie 1974; Wootton 1976). Whether or not the sneaker repertoire was utilized during nest-raid attempts was not reported in these studies.

The observation of behaviors resembling the sneaker repertoire and sneak fertilization observations (or the inference of stolen fertilizations based on behavior similarities or mixed paternity) in these other gasterosteid species provides further support for the placement of sneak fertilization behavior within the ancestral behavioral repertoire of *G. aculeatus*. Ancestral presence of the sneaker repertoire for *G. aculeatus* is the most parsimonious hypothesis, regardless of whether *G. aculeatus* is a more derived group within the Gasterosteidae clade (Keivany & Nelson 2004; Mattern & McLennan 2004; Mattern 2006) or a more ancestral group (Kawahara 2009).

### **(III) The variables influencing sneaking propensity**

There are a number of variables that are commonly associated with expression of alternative reproductive behaviors. These variables are typically associated with male traits subject to intra-sexual selection or inter-sexual selection – including body size/condition (quality), dominance ranking, and territory characteristics (e.g. territory size, inter-nest distance, territory cover). A number of studies in the literature investigate specific variables potentially related to sneak fertilization propensity, either using experimental manipulation or correlative analyses. I attempt to review these studies by grouping results according to variable investigated rather than by individual study. The references for the studies, which are a subset of published articles from the original literature search, and a summary of their results are provided in Table 1.2. Unless otherwise noted, all cited studies are on Threespine Stickleback.

#### ***Body Size/Age***

In an observational study on a natural Japanese population, Mori (1995) detected no difference between the body size of nested males observed to perform nest-raiding and sneaking behaviors and males who did not exhibit these behaviors. Size comparisons between nested sneakers and nested nonsneakers in several European populations – as determined by genetic analyses of nested males in experimental pools – also suggested no significant difference in standard length or weight between the two groups (Le Comber 2003; Candolin & Vlieger 2013). A lack of size differences also was observed for non-nested sneakers and nested courting males in dyadic trials (Vlieger & Candolin 2009). In these paired male trials, Candolin & Vlieger (2009) found no significant size difference between successful and unsuccessful sneakers. However, paternity analyses performed on males from replicate field enclosures from another European population suggested that small males were significantly more successful at performing sneak fertilization (Eizaguirre *et al.* 2009). The size distribution for males unsuccessfully attempting sneak fertilizations in this study is unknown, because the only evidence for sneaking is evidence of successful



attempts. If there is a wide size distribution of males demonstrating the behavior, despite their success, it requires a slightly different interpretation than if this behavior is only displayed or elicited in small males. The former scenario would suggest that males exhibiting sneaking behavior do not differ significantly in size from males that do not exhibit this alternative tactic but only the smallest males are successful. The latter scenario is suggestive of a more constrained condition-dependent strategy.

Size is used as a proxy for age in many organisms with indeterminate growth and some stickleback populations have multiple age classes of males on the breeding grounds. In populations that can have multiple age-classes, younger males may be more likely to adopt the sneaker tactic when social and environmental circumstances decrease their competitive ability (FitzGerald *et al.* 1989; de Fraipont *et al.* 1993). Under laboratory conditions, both 1+ and 2+ males from a biannual Quebec population expressed sneak fertilization behavior, although 1+ males exhibited sneak fertilization behavior significantly more often in dyadic trials (1+: 25% versus 2+: 5%) when deprived of the ability to nest themselves and in more complex rival scenarios (1+: 21% versus 2+: 5%). Younger males also produced more total sperm and more motile sperm than older males when nesting in solitary, noncompetitive situations. The authors suggested that this age-rated difference in sperm traits could simply be a consequence of senescence in 2+ males but could also play a positive role in the sneak fertilization behavior more frequently expressed by younger males. While previously suggesting that territorial defense and parental care may be more costly for younger males (FitzGerald *et al.* 1989), de Fraipont *et al.* (1993) found that females did not express a preference for either age class and males did not express age-related differences in parental care (at least in noncompetitive situations). Thus, when provided similar reproductive resources, age-classes did not differ significantly in measures of reproductive success. Dufresne *et al.* (1990) also found equivocal results related to male age/size and competitive ability. Under laboratory conditions, 2+ males were found to have a competitive advantage over 1+ males when there is a large discrepancy in size. Under field conditions, however, there was no significant

difference in the number of nested 1+ and 2+ males and no difference in reproductive success (as measured by number of eggs) between age groups.

In the general literature on traits associated with alternative reproductive tactics, especially in fish, small size is often characteristic of sneak fertilization tactics (reviewed in Taborsky 2008). An association between small size and sneaking propensity often is observed in species expressing developmentally plastic but sequential tactics (Black goby: Immler *et al.* 2004; Black-faced blennies: De Jonge & Videler 1989; Bluehead wrasse: Warner & Hoffman 1980) or developmentally fixed alternative tactics (Atlantic salmon: Thorpe *et al.* 1998; Bluegill Sunfish: Gross 1982; Plainfin midshipman: Grober *et al.* 1994). Such tactics are often correlated with irreversible morphological polymorphisms. However, there are other species in which differences between males, beyond size or age, are primarily behavioral in nature – such sequential developmental tactics are often reversible – e.g. small non-nested males lack secondary sexual characteristics and are obligatory sneakers while small nest-holding males switch between tactics depending on a variety of factors (Common goby: Magnhagen 1994, 1998; European bitterling: Smith *et al.* 2004; Freshwater blenny: Neat *et al.* 2003; Rose Bitterling: Kanoh 1996). However, there are species in which males exhibit sneaking behavior irrespective of size (e.g. facultative tactic -- pupfish: Kodric-Brown 1986; sandperch: Ohnishi *et al.* 1997; sand goby: Takegaki *et al.* 2012; triplefin blenny: Neat 2001). The flexibility of reproductive tactic expression may depend on the life history variation expressed among stickleback populations. Populations with multiple age/size classes present may be more likely to exhibit sneaking as a reversible sequential developmental tactic. However, opportunistic sneaking behavior may be selected for in populations in which lifespans are shorter and other variables, such as territory availability, allow small males to nest successfully. Future investigations may demonstrate population differences in size correlations with sneak fertilization propensity if a number of population-specific factors affect the significance of male size.

### ***Dominance Ranking***

In male territorial scenarios, male dominance ranking is typically determined by success in aggressive disputes. In a complicated rival scenario, van den Assem (1967) found no correlation between dominance ranking and tendency to sneak. However, there was a negative correlation between egg-stealing and rank – high ranking males were less prone to egg-stealing by rival males. Under similar laboratory conditions, Li & Owings (1978a,b) found that when males exhibited greater differences in status, higher ranking males were more likely to disrupt the courtship of lower ranking males, decreasing probability of courtship success for the low-ranking male. If lower ranking males exhibited successful nest-raiding behavior and sneak fertilization behavior, they experienced a significant increase in status (e.g. territory enlargement or increased nest activities). However, the frequencies of both nest-raiding behaviors and sneak fertilization attempts were highest for paired males of similar dominance rankings or status. Li & Owings (1978b) propose that nest-raiding behavior and sneak fertilization attempts complement dominance-related aggression in that raiding and sneaking are most effective when dominance-related aggression is less effective at creating differences in attractiveness to females. In species with aggressive territorial interactions, size correlations with dominance rank is generally expected. However, none of the studies that have focused on male dominance ranking with respect to sneaking propensity specifically investigated how dominance ranking related to male size or age.

### ***Territory Size***

Under laboratory conditions, males with larger territories experienced lower rates of sneaking by rival males. In complex arenas with large territories (similar to natural conditions), 24% of courtships involved sneak fertilizations, while in small tanks where male territory space was restricted, 44% of courtship observations resulted in sneak fertilizations (van den Assem 1967). van den Assem (1967) attributed this

trend to the fact that males with large territories are more likely to attract females and collect clutches soon after territory and nest establishment, so males with large territories are more likely to stop sneaking earlier than males with small territories. Although not demonstrated directly, van den Assem (1967) proposed that, if males with large territories are entering the parental phase sooner than other males, then the sneak fertilization attempts observed are performed by males with relatively smaller territories. Goldschmidt & Bakker (1990) conducted similar observations under natural conditions and found males with larger territories had higher reproductive success and suffered less egg-stealing – although a direct relationship between territory size and sneaking propensity was not investigated. Le Comber (2003) found similar patterns, in that males with large territories were more likely to dominate spawnings and were less likely to be victims of sneak fertilization. However, there was no difference in territory size of sneakers and sneak victims based on paternity analyses (Le Comber 2003). Mori (1995) also found no difference in territory size between sneaker and nonsneakers under natural conditions.

### ***Close Nesting/Nest Density***

While inter-nest distance has been used as an indirect measure of territory size (FitzGerald & Whoriskey 1985), it more accurately serves as a measure of proximity or density on the breeding grounds. Regardless of overall territory sizes, male nests can still be spread out over the breeding grounds (maximizing distances between nests). Although not demonstrated explicitly, field observations of interactions between nested males led both Kynard (1978) and Mori (1998) to posit that nesting within aggregations (i.e. high densities) may allow males to both attract more females and detect sneakers at greater distances. On the other hand, results of paternity analyses demonstrate that male density is positively correlated with sneaking success under semi-natural conditions (Eizaguirre *et al.* 2009). While this study can account for the correlation between density and sneak fertilization success, it would be

interesting to combine paternity results with observations of all sneak fertilization attempts to determine if the frequency of failed attempts is also related to male aggregation.

Observations under natural conditions in a Japanese population show that, even though neighboring males were more likely to participate in nest raids and sneak fertilization behavior, the probability of sneaking between territorial males was not correlated with male density on the breeding grounds but instead was positively related to the number of neighboring males without eggs (Mori 1995). In other words, frequency of sneak fertilization attempts within a group of males was related to the density of unsuccessful males within the group. Similar results have been observed in other aggregate nesting fish with parasitic reproductive tactics. In the Mediterranean damselfish (*Chromis chromis*), the likelihood of nestholders being parasitized by sneakers was positively correlated both with the number of neighboring nests and the number of neighboring males with empty nests (Picciulin *et al.* 2004).

In a field comparison of several sneaker and nonsneaker populations, Goldschmidt *et al.* (1992) found that, within populations that exhibited the sneaker repertoire, sneak fertilization attempts were more likely to occur when males nested in close proximity. However, comparable inter-nest distances were estimated in populations in which no sneak fertilization attempts were observed. Wootton (1971) observed that males performing raiding (both nest-raiding and sneak fertilization behavior) were generally from territories adjacent to their victims.

While proximity of neighboring males or neighborhood density may be important for determining patterns of sneak fertilization success within populations, it is less certain how sneak fertilization frequency and nest density may be related across populations. The multi-population stickleback study by Goldschmidt *et al.* (1992) demonstrates that nesting density is not a trait predictive of presence or absence of sneak fertilization behavior in a population. In the sand goby (*Pomatoschistus*

*minutus*), sneak fertilization frequency (based on paternity analyses) was not found to differ between two populations with average differences in nest-site density (Jones *et al.* 2001). One might expect high density or close inter-nest distance between neighbors to promote sneaking behavior if sneaking in stickleback is generally an opportunistic tactic performed by neighboring males. But, as mentioned above, this availability of opportunities in high density groups might be countered by higher vigilance or greater visibility of neighbors. In addition, non-nested males or at least males not nested within a group of males exhibit sneak fertilization attempts (Vlieger & Candolin 2009; K. Shaw pers. obs.; Chapter 2). If long-distance sneakers or nonterritorial males contribute greatly towards the sneaker frequency in a population, then inter-nest distance will not be an important variable.

### ***Nest Visibility***

Increased nest concealment was found not only to be preferred by females (Sargent & Gebler 1980; Sargent 1982), but also to reduce the probability of sneak fertilizations under laboratory conditions (Sargent & Gebler 1980). However, the opposite results were observed in a field study of another population – nests in dense vegetation were more likely to be raided and experience sneaking attempts (Mori 1995). Investigating the potential effects of decreased visibility in a population experiencing increased eutrophication, Vlieger & Candolin (2009) found that increased water turbidity, but not increased vegetation, significantly decreased sneak fertilization success. However, neither type of experimental treatment affected the total number of sneaking attempts in their dyadic laboratory assays. In a follow-up study comparing sneaking in experimental pools of nested males in high and low vegetation (visibility) treatments, Candolin & Vlieger (2013) found that more males nested in pools with dense vegetation than sparse vegetation and, based on paternity analyses, a smaller proportion of eggs were sneak fertilized in dense vegetation. However, in this experiment, they were unable to determine

if the lower visibility decreased the total number of sneak fertilization attempts. Males can either reduce their investment in the sneaker tactic under low visibility (few attempts, few successes), which suggests that males are balancing the costs of sneaking against its benefits (Candolin & Vlieger 2013) or males can suboptimally choose to invest effort in a sneaking behavior even though reduced visibility hinders sneaking success (i.e. many attempts, few successes).

It is possible that the effect on nest visibility of sneaking propensity and sneak fertilization success may be related to the type of obstructions (e.g. rocks and rock crevices, vegetation, turbid water), as well as the experience of the sneaker. Nested males may be more familiar with the layout of a neighbor's territory, despite nest concealment, compared to a non-territorial, roaming male.

### ***Territory and Nest Establishment Order***

Males in some threespine populations arrive at the breeding grounds asynchronously (especially pertinent for anadromous populations) and establish territories and nests before courting females. Asynchronous mating behavior provides newly arriving males with the opportunity to steal fertilizations from neighboring established males while establishing their own territory – evidence of this pattern has been observed in several studies on sneaking behavior (Goldschmidt *et al.* 1992; Jamieson & Colgan 1992). Jamieson & Colgan (1992) further suggest that, beyond an early start with territorial establishment and nest-building, earlier established males become physiologically primed to complete the courtship cycle once they gain their first clutch, as males typically court more vigorously following a successful spawning. Thus the pattern of reproductive success typically follows a fixed order of establishment even in instances in which all males have completed a nest (Jamieson & Colgan 1989, 1992; K Shaw pers. obs – Chapter 3). Also, actively denying territorial males access to nesting material in the presence of nested males also elicits sneaking behavior (de Fraipont *et al.* 1993; Vlieger & Candolin

2009). Similar circumstances were found to elicit sneaking behavior in *P. pungitius* – high densities of breeding males resulted in expression of sneaking behavior in nonterritorial males (Morris 1952).

### ***Operational Sex Ratio***

Alternative reproductive tactics generally are assumed to be more prevalent when the operational sex ratio (OSR; Emlen & Oring 1977) is skewed towards a higher abundance of males than females – forcing males of lower competitive ability to seek other ways to gain reproductive success besides the species typical reproductive tactic (Andersson 1994; Shuster & Wade 2003). Goldschmidt *et al.* (1992) used encounter rate between courting males and females as a proxy for OSR in their interpopulation comparison and discovered no relationship between OSR and presence of sneaking behavior – encounter rate was both lowest and highest among the non-sneaking populations. Female availability may affect sneak fertilization propensity, at least within sneaking populations, but this effect could be operating at the local level within the breeding grounds – (e.g. mean crowding, Shuster & Wade 2003; the perceived sex ratio (PSR), Gowaty & Hubbell 2005).

### ***Male Quality***

It has been suggested that variance in reproductive success due to intrasexual competition and/or female choice results in males of low quality using alternative mating tactics to gain access to mates (Dawkins 1980; Hazel *et al.* 1990; Taborsky 2008). Le Comber (2003) observed no significant difference in measures of quality (i.e. SL, red intensity, territory size, and courtship rate) between sneaker males and their victims. As stressed previously for studies in which the determination of sneaker behavior was based on paternity analyses, these results only assessed the quality of successful sneakers. Assuming



that females prefer to spawn with males of higher quality (based on coloration, courtship vigor, and olfactory cues: Mikinski & Bakker 1990; Bakker & Mundwiler 1994; von Hippel 1996; Candolin 1999, 2000, 2003; Milinski *et al.* 2005; Kraak *et al.* 1999; Cubillos & Guderley 2000; Pike *et al.* 2010), a lack of significant differences in quality between sneakers and their victims could indicate that the sneaking tactic is used more successfully by high quality males. Low quality males may be less likely to gain fertilizations using either tactic. Le Comber (2003) predicted that any given sneaker should actually be of higher quality than the sneak victim in order to be successful, especially if territorial vigilance is associated with quality in territorial males (i.e. a compromised ability to defend territory in lower quality males). In experimental breeding pools, Candolin & Vlieger (2013) found that males successful at gaining fertilization via courtship were also successful at gaining paternity via sneaking, according to genetic paternity analyses. In this study, males with high reproductive success via courtship were assumed to be the attractive, preferred males, although no specific analyses correlating male traits with reproductive success were performed. However, their findings support Le Comber's (2003) predictions. This idea that sneaking is primarily performed by high quality males that also predominately perform the primary mating tactic (e.g. territorial courting) runs counter to the typical argument that the occurrence of alternative mating tactics should increase with the proportion of individuals unable to gain access to defendable territory or mates (Shuster & Wade 2003; Taborsky 1998, 2008).

Within populations, sneaking frequency may be higher when more males, including low quality males, are able to gain access to territories and nest because these low quality males are more likely to be the victims of sneak fertilization attempts – possibly by both high and low quality males (Le Comber 2003). In an earlier genetic analysis of sneaking and egg-stealing in a freshwater European population, Largiader *et al.* (2001) found that victims of sneaking were significantly smaller than other parental males and reproductive success (as measured by the #eggs or fry produced) was positively correlated with male body size. These results suggest that these particular parental males were lower quality males,

regardless of whether lower reproductive success was indicative of poor parental care abilities or collection of fewer clutches (e.g. mating with fewer females). Counter to this, Candolin & Vlieger (2013) did not find a size difference between nested males who were and were not sneak victims based on their genetic analysis of an oceanic European population in an experimental laboratory assay. That fact that smaller males may be more likely to be victimized in some populations may be due to lower territory defense abilities. However, it is expected that smaller, less preferred males would have fewer overall mating opportunities. Thus, the probability of successfully sneaking on smaller males would increase when small, low quality nested males are abundant during the breeding season and females are forced to be less choosy.

In terms of olfactory cues, the major histocompatibility complex (MHC), which involves genes (particularly MHC class II) that code for proteins of vital to immune functioning, have been found to play a role in stickleback mate choice (Milinski et al. 2005). Krobbach (2006) investigated the role of allelic diversity in mate choice and found no significant difference in MHC variant number between sneaker males and males chosen as mates by females, and both chosen males and sneaker males had the optimal number of MHC variants (5 alleles). Males with the optimal number of MHC variants also tended to be in better condition, although this result was not significant. In the same population, Eizaguirre *et al.* (2009) investigated MHC differences between sneakers and nonsneakers (determined via paternity analyses) and found a trend towards males lacking a specific MHC haplotype to demonstrate increased sneaking propensity while males with this specific MHC haplotype expressed significantly higher reproductive success via female choice. Although not a statistically significant result, Eizaguirre *et al.* (2009) suggest that males of lesser 'genetic quality' may circumvent female choice by sneaking fertilizations. Interestingly, MHC signals are dependent on the reproductive state of males (i.e. only produced when males are reproductively receptive) and such signals can be costly to produce. Males whose immune system is activated by infection should not be able to produce the excess MHC necessary

for signaling purposes and would stop maintaining a nest or maintain a nest and produce a weak MHC signal (Miliniski *et al.* 2010). An intriguing possibility is that sneaking propensity may be linked to compromised MHC signaling and an inability to effectively attract females.

### **Summary**

The above study descriptions and comparisons (as summarized in Table 1.2) demonstrate that there are different associations between environmental and social variables and expression of sneaking behavior and the strength and direction of these associations vary among populations. Hyatt & Ringer (1989) came to a similar conclusion regarding the relative importance of cannibalism and egg-raiding and the factors promoting these behaviors when observing the diverse environmental conditions, behavioral events, and types of individuals that were associated with these nest-directed behaviors. What types of conditions could be responsible for differential selection pressures on sneaking behavior? Variation in nest cover/concealment or available breeding habitat is associated with higher propensities in some populations compared to others. Higher territorial vigilance due to selective pressures by nest predators (e.g. sculpin or cannibalistic groups) may affect the accessibility of nests for sneakers resulting in differential opportunities across populations. Female availability or nesting synchrony among males may affect the sneaking cost-benefit ratio differently across populations. Lack of associations between variables and sneaking propensity or lack of sneaking behavior expression entirely could occur in populations if there is little variance in the predictor variable under natural conditions. It is also possible that differences in association observed in the literature are due to differences in methodologies (e.g. field versus laboratory studies, different definitions of dominance rank or territory size, etc.). The intriguing interpopulation differences described (Wootton 1971; Goldschmidt & Bakker 1990; Goldschmidt *et al.* 1992), coupled with the equivocal findings regarding potential associations between

variables and sneaking behavior suggests that it would be useful to employ consistent methods for future geographic comparisons.

#### **(IV) Categorizing the threespine alternative reproductive tactic**

Alternative phenotypes can evolve if there is more than one adaptive option in a given functional context (West-Eberhard 2003). The concept of alternative reproductive tactics is that individuals allocate resources to mutually exclusive ways of achieving reproductive success: e.g. territoriality vs. floating, monopolization vs. scramble competition, resource and access investment (bourgeois tactic) vs. reproductive parasitism (sneak/streak fertilization, female mimicry) (Taborsky, Oliveira & Brockmann 2008). As long as alternative phenotypes (either behavioral or morphological) are developmentally and functionally semi-independent of each other, they can be independently subject to selection, as well as differentially expressed among individuals (West-Eberhard 2003). Alternative reproductive tactics can also be considered from an adaptive plasticity perspective, especially when tactic flexibility is observed – male reproductive tactics (e.g. bourgeois male vs. parasite) as adaptations to a changing environment of male-male reproductive competition (Shuster & Wade 2003).

Current categorization of male alternative reproductive tactics mainly separates expression of alternatives based on the extent of spatial and temporal environmental variability that individuals experience during their lifetime (Schlichting & Pigliucci 1998; Taborsky, Oliveira & Brockmann 2008). Developmentally fixed alternatives, based on either genetic polymorphisms (e.g. swordtails, *Xiphophorus spp.*, Zimmerer & Kallman 1989) or genetic monomorphisms with developmental switchpoints (e.g. Atlantic salmon, *Salmo salar*, Aubin-Horth *et al.* 2005), are most likely to arise if the environment is coarse-grained and environmental change is either spatially or temporally rare or unpredictable during a lifetime (Levins 1968; Moran 1992; Schlichting & Pigliucci 1998; West-Eberhard

2003; Taborsky *et al.* 2008). Developmentally fixed alternatives typically exhibit different suites of morphological, physiological, and behavioral traits that are integrated but potentially constrained by this integration (DeWitt *et al.* 1998; Sih *et al.* 2004; Duckworth 2010; Dochtermann & Dingemanse 2013; Snell-Rood 2013). For example, male bluegill sunfish, *Lepomis macrochirus*, either reach sexual maturity when small and become a parasitic sneaker morph with relatively large testes (later transitioning into a female mimic) or grow slowly and mature at a larger size to become a nest-holding bourgeois/parental morph (Gross 1982; Neff *et al.* 2003; Neff 2004). Tactic-specific success is likely constrained by male size at maturity, as growth slows after sexual maturation (small males have low resource-holding potential and large males are unlikely to be successful at sneak fertilizations or female mimicry). Size, rather than age, is predicted to be the underlying mechanism of this ontogenetic switchpoint, considering morph reproduction age varies among populations (reviewed in Taborsky & Brockmann 2010). In terms of behavioral costs and benefits, such developmental behavioral plasticity is slow to form and costly (time and energy invested in feedback during development and in the initial neural networks associated with learning) but can produce a wide range of highly integrated responses (Snell-Rood 2013). However, in terms of developmentally fixed mating tactics, wide-ranging responses are usually reduced to combinations of integrated, alternative traits via selection.

Facultative tactics are based on plastic responses to conditions and are simultaneous or sequential in their expression over the lifetime of an animal. Simultaneous refers to tactic choice dependent on circumstances (Taborsky, Oliveira & Brockmann 2008; Taborsky & Brockmann 2010). This term was originally used to describe simultaneous hermaphroditism, in which individuals switch sex based on immediate environmental conditions or social partners (Brockmann 2001; Taborsky, Oliveira & Brockmann 2008). Use of flexible and reversible alternative mating tactics is advantageous if individuals generally experience fine-grained changes in their physical and social environment or fine-grained changes in their own physical condition (Levins 1968; Moran 1992; Schlichting & Pigliucci 1998;

West-Eberhard 2003). Highly flexible tactics are particularly beneficial when environmental and social conditions are very unpredictable, especially when little information exists concerning the number of potential mates and the quality and number of current competitors (Taborsky, Oliveira & Brockmann 2008; Taborsky & Brockmann 2010). For highly flexible and reversible tactic switches, individual condition or quality (e.g. dominance status, resource-holding potential, body condition) may be context-dependent if social dynamics (social groups and dominance hierarchies) shift frequently within breeding cycles or seasons (Bierbach *et al.* 2014). Plastic responses associated with simultaneous tactics are often behavioral and involve differential activation of neural and physiological networks in response to immediate environments or conditions (Zupanc & Lamprecht 2000; Burmeister 2007; Taborsky, Oliveira & Brockmann 2008; Snell-Rood 2013). The most costly aspect of this activational behavioral plasticity is the maintenance of a large, complex, energetically expensive neural network necessary for fine-range motor responses and large behavioral repertoires (reviewed in Snell-Rood 2013).

Sequential tactics are also plastic but generally involve a developmental switch, which is an optimal response when conditions change with ontogeny (Taborsky, Oliveira & Brockmann 2008). Sequential tactics are more likely to be observed in organisms with indeterminate growth, as alternative tactics become more or less tractable as individuals grow in size (i.e. small males as sneaker, large males as territorial nest-holders). The terminology for this type of plastic trait is originally derived from categories of sequential hermaphroditic strategies (Brockmann 2001). Sequential tactics can be of a fixed sequence (i.e. fixed sequential plastic tactics), such as the ontogenetic switch between parasitic and territorial tactics in the Mediterranean (ocellated) wrasse (*Symphodus ocellatus*, Taborsky *et al.* 1987; Alonzo *et al.* 2000), or they will be reversible over the lifetime of the animal (i.e. reversible sequential plastic tactics). Condition dependence may be relevant to reversible sequential tactics, especially if changes in environmental and social factors influence the link between size and territory-holding potential. While few alternative mating tactics have been categorized as reversible sequential

tactics, behavioral mating tactics or flexible body coloration are more likely to characterize such reversible alternative tactics than morphologically divergent traits that are constitutive or difficult to reverse (Brockmann 2001). A potential example of reversible sequential tactics is the territorial (courtship), semi-territorial (courtship and sneak fertilizations) and non-territorial (sneak fertilizations) tactics of the southern mouthbrooder cichlid (*Pseudocrenilabrus philander*). While the largest males generally adopt the territorial tactic as predicted for an animal with indeterminate growth, laboratory manipulations of male density and social status demonstrate that large males can revert to the semi-territorial and non-territorial tactics, replete with the behavioral and coloration changes associated with those tactics (reviewed in Chan & Ribbink 1990). Such tactics are expected to involve both developmental and activational behavioral plasticity. Fixed and plastic alternative tactics, but sequential tactics especially, should include aspects of both developmental behavioral plasticity that allow for longer-term changes (e.g. changes in hormone levels; Moore 1991; Oliveira *et al.* 2008) and activational behavioral plasticity (e.g. changes in aggressive behavior or territorial defense; Burmeister 2007; Huffman *et al.* 2012; Snell-Rood 2013).

Plastic simultaneous and sequential tactics, especially, are not mutually exclusive classifications of tactics for many populations. For instance, many species have mating systems in which tactic switches generally occur with size (i.e. sequential tactics), although males in intermediate categories typically exhibit more opportunistic use of alternative tactics (i.e. simultaneous tactics). In the common goby (*Pomatoschistus microps*), males of intermediate size switch between territorial courtship tactics and sneaking, while small and large males do not (Magnhagen 1992). The difficulty with interpreting these mixed strategy scenarios is that intermediates expressing simultaneous tactics can simply be an ontogenetic step in a sequential tactic or the genetic threshold for tactic switching itself may differ among individuals (Dominey 1984; Shuster & Wade 2003). However, if there is individual variation in the genetic threshold for tactic switching, a correlation between male size and expression of

simultaneous tactic use should be fairly weak. Examples of mating systems exhibiting genetic variation in tactic-switching thresholds include species with size-based sequential tactics in which opportunistic switching is related to the immediate social situation rather than size – small male Arctic charr (*Salvelinus alpinus*) generally employ the sneaker tactic in the presence of larger males but many will readily switch tactics (and sperm traits) if they find themselves to be the largest male on a spawning site (Rudolfson *et al.* 2006), or opportunistically court a female on a spawning site while the site-holder is preoccupied (Sorum *et al.* 2011).

Reversible facultative tactic switching (simultaneous and sequential) can be categorized along a responsiveness continuum from highly flexible, opportunistic/situational tactic switches to gradual condition-dependent tactic switches that often require hormonal activation and other time-sensitive physiological processes (e.g. body color changes, mass gain). For instance, fluctuations in individual condition may lead to individuals changing tactics across a breeding season, as observed in animals in which body condition is predictive of territory-holding capacity. In the wrinkled toadlet (*Uperoleia rugosa*), males give up the energetically costly territorial tactic when body condition is poor and become silent satellites until they put on mass and regain a territory (Robertson 1986). In this system, the speed of tactic switching is constrained by the speed of physiological changes. Opportunistic tactics are generally under activational behavioral control and involve rapid switches between tactics (Lucas & Howard 2008; Snell-Rood 2013). Examples include the rose bitterling (*Rhodeus ocellatus*), in which territorial males opportunistically utilize the sneaker tactic during neighboring courtships (Kano 1996) and the small sneaker male Arctic charr (*Salvelinus alpinus*) that opportunistically employ the courtship tactic when territory holders are preoccupied (Sorum *et al.* 2011). Opportunistic tactic expression may not be related to obvious differences between males in size, condition, or dominance status. For instance, use of the sneaker tactic by small, non-territorial male rose bitterling (*Rhodeus ocellatus*) is condition-dependent but larger, territorial males will rapidly employ sneaking behavior during



neighboring courtships (Kano 1996). Individuals in a population can vary in their propensity to opportunistically switch tactics – relying on various decision-rules regarding tactic switchpoints (Brockmann 2001; Shuster & Wade 2003; Burmeister 2007; Dingemanse *et al.* 2010). Individual variation in tactic switching is generally implicit under circumstances in which some territorial males, especially those exhibiting lower reproductive success, are not observed to opportunistically adopt alternative reproductive tactics (Wells 1977; Moore & Thompson 1990; West-Eberhard 2003).

Determining the extent of flexibility underlying plastic mating tactics (from transitional switches within or across breeding cycles or seasons to immediate, rapid switches based on opportunity) is essential to then investigating the specific cues that elicit tactic switching and defining the overall contribution of hormonal and neural control underlying the proximate causes of tactic switching (Zupanc & Lamprecht 2000; Oliveira *et al.* 2008). For instance, rapid responses may reflect the presence of immediate social cues while transitional responses may reflect the acquisition of incremental cues regarding changes in group-level and population-level dynamics. If cue use does not appear to be optimized, it could reflect individual genetic variation in tactic-switching thresholds or time-sensitive constraints in physiological responses that are important to consider when studying the evolution of flexible mating tactics. The extent and rapidity of tactic switching is also vital to consider in terms of optimal time and energy allocation within flexible tactics (Candolin & Vlieger 2013).

Examples of reversible facultative reproductive tactics are listed in Table 1.3. This list is not meant to be comprehensive but rather provide a representative sample of vertebrate and invertebrate taxa. In Table 1.3, reversible, facultative tactics are grouped based upon three categories of tactic flexibility rather than based on whether the plastic tactic is simultaneous or sequential to provide a more fine-grained assessment of timescale of tactic flexibility. ‘Situational’ flexibility can be rapid and short-term, primarily affected by immediate opportunity (e.g. courtship behavior by a neighbor).

‘Intermediate’ flexibility refers to tactic switching that primarily occurs over days, potentially requiring repeated exposure to a stimulus to trigger behavioral switches. Descriptions often use the term ‘opportunistic’ to describe instances of tactic-switching but this term has been used to imply both immediate flexibility and gradual tactic-switching over the span of several days (e.g. switching from a nonterritorial sneaking or satellite tactic to a territorial, nest-holder tactic). To emphasize systems that specifically document evidence of immediate (situational) flexibility, I highlight studies that provide time periods of flexible response to opportunistic cues (Table 1.3). ‘Conditional’ flexibility describes reversible mating tactics that exclude some individuals from one tactic due to population level processes that often change over breeding cycles or breeding seasons (e.g. size- or density-dependent use of the sneaking tactic changes if opportunities arise over the breeding season, such as access to nest sites or physiological changes in body condition). For conditional flexibility, tactic switches are typically elicited by repeated cues similar to those that result in intermediate flexibility, however, due to the nature of the population-level processes involved, switches will be restricted for many individuals and there could be a longer duration between switches when they do occur. Intermediate and conditional flexibility are indicative of reversible sequential plastic tactics whereas situational flexibility is indicative of simultaneous (opportunistic) plastic tactics.

While Table 1.3 is by no means an exhaustive list, it highlights the significance of temporal variation in tactic flexibility within and across animal mating systems. In particular, the presence of more than one form of flexibility for a species is indicative of tactic switchpoint variation within a population (Brockmann 2001; Shuster & Wade 2003; Tomkins *et al.* 2004; Dingemanse *et al.* 2010). A common trend observed in Table 1.3 is the coexistence of a condition-dependent strategy and expression of situational tactic flexibility, in which individuals of intermediate condition (e.g. intermediate size or body condition) or territorial individuals (regardless of specific conditional influences) opportunistically switch mating tactics. Examples in Table 1.3 suggest that intermediate tactic flexibility can be associated with cues

regarding mating success, such that territorial males with low success will transition to sneaking behavior (e.g. the herbivorous damselfish, *Stegastes nigricans*; Karino & Nakazono 1993) or cues regarding short-term changes in energy reserves, such that males of intermediate condition will transition between territorial and satellite tactics every few days (e.g. rubyspot damselfly, *Hetaerina americana*; Raihani *et al.* 2008). Such examples demonstrate an association between intermediate tactic flexibility and condition-dependent cues. Certain mating systems may be more conducive to males of intermediate condition transitioning back and forth between tactics on a short time scale. A number of systems that have been designated as having only conditional flexibility may contain individuals with more rapid and opportunistic tactic flexibility but the designations provided here are limited to the extent of detail regarding flexibility provided in the published accounts. In many explicit accounts of situational plasticity, territorial males opportunistically use alternative tactics on neighboring males (e.g. black-faced blenny, Mozambique tilapia, peacock blenny, rose bitterling, sand perch, southern mouthbrooding cichlid, green tree frog, and Hawaiian field cricket). Many of the other explicit accounts of situational plasticity are systems in which the alternative male tactics are not parasitic but rather depend directly on female availability (courtship/mate-guarding vs mate searching: natterjack toad and soapberry bug) or female response to courtship (courtship vs sneak/forced fertilization/copulation: Trinidadian guppy, alpine newt, and striped wolf spider).

The mating tactics of the Threespine Stickleback have been described as facultative in Taborsky *et al.* (2008) and Taborsky (2008) but the extent of tactic flexibility has not been specified. Studies regarding the stickleback sneaker tactic have provided good descriptions of the immediate behaviors involved and the potential variables influencing the expression of the tactic in a number of populations and geographic regions. However, the extent to which males have the ability to switch between the courter and sneaker tactics has not been investigated in detail. There are hints in several studies to suggest this tactic is indeed flexible, given that territorial nested males and non-territorial males alike

will perform sneak fertilization behavior, suggesting that many males can either sequentially or opportunistically switch between tactics. Results of pool experiments (Jamieson & Colgan 1992) are suggestive of sequential tactic use – sneaking is generally performed by males of lower dominance status until males of higher dominance status transition from courting to parental behaviors, thus increasing mating opportunities for males of lower status (Table 1.2). However, it is possible that this result is an artifact of the constrained nature of female choice on a small subset of males with variation in their dominance status. When there is a temporally consistent hierarchical social situation, it may be difficult to reveal individual male potential for situational tactic switching, even though such potential may exist (Howard 1981; Waltz & Wolf 1984). Using a behavioral assay that reduces dominance differences among males may be most effective in eliciting other male-male interactions (e.g. sneak fertilizations and nest-raiding behaviors) that males employ to increase their attractiveness to females and their reproductive success (Li & Owings 1978b).

In Chapter 3, I present results to demonstrate that rapid, opportunistic tactic switching is commonly observed in threespines under laboratory conditions. Such facultative responses demonstrate situational flexibility. While this seems the best way to categorize the reproductive tactics of male stickleback, it is also possible to see a sequential component in some populations with restrictions imposed on territoriality by breeding male density, male condition, and other selective factors either across breeding cycles or breeding seasons (Table 1.3). For example, competition between males belonging to different age-related size classes could produce an added selective pressure for younger, smaller males to adopt an alternative tactic (Dufresne *et al.* 1990; de Fraipont *et al.* 1993). Many stickleback populations might exhibit a combination of simultaneous and reversible sequential mating tactics. In Chapter 3, I also document variation in tactic expression and tactic-switching propensity, which demonstrates variation in the switchpoint for sneaking/courting behavior among males (Wells 1977; Waltz & Wolf 1984; Crowley 2000; Shuster & Wade 2003).

## **(V) The potential influence of the sneaker tactic on trait evolution and selection**

Jones *et al.* (2001) demonstrated that studies of mating systems should consider a number of populations for any given study species, as geographically distinct ecological factors have the potential to affect population genetics, sexual selection, and the evolution of alternative tactics themselves. However, it can be very difficult to ascertain the direction and magnitude of the alternative tactic effects without good information regarding both the mating and the realized reproductive success (e.g. offspring production) of cuckolding and cuckolded males. An added layer of complexity is the fact that spatial and temporal variation in the frequency of alternative tactics and variation in the strength of selection on species-typical and alternative tactics can contribute to the maintenance of sexually selected traits and genetic diversity within a population. Studies limited to a single observation site, point in time, or type of selection could produce equivocal conclusions about how sexual traits (morphological or behavioral) evolved (Cornwallis & Uller 2010). Thus, being as informed as possible about the breeding dynamics of a study population, especially in relation to other populations that may experience divergent selection pressures, is crucial to uncovering the information necessary to make informed inferences about sexual selection and trait evolution.

Le Comber (2003) provided a good overview of ways in which sneak fertilization behavior can affect evolutionary dynamics. Alternative reproductive tactics can affect the number of males that actually reproduce, generally increasing the effective population size for males, with important consequences for population genetics (Chesser 1991; Sugg & Chesser 1994). Changes in effective population size can have consequences for the strength of selection on sex roles within a population. The extent to which individuals of each sex can afford to be choosy is generally a function of how many males and females are available to mate coupled with the extent of sex differences in reproductive rates (Kokko & Monaghan 2001; Kokko & Johnstone 2002). Female stickleback are known to be the choosy

sex, but male stickleback demonstrate individual variation in choosiness when the operational sex ratio (OSR) is skewed to decrease or increase male-male competition (Borg 1985; Wootton *et al.* 1995; Candolin & Salesto 2009). Both males and females may use density of nested males on the breeding grounds as a cue to determine whether to adopt a choosy or indiscriminate behavioral rule for mate choice. If a large fraction of nonterritorial males utilize the sneak fertilization tactic, the relative reproductive success of males that choose, perhaps inaccurately, to court will be greatly reduced.

Sneaking and egg-stealing is costly to females that exhibit strong mate choice (Rowland 1994). If sneaking is frequent and successful, a female will lose some of the benefits of choice (Largiadier *et al.* 2001; Alonzo 2008), particularly if males adopting the sneak fertilization tactic have drastically reduced competitive abilities, parental abilities, or heritable secondary sexual characteristics. On the other hand, if Le Comber's (2003) assessment that many sneakers are high quality males is correct, there could be essentially no cost for female stickleback. Findings in a separate stickleback population support this idea (Candolin & Vlieger 2013). This might explain why female choosiness or male sexual traits have not significantly diminished in populations with seemingly high sneak fertilization frequencies. It is also theoretically possible that sneaker males represent a significantly different but successful, heritable reproductive phenotype and females choose to spawn with males susceptible to sneak fertilization attempts in order to diversify the reproductive tactics of their own offspring.

The prevalence of sneak fertilization behavior can also affect the evolution of parental care behavior. A meta-analysis by Griffin *et al.* (2013) suggests that the benefits of parental care outweigh the costs of potential lost paternity in the Threespine Stickleback. However, these cost-benefits estimates are based on a small subset of populations from which genetic paternity has been calculated. Population-level variation in sneaking propensity and cuckoldry probabilities might produce population-level variation in the extent of parental care. Given that male Threespine Stickleback are not capable of easily

distinguishing cuckolded clutches (Frommen *et al.* 2007; Mehlis *et al.* 2010), perceived risk of cuckoldry alone (e.g. high rate of sneak attempts) could skew cost-benefit ratios in some populations. Such effects may be further complicated since males express individual, consistent differences in parental care behaviors (Stein & Bell 2012), and thus might also express individual differences in response to cuckoldry or the perceived risk of cuckoldry. Carryover effects, due to cuckoldry attempts and interactions with these intruding males, can increase aggression and territorial defense and/or decrease parental effort in victimized males (Foster *et al.* 2008). Both these effects have the potential to reduce a male's reproductive success by decreasing time allocated to egg care. Further investigation is necessary to determine if and to what extent cuckoldry, or the threat of cuckoldry, magnifies loss of reproductive success by eliciting plastic responses in parental effort.

Determining the strength of sexual selection within a population requires measuring and comparing the reproductive success of individuals. If sneak fertilization behavior in a population is frequent (and frequently successful), measures of reproductive success can be seriously flawed (Largiadere *et al.* 2001), particularly if reproductive success is deduced from the number of eggs in a nest (e.g. Bakker & Mundwiler 1994; Kraak *et al.* 1999) - males would be assigned high reproductive success, even if the majority of their eggs are cuckolded or stolen from other males. A combination of genetic information and observational data on behavioral interactions among nested males is the most accurate way to determine the extent male-male interactions and male-female interactions contribute to variation in reproductive success. Observational data is also the best way to determine the extent to which males choose maladaptive reproductive tactics and do not gain reproductive success.

## **1.4 CONCLUSION**

In this introduction, I have reviewed the extent of what is known about alternative reproductive tactics in Threespine Stickleback. Apparent in the literature review is the fact that sneaking has been investigated in a number of populations but rarely in a comparative context (Goldschmidt *et al.* 1992; Foster 1994; Foster 2013b). On the other hand, this broad representation of populations does provide evidence that sneak fertilization behavior and the sneaker repertoire, in general, are fairly ancient components of threespine behavior. This wide geographic sampling of populations also highlights absences of certain behaviors that suggest more thorough investigation of mating tactics and male competition in such populations may provide valuable insights into the evolution and maintenance of behavioral plasticity. The same can be said of the many variables associated with sneaking behavior investigated across population, which deserve further investigation in focused interpopulation comparisons.

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**Table 1.1.** Published accounts of sneak fertilizations and 'nest-raiding' behavior in the Threespine Stickleback (*Gasterosteus aculeatus* L.); Locations within geographic region are ordered chronologically by study. Coded categories include 'POPULATION TYPE': **M** = marine, **A** = anadromous, **FW** = freshwater; 'STUDY TYPE': **F** = field, **G** = genetic/paternity analyses, **L** = laboratory; '**SNEAK**' refers to presence/absence of the sneaker repertoire and specific behaviors observed in conjunction with the sneaker repertoire (**A** = Presence of sneak repertoire investigated and not observed; \* = absence of observation w/ genetic evidence of stolen fertilizations; **A\***= accounts of nest-raiding (stealing eggs, nest material) in the absence of accounts of the sneak repertoire; **SF** = sneak behavior with fertilization attempts; **SNR** = sneak behavior with nest raiding attempts (stealing eggs, nest material); **SFNR** = sneak behavior with fertilization and nest raiding attempts. Superscripts denote population labels in **Figure 1.1**.

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EASTERN NORTH AMERICA				
LOCATION	POPULATION TYPE	REFERENCE(S)	STUDY TYPE	SNEAK
Long Island , NY <sup>LI</sup>	A	Sargent & Gebler (1980); Sargent (1982)	L	SF
Priest Creek, Quebec <sup>PC</sup>	FW	Jamieson & Colgan (1992)	L	SF
Isle-Verte, Quebec <sup>IV</sup>	A	Rico <i>et al.</i> (1992); Blais <i>et al.</i> (2004)	G	*
Isle-Verte, Quebec <sup>IV</sup>	A	De Fraipont <i>et al.</i> (1993); Cubillos & Guderley (2000)	L	SF
EAST ASIA				
Tsuya River, Japan <sup>TR</sup>	A	Mori (1995, 1998)	F	SFNR

**Table 1.1.** (continued)

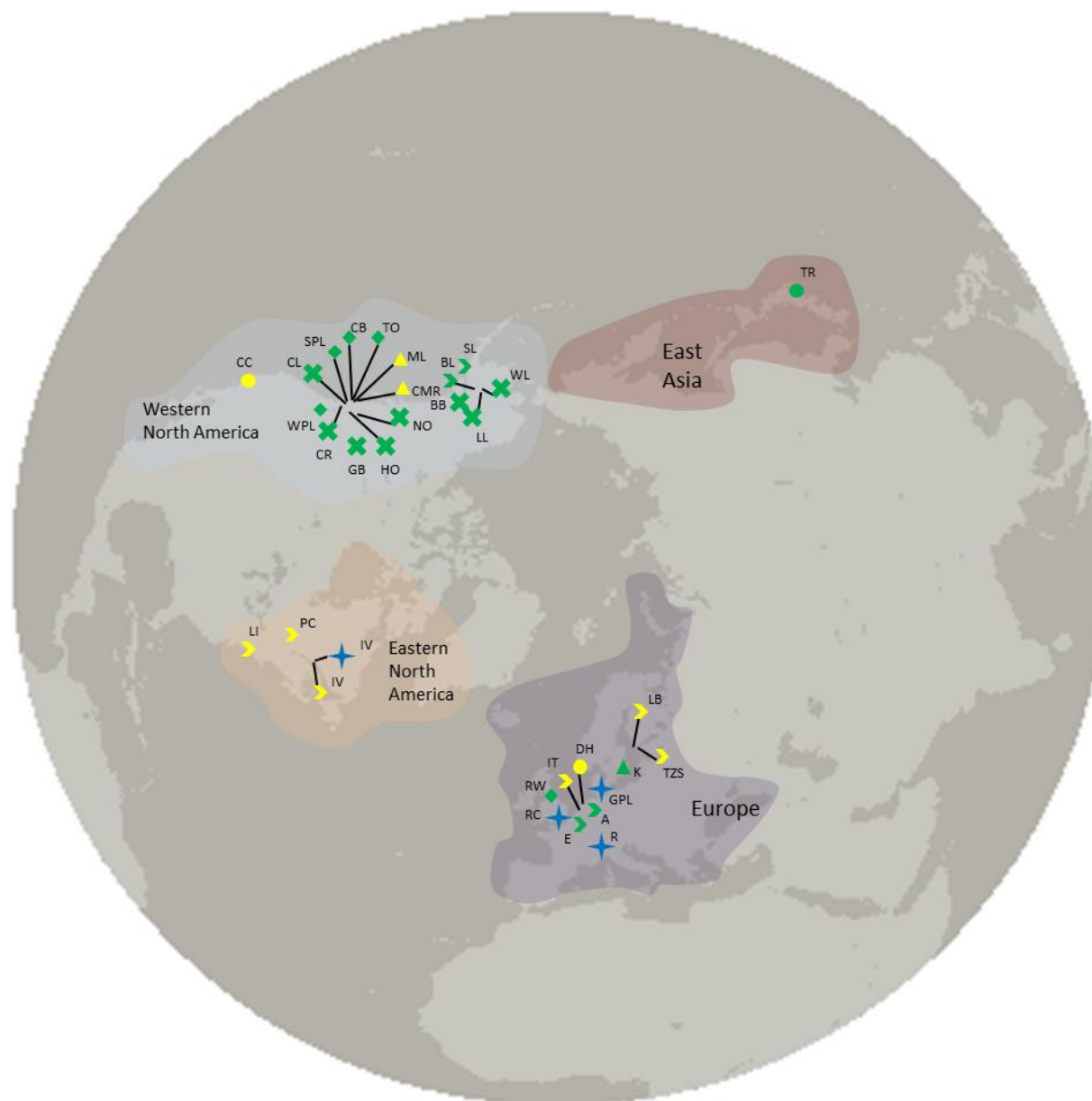
WESTERN NORTH AMERICA				
LOCATION	POPULATION TYPE	REFERENCE(S)	STUDY TYPE	SNEAK
Chesterman Beach, BC <sup>CB</sup>	A	Black & Wootton (1970)	F	SNR
Campbell River, BC <sup>CMR</sup>	A	Black (1971)	L	A*
Tofino, BC <sup>TO</sup>	A	Wootton (1971)	F	SNR
Wapato Lake, WA <sup>WPL</sup>	FW	Kynard (1978)	F	SNR
Conn Creek, CA <sup>CC</sup>	FW	Li & Owings (1978a,b)	L	SFNR
Crystal Lake, BC <sup>CR</sup>	FW	Goldschmidt <i>et al.</i> (1992); Foster <i>et al.</i> (1996)	F	A
Garden Bay Lake, BC <sup>GB</sup>	FW	Goldschmidt <i>et al.</i> (1992); Foster <i>et al.</i> (1996)	F	A
∞ Hotel Lake, BC <sup>HO</sup>	FW	Goldschmidt <i>et al.</i> (1992); Foster <i>et al.</i> (1996)	F	A
North Lake, BC <sup>NO</sup>	FW	Goldschmidt <i>et al.</i> (1992); Foster <i>et al.</i> (1996)	F	A
Cowichan Lake, BC <sup>CL</sup>	FW	Goldschmidt <i>et al.</i> (1992); Foster <i>et al.</i> (1996)	F	A
Sproat Lake, BC <sup>SPL</sup>	FW	Goldschmidt <i>et al.</i> (1992); Foster <i>et al.</i> (1996)	F	SNR
Big Beaver Lake, AK <sup>BB</sup>	FW	Foster <i>et al.</i> (1996)	F	A
Lynne Lake, AK <sup>LL</sup>	FW	Foster <i>et al.</i> (1996)	F	A
Willow Lake, AK <sup>WL</sup>	FW	Foster <i>et al.</i> (1996)	F	A
Bruce, AK <sup>BL</sup>	FW	Foster <i>et al.</i> (1996)	F	SF
Stephan, AK <sup>SL</sup>	FW	Foster <i>et al.</i> (1996)	F	SF
Misty Lake, BC <sup>ML</sup>	FW	Raeymaekers <i>et al.</i> (2010)	L	A*

**Table 1.1.** (continued)

EUROPE				
LOCATION	POPULATION TYPE	REFERENCE(S)	STUDY TYPE	SNEAK
Den Helder, Netherlands <sup>DH</sup>	A	van den Assem (1967)	L	SFNR
River Wear, England <sup>RW</sup>	A	Wootton (1971); Wootton (1972)	F	SNR
Karlskrona, Sweden <sup>K</sup>	M	Borg (1985)	F	A*
Ernst, Netherlands <sup>E</sup>	FW	Goldschmidt & Bakker (1990); Goldschmidt <i>et al.</i> (1992)	F	SF
Apeldoorn, Netherlands <sup>A</sup>	FW	Goldschmidt & Bakker 1990); Goldschmidt <i>et al.</i> (1992)	F	SF
Tvarminne Zool. Station, Finland <sup>TZS</sup>	M	Candolin (2000); Candolin & Vlieger (2013)	L	SF
Roche, Switzerland <sup>R</sup>	FW	Largiader <i>et al.</i> (2001)	G	*
River Cam, England <sup>RC</sup>	A	Le Comber (2003)	G	*
Island of Texel, Netherlands <sup>IT</sup>	A	Bakker <i>et al.</i> (2006)	L	SF
Greater Plon Lake, Germany <sup>GPL</sup>	FW	Krobbach (2006); Eizaguirre <i>et al.</i> (2009)	G	*
Langskar Bay, Finland <sup>LB</sup>	M	Vlieger & Candolin (2009)	L	SF
EAST ASIA				
LOCATION	POPULATION TYPE	REFERENCE(S)	STUDY TYPE	SNEAK
Tsuya River, Japan <sup>TR</sup>	A	Mori (1995, 1998)	F	SFNR



**Figure 1.1.** Distribution of studies related to sneak fertilization behavior and nest-raiding in threespine stickleback; Study type is denoted by color (Green = Field observations; Yellow = Laboratory observations; Blue = Genetic analysis) and study findings are coded by symbol (✕ = No observation of sneaker repertoire; ✦ = No observation of sneaker behavior – evidence of stolen fertilizations; ▲ = No observation of sneaker behavior – observation of nest raiding; ➤ = Observation of sneaker behavior – only involving fertilization attempts (SF); ◆ = Observation of sneaker behavior – only involving nest raiding (SNR); ● = Observation of sneaker behavior – involving both fertilization attempts and nest raiding (SFNR)).



**Figure 1.1.**

**Figure 1.2.** Diagram of stickleback behaviors noted in literature review. Intruding males can approach nest-holder territories with either conspicuous or inconspicuous (i.e. 'sneaker repertoire') nest-directed behavior (primary behavior). Territory approach is followed by different outcomes (secondary behaviors). Each behavior outcome can provide the intruder with different advantages (e.g. direct reproductive success; ability to provide parental care; forceful resource acquisition (for mating or offspring); interference with a rival's nesting cycle). As the arrows suggest, some behaviors can provide more than one advantage to individual intruders.

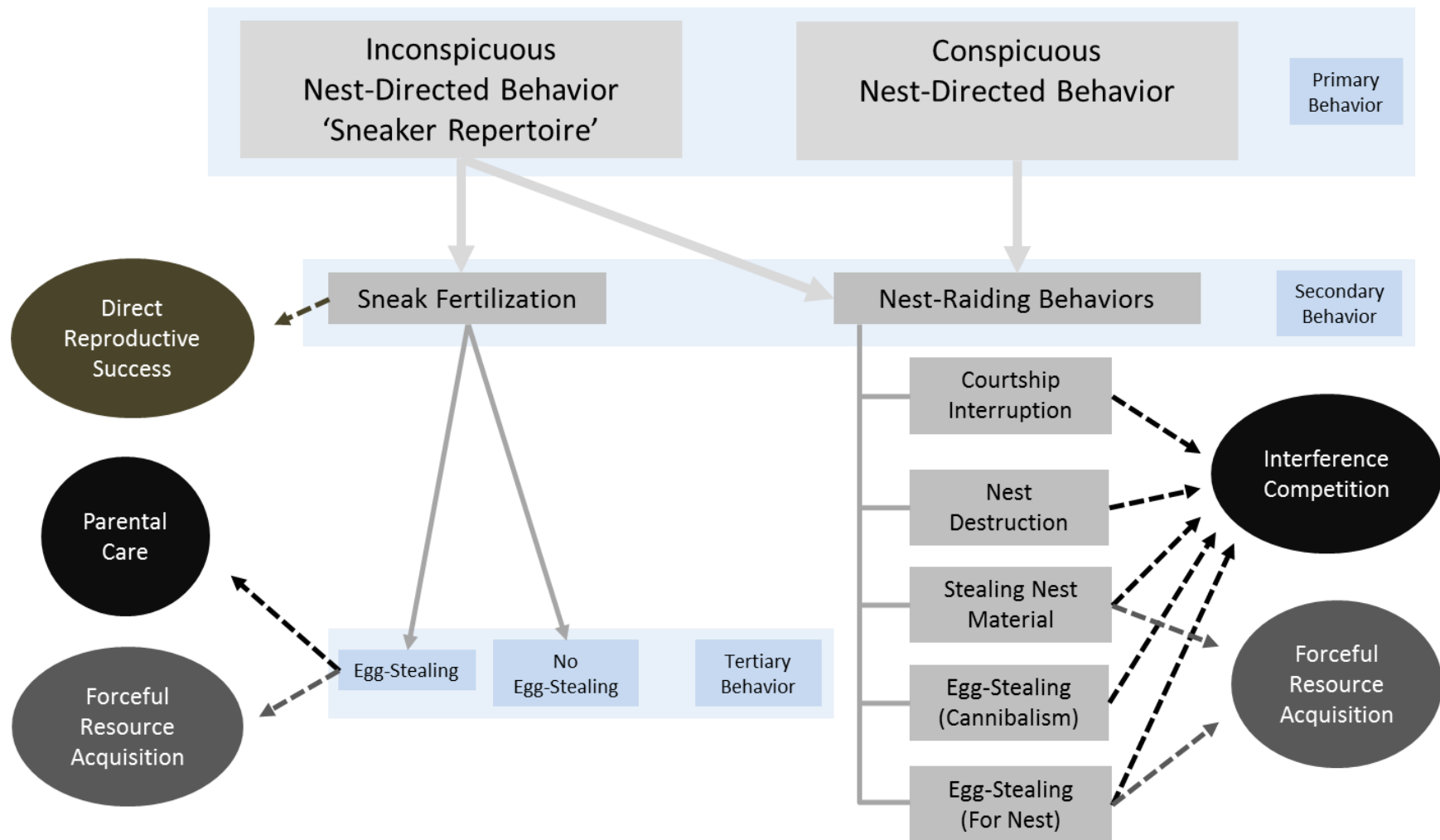


Figure 1.2.

**Table 1.2.** Published accounts of variables investigated in sneaking studies of Threespine Stickleback. Region codes: E = Europe, ENA = Eastern North America, J = Japan, WNA = Western North America. Study Type codes: F = field, L = lab. Relationship refers to the association of the variable and sneaking behavior: + (positive association between variable and sneaking behavior), - (negative association between variable and sneaking behavior, **X** (no relationship, e.g. equivalent between sneakers and nonsneakers).

VARIABLE/REFERENCE	REGION	STUDY TYPE	RELATIONSHIP	RELEVANT FINDINGS
<b>BODY SIZE/AGE</b>				
Mori (1995)	J	F	X	Observational accounts suggest body size did not differ between sneaker and nonsneakers
DeFraipont, FitzGerald & Guderley (1993)	ENA	L	-	Younger males (1+) adopted sneaker behavior significantly more often than (2+) males in dyadic and complex rival laboratory trials.
Le Comber (2003)	E	L	X	Paternity analyses suggest no differences in standard length between nested sneakers and nonsneakers
Eizaguirre <i>et al.</i> (2009)	E	F	-	Paternity analyses suggest that small males were significantly more successful at performing sneak fertilizations
Vlieger & Candolin (2009)	E	L	X	In dyadic assays, nonnested sneakers and nested courters did not differ in standard length or weight; nor did failed and successful sneakers
Candolin & Vlieger (2013)	E	L	X	Paternity analyses suggest no differences in weight between nested sneakers and nonsneakers

**Table 1.2.** (continued)

VARIABLE/REFERENCE	REGION	STUDY TYPE	RELATIONSHIP	RELEVANT FINDINGS
<b>DOMINANCE RANKING</b>				
van den Assem (1967)	E	L	X	In complex rival scenarios, sneak tendency was not correlated with rank in the dominance hierarchy
Li & Owings (1978a,b)	WNA	L	X	In multiple rival scenarios, sneak fertilization attempts most common between males of similar rank or status
<b>TERRITORY SIZE</b>				
van den Assem (1967)	E	L	-	In complex rival scenarios, results imply that small territory size is likely to be associated with higher sneak propensity (not directly measured).
Mori (1995)	J	F	X	Territory size did not differ between sneakers and nonsneakers
Le Comber (2003)	E	L	X	Based on paternity analyses, sneaker males and sneak victims did not significantly differ in territory size

**Table 1.2.** (continued)

VARIABLE/REFERENCE	REGION	STUDY TYPE	RELATIONSHIP	RELEVANT FINDINGS
<b>CLOSE NESTING/NEST DENSITY</b>				
Wootton (1971)	E/WNA	F	+	Sneaking more likely to occur between neighboring males
Goldschmidt, Foster & Sevenster (1992)	E/WNA	F	+	Sneaking more likely to occur when males were in close proximity
Mori (1995)	J	F	+	Sneak propensity positively related to the number of neighboring males without eggs (not nest density per se); Neighboring males more likely to exhibit sneak behavior
Eizaguirre et al. (2009)	E	F	+	Genetic paternity analysis suggests male density enhances sneaking success under semi-natural conditions
<b>NEST VISIBILITY</b>				
Sargent & Gebler (1980)	ENA	L	+	Nest concealment decreases the probability of sneak fertilizations under laboratory conditions
Mori (1995)	J	F	-	Nest concealment increased the probability of sneak attempts under natural conditions
Vlieger & Candolin (2009)	E	L	X	In dyadic assays, water turbidity, but not increased vegetation, significantly decreased sneaking success; neither type of decreased visibility affected the total number of sneak attempts
Candolin & Vlieger (2013)	E	L	+	Based on paternity analyses, vegetation decreases sneak fertilization success in experimental pools

**Table 1.2.** (continued)

VARIABLE/REFERENCE	REGION	STUDY TYPE	RELATIONSHIP	RELEVANT FINDINGS
<b>TERRITORY/NEST ORDER</b>				
DeFraipont, FitzGerald & Guderley (1993)	ENA	L	+	Absence of nest-building resources elicits sneaking behavior in males under laboratory conditions
Goldschmidt, Foster & Sevenster (1992)	E	F	+	Sneaking propensity negatively related to order of territory and nest establishment
Jamieson & Colgan (1992)	ENA	L	+	Late nesting males had lower courtship vigor, lower initial reproductive success, and higher propensity to sneak
Vlieger & Candolin (2009)	E	L	+	Absence of nest-building resources elicits sneaking behavior in males under laboratory conditions
<b>OPERATIONAL SEX RATIO</b>				
Goldschmidt, Foster & Sevenster (1992)	E/WNA	L	X	In interpopulation comparisons, OSR was not related to the presence of sneak fertilization behavior
<b>MALE QUALITY</b>				
Le Comber (2003)	E	L	X	Based on paternity analyses, sneaker males and sneak victims did not significantly differ in measures of quality
Krobbach (2006)	E	F	X	Paternity analyses suggest no significant difference in the number of MHC variants between sneakers and males successfully reproducing based on female choice
Eizaguirre <i>et al.</i> (2009)	E	F	-	Paternity analyses suggest that males with a specific MHC haplotype had increased reproductive success via female choice, while those without it tended towards increased sneaking propensity



**Table 1.3.** Examples of species that use reversible, facultative mating tactics. 'Extent of Flexibility' refers to the temporal scale of tactic switching categorized to the best extent possible based on the information published for a given system. 'Situational' describes reversible mating tactics elicited as an immediate response to a mating opportunity; \* = 'Situational' tactic accounts which explicitly indicate that males can switch rapidly between mating tactics in an opportunistic manner (e.g. behavioral changes observed within minutes); 'Intermediate' describes reversible mating tactics that may change over the time frame of a few days -- may involve repeated exposure to a stimulus to elicit behavioral switches; 'Conditional' describes reversible mating tactics that exclude some individuals from one tactic due to population-level processes that may change over breeding cycles or breeding seasons (e.g. size- or density-dependent use of sneaking tactic that may switch to territoriality if opportunities arises over breeding season, such as access to nest sites); 'Developmental' describes irreversible, and typically polymorphic, mating tactics that are determined at some point during development resulting in divergent developmental trajectories (e.g. territorial versus sneaker morph). When variation in tactic use exists for a mating system, more than one form of flexibility may be listed (e.g. sneaking tactic may be condition-dependent for some portion of males and situationally flexible for other males within a system).

Table 1.3.

SPECIES	MODE OF FERTILIZATION	EXTENT OF FLEXIBILITY	REFERENCE(S)	DESCRIPTION OF TACTICS
FISH				
Arctic Charr ( <i>Salvelinus alpinus</i> )	External	Situational*/ Conditional	Rudolfson <i>et al.</i> (2006); Sorum <i>et al.</i> (2011)	Courting and mate-guarding behavior by territorial males is primary mating tactic on lekking grounds; size- and hierarchy-dependent use of sneaking at lekking sites by small males; males can switch tactics and sperm traits upon hierarchical disturbances; males utilizing the sneaker tactic also observed to opportunistically court females on the lek sites of other males when guarding males are preoccupied
Black-faced Blenny ( <i>Tripterygion tripteronotus</i> )	External	Situational*/ Conditional	Wirtz (1978); De Jonge & Videler (1989)	Courting by nest-holders is the primary mating tactic; size- and density- dependent use of female mimicry by non-territorial males; opportunistic color change and use of female mimicry by territorial males in the presence of courting neighbors
Common Goby ( <i>Pomatoschistus microps</i> )	External	Situational/ Conditional	Magnhagen (1992, 1994, 1998)	Courting by nest-holders is the primary mating tactic; size- and density- dependent use of aggressive sneaker and inconspicuous drab sneaker tactic; males of intermediate size switch between sneaker and courter tactic; some nest-holders <b>aggressively</b> steal fertilizations
Desert Pupfish ( <i>Cyprinodon macularius</i> )	External	Conditional	Kodric-Brown (1986)	Courting by nest-holders is the primary mating tactic; frequency of individuals adopting courting satellite tactic varies depending on size, population density, availability of oviposition substrates and breeding area size; sneak spawning is adopted by males of any size unable to invest energy in either the territorial or satellite tactic

**Table 1.3.** (continued)

SPECIES	MODE OF FERTILIZATION	EXTENT OF FLEXIBILITY	REFERENCE(S)	DESCRIPTION OF TACTICS
FISH				
Freshwater Blenny ( <i>Salaria fluviatilis</i> )	External	Intermediate/ Conditional	Neat <i>et al.</i> (2003)	Courting by nest-holders is the primary mating tactic; size dependent use of sneaker tactic by non-territorial males; use of sneaker tactic by temporarily non-territorial males
Herbivorous Damselfish ( <i>Stegastes nigricans</i> )	External	Situational/ Intermediate	Karino & Nakazono (1993)	Courting by nest-holders is the primary mating tactic; males may turn drab and sneak on spawning neighbors, typically after failed spawning attempts or failure to attract females
Longear Sunfish ( <i>Lepomis megalotis megalotis</i> )	External	Situational/ Conditional	Keenleyside (1972)	Courting by nest-holders is the primary mating tactic; the majority of sneak fertilizations are attributable to non-territorial, non-nested males but also performed by some neighboring territorial males
Mediterranean Damselfish ( <i>Chromis chromis</i> )	External	Situational/ Conditional	Picciulin <i>et al.</i> (2004)	Courting by nest-holders is the primary mating tactic; differences in courtship behavior expressed by non-territorial males and territorial males with small clutches (compared to courtship behavior of successful nestholding courtiers) results in the use of the sneak tactic by unsuccessful males; High breeding synchrony and female availability likely influences density of unsuccessful males
Mozambique Tilapia ( <i>Oreochromis mossambicus</i> )	External	Situational*/ Conditional	Oliveira & Almada (1998)	Courting by territorial males is the primary mating tactic; size- and hierarchy- dependent use of sneaker tactic by drab non-territorial, non-courting males ; some males observed to adopt a semi-territorial "floater" tactic -- courting females in territories of other males during their brief absence; dominant territorial males observed to opportunistically sneak on neighboring courtships
Peacock blenny ( <i>Salaria pavo</i> )	External	Situational*/ Conditional	Goncalves (pers. obs.); Oliveira <i>et al.</i> (2001)	Courting by nest-holders is the primary mating tactic; size- and density- dependent use of female mimicry tactic by non-territorial males; opportunistic switching between use of female mimicry and courting behavior by territorial males within minutes

**Table 1.3.** (continued)

SPECIES	MODE OF FERTILIZATION	EXTENT OF FLEXIBILITY	REFERENCE(S)	DESCRIPTION OF TACTICS
FISH				
Pecos River Pupfish ( <i>Cyprinodon macularius pecosensis</i> )	External	Intermediate/ Conditional	Kodric-Brown (1977, 1986)	Courting by nest-holders is the primary mating tactic; frequency of individuals adopting drab satellite and sneaker tactic varies depending on population density, availability of oviposition substrates and breeding area size; proportions of males using alternative tactics varied across days
Plainfin Midshipman ( <i>Porichthys notatus</i> )	External	Situational/ Developmental	Grober <i>et al.</i> (1994); Lee & Bass (2004)	A polymorphic system in which a large territorial, courting and small non-territorial, sneaking morph is determined based on a permanent difference in developmental trajectory; however, large territorial morph males have been observed to <b>aggressively</b> steal fertilizations
81 Rose Bitterling ( <i>Rhodeus ocellatus</i> )	External	Situational*/ Conditional	Kanoh (1996)	Courting by territorial males is the primary mating tactic; size- and density- dependent use of sneaker tactic by non-territorial males; opportunistic use of sneaker tactic by territorial males during neighboring courtships
Sailfin Molly ( <i>Poecilia latipinna</i> )	Internal	Situational/ Developmental	Farr <i>et al.</i> (1986); Trexler <i>et al.</i> (1990); Seda <i>et al.</i> (2012); Fraser <i>et al.</i> (2014)	A polymorphic system in which large males perform courtship displays and small males adopt sneak copulation behavior based on a permanent difference in developmental trajectory; intermediate-sized males switch between tactics depending on the presence of competitors
Sand Goby ( <i>Pomatoschistus minutus</i> )	External	Conditional	Malavasi <i>et al.</i> (2001); Takegaki <i>et al.</i> (2012)	Courting by nest-holders is the primary mating tactic; size- and density-dependent use of inconspicuous drab sneaker tactic; small drab sneaker males will become territorial courtiers when large competitive males removed
Sandperch ( <i>Paraperca synderi</i> )	External	Situational*	Ohnishi <i>et al.</i> (1997)	Courting by harem holders is the primary mating tactic; neighboring harem holders observed to sneak fertilizations typically after completing matings in their own harem

**Table 1.3.** (continued)

SPECIES	MODE OF FERTILIZATION	EXTENT OF FLEXIBILITY	REFERENCE(S)	DESCRIPTION OF TACTICS
FISH				
Southern Mouthbrooder Cichlid ( <i>Pseudocrenilabrus philander</i> )	External	Situational*/Conditional	Chan & Ribbink (1990)	Courting by territorial males is the primary mating tactic; size- and hierarchy- dependent use of sneaker tactic by drab non-territorial, non-courting males and opportunistic use of sneaker tactic during neighboring courtships by semi-territorial, courting males
Trinidadian Guppy ( <i>Poecilia reticulata</i> )	Internal	Situational*	Baerends et al. (1955); Liley (1966); Farr (1989); Houde (1997)	Courting is the primary mating tactic used; males may also attempt sneak copulations using gonopodial thrusts; individual males have been observed to use both tactics -- even during the same courtship attempt; frequency of tactic use can be due to environmental factors or may be condition- or density dependent
Triplefin Blenny ( <i>Axoclinus nigricaudus</i> and <i>A. carminalis</i> )	External	Situational/Conditional	Neat (2001)	Courting by nest-holders is the primary mating tactic; size- and density- dependent use of sneaker tactic by non-territorial males; opportunistic use of sneaker tactic by territorial males (more prevalent in <i>A. carminalis</i> )
AMPHIBIANS				
Alpine Newt ( <i>Triturus alpestris alpestris</i> )	Internal	Situational*	Denoel et al. (2001)	Males utilize either courtship plus waiting or a sneaky luring tactic during the sperm transfer phase of mating; the same males can utilize either tactic in an opportunistic manner during the same courtship bout depending on female receptivity
Bullfrog ( <i>Rana catesbeiana</i> )	External	Conditional	Howard (1984)	Signaling by territory holders is primary mating tactic; 1 yr old males observed to switch between territorial and satellite behavior based on condition or age, while older males are typically only observed to use the territorial tactic

**Table 1.3.** (continued)

SPECIES	MODE OF FERTILIZATION	EXTENT OF FLEXIBILITY	REFERENCE(S)	DESCRIPTION OF TACTICS
AMPHIBIANS				
Great Plains Toad ( <i>Bufo cognatus</i> )	External	Conditional	Sullivan (1982)	Signaling by territory holders is primary mating tactic; switch to satellite behavior is density-dependent -- males adopting satellite positions became more frequent at higher densities and removal manipulations of calling males suggests that all males are capable of switching between behaviors in respond to aggregation densities
Green Tree Frog ( <i>Hyla cinerea</i> )	External	Situational*/ Conditional	Perrill <i>et al.</i> (1982); Humfield (2008)	Signaling by territory holders is primary mating tactic; Territorial or satellite status is likely determined by a combination of several context and condition-dependent factors; some satellite males observed to opportunistically assume the calling tactic if calling males are removed and territorial calling males have been observed to use satellite behavior upon exposure to conspecific mating calls, independent of size
Natterjack Toad ( <i>Bufo catesbeiana</i> )	External	Situational*/ Conditional	Arak (1983)	Signaling by territory holders is primary mating tactic; Switch between territorial or searching behavior is density-dependent as well as size-dependent, given that larger males are more likely to use to calling tactic and small males are more likely to opportunistically switch between tactics
Quacking Frog ( <i>Crinia georgiana</i> )	External	Situational/ Conditional	Roberts <i>et al.</i> (1999); Byrne (2002); Bryne & Roberts (2004)	Signaling by territory holders is primary mating tactic; Switch between territorial, searching behavior, and satellite behavior is density-dependent as well as size-dependent -- both large and small males may switch to searching or satellite tactic although small searching or satellite males have less mating success when competing against with large males utilizing searching and satellite tactics

**Table 1.3.** (continued)

SPECIES	MODE OF FERTILIZATION	EXTENT OF FLEXIBILITY	REFERENCE(S)	DESCRIPTION OF TACTICS
AMPHIBIANS				
Wrinkled Toadlet ( <i>Uperoleia rugosa</i> )	External	Conditional	Robertson (1986)	Signaling by territory holders is primary mating tactic; territorial calling is energetically costly, such that territorial males that lose mass may give up calling and become silent satellites near calling males while putting on mass; satellite males that regain mass typically regain a territory and resume calling
ARTHROPODS				
Dot-tailed whitefaced dragonfly ( <i>Leucorrhinia intacta</i> )	Internal	Intermediate/ Conditional	Campanella & Wolf (1974); Waltz & Wolf (1984)	Territoriality is the primary mating tactic; tactic choice likely density-dependent with males either hold territories or are site-fixed satellites; satellites are either resident satellites or transient satellites -- switching tactics daily or every few days
84 Hawaiian field cricket ( <i>Telegrillus oceanicus</i> )	Internal	Situational*	Rowell & Cade (1993); Hack (1998)	Signaling by territory holders is primary mating tactic; males have been observed to switch between the primary calling tactic and a silent satellite tactics over the course of a night
Horseshoe crab ( <i>Limulus polyphemus</i> )	External	Intermediate/ Conditional	Brockman & Penn (1992); Brockmann (2002)	Attachment to spawning females is the primary mating tactic; tactic use typically conditional -- attached males are usually younger and in better condition than males acting as satellites; males of intermediate condition observed to switch between tactics across observation weeks
Rock shrimp ( <i>Rhynchocinetes typus</i> )	Internal	Conditional	Correa <i>et al.</i> (2003)	Mate guarding is the primary mating tactic -- males of all ontogenetic stages/size classes are observed to perform mate guarding under a competition-free situation but under most natural conditions, a size-dependent dominance hierarchy results in smaller males utilizing sneaky insemination tactics
Rubyspot damselfly ( <i>Hetaerina americana</i> )	Internal	Intermediate/ Conditional	Raihani <i>et al.</i> (2008)	Territoriality is the primary mating tactic; tactic use is size and conditional dependent but males with intermediate fat reserves can switch between territorial and nonterritorial tactics over a period of several days

**Table 1.3.** (continued)

SPECIES	MODE OF FERTILIZATION	EXTENT OF FLEXIBILITY	REFERENCE(S)	DESCRIPTION OF TACTICS
ARTHROPODS				
Soapberry bug ( <i>Jadera haematoloma</i> )	Internal	Situational*	Carroll & Corneli (1999)	Males typically utilize mate guarding behavior when sex ratio is male biased; males in a population with relatively large fluctuations in sex ratio observed to readily switch between mate guarding and searching tactics compared with populations with more stable sex ratios
Striped wolf spider ( <i>Rabidosa punctulata</i> )	Internal	Situational*/ Conditional	Nicholas (2007)	Courting is the primary mating tactic used; tactic use typically conditional, with high condition males using forced copulation attempts and poor condition males using the courtship tactic; however a percentage of males attempt forced copulation and courtship tactics during the same interaction with a female
MAMMALS				
Bighorn sheep ( <i>Ovis canadensis</i> )	Internal	Conditional	Hogg & Forbes (1997)	The primary mating tactics utilized by males involves defense and cooperative mate guarding; within a single breeding season males have been observed to switch between mate guarding and harassment (coursing) tactics; switching likely due to changes in male dominance hierarchy or changes in female receptivity
Fallow deer ( <i>Dama dama</i> )	Internal	Conditional	Thirgood (1991); Apollonio <i>et al.</i> (1992)	Within a single breeding season males have been observed to switch between satellite and lekking behavior and satellite and solitary defense but switches from solitary territoriality and lekking rarer; switching likely condition- and density-dependent
Uganda kob ( <i>Kobus kob thomasi</i> )	Internal	Conditional	Balmford (1990)	Within a single breeding season males have been observed to switch between lekking and solitary territorial behavior; switching likely condition- and density-dependent



## CHAPTER 2

# REGIONAL & POPULATION-LEVEL DIFFERENCES ACROSS THE PACIFIC NORTHWEST IN THE EXPRESSION OF AN ANCESTRAL TENDENCY: SNEAKING BEHAVIOR IN MALE THREESPINE STICKLEBACK (*GASTEROSTEUS ACULEATUS* L.)

## 2.1 ABSTRACT

Since context-dependent, alternative reproductive tactics are primarily influenced by immediate environmental and social conditions, interpopulation comparisons can provide insights regarding selective pressures shaping such facultative behavioral tactics. This study focuses on population-level differences in the expression of sneaking behavior, an alternative reproductive tactic, in Threespine Stickleback (*Gasterosteus aculeatus* L.) of the Pacific northwest of North America. Combining behavioral observations collected *in situ* over a 25 yr period, the data suggest that populations differ in the propensity to express sneaking behavior, with special attention drawn to a number of freshwater British Columbia populations that have consistently demonstrated an absence of the sneaker repertoire during the period of data collection. Other populations observed across multiple breeding seasons either demonstrate a fairly constant or an increasing frequency of sneaking observations across years.

General comparisons among sneaking populations suggest intra- and interpopulation variation exists in both the social contexts eliciting sneaking behavior and the level of nest-holder aggression in response to sneaking outside the courtship context. Sneakers are typically more likely to sneak on males in the courtship stage versus the nest-building phase or the parental phase of the breeding cycle. However, sneaking behavior was also observed outside of the courtship context in many populations.

Among those populations that do express sneaking behavior outside of courtship, sneakers are equally as likely to perform sneaking behavior in response to courting/spawning behaviors, nest-directed behaviors, or guarding behaviors of territorial males. The most common response of nest-holder males to sneaker territorial intrusions occurring outside courtship is aggressive chasing. For populations with high sneaker frequencies, such territorial intrusions may be costly to nest-holders in terms of time and energy budgeted for territorial defense.

The geographic variation in expression of sneaking behavior observed in this study emphasizes the importance of accounting for population-level variation in sneaking behavior in future behavioral studies and offers an opportunity to provide more accurate and complete investigations of selective pressures, reproductive success, and evolutionary maintenance of plastic reproductive tactics.

**KEYWORDS:** Threespine Stickleback, *Gasterosteus aculeatus*, geographic variation, behavioral plasticity, sneaking, alternative reproductive tactic.

## 2.2 INTRODUCTION

A diverse range of animal taxa express alternative reproductive tactics, ranging from genetically fixed morphological alternatives to context-dependent behavioral flexibility (Oliveira *et al.* 2008). Although the best known examples of alternative reproductive tactics are characterized by genetic polymorphisms (e.g. marine isopods: Shuster & Wade 1991; side-blotched lizards: Sinervo & Lively 1996), the majority of documented alternative reproductive tactics actually reflect conditional responses to short-term social contexts (Brockmann *et al.* 2008; Bergmuller & Taborsky 2010). Behavioral plasticity in reproductive tactics is expected to evolve when the environmental changes that influence mating success occur within individual lifetimes and when circumstances in which successful matings occur are

highly variable (e.g. asynchronous receptivity and spatial clumping of females). Highly flexible and reversible behavioral tactics are often observed in mobile, iteroparous species, in which both males and females can rapidly change behaviors to exploit mating opportunities as they arise (Shuster & Wade 2003; Taborsky *et al.* 2008).

Alternative reproductive tactics have the potential to influence population dynamics and selective pressures within populations (Smith & Sibly 1985; Chesser 1991; Sugg & Chesser 1994; Magurran 1996, 1998; Moran & Garcia-Vazquez 1998; Alonzo & Warner 2000; Jones *et al.* 2001a). However, relatively little is known about the fitness consequences of highly flexible tactics which allow individuals to track changes in environmental, social, or age-related conditions that affect fitness (Moran 1992; Taborsky, Oliveira & Brockmann 2008). While genetic variation is likely to result in variation in behavioral plasticity among individuals, the extent of genetic and environmental contributions to such flexible tactics have not been widely documented (Shuster & Wade 2003). Population and regional comparisons may inform investigations regarding selective pressures and fitness consequences of flexible alternatives because such tactics are so closely tied to the immediate environmental and social conditions that are likely to vary among populations (Foster 1994; Carroll & Corneli 1999; Jones *et al.* 2001b; Cornwallis & Uller 2010; Foster 2013a,b). A number of species with genetic polymorphisms or monomorphic developmental/conditional switchpoint tactics (Bluegill Sunfish: Gross & Charnov 1980; European Earwig: Tomkins & Brown 2004; Atlantic Salmon: Moran & Garcia-Vazquez 1998; Martinez *et al.* 2000; Aubin-Horth *et al.* 2006; Side-blotched Lizard: Corl *et al.* 2010) and context-dependent, facultative tactics (e.g. Threespine Stickleback: Goldschmidt *et al.* 1992; Trinidadian Guppy: Kelly *et al.* 1999; Sand Goby: Jones *et al.* 2001b; freshwater Blenny: Neat *et al.* 2003) demonstrate geographic variation in tactic expression.

The Threespine Stickleback is a promising model organism for investigating the causes and consequences of highly plastic reproductive tactics. Males typically establish territories, build nests, and court and spawn with multiple females. They then provide all parental care for young in the nest (Foster 1994a Rowland 1994 for review). However, males may employ a set of simultaneous alternative reproductive tactics (*sensu* Taborsky 2008) whereby non-nesting, nest-building or courting males will attempt opportunistic sneak fertilizations on spawning, nest-holding males. Males performing this behavior typically exhibit stereotypical drab coloration and inconspicuous substrate gliding referred to as sneaking (van den Assem 1967) – specifically referred to as the ‘sneaker repertoire’ here. Not only do individuals express reversible, context-dependent reproductive tactics (courtship vs sneaking) but they also demonstrate variation in the propensity to express courtship or sneaking behavior. This differential responsiveness suggests genetic variation in tactic expression within, and potentially among, populations (Chapter 3). The expression of the sneaker repertoire has been attributed to a variety of condition- and context-dependent factors (e.g. male age or size, nest location, inter-nest distance, nesting order: van den Assem 1967; Li & Owings 1978b; Sargent & Gebler 1980; Goldschmidt *et al.* 1992; Jamieson & Colgan 1992; de Fraipont *et al.* 1993).

The extensive geographic range of stickleback populations with well-documented differences in environmental and social conditions provides great potential to discern variation in tactic frequency in multiple populations. However, there have been few direct field (e.g. Black & Wootton 1970; Kynard 1978; Mori 1995) or comparative studies of this behavior (e.g. Wootton 1971; Goldschmidt *et al.* 1992; Foster *et al.* 1996). Preliminary observations suggest there is population level variation in the frequency of sneaking behavior, particularly in the Pacific Northwest (Goldschmidt *et al.* 1992; Foster *et al.* 1996). Presence of the sneaking repertoire has been noted in both anadromous populations of the Cook Inlet region, Alaska (AK) and several anadromous populations in British Columbia (BC). However, sneak fertilization behavior has not been detected in freshwater populations in southern BC, despite extensive

observation, and never observed during fertilization opportunities in these freshwater BC populations (Black & Wootton 1970; Wootton 1971; Goldschmidt *et al.* 1992; Foster *et al.* 1996). In the Cook Inlet region, occurrence of sneaking appears to be variable across freshwater populations (Foster *et al.* 1996). Assuming that presence of the sneaker repertoire is the ancestral condition in the postglacial radiation of Threespine Stickleback, it is possible that the absence of this character in BC freshwater populations is due either to loss of sneaking behavior in some populations or to extreme reduction in the frequency of expression (Foster *et al.* 1996; Foster 2013b). Regional differences among oceanic populations appear to have influenced behavioral evolution in derived, freshwater populations (reviewed in Foster 2013b), but the complex metapopulation structure of the oceanic ancestor makes it difficult to predict the array of behavioral phenotypes in many derived populations. The high prevalence of plasticity in behavioral traits makes this task more challenging, as differences in behavioral expression across populations may stem from purely genetic influences to purely environmental influences, or population-level genetic variation in plasticity itself (Foster 2013b).

An important objective of this study is to broaden the scope for discovering potential variation in tactic expression, its consequences, and utility in microevolutionary studies by describing the patterns of geographic variation in sneaking propensity across Pacific Northwest populations. Another study objective is to characterize contextual cues eliciting the sneaker tactic and responses to the sneaker tactic among populations. I focus on a subset of sneaker populations with large samples of sneakers ( $n \geq 20$ ) to investigate population-level variation in cues eliciting sneaking behavior, in particular the breeding stage of sneak victims and activities evoking the sneaker repertoire. I also investigate one aspect of how sneaking may affect nest-holder behavior, specifically, whether sneaking elicits aggressive responses.

## 2.3 METHODS

### 2.3.1 Study Sites

This study includes observational data from seven oceanic and seventeen freshwater populations of Threespine Stickleback in northwestern North America collected between 1985 and 2009. Fifteen populations are from the Cook Inlet region of Alaska (between 59 and 63 degrees North latitude) and nine are from southern British Columbia (between 48 and 50 degrees North latitude) (Figure 2.1; descriptions in Appendix 2A).

### 2.3.2 Behavioral Observations

All study sites were sufficiently clear to permit *in situ* observation from the shoreline (e.g. all oceanic populations and several freshwater populations) or using mask and snorkel (e.g. many freshwater populations). The sticklebacks did not respond to the presence of an observer at any of the sites, unless the observer moved suddenly. Because observers were stationary for long periods, individual males could be viewed engaging in uninterrupted nest building, courtship and parental behavior.

For ten populations, observations were made across several years between 1985 and 2009. Information on which populations were observed across multiple years as well as estimates of observation hours spent in each population can be found in Appendix 2B. Many AK and BC populations were observed for a minimum of 12 hours using 3-min or 10-min spot observations on either randomly selected nested (focal) males or nested (focal) males mapped along a transect. However, the data set includes a number of populations observed between 1985 and 2009 for which full behavioral profiles were recorded during daily 3-min or 10-min observation periods on males located in mapped grids or

transects and such populations often have a greater number of observation hours (e.g. 700 hrs in Crystal Lake during 1985 and 400 hrs in Garden Bay during 1986).

For population with full behavioral profiles, all sneaking attempts occurring in the presence of the focal males were recorded across all stages of the reproductive cycle (nest-building, courtship, and parental). Sneaker color and behavior were recorded when possible. In a number of study populations, the focus was on courting males, as sneaking had been described in this context only. The color and behavior of all sneakers that approached a male during courtship were recorded and described in detail. While such data could be used to assess the frequency of sneaking during courtship interactions, these populations were omitted from any analyses focused on comparing the frequency of sneaking attempts across male breeding stages.

For populations in which observations were collected on males in multiple breeding stages, any sneaking attempts directed at focal males were recorded during the observational periods, whether during courtship or when the focal male was engaged in other activities such as nest maintenance or territorial defense. Collection of these observations provides an opportunity to investigate the frequency with which sneaking is employed in contexts outside of courtship.

Field observations of behavior often can be incomplete due to the difficulty of recording many occurrences rapidly. We included in this analysis only descriptions of sneaking that clearly indicated that the sneaker had sunk to the substrate or into nearby aquatic vegetation and was oriented towards the focal male and either (1) began moving towards the focal male or focal male's nest by slowly gliding over and around the substrate and objects along the substrate or (2) rushed towards focal male or focal male's nest from a hiding position. Such descriptions became our primary definition of a sneaking event (i.e. the sneaker repertoire). Often, males performing such behaviors took on characteristic drab coloration, aiding in the recognition of an intruding conspecific as a sneaker. We excluded references to

chasing of drab individuals when no further description was provided since individuals with sneaker coloration often swam through territories and were chased by males without exhibiting sneaking behavior.

### *2.3.3 Population Comparison of Sneaker Observations*

I collected descriptions of the sneaker repertoire and potential sneak fertilizations or sneak fertilization attempts occurring during focal male observations, specifically focusing on attempts that occurred during nest showing, a behavior performed by a male during courtship to encourage a gravid female to enter the nest and spawn. The extent to which sneak fertilization attempts outside the context of spawning actually results in successful gains in extrapair paternity is unclear (e.g. Sevenster-Bol 1962). Thus, performance of sneaking during nest showing behavior serves as a conservative estimate of sneak fertilization attempts as it occurs during a time considered most relevant to reproductive success. However, the sneaking repertoire has also been observed to occur in response to other actions performed by focal males. Instances of sneaking behavior performed outside of courtship nest showing are reported for a subset of sneaker populations (See *2.3.4 Population Comparisons of Sneaker Characteristics*).

Proportions of observed complete courtships (i.e. courtships which involve the focal male showing the nest entrance to a gravid, courting female) in which sneaker observations were recorded were tallied for each oceanic and freshwater AK and BC population ('All Complete Courtships', Table 2.1). Depending on the population, these proportions may include multiple courtship observations for individual males (e.g. Stepan Lake). Given the possibility that specific courting males may attract multiple sneakers or, more problematic, specific courting males may attract the same sneaker repeatedly, one random observation from each courting male was chosen in order to calculate a more conservative



estimate of prevalence of sneaking tactic use among males ('1 Complete Courtship/Male', Table 2.1). Statistical analyses were conducted on both sets of proportions to determine whether there were significant differences in results based on the method used to determine sneaker prevalence. When the relevant information was present, sneaker observations were tallied for each population in a similar fashion for both incomplete courtships and all courtships (complete and incomplete combined). In addition, all instances of sneaking occurring outside of courtships were tallied when such information was collected in population observations. These other tallied counts can be found in Appendix 2B.

#### *2.3.4 Population Comparisons of Sneaker Cues and Sneak Victim Responses*

Subsets of populations were analyzed to investigate potential geographic variation in populations with respect to contextual cues eliciting the sneaker repertoire and responses to the sneaker repertoire. A subset of populations in which focal observations were taken on males in different breeding stages within grids or transects were used for comparison of (I) sneaker attempts on males in different stages of the breeding cycle (e.g. nest-building, courtship or parental stages; n = 6 populations) and (II) sneaker attempts on territorial males during different territorial activities (e.g. courtship/fertilization, nest-directed activities, or territorial defense; n = 6 populations). Another subset of populations with observations that included specific details regarding sneak victim behavior was used for comparison of (III) nest-holder responses to sneaking outside of courtship (e.g. during nest-activity or territorial defense; n = 5 populations). Only populations with at least 20 sneaker observations were used for each analysis.

For comparison I, sneaker attempts (counts) on males in different stages of the breeding cycle, three categories were compared -- focal males who were the victims of sneaking during the nest-building stage, courtship stage, or parental stage of the breeding cycle. Male breeding stage (nestbuilding,

courtship, or parental) was determined based on a combination of information regarding behavioral repertoire, nest stage, and egg presence (Foster *et al.* 2008).

For comparison II, sneaker attempts (counts) on territorial males during different territorial activities, focal male territorial activities were condensed into three categories -- focal males who were the victims of sneaking during courtship/fertilization (both complete and incomplete courtships), nest-directed activity (fanning, digging, gluing, and creeping thru), and territorial defense (patrolling territory or chasing/following territorial intruders out of territory).

For comparison III, sneak victim responses following sneaker attempts outside of courtship scenarios (counts) were condensed into three categories – instances in which sneakers left without detection, instances in which sneakers left upon detection without escalation (i.e. low aggression responses involving sneak victims using head-down displays and peering at sneakers until they leave their territory), and instances in which sneak victims chased sneakers out of their territory (i.e. high aggression responses).

Given that these data sets often included sneaker observations from known males within grids or transects, a handful of observations may involve the same sneaker or sneak victim. However, it is not possible to determine possible repeat observations for unknown sneakers, so we decided to use observation, rather than individual as the unit of replication for these comparisons. Data included in these analyses were collected from multiple grids in each population and we assume individual observations are drawn from a large pool of individuals from each location.

To determine whether inclusion of multiple observations for known sneakers has an effect on data structure, a second data set including all the observations from unknown sneakers but only a single, random observation per known sneaker male was compared to the total set of sneaker counts for each of the three data sets described above (I, II and III). Mantel tests were conducted in PC-ORD (v4.20,

McCune & Mefford 1999) to determine whether randomized data would be a better fit to the data set than the subset with single observations for known males. The Mantel test conducts matrix correlations with permutations to determine whether the first matrix is more similar to the second matrix than any randomized version of the second matrix. The significance level of the Mantel correlation is the proportion of permutations that lead to a higher correlation coefficient (Mantel 1967; Sokal & Rohlf 1995). Using the Euclidean distance measure and 900 runs for the Monte Carlo Randomization Test, the analyses suggested that the larger data sets were not biased by observations of single known males -- in all three cases the two matrices were significantly correlated (standardized Mantel statistic ( $r$ ) ranged from 0.644 to 0.785,  $p < 0.05$ ) despite the fact that small sample sizes resulted in a small number of possible permutations of the data. Thus, complete data sets were used to investigate population variation for comparisons.

#### *2.3.5 Statistical Analyses for Sneaker Cues and Sneak Victim Responses*

Due to low observation counts for some behavioral categories, population comparisons were performed in R (v2.15.0, R Development Core Team), using a variation of the Fisher's exact test (Sokal & Rohlf 1995). Monte Carlo estimates of p-values (10,000 replicates, R *Stats* package) were used for proportion comparisons given that the contingency tables were larger than 2 x 2. When applicable, unplanned comparisons were used to determine if specific populations were driving significant differences and significance levels were adjusted accordingly (Sokal & Rohlf 1995).

General comparisons were also made between the categories within the three data sets. Instead of investigating population differences in sneaking propensity across categories, the goal was to identify aspects of sneaker behavior (I, II) or response to sneaker behavior (III) that may be prominent regardless of population variation. For these analyses, populations were treated as a group of independent samples

and nonparametric comparisons were performed on the proportional totals (PASW Statistics GradPack v18.0, SPSS Inc. 2009). When significant differences were found in the overall category comparison, post-hoc pairwise comparisons were performed with Bonferroni correction for multiple tests.

### *2.3.6 Descriptions of Sneaking Behavior Within and Across Regions*

Descriptions of the sneaker repertoire observed in populations of the different regions (AK and BC) and the different population types (Oceanic and Freshwater) are presented to provide comparisons of similarities and differences among populations. In particular, the general description of sneaker behavior focuses on 1992 observations from Stepan Lake in which data were collected throughout most of the breeding season (June 1-June 22) with 200+ hours of observation performed on males in grids. A multi-year comparison (1992, 2008, 2009) of sneaker and sneak victim behaviors (e.g. comparisons I, II, and III) is provided for Stepan. Given the potential lack of independence between 2008 and 2009, Fisher's exact tests are used to compare proportions for 1992 vs 2008 and 1992 vs 2009 only. Sneaker behavior observed in other populations is then compared to that observed in Stepan to discuss population-level similarities and differences.

## **2.4 RESULTS**

### *2.4.1 Population Comparison of Sneaker Observations*

Overall the sneaker frequencies using the conservative estimate based on a random courtship per focal male were very similar to the sneaker frequencies based on the complete data set (Table 2.1). A Spearman rank-order test demonstrated a significantly positive relationship between 'all complete

courtship' and '1 complete courtship/male' sneaking frequencies ( $r_s = 0.981$ , d.f. = 22,  $p < 0.001$ ), suggesting that multiple sneaking attempts directed at single males were not frequent enough to bias the counts. Given the similarity between these sets of proportions, discussion of the results focuses on the complete data set.

Regional variation in the use of the sneaker repertoire during courtship is presented in Table 2.1. The proportions presented are based on the single year with the greatest number of observation hours for each population. It is assumed that for populations with multiple observation years, the year with the most intensive sampling effort is likely to best represent the average sneaker frequency for that population. An expanded table that includes multiple years of proportions per population and multiple categories for sneaker frequencies can be found in Appendix 2B.

The percentage of complete courtships that involved sneaker observations ranged from 11-60% for AK oceanic populations and from 0-33% for AK freshwater populations. For BC oceanic populations, the range of percentages fell within those described for the AK populations (0-20%), whereas we did not observe sneaking behavior for any BC freshwater populations, despite similar numbers of observation hours (Appendix 2B) and comparable numbers of complete courtships observed. We also did not observe sneaking behavior during incomplete courtships or outside courtship in BC freshwater populations (Appendix 2B).

#### *2.4.2 Population Comparisons of Sneaker Cues and Sneak Victim Responses*

##### **(I) Sneaker attempts on males in different stages of the breeding cycle**

The proportions of sneaking observations on nest-building, courting, and parental males differed significant among populations (Figure 2.2; Fisher's exact test with simulated p-value,  $p < 0.001$ ).

Removal of Francis Peninsula (BC) from the comparison, however, results in no significant difference in proportions between the remaining populations ( $p = 0.06$ ). A high proportion of males in the courtship stage of the breeding cycle and a low proportion of males in the parental cycle encountered sneakers in Francis Peninsula Lagoon compared to other populations (Figure 2.2).

The overall differences in the frequency of sneaking across the three breeding stages does differ significantly across the three categories (PASW; Kruskal-Wallis  $X^2 = 11.802$ , d.f. = 2,  $p = 0.003$ ), with sneaking observations on males in the courting phase being significantly more frequent than sneaking observations on males in the other two stages (Mann-Whitney U test, PASW; Table 2.2).

## **(II) Sneaker attempts on territorial males during different territorial activities**

Analysis of the overall differences in the frequency of sneaking in response to courtship/fertilization behavior, nest-directed activity, and other territorial activity (regardless of sneak victim breeding stage) indicates that sneaking frequency does not differ significantly across the three categories (PASW; Kruskal-Wallis  $X^2 = 4.826$ , d.f. = 2,  $p = 0.09$ ). In general, sneaking occurs in response to courting behaviors just as frequently as it occurs in response to males performing nest-directed activities or actively guarding a territory.

However, populations vary in the proportions of sneaking observations across the three categories of territorial male activity (R: Fisher's exact test, simulated p-value with 10,000 replicates:  $p < 0.001$ , Figure 2.3). Contingency tables were subdivided for pairwise comparison of activity categories across populations (Fisher's exact tests; courtship/fertilization category removed:  $p = 0.003$ ; nest-directed activity category removed:  $p = 0.05$ ; territorial activities category removed:  $p = 0.008$ ). Adjustment of error rate for these three unplanned comparisons ( $\alpha = 0.017$ ; Sokal & Rohlf 1995), suggests that variation in proportions within the category of nest-directed behavior is driving population-level differences.

### **(III) Nest-holder responses to sneaking outside of courtship**

Populations exhibit significant differences in the proportion of nestholders not responding, or responding with low or high aggression to sneaking males (R: Fisher's exact test, simulated p-value with 10,000 replicates:  $p < 0.003$ , Figure 2.4). Combined removal of Big Beaver Lake and Big Lake Creek from the comparison, however, results in no significant difference in proportions among the remaining populations ( $p = 0.214$ ).

In the analysis of the overall differences, the frequencies of nest-holder reactions do differ significantly across the three categories (PASW; Kruskal-Wallis  $X^2 = 10.5$ , d.f. = 2,  $p = 0.005$ ), with high aggression responses (chases) occurring significantly more often than low aggression peering behavior or the absence of detection (Mann-Whitney U test, PASW; Table 2.3).

#### *2.4.3 Descriptions of Sneaking Behavior Within and Across Regions*

##### **(a) Freshwater Alaskan Populations**

Sneaking behavior was observed in ten of eleven freshwater lakes for which behavioral observations were available. However, despite long-term observation in some lakes, episodes of sneaking were mostly rare. In Whale Lake, no sneaking behavior was observed. Of the ten populations in which sneaking was observed during complete courtship, it was most common in Big Lake and Stepan Lake ('All complete courtships': 31% and 33%, respectively, Table 2.1).

A larger number of full behavioral profiles on focal (territorial) males were obtained for Stepan Lake in 1992. Observations from this data set will be used to discuss general sneaker attributes observed in Alaskan freshwater populations with notes from other populations surveyed less extensively. Prior to a sneaking attempt, sneaker males in Stepan Lake typically exhibited behaviors described in the earlier

literature (e.g. van den Assem, 1967; Wootton, 1971; Li & Owings, 1978a, b; Jamieson & Colgan, 1992; Goldschmidt *et al.* 1992). Males that made repeated sneaking attempts on one or more territorial males often swam widely over the breeding area, typically high in the water column, periodically sinking to the bottom. Males sunk to the substrate and slowly moved close to a male's nest at short intervals. A sneaker often utilized vegetation or other obstructions to shield movement from the territorial male. In Stepan and other AK freshwater populations in which males sometimes nested close to vegetation, sneakers often perched high in vegetation and peered down at the focal male's territory before either sinking to the substrate or rushing towards the nest from the perched position (K Shaw, pers. obs.). This common behavior was not specifically described in previous accounts of the sneaker repertoire. Sneaker males for which coloration data were obtained displayed a range of substrate-matching coloration from drab and mottled brown to drab coloration typically described as either a zebra stripe (vertical barring) or checkered pattern.

In daily observations of focal males conducted in 1992 (June 1-22), 24 out of 97 males in gridded areas were observed to perform typical sneaking behavior at least once. All 24 males performed the sneaking repertoire within the first 1-2 days of settlement in a territory. Only 33% (8/24) of these territorial males completed nest-building and courting and then began the parental stage of the nesting cycle and only 50% (4/8) of those males produced fry. Males that did not express the sneaker tactic during daily observations ( $n = 64$ ) typically followed nest-building with courtship. Of these males, 58% (37/64) began the parental stage, although only 40.5% (15/37) produced fry. Of those territorial males that successfully produced fry, 16% (4/24) originally expressed the sneaker tactic and transitioned to the courtship tactic while 23% (15/64) were only observed to express the courtship tactic. These tactic success rates are not significantly different (R; Fisher's exact test with 10,000 replicates,  $p = 0.573$ ), especially if several other factors are taken into account, such as (1) the possibility that unsuccessful males exhibiting either tactic nest and complete the breeding cycle elsewhere; (2) the potential for



males exhibiting sneaking to gain extra paternity via stolen fertilizations; and (3) the potential for males not observed to exhibit the sneaking tactic to do so outside of the observation period.

All instances in which sneakers were successful in creeping through a nest occurred after the courting male showed the nest entrance – either after the female deposited eggs in the nest or the courting male fertilized the eggs in the nest. Of the 18 shows during which sneakers were present (Table 2.1), two (11%) resulted in sneaker males entering the nest either before or after the focal male could follow the female through the nest, indicating that sneaker success is relatively rare.

Successful sneakers have been observed to steal eggs and deposit them in their nests following fertilization (van den Assem, 1967; Jamieson & Colgan, 1992; K Shaw & S Foster, pers. obs.). This was observed in the field if the sneaker male was followed closely. In one instance, two drab sneakers simultaneously stole a fertilization by entering the nest as the female exited but prior to the courting male entering. One of these two sneakers returned to the nest entrance, obtained a mouthful of eggs and returned to his own nest and deposited the eggs. This sneaker male, for which daily focal observations were obtained, attained brighter coloration within 30 min of the stolen fertilization. He then completed the courting phase and entered the parental phase of the breeding cycle within 4 days. However, no further observations of reproductive success were recorded, suggesting that this male did not complete the reproductive cycle. Another drab sneaker entered a nest following both the female and courting male. This male then removed eggs from the nest entrance, with no aggression exhibited by the nest-owner, and deposited them in his own nest. This male remained drab and continued performing sneaking behavior on courting males over the next two days, after which he abandoned his territory.

Typical sneaking behavior was observed to occur in Stepan Lake even when territorial males were not engaged in courtship. Males with sneaker coloration and lying close to the substrate were

observed around territories of males either performing nest maintenance or territorial defense (Figure 2.3, Stepan Lake 1992,  $n = 100$  sneaker observations). In such instances, territorial males either chased these sneaker males away or the sneaker males would rush into the territory, tapping the nest with their snout or performing rooting behavior in the nest. During five focal observations, males performing rooting behavior in a nest stole nesting material.

Stepan observations from 1992 are in agreement with the general results of this study -- sneaking is more commonly directed towards males in the courtship stage of breeding rather than the nest-building or parental stage (i.e. Figure 2.2; Table 2.2). Daily counts of sneaker males and parental males observed with fry demonstrate that there is a significant difference in the peak timing in abundance of these two behavioral types (Figure 2.5) -- in particular, peak abundance of sneaker males occurs 10 days prior to the peak abundance of parental males with fry. Given that the parental period between courtship and fry hatching can range from 6-12 days (van Iersel 1953; Swarup 1958), this suggests that sneakers were more abundant when the majority of males who later produced fry were still in the courtship stage of the breeding cycle.

Sample sizes were large enough ( $n > 20$  observations) to conduct comparisons of sneaker and sneak victim characteristics (e.g. comparisons I, II, and III) across multiple years in Stepan Lake, AK (1992, 2008, and 2009; Appendix 2B). Observation proportions were compared separately for 1992 vs 2008 and 1992 vs 2009. Proportions for 2008 and 2009 were not compared directly because use of consecutive years may violate the assumption of independent samples for this iteroparous population. In general, the results of these comparisons highlight the presence of inter-annual variability in sneaking behavior and responses to sneaking behavior within this population.

Proportions of sneaker observations occurring during complete courtships vs incomplete courtships and outside of courtships differ significantly across years (R; Fisher's exact tests with 10,000

replicates; 1992 vs 2008:  $p = 0.004$ ; 1992 vs 2009:  $p = 0.001$ ; Figure 2.6). For both comparisons, subdividing contingency tables (Sokal & Rohlf 1995) show that the higher proportion of sneaking observations during complete courtships in 1992 and the higher proportions of sneaker observations outside of courtship in both 2008 and 2009 are driving results (comparisons are not significant when each of these categories are removed).

The proportion of sneaking attempts on males in different breeding stages (i.e. comparison I: nest-building, courtship, and parental) differ significantly between years (1992 vs 2008:  $p > 0.001$ ; 1992 vs 2009:  $p > 0.001$ ). The higher proportions of sneaking observations occurring for nest-building males in both 2008 and 2009 are primarily responsible for divergence from 1992 proportions (Figure 2.7a).

The proportion of sneaking attempts on males during different territorial male activities stages (i.e. comparison II: courtship/fertilization, nest-directed activity, other territorial activity) differ significantly between years (1992 vs 2008:  $p > 0.001$ ; 1992 vs 2009:  $p > 0.001$ ). The higher proportion of sneaking observations during nest-directed activity in 2008 and 2009 versus 1992 is significant in subdivided contingency tests but the higher proportion of sneaking observations during courtship and fertilization behavior in 1992 is only influential in the comparison with 2008 observations (Figure 2.7b).

The proportion of low and high aggression nest-holder responses to sneaking behavior (i.e. comparison III: no detection, detection with low aggression; detection with high aggression) differ significantly between years (1992 vs 2008:  $p > 0.002$ ; 1992 vs 2009:  $p > 0.001$ ). For the 1992 vs 2008 comparison, both the difference in proportion of low aggression (sneaker leave after detection) and high aggression (chasing) responses are influential. For the 1992 vs 2009 comparison, the proportional differences are mainly driven by the difference in proportion of low aggression responses between years (Figure 2.7c).

General sneaking behavior and coloration patterns were similar in Alaskan freshwater populations exhibiting lower levels of sneaking during courtship. However, several deviations from characteristic sneaking behavioral patterns have been observed in other freshwater sneaking populations of Alaska. During 1992, out of 12 sneaker observations in Stephan Lake, none of the sneaker males appeared to have their own nest or territory. Similar accounts of a large number of males without territories or nests performing sneaking behavior is apparent in the 1992 accounts from Big Lake, in which only 2 of 12 sneaker males were noted to be nest-holders. In 2009, just 5 of 30 sneakers were observed to be nest-holders in Big Lake Creek. In other populations, sneaker observations are typically split in half between territorial males with nests and territorial males without nests or males with unknown territorial status (typically due to a failed attempt to follow the male after sneaking). Given that males may travel several meters to perform sneaking behavior, it is possible that a greater number of unknown males have territories at further distances from the observation sites or grids.

Males performing typical sneaker behaviors in Y Lake were often observed to exhibit these behaviors outside of the courtship context in both 1992 and 2009 -- attempting to sneak while the territorial male is performing either nest maintenance or territorial defense. In the two instances in which sneaker males performed a successful sneak fertilization and egg-stealing in 1992, neither occurred when the territorial males was courting -- one instance occurred while a male was performing territorial defense and another while a male had left in territory in pursuit of a gravid female. Similar instances of sneaker "success" in gaining entry to the nest outside of courtship and the potential utility of this behavior are discussed for Francis Peninsula Lagoon, BC (described below).

In general, sneaker coloration is typically drab (93% of males in populations with  $n \geq 20$  sneakers). However, there is variability within populations across years. Sneaker males observed in Y Lake during 2009 were all drab in coloration, however, 42% (8/19) of the observed sneakers had bright

body coloration in 1992. Bright males were described as having golden body coloration and pinkish-red throats similar to the courting males observed in this population.

#### **(b) Oceanic Alaskan Populations**

Sneaking frequencies in oceanic Alaskan populations were comparable to those in many of the derived freshwater populations of this region (Table 2.1). The general behavioral repertoire expressed and the sneaker coloration were similar to that described for AK freshwater populations. Observations from these populations demonstrate that sneaker males of unknown territorial status can be common. During 1995 observations in Anchor River and 2009 observations in Rabbit Slough, none of the sneakers was observed to be a territory-holder.

#### **(c) Freshwater British Columbia Populations**

Sneaking during courtship has not been observed in the six freshwater BC populations, despite equivalent, and in some cases, more intensive observation in these populations compared to freshwater AK populations. Despite several years of observations collected in Hotel and North Lake, none of the observations ranging from 1985 to 2009 indicate any definitive evidence of sneaking during courtship or even outside courtship (for populations in which observational data was collected on males outside of courtship).

#### **(d) Oceanic British Columbia Populations**

Frequencies of sneaking behavior observed in BC oceanic populations are comparable to those observed in both the oceanic and freshwater AK populations. The frequencies of drab sneakers observed in Francis Peninsula Lagoon during both 2008 and 2009 are comparable to the frequencies observed in AK populations (Table 2.1). Interestingly, much like the observed sneaker successes in Y Lake, AK, the three instance of sneaking success in Francis Peninsula Lagoon during 2009 involved sneaker males

successfully creeping through the nests of other males while the males were either preoccupied with territorial defense or while the territorial male was out of the territory.

#### *2.4.4 General Descriptions of Successful Sneaker Repertoire Use*

Of all observations recorded from 1985-2009 there were 11 instances of successful use of the sneaker repertoire, meaning a male performing sneaking behavior was observed to creep through a nest in a manner characteristic of a fertilization attempt. The majority of these successful sneaking observations occurred in AK freshwater populations (Stepan Lake (1992) = 2, (2008) = 1; Big Lake (1992) = 3; Y Lake (1992) = 2) and several occurred in an anadromous BC population (Francis Peninsula Lagoon (2009) = 3, described under 'Oceanic British Columbia Populations'). Combined, these 11 involved 13 successful sneaker males.

Of the 11 successful observations, 10 were performed on males in the courtship stage but only 6 of the 10 successful sneaking events occurred during a complete courtship event, in which the nest owner spawned in the nest. In 5 of these 6 post-spawn sneaking events, sneakers stole eggs. Of the other 4 observations, 3 sneak fertilizations were performed while the nest-owner was away from the nest – often preoccupied with chasing territorial intruders or patrolling his territory (Francis Peninsula Lagoon (2009)). The other observation involved a male sneaking to a nest to steal eggs while the nest-owner was preoccupied with intruders and then returning to sneak a fertilization while the nest-owner was performing nest activities in response to the eggstealing event (Y Lake (1992)). One successful sneak observation was performed on a male in the early parental stage (Y Lake (1992)). During this observation, the sneaker crept through the nest and stole eggs while the nest-owner was patrolling his territory.

Of the 9 successful sneakers for which color information was collected, 7 exhibited drab coloration and 2 expressed some typical nuptial coloration. In both cases the successful sneakers expressing nuptial coloration had nests, suggesting that these males may have been performing courtship behavior and utilizing the sneaking tactic opportunistically (Y Lake (1992) and Stepan Lake (2008)). Overall, 7 of the 13 successful sneakers were observed to be territorial nest-owners. The other 6 successful sneakers could not be followed post-sneaking so it is uncertain whether these males were also nest-owners or at least territorial.

In total 7 of the 13 successful sneakers also engaged in egg-stealing. Of these males, 5 were observed to bring the stolen eggs back to their territory and put them in their own nest. During the same observation day, 4 of these 5 males began to exhibit nuptial coloration. Similar rapid color changes following egg-stealing have been observed under laboratory conditions (K Shaw, pers. obs.)

## **2.5 DISCUSSION**

### *2.5.1 Geographic Variation in Sneaking Frequency of Pacific Northwest Stickleback*

The comparison of sneaking frequencies across multiple populations of the Pacific Northwest demonstrates that the sneaking repertoire is absent, or exhibited at such low frequencies that it is undetectable, in the BC freshwater populations investigated in this study. Sneaking frequencies in AK oceanic and freshwater populations are very similar, indicating an influence of ancestral expression of the repertoire in AK oceanic populations on the presence of the sneaker repertoire in the derived freshwater populations of this region. Given that the presence of the sneaker repertoire is the ancestral state across the Threespine Stickleback distribution (Foster *et al.* 1996; Chapter 1), it is difficult to explain why the current presence of the sneaker repertoire in oceanic BC populations is not also reflected in the

derived freshwater populations of the region. Although there is the potential for regional structure to produce trait variation between oceanic AK and BC populations (Foster 2013b), ranges of sneaker frequencies overlap between the two regions. Differences in social and environmental cues experienced during the breeding season are most likely responsible for the variation in sneaking frequencies observed among the oceanic populations. The physical attributes of the breeding areas for these marine and anadromous populations can be quite different (Appendix 2A). For freshwater populations, variation in sneaking frequency should be contingent on both the genetic makeup of individuals that originally seeded different freshwater populations and any subsequent selection on the expression of alternative mating tactics within newly established populations, as observed among freshwater AK populations (Table 2.1). However, given similar sneaking frequency between some of the oceanic populations of the AK and BC regions, we would expect the variation in sneaking frequency in freshwater BC populations to be similar to that among freshwater AK populations.

What are the causes of the disparity between oceanic and freshwater BC sneaking frequencies, especially the lack of any observations of sneaker repertoire in freshwater BC populations? Since there is no significant difference in observation hours spent between BC freshwater populations and other populations in which the sneaker repertoire has been reported, absence cannot be due to lower sampling effort at these sites (see Appendix 2C). It is also unlikely that the absence of this repertoire is due to appreciable temporal fluctuations in frequency of this behavioral repertoire either within or across breeding seasons: freshwater BC populations have been extensively studied within and across multiple breeding seasons in some instances (see Appendix 2B for observation dates and years). This leaves several hypotheses for the absence of the sneaker repertoire in freshwater BC populations: 1) a lack of the sneaker repertoire in colonizing ancestors; 2) the loss of sneaking in the colonized freshwater populations; or 3) environmentally contingent expression of sneaking.



### 1) A lack of the sneaker repertoire in colonizing ancestors

Assuming that the sneaker repertoire is to some extent under genetic control, which is likely given its stereotyped components (Foster *et al.* 1995, 1996), it may be that the absence of the sneaker repertoire in a handful of freshwater BC populations is due to absence of the sneaker repertoire in the colonizing oceanic fish. Given the molecular evidence suggesting high gene flow within a very large regional admixtures of marine *G. aculeatus* populations (Withler & McPhail, 1985; Taylor & McPhail 1999, 2000; Cresko, 2000; Schluter & Conte 2009; Hohenlohe *et al.* 2012) and the widespread evidence that the sneaker repertoire is the ancestral state in this species (Foster *et al.* 1996; Chapter 1), the hypothesis of multiple colonization events by marine fish with no propensity to perform the sneaker repertoire is unlikely unless it was a fairly ancient event. One possible scenario is that haplotypes that vary in their distribution across the Pacific Northwest also vary in their propensity to exhibit sneaking behavior. An ancient Japanese mtDNA lineage, TNPC, is rare in the Pacific Northwest and has just recently begun to spread from western Pacific waters (Orti *et al.* 1994; Deagle *et al.* 1996; Thompson *et al.* 1997; Taylor & McPhail 1999). Johnson & Taylor (2004) found a correlation between the lack of TNPC haplotypes and higher elevations across the Pacific Northwest, suggesting that populations at higher elevations were not secondarily inundated during post-glaciation isostatic rebound. A link between the Japanese haplotypes and sneaking propensity based on elevational gradients is unlikely, however, because a comparison of lake elevations from both AK and BC populations listed in Table 2.1 suggests no association between sneaking frequency and population elevation (Appendix 2D).

On the other hand, a relationship between the Japanese haplotypes and sneak propensity based on longitudinal gradients is more plausible. The current haplotype distributions suggest that northern Vancouver Island represents the southernmost extent of TNPC dispersal in the western Pacific Ocean

after migration across the Bering Sea (Johnson & Taylor 2004). All BC populations in the sneaking analysis are located more southerly than the two most southern populations included in the Johnson & Taylor (2004) study – both of which lacked TNPC haplotypes. Combined with the observation that sneaking frequencies have been observed to increase across several sampling years in the oceanic population of Francis Peninsula Lagoon (Appendix 2B), it is possible that the TNPC haplotypes are migrating southerly through the Strait of Georgia and are related to increased expression of sneaking behavior. The actual presence and frequency of TNPC haplotypes in the sneaking study populations is unknown, but warrants further investigation given the known patterns of TNPC haplotype distribution and potential dispersal along the coast of British Columbia and the potential for ancient DNA lineages, such as TNPC, to be associated with distinct phenotypic traits (Haglund *et al.* 1992; Lavin & McPhail 1993; Orti *et al.* 1994; Appendix 2D).

## **2) The loss of sneaking in the colonized freshwater populations**

Another possible explanation is that drift has been important, in the form of founder events and random trait loss. It is possible that only a small proportion of oceanic fish colonizing both mainland BC (Garden Bay, Hotel Lake, North Lake) and Vancouver Island (Cowichian Lake, Crystal Lake, Sproat Lake) carried alleles associated with sneaking, leading to loss via drift, even if sneaking behavior was a favorable mating tactic (Foster 2013b). While loss of a favorable trait at low frequencies due to founder effects is possible, it is also possible for low frequency alleles to spread within a new population – given strong positive selection for a trait and a large enough initial population size during colonization. For instance, an increase in the frequency of alleles associated with low-plate morphs in freshwater threespine populations lacking piscivorous predation pressure is evident when compared to the low frequency of these alleles in the standing genetic variation of oceanic threespines (Colosimo *et al.* 2005).

One might expect a similar positive response in alleles directly associated with reproductive success, such as behaviors associated with alternative reproductive tactics, during colonization events. In some cases, the presence of alternative reproductive tactics has actually been associated with the maintenance of genetic diversity in small populations, allowing for population persistence (Moran & Garcia-Vazquez 1998; Garcia-Vazquez *et al.* 2001; Juanes *et al.* 2007).

However, if novel environmental conditions do not elicit the expression of alternative reproductive behaviors, these behavioral phenotypes may be lost as a consequence of drift (Moran 1992; Masel *et al.* 2007; Foster 2013b). Such an extreme scenario, however, is an unlikely outcome (Foster 1995; Foster *et al.* 1996). There is evidence for maintenance of ancestral behavioral traits despite long periods of disuse across many taxa (reviewed in Foster 2013b) – stickleback-specific examples include anti-predator behavior in predator-naïve stickleback populations (Messler *et al.* 2007; Lahti *et al.* 2009) and diversionary display behavior in populations lacking cannibalistic groups (O’Neil 2012). Plus, the potential for this particular behavior to be lost in multiple disparate populations in BC is highly unlikely, unless these colonized freshwater environments all share similar selective regimes with respect to social interactions, resulting in parallel loss or change upon colonization (West-Eberhard 2003; Foster 2013b).

Multiple losses or changes of ancestral expression patterns have been observed for both display (e.g. conspicuous ritualized behaviors, such as diversionary behaviors and courtship) and nondisplay behaviors (e.g. sneaking, foraging, cannibalistic, and anti-predator behaviors) across populations of stickleback, although display behaviors are generally more strongly phylogenetically conserved (Foster 1995, Foster *et al.* 1996). However, nondisplay behaviors may also be evolutionarily constrained because of functional requirements associated with the behavior, such as remaining undetected during a sneak fertilization attempt; there may only be a few specific combinations of morphological and behavioral

characteristics that are effective to produce efficiency of the behavior (Foster 1995). In general, motor patterns that are utilized in several different behaviors may be more likely to be retained across populations, depending on the contexts in which the shared motor pattern is utilized. Freezing and sinking, for instance, have also been observed in antipredator behaviors, especially in response to piscivorous predators (Giles & Huntingford 1984; Foster & Ploch 1990; K Shaw pers. obs.). Relaxed selection in one or both of the contexts in which specific behavioral patterns are utilized may increase the probability of trait loss in a population (Lahti *et al.* 2009). However, such a scenario is unlikely to apply to the absence of sneaking behavior in BC freshwater populations because many of these populations do have piscine predators that should select for the maintenance of motor patterns shared in the antipredator response and the sneaking repertoire (e.g. Garden Bay and Crystal Lake; Foster & Ploch 1990). It would be interesting to test the extent to which individuals utilize these sneak-related motor patterns in response to chasing piscine predators in non-sneaking BC freshwater populations versus sneaking AK freshwater populations with similar predator regimes. In Stepan Lake, AK, sinking and freezing behavior observed in response to trout was indistinguishable to those motor patterns expressed in the sneaker repertoire (K Shaw, pers. obs.)

### **3) Environmentally contingent expression of sneaking**

The best explanation for the lack of sneaking observations in freshwater BC populations is that expression of sneaking behavior is environmentally contingent. Opportunistic mating tactics may not be observed when environmental and social factors result in low levels of competition (Cade 1980; Perrill *et al.* 1982; Warner & Hoffman 1980; Sullivan 1982; Schradin & Lindholm 2011). It is also possible that, in the case of BC freshwater populations, certain environmental or social cues necessary to elicit the behavior may be missing or altered.

In general, it is incorrect to assume that population differences have a genetic basis given that environmental differences between populations may cause behavioral differences even if populations do not differ genetically (Carroll & Corneli 1999). It is possible that this repertoire is retained in populations for which there is no evidence of expression but the environmental/social cues necessary to elicit the behavior may be infrequent or lacking (Shuster & Wade 2003; Foster 2013b). For instance, factors such as high female availability, low male-male competition, or high breeding synchrony may eliminate particular social cues that typically elicit sneaking behavior. Different combinations of cues related to male size/condition or territory attributes may elicit sneaking behavior in different populations (e.g. van den Assem 1967; Li & Owings 1978b; Sargent & Gebler 1980; Goldschmidt *et al.* 1992; Jamieson & Colgan 1992; de Fraipont *et al.* 1993; reviewed in Chapter 1). For instance, while inter-nest distance was a factor found to be comparable in sneaking European freshwater populations and non-sneaking BC freshwater populations (Goldschmidt *et al.* 1992), other important cues may be absent.

Another potential scenario is a change in sensitivity to a given cue – individuals of different populations may be more or less sensitive to the same cues that elicit reproductive tactic changes (Shuster & Wade 2003; Tomkins & Brown 2004; Emlen 2008). As sneaking behavior is an activational behavioral response, contextual environmental and social cues result in the elicitation of the sneaker repertoire in a threshold fashion based on complexes of neural and physiological networks (Carroll & Corneli 1999; Tomkins & Hazel 2007; Dingemanse *et al.* 2010; Stamps & Goothuis 2010b; Snell-Rood 2013). It is possible that BC freshwater populations are less sensitive to the same cues that readily elicit sneaking in other populations. Selection towards a higher response threshold for elicitation of sneaking behavior would be advantageous if reproductive payoffs are generally low for the sneaker tactic, which may occur if territorial males are more vigilant or aggressive in response to sneakers or more likely to cannibalize mix paternity clutches in some populations.

Common garden laboratory assays presented in Chapter 3 are used to investigate the extent of environmental and genetics effects on the use of the sneaking repertoire across a subset of AK and BC populations described in this study. Lab-reared individuals who have no prior experience with the cues in their natural habitat are used to determine the extent to which the lack of the sneaker repertoire in BC freshwater populations under field conditions is genetically determined (and under the most extreme circumstances, potentially loss) and the extent the same cues elicit the sneaking repertoire in different sneaking populations. Such an experiment will set the stage for further investigations into the genetic underpinnings of alternative mating tactics in *G. aculeatus*. If an underlying genetic component exists for sneaking propensity, it is possible that this reproductive tactic is maintained in a population due to the effects of GxE interaction -- where it is maladaptive if expressed under certain conditions but beneficial under other conditions (i.e. ecological crossover, Greenfield & Rodriguez 2004).

### *2.5.2 Generalities regarding sneaking behavior elicitation*

Comparisons across populations that exhibit sneaking behavior suggest that males typically sneak on territorial males in the courtship phase. Interestingly, sneaking observations are not significantly more frequent during courtship/fertilization activity compared to the other two categories of territorial male activity (nest-building and territorial defense) and the differences across populations are mainly driven by variation in propensity to sneak on males performing nest-directed activities. It might be assumed that sneaker males would utilize courtship or fertilization behavior as an immediate cue to perform sneaking. However, it is possible that the direct behavioral cues used by sneaker males are not exclusive to courtship behavior. For instance, sneaker males may frequently mistake nest-building activity for spawning because several very conspicuous nest-directed behaviors (e.g. creeping through, digging in entrance, fanning) are performed during regular nest maintenance activity and during courtship

behavior. In laboratory observations of sneaking behavior, Jamieson & Colgan (1992) observed that males occasionally demonstrated sneaker behavior whenever focal males exhibited nest-directed behaviors resembling spawning. Sneaking fertilization attempts performed on males in breeding stages other than courtship (e.g. nest-building or parental) were observed and are likely a result of misjudging territorial male status or behavior, as both nesting-building males and early parental males express high levels of nest-directed behaviors.

An important question to consider is why sneaker males may not use more reliable courtship-specific cues to recognize a potential sneak fertilization opportunity. The presence of a gravid female at a territorial males nest may serve as the best indication of a potential spawning event, but there are several reasons why it may not be advantageous to rely on the presence of a gravid females as the best indicator of a potential spawning event: (1) in some populations, females remain at a distance away from the nest during courtship, only approaching right before rejecting or accepting and spawning in a male's nest (K Shaw, pers. obs.) -- in such cases the territorial male's behaviors may be a more conspicuous indicator of potential spawning and (2) a sneaker male may approach a nest with a spawning female already inside; again the conspicuous male behavior directed at the nest (and similar to some nest-directed behaviors expressed outside of courtship) would be the most reliable cue available.

### *2.5.3 The significance of sneak fertilization outside of courtship*

While it is plausible that sneaker males mistake certain nest-directed activities performed by non-courting males as potential courtship cues, it is unlikely that they mistake the aggressive territorial activities of many males as courtship behaviors. As mentioned for Y Lake, AK and Francis Peninsula Lagoon, BC, sneaker males may utilize the opportunity offered by preoccupied territorial males (e.g.

chasing unwanted conspecifics and heterospecifics out of territories), to inconspicuously gain access to the nest and perform pre-oviposition ejaculations (*sensu* Sevenster-Bol 1962).

Pre-oviposition ejaculation is common in species with highly localized and predictable spawning sites (Taborsky 1998 -- e.g. European ocellated wrasse (*Symphodus ocellatus*; Taborsky *et al.* 1987), European bitterling (*Rhodeus sericeus*; Smith *et al.* 2002, 2003, 2004), rose bitterling (*Rhodeus ocellatus*; Kanoh 1996), and the grass goby (*Zosterisessor ophiocephalus*, Ota *et al.* 1996). Pre-oviposition ejaculation is often performed by territorial male threespines after completion of nest-building or in response to rival male presence and is typically associated with "creeping through" the nest, even in the absence of a gravid, courting female (Sevenster-Bol 1962). Such behavior may be an advantageous response to potential sperm competition given that stickleback sperm can lie dormant in the nest with the potential to be reactivated by female ovarian fluid upon spawning (Elofsson *et al.* 2003; LeComber *et al.* 2004). Sneaker males can utilize this strategy to gain paternity in another male's nest, as demonstrated by the sneak fertilization behavior outside of courtship observed in Y Lake, AK, and Francis Peninsula Lagoon, BC. This is an advantageous tactic, especially if risk of performing creeping through behavior in the absence of the territorial male is quite low for the sneaker male, allowing a male to circumvent potential aggressive behavior from the nest-holder. It may also decrease the chances that the nest-holder will abandon his nest or cannibalize the eggs in the nest. It is unlikely that male threespines can use specific olfactory cues to recognize their eggs (Mehlis *et al.* 2010). Without visual confirmation of a potential sneaking incident, a cuckolded nest-holder may be unaware that rival sperm has been deposited in the nest. Further investigation of the frequency and the potential success of the sneaker repertoire in contexts outside of courtship, especially in the absence of nest owners, would be necessary to determine if use of pre-oviposition fertilization is common in stickleback populations.



#### 2.5.4 Sneak Victim Responses

Sneak fertilization success can directly influence the reproductive success of a cuckolded male. Sneak fertilization attempts (whether successful or not) can indirectly influence the reproductive success of nest-holders. Territorial males decrease their expression of conspicuous courtship and nest-directed behavior in the presence of rivals (Ward & FitzGerald 1988; LeComber *et al.* 2003; Dzieweczyński & Rowland 2004) and the presence of intruders in general may increase the time males devote to territorial defense behavior (van den Assem 1967; Foster *et al.* 2008). Rival males perform several types of behaviors that can be considered interference competition -- including egg-stealing (and cannibalism), stealing nest material, and nest destruction. Such behaviors serve to disrupt male activities (Li & Owings 1978b). Li & Owings (1978b) suggested that the high frequency of sneaking observations they observed outside of courtship and fertilization (when sneaking is generally assumed to gain sneakers extra paternity) under laboratory conditions, represents interference competition. Similarly, van den Assem (1967) proposed that use of the sneaking repertoire by males in the nest-building phase to damage the nests of other males may serve to lower the position of competitors. Use of inconspicuous behavior during interference competition is advantageous in that it decreases the chances of aggressive responses by territorial males.

Many of the behaviors categorized as interference competition by Li & Owings (1978b) were observed on occasion in both AK and BC populations. There were instances of egg-stealing, sometimes accompanied by egg cannibalism, as well as instances of nest destruction and stealing of nest material, corroborating previous observations of such behaviors under natural conditions (e.g. Wootton 1972; Kynard 1978; Mori 1995; Goldschmidt *et al.* 1992). However, during the majority of observations in this current study, egg-stealing or stealing of nest material was not prefaced by the inconspicuous sneaker repertoire but motor patterns similar to those expressed in a diversionary display (K. Shaw, pers. obs.),

a conspicuous display behavior (Foster 1988). Such conspicuous interference behaviors typically divert a rival male's attention away from territorial activities, possibly increasing nest-holder aggression at the cost of courtship opportunities.

The question is to what extent seemingly inconspicuous sneaking attempts outside of the courtship context represent purposeful attempts at interference competition rather than mistaken opportunities to attempt a sneak fertilization. Across the sneaking populations investigated inconspicuous sneaker males are overwhelmingly detected by the nest-holder and the primary response of territorial males to sneaking outside of the courtship context is to aggressively remove the sneaker from the territory, typically by chasing (Figure 2.4). Highly aggressive responses suggests the presence of a sneaker is perceived as a threat. Although our results do not specifically investigate sneaker intentionality, the high frequency of sneaking on nest-directed activity, regardless of breeding behavior category, supports misinterpretation of cues over intentional competitive interference. While the main function of the sneaking repertoire is to steal a fertilization undetected, even inadvertent detection of sneakers will influence nest-holder behavior and potentially lower their competitive ability. Thus, whether the disruptions are intentional or not, these interactions have the ability to produce carry-over effects for the victimized males, especially involving increased courtship and territorial aggression and territorial vigilance following an aggressive encounter with a sneaker (Ward & FitzGerald 1987; Dzieweczynski & Rowland 2004). For populations with high sneaker frequencies, such territorial intrusions may be costly to nest-holders in terms of time and energy budgeted for territorial defense. It is possible that a response to potential sneaking is nest abandonment by parental males, as observed in bluegill sunfish (Neff 2003); this would account for the high number of parental males disappearing from their territory between observation periods. To further understand potential social consequence of the sneaker tactic, the extent to which high sneaking propensity alters the behavior of territorial males should be more thoroughly investigated. Of particular interest for this highly flexible tactic is whether

the propensity for a nest-holder to express sneaking behavior facultatively influences time-budgeting for courtship and territorial defense, as well as expression of territorial aggression.

#### *2.5.5 The significance of geographic variation in threespine reproductive tactics*

Sneaking is often described as if it is a static component of the stickleback reproductive repertoire, which gives the impression that it occurs at similar frequencies across populations (e.g. Wootton 1971; Ostlund-Nilsson 2006; but see Goldschmidt *et al.* 2002). It also has been inadvertently marginalized, treated as a behavior of little consequence when it comes to inferences regarding female choice and male reproductive potential and success mainly because the prevalence of this reproductive tactic in many study populations is unknown (e.g. Kraak *et al.* 1999; Candolin 2004; Heuschele & Candolin 2010). However, geographic variation in the prevalence of this behavioral trait is likely to have profound consequences for the strength of female preference on male sexually selected characters, effective population size, and measures of tactic-specific reproductive success (Sugg & Chesser 1994; Magurran 1996, 1998; Jones *et al.* 2001a; Lurgiader *et al.* 2001; Le Comber 2003; Shuster & Wade 2003; Juanes *et al.* 2007; Morris *et al.* 2010). For instance, it is often assumed that female preference generally selects for bright, vigorous courting males (e.g. McLennan & McPhail 1990; Milinski & Bakker 1990; von Hippel 1996, 2000), but depending on the characteristics of sneakers and the extent to which these characteristics are heritable, a high prevalence of sneak fertilizations in a population has the potential to dampen the effects of female preference on sexually selected traits in males. Geographic comparisons can inform potentially biased or stereotyped paradigms that are often perpetuated (e.g. “species-typical” behavior). For instance, Cornwallis & Uller (2010) point to a potential problem that often arises in microevolutionary studies – the non-random choice of study populations from habitats in which abundance is high and data collection is most efficient can result in biased perception of both variation

in phenotypes and the extent of environmental variation. Such biases in data collection can lead to the formation of incomplete conclusions regarding trait evolution and genetic and environmental contributions to phenotypic variation.

As the results of the regional comparison of sneaking frequencies indicate, not only are there regional differences in sneaking propensity, but even populations in close proximity to one another show variation in frequencies of sneaking observations both during and outside courtship (e.g. AK freshwater populations). However, use and success of alternative reproductive tactics can fluctuate within seasons and across seasons (e.g. Gross 1996; Henson & Warner 1997; Alonzo & Warner 2000; Calsbeek *et al.* 2002; Aubin-Horth *et al.* 2006; Rios-Cardenas *et al.* 2007; Chapman *et al.* 2009; Schradin & Lindholm 2011). Based on the inter-annual variation in sneaking frequencies and other aspects of sneaking behavior observed in Stepan Lake, it is plausible that more extensive within-season and across-year sampling will reveal similar patterns of variability in sneaker frequencies, particularly for neighboring populations experiencing similar environmental conditions. For instance, depending on timing of within-season sampling, peak frequencies of sneaking may be missed. These peaks in sneaker abundance can be short-lived, as demonstrated by the 4 day peak in Stepan Lake.

It is also important to note that interpopulation variation in observed sneaking propensity may not reflect the extent of actual sneaker reproductive success. There could be greater similarities in sneak fertilization tactic success among populations than what might be suggested based on observed attempts. Males in some populations may be more active seekers of sneak fertilizations opportunities but not more successful in terms of gaining stolen fertilizations than sneakers in other populations that may only use the behavior when specific courtship opportunities arise but also utilize the repertoire more effectively (K Shaw, pers. obs.; Chapter 3). Detailed comparisons of male reproductive success are required to determine how the propensity of performing sneaking behavior actually translates into

fitness and whether this relationship differs across populations. Besides the extent to which individual propensity to express sneaking behavior translates into successful cuckoldry and direct fitness gains, there may be indirect fitness consequences for sneakers and sneak victims in terms of time and energy budgeted for scouting sneak victims and territorial defense in response to sneaking.

Given the stickleback's status as a model organism in the study of behavioral ecology (Bell 1995; Foster 2013b), examining the extent of population-level differences in tactic frequency provides novel insights into other areas of stickleback biology -- including mating behavior, female preferences, and the selective pressures of mating tactics on morphology, behavior and life history traits. Overall, this study provides a useful first-step for future studies regarding alternative reproductive tactics of threespines by documenting populations that would benefit from more thorough, long-term, *in situ* investigations of sneaking behavior and the effects of this behavior (or absence/rarity of this behavior) on population dynamics. Accounting for variation in prevalence of alternative mating tactics among populations will allow for a better understanding of the interplay between the frequency of mixed paternity and the strength of female mate choice and the effects of this interaction on male parental care and male sexual traits, such as ornaments and behavior (Cornwallis & Uller 2010). Population specific estimates of sneaking propensity will also help discern the extent to which this alternative reproductive behavior affects male aggression, territoriality and territory choice, and frequency of conspicuous vs. inconspicuous courtship by breeding males. Inter-population variation in this alternative reproductive tactic is particularly intriguing because of the opportunistic nature of sneaking behavior and the variation in tactic flexibility expressed by males within and across populations (Chapter 3) and population comparisons will provide valuable insights into the evolution and maintenance, as well as potential costs, of such highly plastic behavioral strategies.

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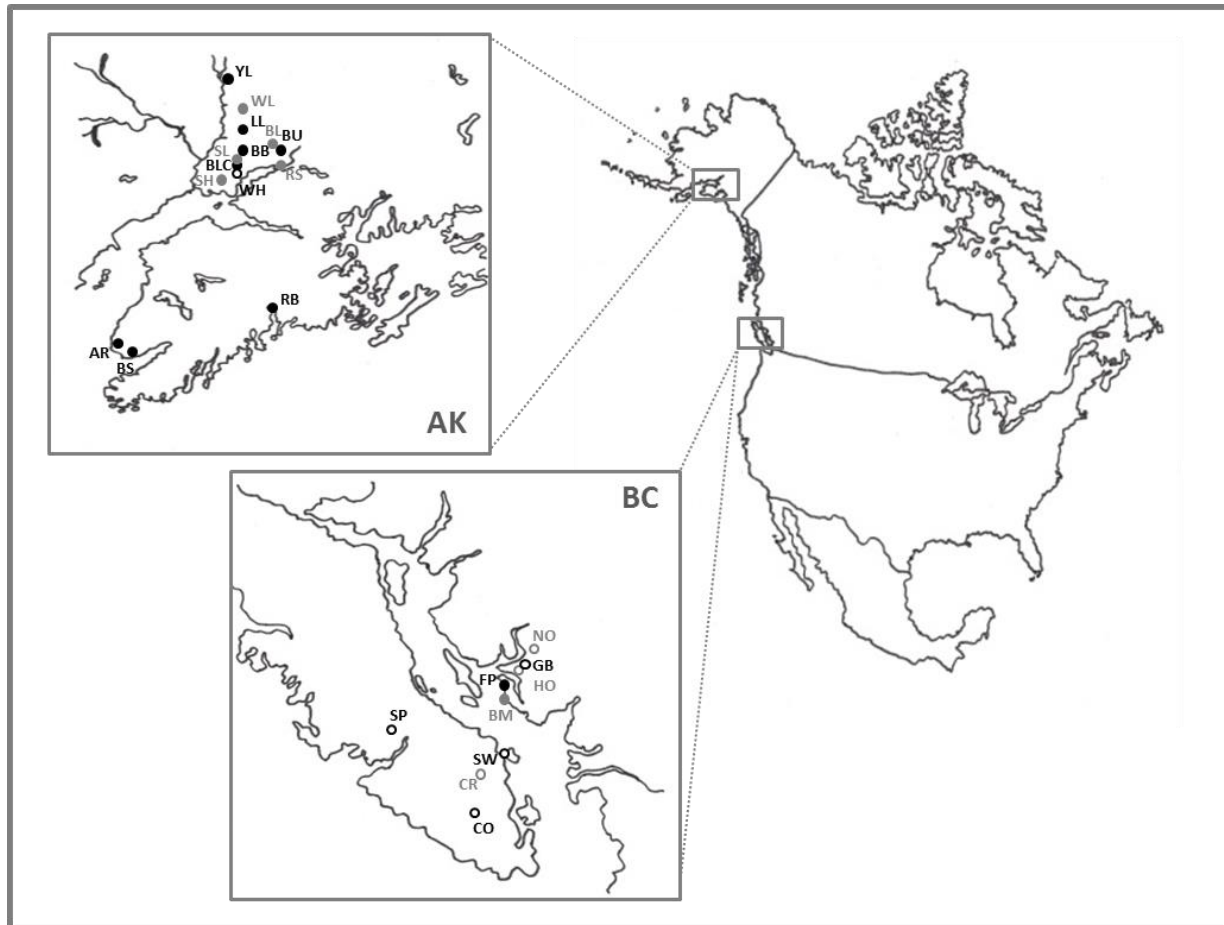
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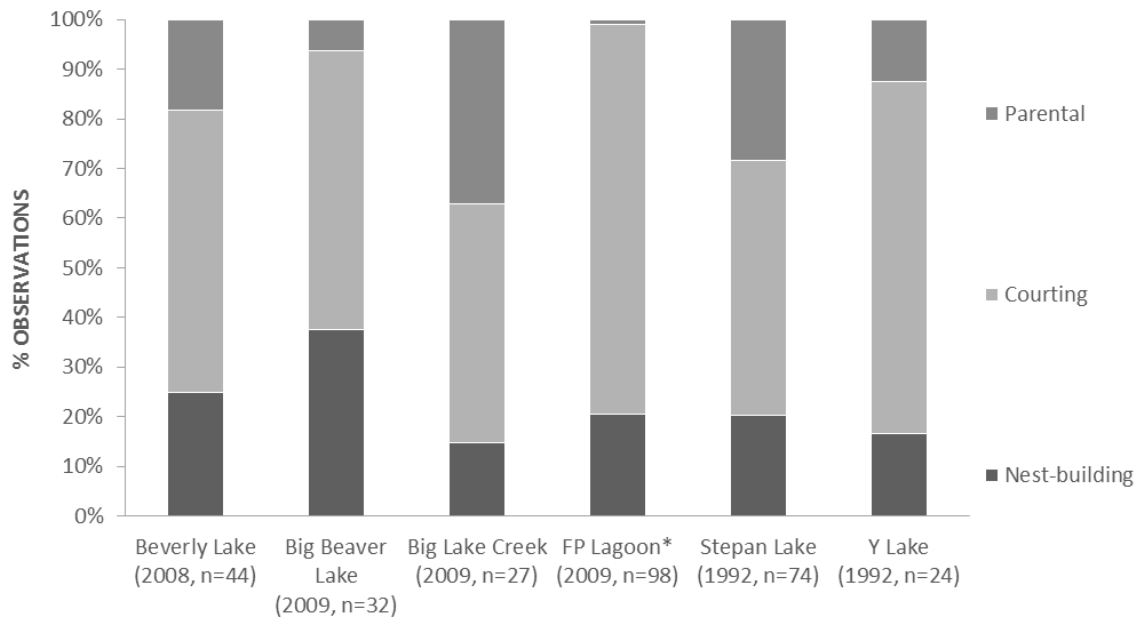
**Figure 2.1.** Geographic distribution of populations used in the study. Symbols indicate presence/absence of sneak repertoire observations -- sneaking observed ( • ) versus sneaking not observed ( ◦ ). Grayscale symbols and population codes used to differentiate populations in close proximity to others. Alaskan (AK) oceanic populations include: Anchor River (AR), Beluga Slough (BS), Rabbit Slough (RS), and Resurrection Bay (RB). AK freshwater populations include: Beverly Lake (BL), Big Beaver Lake (BB), Big Lake/Big Lake Creek (BLC), Bruce Lake (BU), Lynne Lake (LL), Stepan Lake (SL), Stephan Lake (SH), Whale Lake (WH), Willow Lake (WL), and Y Lake (YL). British Columbia (BC) oceanic populations include: Buccaneer Marina (BM), Francis Peninsula Lagoon (FP), and Swy-A-lana (SW). BC freshwater populations include: Cowichan Lake (CO), Crystal Lake (CR), Garden Bay Lake (GB), Hotel Lake (HO), North Lake (NO), and Sproat Lake (SP).

**Table 2.1.** The number of complete courtships (defined as a courtship in which the male showed the nest entrance to the female) in which sneaking did, and did not occur. Value in parentheses is the proportion of courtships involving sneaking. The column headed “1 Complete Courtship/Male” is a subset of the data in which a single courtship was selected at random for any males for whom we had multiple observations. Each population is represented by either the single year in which observations were obtained or the year with the greatest number of observation hours.

**Table 2.1.**

<b>Populations<sup>1</sup></b>	<b>All Complete Courtships</b>	<b>1 Complete Courtship/Male</b>
<b>Oceanic, Alaska</b>		
Anchor River <sup>g</sup>	4/12 (0.33)	4/11 (0.36)
Beluga Slough <sup>f</sup>	1/9 (0.11)	1/7 (0.14)
Rabbit Slough <sup>d</sup>	1/7 (0.14)	1/7 (0.14)
Resurrection Bay <sup>l</sup>	3/5 (0.60)	2/3 (0.67)
<b>Freshwater, Alaska</b>		
Beverly Lake <sup>k</sup>	2/12 (0.17)	1/7 (0.14)
Big Beaver Lake <sup>l</sup>	3/24 (0.13)	2/13 (0.15)
Big Lake <sup>d</sup>	5/16 (0.31)	4/14 (0.29)
Big Lake Creek <sup>l</sup>	1/17 (0.06)	1/10 (0.10)
Bruce Lake <sup>e</sup>	1/29 (0.03)	1/24 (0.04)
Lynne Lake <sup>f</sup>	1/17 (0.06)	1/16 (0.06)
Stepan Lake <sup>d</sup>	18/54 (0.33)	14/40 (0.35)
Stephan Lake <sup>d</sup>	4/46 (0.09)	4/34 (0.12)
Whale Lake <sup>e</sup>	0/35 (0)	0/18 (0)
Willow Lake <sup>d</sup>	1/24 (0.04)	1/20 (0.05)
Y Lake <sup>d</sup>	3/18 (0.17)	3/17 (0.18)
<b>Oceanic, Canada</b>		
Buccaneer Marina <sup>e</sup>	2/21 (0.10)	2/20 (0.10)
Francis Peninsula Lag. <sup>l</sup>	20/102 (0.20)	11/30 (0.37)
Swy-A-Lana <sup>h</sup>	0/14 (0)	0/14 (0)
<b>Freshwater, Canada</b>		
Cowichan Lake <sup>c</sup>	0/20 (0)	0/19 (0)
Crystal Lake <sup>a</sup>	0/72 (0)	0/24 (0)
Garden Bay Lake <sup>b</sup>	0/25 (0)	0/21 (0)
Hotel Lake <sup>l</sup>	0/25 (0)	0/18 (0)
North Lake <sup>c</sup>	0/19 (0)	0/16 (0)
Sproat Lake <sup>c</sup>	0/161 (0)	0/60 (0)

<sup>1</sup>Sample years = (a) 1985; (b) 1986; (c) 1989; (d) 1992; (e) 1993; (f) 1994; (g) 1995; (h) 1996; (i) 1997; (j) 2006; (k) 2008; (l) 2009

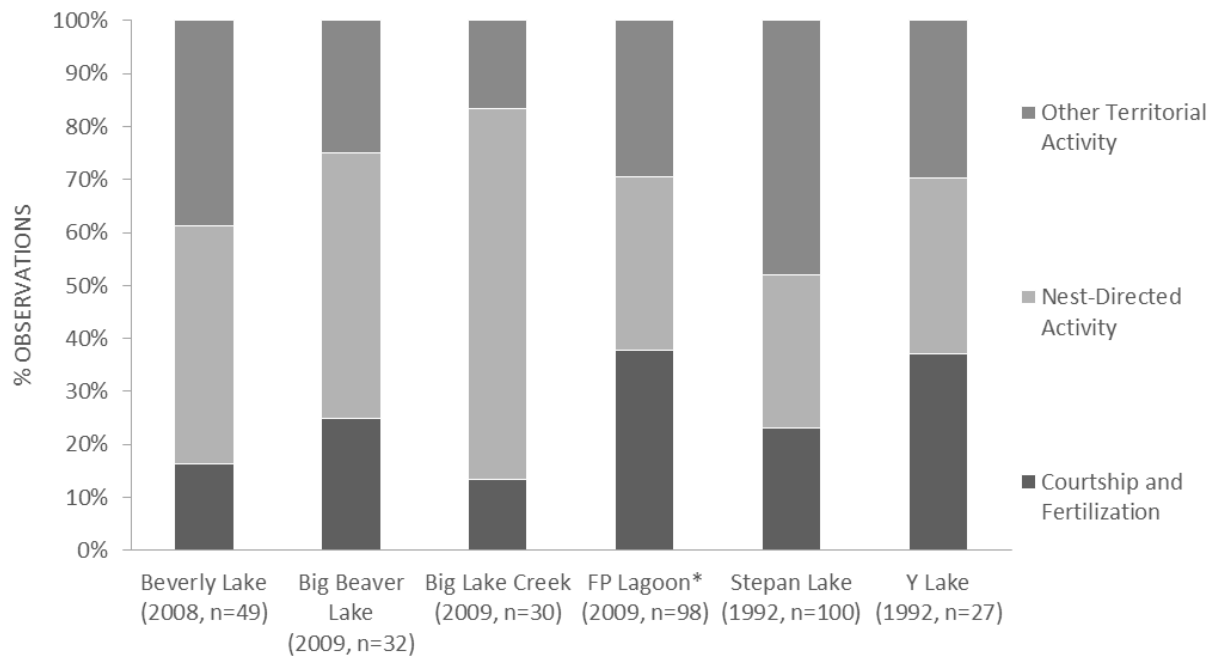


**Figure 2.2.** Frequency of sneaking attempts on territorial males of different breeding stages (i.e. % sneaking attempts on males in the nest-building, courting or parental stage of breeding), regardless of immediate territorial male behavior (e.g. nest-activity, territory defense). Includes a subset of populations for which there were observations of focal males in all breeding stages. All populations are Alaskan Freshwater except Francis Peninsula Lagoon (denoted by \*), which is a BC oceanic population.

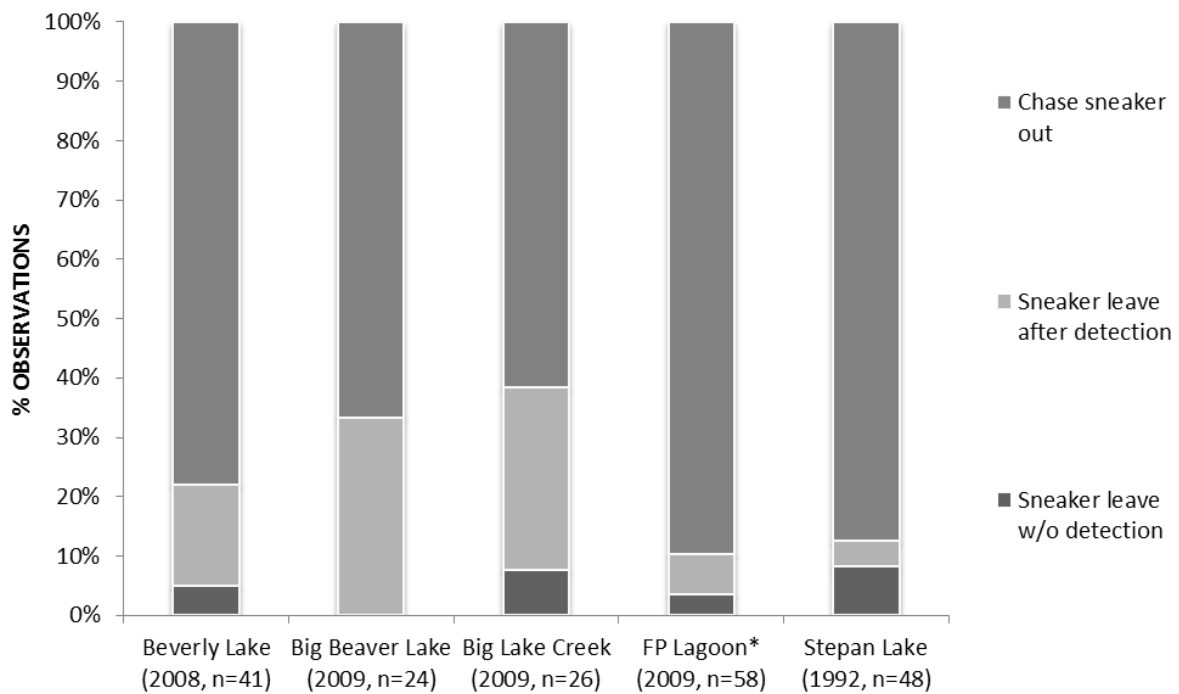
**Table 2.2.** Multiple comparisons of sneaking proportions on males of different breeding stages using the nonparametric Mann-Whitney Test. Populations were treated as a group of independent samples.

Comparison	Mann-Whitney U	Wilcoxon W	Z	p <sup>a</sup>
Nest-building vs Courting	0	21	-2.887	0.004*
Nest-building vs Parental	12	33	-0.962	0.336
Courting vs Parental	0	21	-2.882	0.004*

<sup>a</sup> Test significance based on two-tailed test; Bonferroni correction used to account for multiple comparisons -- significance set at  $p \leq 0.017^*$



**Figure 2.3.** Frequency of sneaking attempts during different territorial male behaviors. Includes a subset of populations for which there were observations of focal males in all breeding stages. All populations are Alaskan Freshwater except Francis Peninsula Lagoon (denoted by \*), which is a BC oceanic population.



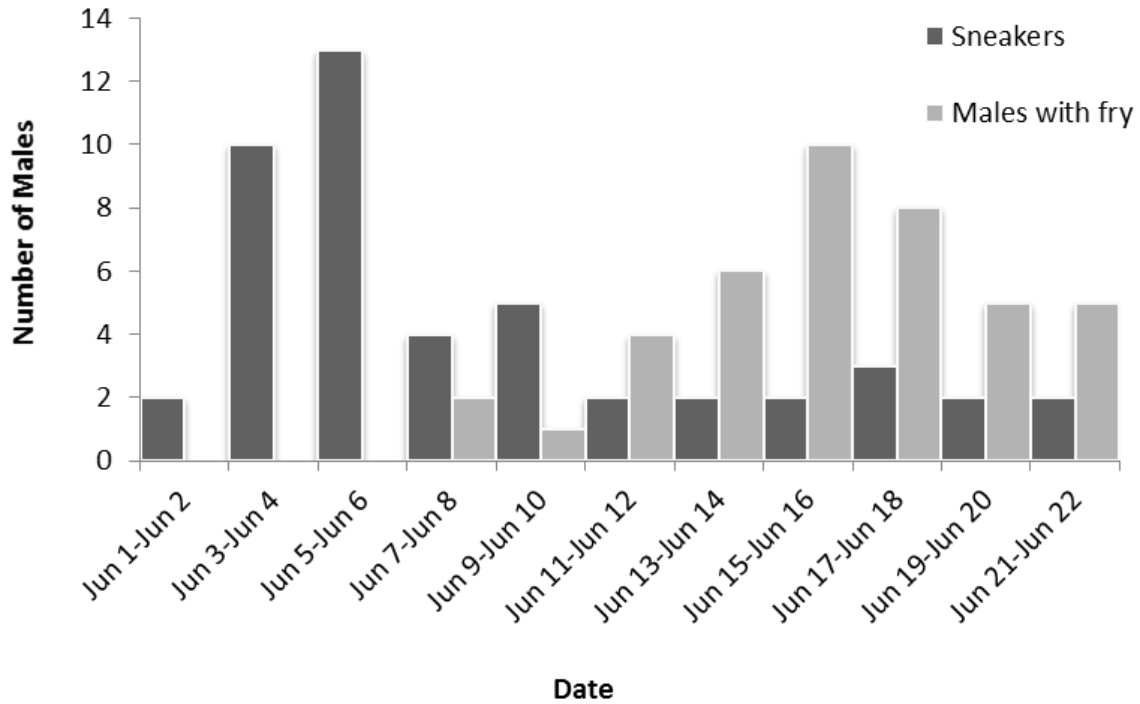
**Figure 2.4.** Frequency of nest-holder responses to sneaking attempts outside of courtship (e.g. during nest-activity or territorial defense). Responses to sneaking attempts are either initiated by the sneaking male ('Sneaker leave w/o detection' = sneaker leaves territory without detection by territorial male or 'Sneaker leave after detection' = sneaker leaves territory after detection but involves no active response by the territorial male) or responses initiated by the territorial male ('Chase sneaker out' = any active and aggressive behavior used by the territorial male to remove the sneaker from the territory). Sneakers leaving without detection represents 'no detection' by nest-holder, while sneaker leaving after detection without active nest-holder response and with active nest-holder response represent 'low aggression' and 'high aggression' responses, respectively. All populations are Alaskan freshwater except Francis Peninsula Lagoon (denoted by \*), which is a BC oceanic population.

**Table 2.3.** Multiple comparisons of nest-holder responses to sneaking attempts outside of courtship (e.g. during nest-activity or territorial defense) using the nonparametric Mann-Whitney Test. Populations were treated as a group of independent samples. Sneakers leaving without detection represents ‘no detection’ by nest-holder, while sneaker leaving after detection without active nest-holder response and with active nest-holder response represent ‘low aggression’ and ‘high aggression’ responses, respectively.

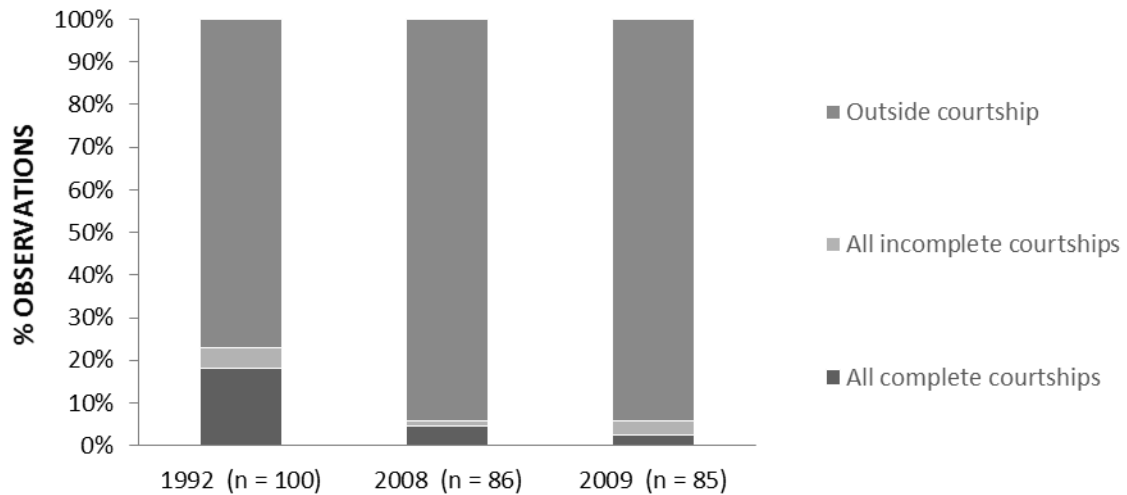
Comparison	Mann-Whitney U	Wilcoxon W	Z	p <sup>a</sup>
No detection vs low aggression response	5	20	-1.567	0.117
No detection vs high aggression response	0	15	-2.611	0.009*
Low aggression vs high aggression response	0	15	-2.611	0.009*

<sup>a</sup> Test significance based on two-tailed test; Bonferroni correction used to account for multiple comparisons -- significance set at  $p \leq 0.017^*$



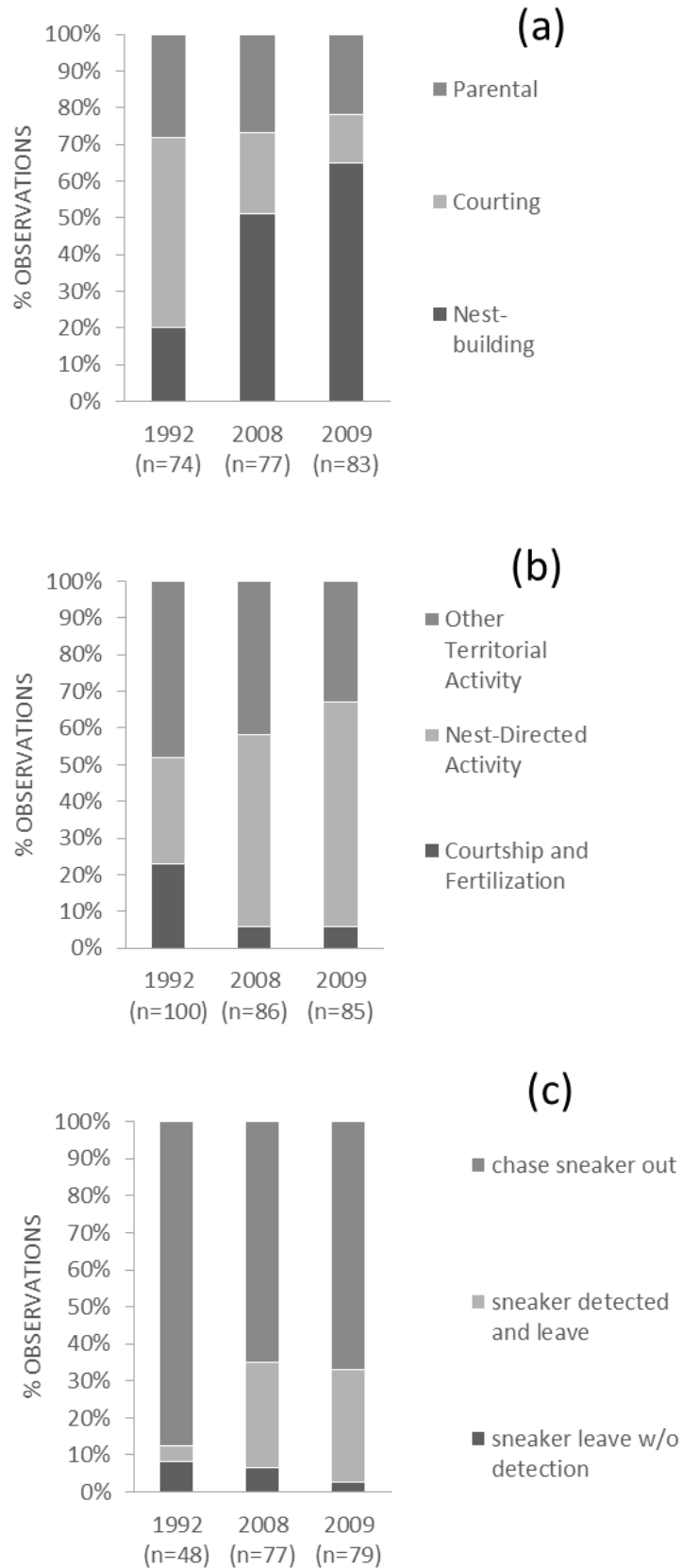


**Figure 2.5.** Difference in timing between abundance of sneakers and the abundance of parental males with fry over the course of a breeding cycle during the 1992 breeding season in Stepan Lake, AK. There is a significant difference in the median date between the two male types – peak abundance of sneakers occurs on June 6<sup>th</sup> while peak abundance for parental males with fry occurs on June 16<sup>th</sup> (Two-sample Wilcoxon test of medians,  $W = 1560.5$ ,  $p \leq 0.0001$ ).



**Figure 2.6.** Frequency of sneaking observations during complete courtships vs incomplete courtships and outside courtships (e.g. performed on males conducting other territorial behaviors) compared across multiple years in Stepan Lake, AK. Sample sizes for the different years can be found in Appendix 2B.

**Figure 2.7.** Stepan multi-year comparisons for sneaker cues and sneak victim responses: **(a)** Frequency of sneaking attempts on territorial males of different breeding stages (i.e. % sneaking attempts on males in the nest-building, courting or parental stage of breeding), regardless of immediate territorial male behavior (e.g. nest-activity, territory defense). **(b)** Frequency of sneaking attempts during different territorial male behaviors (i.e. courtship/fertilization, nest-directed activity, other territorial activity), regardless of male breeding stage. **(c)** Frequency of responses to sneaking attempts on territorial males outside of courtship (e.g. during nest-activity or territorial defense). Responses to sneak attempts are either initiated by the sneaking male ('Sneaker leave w/o detection' = sneaker leaves territory without detection by territorial male or 'Sneaker leave after detection' = sneaker leaves territory after detection but involves no active response by the territorial male/low aggression response) or responses initiated by the territorial male ('Chase sneaker out' = any active and aggressive behavior used by the territorial male to remove the sneaker from the territory/high aggression response).



**Figure 2.7.**

## CHAPTER 3

# A COMMON GARDEN STUDY OF THE POPULATION-LEVEL DIVERGENCE AND INDIVIDUAL VARIATION IN SNEAKING PROPENSITY OF THREESPINE STICKLEBACK (*GASTEROSTEUS ACULEATUS* L.)

### 3.1 ABSTRACT

The sneaker repertoire, often utilized by males to inconspicuously steal fertilizations from spawning males, is an ancestral behavioral trait that has been described in multiple oceanic and freshwater populations across the distribution of Threespine Stickleback. *In situ* field observations reveal sneaking behavior in oceanic populations of British Columbia and oceanic and freshwater populations of Alaska, but the sneaker repertoire is rarely observed in freshwater populations of British Columbia. To investigate whether this population-level variation in sneaker propensity has a genetic basis, a standardized repeated trial behavioral assay was performed in attempts to elicit sneaker behavior in lab-reared fish from oceanic and freshwater populations of both British Columbia and Alaska. Results suggest that propensity to perform the sneaker repertoire is comparable across all populations tested, demonstrating that absence of expression under natural conditions is not due to purely genetic differences in tactic expression. Such findings highlight the potential difficulties of using behavior observed under laboratory conditions as a proxy for behaviors expressed under natural conditions.

Observations of sneaking behavior across all populations were combined in this study to provide general insights regarding repertoire propensity, plasticity, and success. Individuals demonstrated variation in extent of courting and sneaker tactic expression along a continuum from high courtship/low

sneaking expression to low courtship/high sneaking expression, plus individuals specializing in one tactic or the other.

With the repeated-trial design of the assay, we were able to show sneakers vary in consistency of tactic use across trials, and highly consistent sneakers demonstrated a higher rate of sneak fertilization success. Sneaker males also varied in their behavioral flexibility – the ability to switch between the courtship and sneaker tactics within and across trials. These results corroborate previous observations of opportunistic reproductive tactic use in threespine populations.

**KEYWORDS:** Threespine Stickleback, *Gasterosteus aculeatus*, geographic variation, behavioral plasticity, behavioral flexibility, sneaking, alternative reproductive tactic.

### 3.2 INTRODUCTION

Species-specific alternative reproductive tactics are based on combinations of alternatives from three distinct evolutionarily significant levels: determination (the extent of genetic and environmental contributions to reproductive phenotypes), plasticity (the extent to which reproductive phenotypes are condition-dependent and reversible), and selection (the extent to which alternative tactics have equal fitness, Taborsky 1998; Brockmann, Oliveira, and Taborsky 2008). Categorizing alternative reproductive tactics for a species is an important first step towards understanding how variation is maintained within populations and how evolutionary trajectories can diverge across populations (Brockmann, Oliveira, and Taborsky 2008). This involves documenting the form of individual tactic expression (e.g. developmentally fixed versus plastic) and the extent of variation in tactic expression (e.g. tactic use consistency and flexibility/reversibility). It is also important to determine whether purely genetic, purely environmental (i.e. plasticity), or a combination of the two mechanisms (i.e. genetic differences in

plasticity) underlie population-level differences in tactic expression if they exist. Within species, geographic variation in environmental attributes, population dynamics, and expression of alternative reproductive tactics can synergistically produce different evolutionary trajectories among populations (Tomkins & Brown 2004; Aubin-Horth *et al.* 2005a, 2006). Within populations, individual variation in alternative reproductive tactic expression can have direct effects on individual fitness, as well as interact with social dynamics and sexual selection (Smith & Sibly 1985; Chesser 1991; Sugg & Chesser 1994; Magurran 1996, 1998; Alonzo & Warner 2000; Jones *et al.* 2001; Rio-Cardenas *et al.* 2007; Morris *et al.* 2010). The magnitude of such effects can be influenced by the extent to which alternative reproductive tactics are plastic (e.g. responsive to environmental and social influences; Shuster & Wade 2003; Fawcett *et al.* 2011).

Threespine Stickleback males employ a set of plastic alternative reproductive tactics whereby nest-building or courting males, exhibiting stereotypical drab coloration and inconspicuous behavior, attempt opportunistic sneak fertilizations on spawning, nest-holding males. This repertoire has been observed in the field and in wild-caught fish under laboratory conditions, and its expression has been attributed to a number of environmental and social factors (e.g. male age, nest location, inter-nest distance, nesting order: van den Assem 1967; Li & Owings 1978b; Sargent & Gebler 1980; Goldschmidt *et al.* 1992; Jamieson & Colgan 1992; de Fraipont *et al.* 1993; See Chapter 1 review). Due to the wide distribution of the Threespine Stickleback, there is great potential for discerning variation in tactic frequency and tactic switching in multiple populations; however, there have been few direct field (Black & Wootton 1970; Kynard 1978; Mori 1995) or comparative studies of this behavior (Wootton 1971; Goldschmidt *et al.* 1992; Foster *et al.* 1996).

A recent geographic comparison of *in situ* sneaking behavior indicates variation among populations of the Pacific Northwest in the use of this alternative reproductive tactic and highlights the

absence or rarity of expression of the sneaker repertoire in a number of freshwater British Columbia (BC) locations (See Chapter 2, Foster 2013b). These geographic differences may reflect genetically fixed differences among populations, purely plastic behavioral responses to local environmental differences without underlying genetic differentiation, or variation in norms of reaction (genotype x environment interactions) due to divergent local conditions (reviewed in Foster 2013a,b). Determining whether individuals in BC freshwater populations can exhibit sneaking behavior allows for future comparisons of this plastic behavioral repertoire across ancestral and derived populations, and in varying environmental and social contexts that may provide important developmental or activational cues for sneaking (Snell-Rood 2013).

The primary goal of this study is to test hypotheses regarding population-level variation in expression of the sneaker tactic observed under natural conditions. Lab-reared fish were used to determine the relative contribution of genetic differentiation and environmental effects on sneaking propensity in oceanic and freshwater populations from the Pacific Northwest. Individuals were subjected to a common garden behavioral assay designed to elicit sneaking behavior during courtship. If sneaking cannot be elicited in BC populations with lab-reared fish, this may be a result of loss of the sneaking repertoire upon colonization of freshwater, which could occur if the reproductive benefits of this alternative reproductive tactic are not exposed to selection (West-Eberhard 2003; Foster 2013b). If sneaking can be elicited in the lab-reared fish from BC freshwater populations and there are similarities in frequency of sneaking behavior observed across populations, this suggests there is variation in the presence of important proximate cues which trigger sneaking behavior under natural conditions (Foster 2013b). However, if there is variation in aspects of sneaking behavior observed across populations, this suggests populations-specific selective pressures have caused changes in the strength of the stimulus required to elicit the sneaker response, i.e. genetic accommodation of cue responsiveness (e.g. shift in slope or elevation of plastic response; Schlichting & Pigliucci 1998; West-Eberhard 2003; Shuster & Wade



2003). It is possible the cue threshold for sneaking behavior is so high in certain populations that it may take multiple rounds of exposure to the mating context to elicit the behavior in individuals from such populations. This study uses repeated courtship trials on individuals to ensure any potential variation in cue responsiveness across populations will be captured (Biro 2012). Expression of sneaking behavior by males in populations in which this behavior is not observed under natural conditions will provide the basis for future investigations of population-level variation in the cues present under natural conditions and in the sensory systems used to detect relevant cues.

While populations vary in the frequency of sneaking behavior, individuals within populations also vary in their responsiveness to cues and behavioral plasticity within and across contexts (Crowley 2000; Shuster & Wade 2003; Dingemanse *et al.* 2010; Stamps & Groothuis 2010a,b; Taborsky & Oliveira 2012; Dingemanse & Wolf 2013). Populations can demonstrate genetic variation for tactic switchpoints (Lucas & Howard 2008; Buzatto, Simmons & Tomkins 2012) and, with the aid of balancing selection, multiple tactic thresholds may be retained in a population (Aubin-Horth *et al.* 2005a; Lichocki *et al.* 2012). For instance, the same female distributions under natural conditions may elicit courtship behavior in some males and sneaking behavior in other males (Shuster & Wade 2003). In terms of within-population variation, an important goal is to clearly define the range of variation in individual consistency of use and reversibility of sneaking behavior, as the ability to optimize cue detection and respond accordingly has the potential to influence fitness gains via successful sneak fertilizations.

Individual behavioral expression can be extremely stable and consistent within contexts (e.g. Dingemanse *et al.* 2002; Nakagawa *et al.* 2007; Stein & Bell 2012). The repeated design of the behavioral assay is used to investigate if individual sneaker tactic use is consistent or predictable across multiple mating opportunities that closely approximate the same social context (Stamps *et al.* 2012; Biro & Adriaenssens 2013).

In addition to variable expression of the sneaking tactic, individual males sometimes vary in their propensity to switch between mating tactics (e.g. behavioral flexibility, Taborsky & Oliveira 2012). Male stickleback are known to adjust nuptial coloration accordingly under risk of predation (Candolin 1998), so it is plausible to assume males can assess cues with respect to competition and sneak fertilization opportunities and adjust reproductive effort accordingly. Sneaking behavior in threespines is considered to be a facultative or opportunistic tactic (Taborsky, Oliveira & Brockmann 2008; Taborsky & Brockmann 2010), but the extent to which this reproductive tactic is reversible and the extent to which males vary in their flexibility has not been clearly documented. Males may switch between sneaking and courtship tactics across a scale of minutes, hours, or days. It is also possible that some males use the sneaking and courting tactics in a condition-dependent rather than opportunistic fashion – primarily expressing the sneaking tactic at a younger age or smaller size and switching to the courtship tactic when larger and more competitive (de Fraipont *et al.* 1993). Males paired in the common garden assay were matched for size and condition to control for condition-dependent tactic expression and were allowed to freely interact with females and decide whether to opportunistically switch tactics, such that the variation in flexible tactic use among males and within males could be examined.

### **3.3 METHODS**

#### *3.3.1 Crosses, Fish Maintenance, and Assay Population Descriptions*

Breeding adult stickleback were collected in minnow traps during 2008 and 2009 spring and summer months from multiple populations in British Columbia (BC) and Alaska (AK). Table 3.1 lists the populations and years the crosses were made for each population and Figure 3.1 shows the geographic distribution of the study populations. British Columbia freshwater populations (BCFW) include: Crystal

Lake on Vancouver Island, and three populations on the Sechelt Peninsula -- Garden Bay Lake, Hotel Lake, and North Lake. British Columbia oceanic populations (BCO) include two populations that breed in shallow lagoons on the Sechelt Peninsula -- a marine population (Buccaneer Marina) near Secret Cove and a marine population (Francis Peninsula Lagoon) near Madeira Park. AK freshwater populations (AKFW) include Stepan Lake, which is part of the Meadow Creek tributary located in the Mat-Su Valley. Alaskan oceanic populations (AKO) include a fully marine population (Mud Bay) that breeds in tide pools near Homer, Alaska, a marine population that breeds in tide pools in Resurrection Bay, and an anadromous population that breeds in a freshwater creek (Rabbit Slough) near Wasilla, Alaska.

Multiple full-sib families, based on single male-female pairings, were made in the field for each population (Table 3.1) and then delivered overnight to Clark University in Worcester, MA. The crossing and fry rearing techniques used for the populations in this study are the same protocols described in Wund *et al.* (2012). Once fish reached adulthood, families were held separately in 37-L aquaria within a recirculating system. The majority of populations experienced two repetitions of seasonal cycles (short day: 8/16h light-dark cycle at 17°C, long day: 16/8h light-dark cycle at 20°C), with assay trials beginning during the second long day cycle when the fish were two years of age and coming into breeding condition. However, fish from Rabbit Slough, AK were tested in the assay trials during the first long day cycle, when the fish were one year of age (see Table 3.1). Individuals from this population were tested at an early age because of the fast growth rate and shorter lifespan experienced by this population when reared under laboratory conditions. Fish from the other populations could not be used during the first year of rearing because their body size was too small for handling and monitoring during video analysis of trials. All adult fish were fed chironomid larvae until satiation during holding in the recirculating tanks and during use in the assay trials.

### 3.3.2 Sneaker Assay Protocol

The experimental design for the sneaker assay allowed for dyadic interactions between males, and between males and gravid females. In May-July of 2010 and 2011, males prepared for reproduction with exposure to long day light cycles (16/8h light-dark cycle at 20°C) were used in week-long behavioral assay trials. Individuals were chosen when they had developed any male throat or eye coloration. Males paired together did not necessarily have the same degree of nuptial coloration when first paired, although many pairs developed equivalent nuptial coloration intensity during the trials.

Although other physiological and morphological components may influence aggressiveness and dominance rank of individuals, standard length (SL) is an important determinant of rank. Li & Owings (1978b) found sneaking behaviors to be more frequent between males of similar dominance rank. Males from different families were sized-matched (to within 5 mm SL) for the assay trials to increase the likelihood that sneaking behavior would be expressed. Before pairs of males were introduced into assay tanks, each was weighed and photographed (lateral view with ruler on a moist sponge in a clear plastic container) either before feeding or several hours after feeding. Standard length (SL) from digital images was later determined using ImageJ (NIH, v1.44) to verify size-matching. Two separate SL measurements were averaged for each male. A paired t-test confirmed no significant differences in male SL for male pairs (M1:  $\bar{x} = 4.35 \text{ cm} \pm 0.44 \text{ cm}$ ,  $n = 118$ ; M2:  $\bar{x} = 4.36 \text{ cm} \pm 0.47 \text{ cm}$ ,  $n = 118$ ; absolute difference:  $\bar{x} = 0.19 \text{ cm} \pm 0.16 \text{ cm}$ ;  $t = -0.710$ ,  $d.f. = 117$ ,  $p = 0.48$ ). Pre-assay SL and weights were used for estimation of body condition for later analyses.

Each size-matched pair was simultaneously introduced to a 76-L long aquarium. These large aquaria ensured that males had average territory sizes (38-L) that promoted nest building and courtship and decreased male-male aggression (Rowland 1994). Assay tanks contained nesting dishes on opposite sides of the aquarium and a row of large plastic aquarium plants down the center of the tank. Vegetation

cover has been associated with reduced male-male aggression (Rowland 1994; Candolin & Voigt 2001; Dziewieczynski & Rowland 2004; Candolin *et al.* 2008) and is used as cover by sneaking males under natural conditions (K Shaw, pers. obs). The bottom of each assay tank was covered with sand, small to medium size rocks, and a single large rock on each side to provide camouflage and cover if sneaking occurred. Lastly, equal amounts of nesting material were placed on each side of the tank. Figure 3.2 shows an example of the assay tank setup.

Once introduced to the assay tanks, males were encouraged to build nests by exposure to gravid females in a clear plastic, floating breeder container, set above the aquatic plants in the middle of the tank. Males encountered up to three different gravid females for 10 minute periods every day until at least one of the two males completed a nest. Trials began when at least one of the paired males had a nest with a clearly defined entrance – an average of 2 days.

Each assay trial involved introduction of a plexiglass container in the center of the tank above the aquatic plants and the placement of a large aquarium light (24"/60.96 cm, 20W Fluorescent, Hagen Aqua-Glo) over the top of the tank. After 10 minutes a gravid female was placed in the plexiglass container. The female and males were given 1-minute to acclimate before the female was released using a wire attached to a door on the plexiglass container. Assay trials generally lasted 10 minutes, although the trial time was extended if a male and female were in the process of spawning when the 10 minutes ended, in order to observe any sneaking behavior during or after spawning. The trial ended with removal of the female to a holding tank. Trials, including the 1 min acclimation time, were recorded via remote control on a digital camera (JVC Everio HDD camcorder, model GZ-MG670BU). Trials were observed on a monitor and general interactions, including spawning and sneak fertilizations, were verified in real time. Two trials were performed each day for a pair of males. Trials were separated into morning and afternoon to account for time of day and to allow for a minimum aggressive refractory period that occurs

post-spawning (1-2 hrs; Van Iersel 1953). Six trials per pair were carried out over a three-day period to ensure that spawning males did not transition to the parental phase and stop courting before the six trials were complete. Non-nested males were left alone in the tank following completion of the 6 trials to specifically determine whether they would invest energy into nesting and courtship and were subjected to a 10 min courtship trial the day following tankmate removal.

The setup simulated natural conditions as closely as possible to attain reliable conclusions about the functional aspects of the behavioral responses, as well as allow elicitation of the full range of behaviors associated with the sneaker repertoire. Both males were allowed access to nests with equivalent amounts of nesting material and free access to females (females released at midline of territories), allowing opportunity for mate choice. Although model females would provide a more consistent stimulus, live stimuli elicit stronger behavioral responses (Dzieweczynski & Forrette 2011; Bell & Peeke 2012; K Shaw, pers. obs.). Trials also allowed males to spawn and maintain nests; this would be the natural behavior of males under field conditions and may influence the propensity of males to sneak or court. In order to complete 6 trials with at least one nested, courting male per pair, it was necessary to allow males to spawn, because males prohibited from completing fertilizations with receptive female often stop courting, become aggressive, and/or destroy their nests (K Shaw, pers. obs.).

Gravid females for trials were unrelated to either of the assay males being tested, and were size-matched to each male pair. Often times, size-matched oceanic or anadromous females were utilized for both oceanic and anadromous population trials, as well as freshwater population trials. Pilot trials in 2008-2009 suggested that oceanic and anadromous females were just as likely to court and spawn with freshwater males, especially if equivalent in size. Gravid females from freshwater populations, however, were only utilized with male pairs from their own population, as the pilot trials suggested ecotype-specific behaviors (e.g. conspicuous vs. inconspicuous courtship) that could influence a freshwater

female's willingness to court and spawn (see also Foster *et al.* 2008). Gravid females were held in small 18.9-L tanks and allowed to view nested, courting males and receptivity was determined by a head-up response to these nested males. After trials, spawned females were returned to their home tanks and potentially used again during their next spawning cycle. Any unspawned females were returned to the holding tanks to be used in an assay trial with a different male pair until they spawned or dropped their eggs.

### *3.3.3 Ethical Note*

All husbandry and behavior assay protocols followed Clark University IACUC No. 005R protocol (2004-2012) with reciprocation by the University of Connecticut IACUC No. R09-001 (2008-2011). We held permits from the Alaska Department of Fish and Game and the British Columbia Ministry of Forests, Lands and National Resource Operations for collections (2008-2010).

### *3.3.4 Assay Behavior Scoring*

Each trial allowed males the opportunity to perform courtship towards the gravid female, perform sneaking behavior in response to a courting tankmate, or switch between the courtship and sneaking tactic. Every male received a categorical score for courtship and sneaking intensity (0 = low intensity, 4 = high intensity). While it is possible to score some aspects of courtship intensity on a continuous scale (i.e. zig-zags/min), it is difficult to score sneaking behaviors in this manner -- a male may hide and sneak very quickly or sneak very slowly and be successful at stealing a fertilization. Comparing the categorical scores for individuals across trials will provide information on a male's propensity to perform certain courtship and sneaking behaviors.

Both zig-zagging and dorsal-pricking were classified as courtship behaviors. The rankings of courtship intensity for males, from lowest to highest intensity, are as follows: (0) no nest activity/courtship behavior observed; (1) nest activity but little or no courtship behavior observed; (2) courtship attempts observed but no nest showings and no spawns or spawn attempts; (3) courtship with nest showings observed but no spawns or spawn attempts; (4) courtship with spawns or spawn attempts.

The following behaviors are indicative of sneaking -- sinking low to substrate, bifocal orientation towards courting or spawning pair, slow body movement along substrate toward courting or spawning pair with little pectoral fin movement, attempts to hide using objects in tank, creeping or rushing to nest opening during nest showing or spawning attempt. Males were scored conservatively as sneaking if they demonstrated at least three of these behaviors during a trial -- e.g. males only sinking low to substrate while focused on a courting or spawning pair would not fulfill the requirements. Components of the sneaking repertoire are utilized to define sneaking behavior rather than tactic success itself, because small tank size and constant vigilance of territorial males may make sneaker success more difficult than under natural conditions (van den Assem 1967; K Shaw pers. obs.). Sneaking intensity scores were based on the timing and location of sneaking behavior -- i.e. sneaking behavior during courtship or closer to the nest were scored as more intense expressions. The rankings of sneaking intensity for males, from lowest to highest intensity, are as follows: (0) no sneaking behavior observed; (1) sneaking behavior observed outside of tankmate's courtship -- e.g. during tankmate's nest activity; (2) sneaking behavior at or near vegetation during courtship, nest showing, or spawning; (3) sneaking behavior near nest during courtship, showing, or spawning; (4) sneak fertilization or sneak fertilization attempt observed (sneaker in tankmate's nest before, during or after spawning occurred). In this study, sneakers are defined as any male that scored 1-4 in at least one assay trial.



The total sample size was 118 pairs of males (236 individuals; see Table 3.1). Although males were subjected to paired trials, males were considered as independent data points for a number of statistical analyses because sneaking behavior does not preclude courting behavior or vice versa, either within or across trials. It was assumed that males chose whether or not to perform sneaking or courting behavior, and that this choice was to a large extent independent of the tankmate's choice (this point becomes more salient when considering males sneaking outside of courtship or pairs in which both males court and neither male sneaks).

### **3.4 ANALYSES AND RESULTS**

Both population differences and general sneaker characteristics were of interest in this study. Analyses were first performed on males pooled across populations (*Combined Population Analyses*) to investigate general traits associated with sneaking behavior. In these analyses, population was used as a factor whenever it may have been an important defining variable. These analyses are followed by more specific analyses focused on assessing population-level variation in sneaking behavior (*Population-level Comparative Analyses*). Parametric tests were used whenever the data fit the assumptions. Analyses were performed in PASW (SPSS Inc., 2009, v 18) and R (R Development Core Team, v 2.15.0).

#### *3.4.1 Combined Population Analyses:*

708 assay trials were conducted on 118 male pairs. 40% of the 236 individuals tested exhibited sneaking behavior in at least 1 of 6 trials. 29% of males expressing sneaking behavior were successful at

performing a sneak fertilization or sneak fertilization attempt (category 4) in at least 1 of 6 trials. Results are summarized in Appendix 3A.

Of 118 dyadic pairs observed, ~62% were sneaker-nonsneaker dyads, ~29% were nonsneaker-nonsneaker dyads, and ~9% were sneaker-sneaker pairs. No tank-side bias (R vs. L) was observed for the sneaker-nonsneaker subset (52% nested on the left, 48% on the right; exact binomial test,  $p = 0.82$ ).

If sneaking were a purely innate tactic, i.e., males are hard-wired to either perform sneaking behavior or not regardless of context, then a null hypothesis of expected dyad types can be calculated based on the overall proportion of males exhibiting sneaking behavior, and actual proportions of dyad types can be tested for divergence from expected. Overall sneaking frequency was 0.40. Given three different combinations of sneakers (s) and nonsneakers (n), 48% are expected to be sneaker-nonsneaker pairs ( $2sn$ ), 36% are expected to be nonsneaker-nonsneaker pairs ( $n^2$ ), and 16% are expected to be sneaker-sneaker pairs ( $s^2$ ). The observed frequencies differ significantly from those expected based on a hypothesis of innate expression of sneaker tactics ( $\chi^2 = 9.707$ ,  $df = 1$ ,  $p = 0.002$ ).

#### **3.4.1.1 Body Condition Comparisons**

While males were initially matched for SL, no purposeful attempt was made to control for body condition. To calculate condition, I used a measure employed in other stickleback studies (e.g. Frischknecht 1993; Kurtz *et al.* 2006; Kalbe *et al.* 2009):

$$\text{Condition Factor (CF)} = 100 \times W/L^b$$

In which  $W$  is fish weight (g),  $L$  is fish length (cm) and  $b$  is the regression coefficient calculated from the log-transformed values of length and weight (Bolger & Connolly 1989).  $b$  was determined to be 2.428 for length and weight measurements taken prior to trials ( $n = 236$ ). Removing a single outlier

did not substantially alter the regression coefficient ( $b = 2.405$ ) or the calculated condition factors so  $b = 2.428$  was used. A paired t-test suggested no overall differences in condition between paired males -- M1:  $\bar{x} = 2.92 \pm 0.50$ ,  $n = 118$ ; M2:  $\bar{x} = 2.99 \pm 0.72$ ,  $n = 118$ ; absolute difference:  $\bar{x} = 0.28 \pm 0.30$ ;  $t = -1.744$ , d.f. = 117,  $p = 0.08$ ). Thus size-matched fish also demonstrated comparable body condition.

Overall body condition was also compared between males that exhibited the sneaker repertoire and males that did not, while accounting for potential population and regional differences. Ecotype (freshwater vs oceanic) was also included in the analysis as a fixed factor, because it is likely that body shape and size differs significantly among individuals from these different habitats. Population and region (AK vs BC) were included as random effects. A variance components covariance structure, which assumes constant variance (scaled identity) for each random effect was utilized, as there were no a priori predictions regarding the covariance structure (PASW). AIC values were compared for a full linear mixed model and for those with stepwise removal of interactions and random effects. The best model was one without interactions between the fixed effects and retained only population as a random effect. The condition of sneakers and nonsneakers did not differ significantly (sneakers:  $\bar{x} = 3.03 \pm 0.60$ ,  $n = 95$ ; nonsneaker:  $\bar{x} = 2.90 \pm 0.45$ ,  $n = 141$ ,  $F_{1,228} = 0.001$ ,  $p = 0.98$ , PASW), but ecotype (freshwater vs oceanic) had a significant influence on condition (freshwater:  $\bar{x} = 2.72 \pm 0.36$ ,  $n = 148$ ; oceanic:  $\bar{x} = 3.34 \pm 0.52$ ,  $n = 88$ ,  $F_{1,6} = 10.539$ ,  $p = 0.02$ , PASW), unsurprising because body shape and size can differ significantly among oceanic and freshwater ecotypes (e.g. Walker & Bell 2000; Wund *et al.* 2012). The random effect of population did not contribute much to the overall variability in condition scores (population ID covariance estimate = 0.059, s.e. 0.037), but the model accounting for this variable was significantly better (present: AIC = 231.84; removed AIC = 280.46).

If condition is related to sneaking behavior in any way, it is possible that the magnitude of difference in condition between males within pairs is the most relevant measure. For the sneaker-

nonsneaker subset, a paired t-test suggests that the paired males do not differ significantly in condition (sneakers:  $\bar{x} = 2.93 \pm 0.51$ ,  $n = 73$ ; nonsneaker:  $\bar{x} = 2.96 \pm 0.47$ ,  $n = 73$ ;  $t = -0.723$ , d.f. = 72,  $p = 0.47$ ). This is confirmed with a binomial test: sneakers had higher condition scores in 32 of 73 pairs (42%), not significantly different from 0.5 (binomial test (R):  $\bar{x} = 0.5$ , 95% CI = 0.322, 0.559,  $p = 0.35$ ).

Differences in condition were also compared for pairs across the three different groups of paired males: (1) sneaker vs nonsneaker pairs ( $n = 73$ ), (2) nonsneaker vs nonsneaker pairs ( $n = 34$ ), and (3) sneaker vs sneaker pairs ( $n = 11$ ). This was done to determine whether a larger difference was apparent for any of the three groups of paired males. However, there was no significant difference between groups in the magnitude of absolute condition differences for paired males (Kruskal-Wallis Test:  $H = 0.834$ , d.f. = 2,  $p = 0.66$ , PASW), suggesting that the magnitude of difference in condition between paired males is not related to dyad type.

#### **3.4.1.2 Effect of tankmate behavior on male sneaking behavior: sneaker vs nonsneaker pairs**

To investigate what variables are important in determining sneaker behavior across trials for sneaker vs nonsneaker pairs ( $n = 438$ ), it is important to account for the sneaking and courting behaviors of the sneaker males, as well as the courting behaviors of the tankmate. A general additive model was built in R (v 2.15.0) to account for the fact that sneaker male behavior was scored using a categorical scale (logit link family, see section 3.3.4 for description of categories). The category of sneaking behavior observed in the sneaker males (M1S) was set as the dependent variable, and population (POP), the courtship category scores of the sneaker males (M1C) and the courtship category scores of the nonsneaker males (M2C) were set as fixed effects. TRIAL was a repeated measure, defined as a random effect in the model. Male pair (PAIR) was also defined as a random effect. The best-fit distribution to the M1 sneaker scores

was a zero-inflated Poisson (R: `gamlss`). This distribution family was applied to the model (R: `glmmadmb`):

$$M1S = POP + M1C + M2C + TRIAL (random) + PAIR (random)$$

The best-fit model after step-wise AIC scoring was to retain only M2C, the courtship category of the nonsneaker male. While models including M1C and the random effect of male pair (PAIR) were within 2  $\Delta$ AIC values of the best fit model (Table 3.2), the simplified model was chosen to best represent the relationship (Arnold 2010). Higher intensity courtship performed by the tankmate generally elicited sneaking that, on average, included vegetation cover (sneak category 2; Figure 3.3). However, as the range of categorical sneaker responses among individuals suggests, high intensity courtship by the tankmate did not always elicit sneaker responses within individuals. Based on this result, males were assigned a sneaker consistency score (see *Section 3.4.1.4*) to investigate other variables that may influence a male's decision to sneak that may not have been considered in this model.

#### **3.4.1.3 Intra- and Inter-dyad comparisons of Sneaking and Courting Propensities**

Sneaking propensity (the number of trials in which males performed sneaking) and courtship propensity (the number of trials in which males performed courtship) were tallied for each male to compare the frequency of sneaking and courting behavior between paired males. Each pair type (sneaker-nonsneaker, nonsneaker-nonsneaker, sneaker-sneaker) was analyzed separately for sneaking frequencies and/or courting frequencies. For courtship frequencies, both the number of total courtships and the number of high intensity courtships (courtship categories 3 & 4) were analyzed, as it is likely that the number of high intensity courtships are more functionally significant in terms of eliciting sneaker

behavior and demonstrating male courtship motivation (see Section 3.4.1.2: *Effect of tankmate behavior on male sneaking behavior: sneaker vs nonsneaker pairs* - above).

*(a) Comparisons of Intra-dyad Courting Propensity*

To determine if one male for each given dyad type generally demonstrated more courtship, courtship propensity was compared between paired males in each of the three dyad types using the Wilcoxon signed ranks test (SRT, PASW) – a nonparametric analogue of the paired t-test.

The number of trials with courtship and the number of trials with high intensity courtship (courtship categories 3 or 4) were compared between males in sneaker-nonsneaker pairs ( $n = 73$ ) to determine whether sneaker males tend to court with the same propensity as their paired nonsneaker. Overall, nonsneaker males within pairs were found to court during significantly more trials than males that also express sneaker behavior ( $Z = -6.177$ ,  $p < 0.001$ ,  $r = 0.72$ ; Wilcoxon SRT), and these males also have a significantly greater number of high intensity courtship trials (courtship categories 3 & 4:  $Z = -6.891$ ,  $p < 0.001$ ,  $r = 0.81$ , Figure 3.4a).

For nonsneaker-nonsneaker pairs, ( $n = 34$ ), a one-sample Wilcoxon SRT was conducted on the absolute values of the difference in the number of trials with courtship, with the null hypothesis that the median difference is zero. One male of each pair tended to have a higher courtship propensity than the other ( $T_s = 3.767$ ,  $n = 34$ ,  $p < 0.001$ ), and more high-intensity courtships (categories 3 & 4:  $T_s = 4.220$ ,  $n = 34$ , SRT  $p < 0.001$ ; Figure 3.4b).

For sneaker-sneaker pairs ( $n = 11$ ), one male of the pair courted more often over the six trials ( $T_s = 2.00$ ,  $n = 11$ , SRT  $p = 0.046$ ), and more high intensity courtships ( $T_s = 2.825$ ,  $n = 11$ , SRT  $p = 0.005$ ; Figure 3.4c). Conversely, one male in sneaker-sneaker pairs also exhibited higher levels of sneaking

behavior ( $T_s = 2.980$ ,  $n = 11$ , SRT  $p = 0.003$ ; Figure 3.4d) – however, the male exhibiting the lower courtship propensity (Sneaker 1: Figure 3.4c) was not always the male exhibiting higher sneaking propensity (Sneaker 1: Figure 3.4d)

*(b) Comparison of courtship propensity for Nonsneakers in Sneaker-Nonsneaker Pairs and in Nonsneaker-Nonsneaker Pairs*

If males exhibited similar cue thresholds that trigger sneaking behavior, one might expect all males subject to similar courtship propensity to express sneaking behavior. It is possible individuals capable of expressing sneaking behavior did not exhibit the behavior because they were paired with a male expressing low courtship propensity. To investigate this possibility I tested whether nonsneaker males in the sneaker-nonsneaker pairs and the nonsneaker-nonsneaker pairs are fundamentally different in their courtship propensity.

All the nonsneakers from the sneaker-nonsneaker pairs ( $n = 73$ ) were compared with the nonsneaker males from the nonsneaker-nonsneaker group ( $n = 34$ ) that had the higher courting propensity scores for each pair (Mann-Whitney U statistic). There were no significant differences between the distribution of the number of courtship trials for nonsneakers from the 2 pair types: (S-N nonsneakers:  $\bar{x} = 5.89 \pm 0.36$ ,  $n = 73$ ; N-N high courtship propensity nonsneakers:  $\bar{x} = 5.91 \pm 0.38$ ,  $n = 34$ ;  $W = 3898.5$ ,  $U = 1197.5$ ,  $Z = -0.605$ ,  $p = 0.55$ , PASW). A comparison of the frequency of high intensity courtships (behavioral categories 3 & 4) also found no significant differences between nonsneakers in the 2 pair types (S-N nonsneakers:  $\bar{x} = 3.82 \pm 1.79$ ,  $n = 73$ ; N-N high courtship propensity nonsneakers:  $\bar{x} = 3.30 \pm 2.24$ ,  $n = 34$ ;  $W = 1585$ ,  $U = 990$ ,  $Z = -1.708$ ,  $p = 0.09$ ).

*(c) Comparison of high courtship propensity between Nonsneakers in Sneaker-Nonsneaker Pairs and Sneakers in Sneaker-Sneaker Pairs*

Is high courtship propensity comparable between nonsneakers of sneaker-nonsneaker pairs and courting sneakers of sneaker-sneaker pairs? There were no significant differences between the distribution of courtship propensity for nonsneakers of sneaker-nonsneaker (S-N) pairs and courting sneakers of sneaker-sneaker (S-S) pairs (S-N nonsneakers:  $\bar{x} = 5.89 \pm 0.36$ ,  $n = 73$ ; S-S high courtship propensity sneakers:  $\bar{x} = 5.91 \pm 0.30$ ,  $n = 11$ ;  $W = 3100$ ,  $U = 399$ ,  $Z = -0.065$ ,  $p = 0.95$ ). Comparison of the frequency of high intensity courtships (behavioral categories 3 & 4) between these groups revealed no significant difference in the number of high intensity courtship trials for the sneaker-sneaker paired males with the greater high intensity courtship when comparing these two groups (S-N nonsneakers:  $\bar{x} = 3.82 \pm 1.79$ ,  $n = 73$ ; S-S high courtship propensity sneakers:  $\bar{x} = 4.27 \pm 1.19$ ,  $n = 11$ ;  $W = 3064.5$ ,  $U = 363.5$ ,  $Z = -0.517$ ,  $p = 0.61$ ).

*3.4.1.4 Sneak Consistency Scores: Comparing sneak opportunities with realized sneaking frequencies*

Although courtship involving nest-showing and fertilization attempts typically serves as a proximate cue for expression of sneaking behavior, high intensity courtship did not always elicit sneaking behavior in males. High intensity courtship (categories 3 + 4) was exhibited in 75% (531/708) of trials but males expressed sneaking behavior in only 36% (252/708) of trials. While it would be informative to determine repeatability of sneaking behavior across trials (*sensu* Boake 1989), intraclass correlations cannot be calculated for a categorical data set and there is no apparent continuous metric suitable for linearly categorizing sneaking behavior. Also, given the behavioral contingency (intra-pair dependency) revealed in these behavioral assays, a repeatability analysis may not accurately measure the consistency of male sneaking behavior. To obtain a proxy of sneak consistency, we recorded the number of all high intensity



courtship trials (trials with courtship scores 3 & 4) for nonsneaker males of the sneaker-nonsneaker group and all sneaker males of the sneaker-sneaker group. Although all trials included some level of courtship, low intensity courtships might not provide cues or opportunity for sneak fertilizations. Thus, only trials where tankmates performed 5 to 6 high intensity courtship trials are included to serve as a conservative proxy for "sneak consistency".

For this subset of sneaker males ( $n = 41$ ), the average sneaker male exhibited sneaking behavior during about 55% ( $\pm 27\%$ ) of the contests in which the stimulus cue was encountered (Figure 3.5). Individual sneak consistency scores are used as a variable of interest in later analyses, including sneaker nesting latencies and sneak fertilization success. For several analyses, sneakers are grouped into consistency categories based on their sneak consistency score: low ( $< 50\%$ ), medium (50-75%), high ( $> 75\%$ ).

#### *3.4.1.5 Determining Nesting Latencies & Related Behaviors*

##### *(a) Individual, Dyad Type, and Population Differences in Nesting Latencies*

Nesting latency is defined as the number of days from introduction into the assay tank until a completed nest was observed for a given male. Nesting latencies were compared within male dyads to determine whether there were delays between nesting times for males within a dyad, whether sneakers were less likely to be the first of a dyad to complete nesting, and whether pair types (sneaker-nonsneaker pairs, nonsneaker-nonsneaker pairs, and sneaker-sneaker pairs) and populations differed in nesting latency delays.

33% (39/118) of dyads included a nested male and a non-nested male. 64% (25/39) of these nested-nonnested pairs were sneaker-nonsneaker pairs and the remaining 36% (14/39) were nonsneaker-

nonsneaker pairs. Within a single day of the nesting tankmate's removal, 74% of these nonnested males (29/39) built a nest ( $n = 11$ ) or adopted their tankmate's nest ( $n = 18$ ). All males in sneaker-sneaker pairs nested prior to or during trials.

To determine whether there were significant differences in nesting latency (DAYS, square-root transformed) between males within dyads (MALE), males within pair type (PAIRCODE, e.g. sneaker-nonsneaker pairs, nonsneaker-nonsneaker pairs, and sneaker-sneaker pairs) and across populations (POP), a linear mixed model with repeated measures was performed in PASW. Only pairs for which both males had a nesting date were used to compare nesting latencies, with each male designated as M1 or M2 for the repeated effect. For nonsneaker-nonsneaker pairs, and sneaker-sneaker pairs, the first male to nest in a dyad was designated as M1. For sneaker-nonsneaker pairs, the nonsneaker was always designated as M1 in order to provide information on the identity of the sneaker in the analysis. Given no *a priori* assumptions regarding covariance structure, a repeated covariance type assuming constant variance (scaled identity) was specified for the analysis.

For the nested-nested pairs ( $n = 158$  individuals), the best-fit linear mixed model (with repeated measures) for nesting latency was simplified to retain only the fixed factors:

$$\text{DAYS} = \text{MALE} + \text{PAIRCODE} + \text{POP}$$

None of the two-way or three-way interactions were significant and were removed. In the simplified model, both the MALE and POP effects were significant (Table 3.3).

The significant POP effect indicates different nesting latencies among populations (Figure 3.6), with males from Crystal Lake, BC and Stepan Lake, AK taking longer to complete nests. The significant MALE effect suggests that one male typically nested significantly later than the other male (Figure 3.7). For sneaker-nonsneaker dyads this means that sneaker males (M2) generally completed nesting

significantly later than their nonsneaker tankmates (blue bars in Figure 3.7). The nonsignificant POP\*MALE interaction suggests that populations do not differ in the nesting latency relationship between the two males in dyads.

*(b) Relationship between Nesting Latency and Courtship Propensity*

Males who nest early may be primed for courtship. If so, they may be expected to have a greater number of high intensity (categories 3 & 4) courtships trials. Latency to nest was compared to male courtship propensity during trials.

The nesting latencies for males that did nest during the trials ( $n = 197$ ) and corresponding courtship propensity scores for high intensity courtship (categories 3 & 4) were negatively correlated (Spearman's  $\rho = -0.36$ ,  $n = 197$ ,  $p < 0.001$ ). This result indicates that males that nest early tend to perform a greater number of high intensity courtship trials. However, it is not a very strong correlation, so many early nesting males do not perform high intensity courtship while many late-nesting males do.

*(c) Relationship between Nesting Latency and Sneak Consistency Scores*

Sneaker males are often assumed to be nonterritorial males that do not nest. If true, males with higher sneaker propensity should be less likely to build a nest. Comparison of sneak consistency scores between nonnested ( $n = 9$ ) and nested males ( $n = 32$ ) within the same restricted sneaker subset reveals however that nested males have significantly higher consistency scores (Mann-Whitney U test,  $Z = -1.986$ ,  $p = 0.047$ , effect size = 0.310). In addition, there was no significant difference in nesting latency between males with low, medium, and high sneaking consistency across trials (Kruskal-Wallis Test:  $H = 2.384$ , d.f. = 2,  $p = 0.304$ , Figure 3.8).

#### *3.4.1.6 Relationship between Sneak Latencies and Sneak Consistency Scores*

Sneak latency is scored as the number of tankmate courtship trials that occur before sneaker males exhibit sneaking behavior. Variation in latency scores may indicate underlying differences in the cue threshold that triggers sneaking behavior. The subset of sneaker individuals with tankmates exhibiting 5-6 high intensity courtship trials ( $n = 41$ ) was analyzed; these high intensity courtship trials should involve the typical cues males would observe during sneak fertilization opportunities and the sneak propensity scores for these males are more reliable. Sneaker males categorized as low, medium, and highly consistent sneakers differed significantly in the distribution of their sneak latencies (Kruskal-Wallis Test:  $H = 14.332$ , d.f. = 2,  $p = 0.001$ , PASW). Distributions of sneak latencies for males with low sneak consistency scores differed significantly from that of medium and highly consistent sneakers (Mann-Whitney; Low vs High:  $W = 130.5$ ,  $U = 39.5$ ,  $Z = -2.921$ ,  $p = 0.003$ ; Low vs Medium:  $W = 104$ ,  $U = 26$ ,  $Z = -3.362$ ,  $p = 0.001$ ; Medium vs High:  $W = 148$ ,  $U = 70$ ,  $Z = -0.487$ ,  $p = 0.69$ , PASW). In particular, males with low sneak consistency score generally demonstrated sneaking behavior after several tankmate courtship trials rather than sneaking only in early trials (Figure 3.9).

#### *3.4.1.7 Relationship Between Sneaker Success, Body Condition, & Sneak Consistency Score:*

31% (29/95) of sneaker individuals performed a successful sneak fertilization attempt in at least 1 of 6 trials. 38% (11/29) of these were successful in more than a single trial (45 total successful sneaking attempts). 79% (23/29) of successful sneaker males exhibited sneaking behavior in multiple trials. 55% (16/29) of the successful sneakers also successfully stole eggs. In total, 42% (19/45) of all the sneak fertilizations observed in the assay concluded with successful egg-stealing.

It is possible that sneak fertilization success is related to sneak consistency score, sneak latency, and body condition (CF). Because the number of sneakers with multiple successes was small, sneaker males were categorized as either unsuccessful (0) or successful (1) and analyzed using a binary logistic regression model to determine if sneaker consistency, sneaker latency, or the magnitude of difference in condition between the sneaker and the tankmate (CF sneaker male - CF tankmate) significantly affected sneaker success. The subset of sneaker males with tankmates performing 5-6 high intensity courtships trials was used for this analysis ( $n = 41$ ). Only sneak consistency had a significant effect on sneak fertilization success. The model was simplified to only include sneak consistency score (AIC = 50.378), which classified 75.6% of the cases correctly ( $b = 0.036 \pm 0.014$ , d.f. = 1,  $p = 0.013$ ).

Not only was the probability of success higher for consistent males, but 46% of highly consistent males gained multiple sneak fertilizations. However, to determine if males with a higher sneak consistency actually gain significantly more sneak fertilizations by sneaking more often, we determined the relationship between sneak consistency scores and percent successes, to adjust for total possible successes for each male. Sneaker males categorized as low, medium, and high consistency differed significantly in the frequency of sneak observations with successful fertilizations (Kruskal-Wallis Test:  $H = 7.104$ , d.f. = 2,  $p = 0.029$ , PASW). Males in the high consistency category had a significantly greater success rate than males in the low and medium consistency categories (Mann-Whitney; Low vs High:  $W = 195.5$ ,  $U = 59.5$ ,  $Z = -2.188$ ,  $p = 0.029$ ; Low vs Medium:  $W = 230$ ,  $U = 94$ ,  $Z = -0.129$ ,  $p = 0.90$ ; Medium vs High:  $W = 118.5$ ,  $U = 40.5$ ,  $Z = -2.205$ ,  $p = 0.027$ , Figure 3.10).

#### *3.4.1.8 Within and between trial flexibility in reproductive tactics*

Tactic flexibility was measured as the number of switches between courtship behavior and the sneak repertoire within a single trial. In sneaker-nonsneaker pairs, ~73% (53/73) of the sneakers switched

tactics in at least 1 trial and ~38% (20/53) switched in 3 or more, and in sneaker-sneaker pairs, ~96% (21/22) of males switched tactics within at least 1 trial, and ~33% (7/21) switched in 3 or more. A significantly greater proportion of sneaker males switched behaviors in sneaker-sneaker pairs compared to sneaker-nonsneaker pairs for individuals that switched in at least 1 of 6 trials (Fisher's Exact test,  $p = 0.037$ ). However, there is no significant difference in the proportions of sneaker males switching in multiple (3+) trials (Fisher's Exact test,  $p = 0.79$ ).

Flexibility of behavioral repertoires can also be investigated across trials. For example, how many sneaker males successfully gain a fertilization through courtship rather than sneaking and how many of these males revert to sneaking behavior following successful courtship? It might be expected that males who attempted and failed fertilization might revert to sneaking in later trials. After excluding instances in which the unsuccessful courtship trial was the last trial, 20% (13/65) of males that had a failed spawn attempt did express the sneaker tactic in later trials, although 38% (5/13) of these males also demonstrated sneaking behavior in trials prior to the failed spawn attempt. It might be expected that males express the sneaker tactic despite previous courtship success if the tactics are being employed opportunistically. Of the males that successfully spawned before the last trial, 18% (19/106) did express sneaking in later trials, the majority of these males using the sneaker tactic prior to spawning success (68%; 13/19).

#### *3.4.2 Population-level Comparative Analyses:*

Sneaking individuals from each population were divided into subsets of sneaking intensity (categories 1-4), as described in the section *Sneaking Assay Protocol*. In addition to determining the overall frequency of sneaking males for each population, several aspects of sneaking behavior were also compared across populations. Due to low sample sizes for many variables of interest, a variation of Fisher's exact test with

Monte Carlo estimates of p-values (10,000 replicates; stats package, R Core Team) were used for proportion comparisons across multiple populations (i.e. contingency tables larger than 2 x 2). Results are summarized in Appendix 3B.

*(a) Population-level comparison of sneaking behavior*

At least a small percentage of males from every population exhibited sneaking behavior in the lab (Table 3.4), including individuals from freshwater BC populations. The percentage of individuals performing successful sneak fertilizations for a given population range from 4% to 33%. A Fisher's exact test (R; simulated p-value with 10,000 replicates) suggested significant differences across populations in the proportion of individuals expressing sneaking behavior ( $p = 0.02$ , Figure 3.11). As an alternative approach, we used the overall sneaking proportion across populations, 40%, as an expectation for each individual population. An exact binomial test was performed to test for deviation from this 'expected' value. Several populations had either a significantly higher (Buccaneer Marina, BC) or lower number of sneakers (Garden Bay Lake, BC) than expected by 'chance' (Figure 3.11).

When sneaker males are categorized based on their maximum sneaking intensity (i.e. the most intense sneaking category demonstrated by each sneaker male; Figure 3.12), there are no significant differences across populations in the distribution of males among sneaker categories (Fisher's exact test with 10,000 replicates,  $p = 0.58$ ).

The distribution of males among dyad categories (sneaker-nonsneaker, nonsneaker-nonsneaker, and sneaker-sneaker pairs) varied significantly among populations (Fisher's Exact Test with 10,000 replicates,  $p = 0.004$ , Figure 3.13). Generally, the sneaker-nonsneaker pair category was the most common dyadic relationship. For Garden Bay Lake, BC, nonsneaker-nonsneaker pairs were most

abundant, but in two populations (Buccaneer Marina, BC and Rabbit Slough, AK), the nonsneaker-nonsneaker pair was rare. Sneaker-sneaker pairs were rare or absent in a number of populations.

*(b) Population Comparison of Nesting Latency*

Populations differ in nesting latencies (Table 3.3), with males from Crystal Lake, BC and Stepan Lake, AK taking longer to complete nests (see results for *Determining Nesting Latencies & Related Behaviors*: section 3.4.1.5 (a) and Figure 3.6).

*(c) Population comparison of sneak consistency*

Since intra-population sample sizes are low in the subset of data used to determine sneak consistency, we combined population proportions to compare low, medium, and high consistency scores across grouped BC freshwater (BC FW), BC Oceanic (BC O), and Alaskan (AK) populations. These groupings do not differ significantly in proportions of males with low, medium, or high consistency (Fisher's Exact test, 10,000 replicates,  $p = 0.12$ , Figure 3.14a). Many population samples do contain a mixture of consistency expression (Figure 3.14b).

*(d) Population comparison of sneak latency*

Sneak latency scores (number of tankmate courtship trials prior to expression of sneaking behavior) are based on the subset of sneaker individuals with tankmates exhibiting 5-6 high intensity courtship trials ( $n = 41$ ). Intra-population sample sizes are low for this data subset so population proportions were combined into groups of BC freshwater (BC FW), BC Oceanic (BC O), and Alaskan (AK) populations.



These three population groupings differed significantly in the distribution of their sneak latencies (Kruskal-Wallis Test:  $H = 6.307$ , d.f. = 2,  $p = 0.043$ , PASW). BC O males demonstrated significantly lower sneak latencies than BC FW or AK males (Mann-Whitney; BC FW vs BC O:  $W = 112.5$ ,  $U = 46.5$ ,  $Z = -1.991$ ,  $p = 0.046$ ; BC FW vs AK:  $W = 214$ ,  $U = 94$ ,  $Z = -0.793$ ,  $p = 0.428$ ; BC O vs AK:  $W = 106$ ,  $U = 40$ ,  $Z = -2.324$ ,  $p = 0.02$ , PASW). However, after accounting for multiple comparisons with a Bonferroni correction, these differences were no longer significant ( $\alpha = 0.017$ ; Figure 3.15).

*(e) Population comparison of sneaker success*

There are no significant differences across populations in the proportion of sneaker males performing successful sneak fertilizations (Fisher's Exact test, 10,000 replicates,  $p = 0.35$ , Figure 3.16). However, Rabbit Slough, AK, has a higher successful sneaker frequency than expected (exact binomial test,  $p = 0.018$ ) from the combined population dataset (31% successful sneaker males, Figure 3.16). For Rabbit Slough, more sneaker males performed sneak fertilization behavior than all other sneak behavior categories combined.

*(f) Population comparison of within-trial reproductive tactic flexibility*

Although intra-population sample sizes are low, some population-level variation in tactic plasticity can be visualized. Every population has at least one male demonstrating within trial tactic plasticity. Populations differed somewhat in the proportion of sneaker males that switched behaviors in at least 1 of 6 trials (Fisher's Exact test with 10,000 replicates,  $p = 0.05$ ; Figure 3.17a) but there were no significant differences in the proportions of sneaker males switching in multiple (3+) trials when the populations were compared (Fisher's Exact test with 10,000 replicates,  $p = 0.10$ ; Figure 3.17b). No populations

differed significantly from the overall percentage of tactic-switching sneaker for either 1+ trials (78%) or 3+ trials (28%).

## 3.5 DISCUSSION

### *3.5.1 Maintenance of the sneaker repertoire in BC freshwater populations: potential causes and consequences*

A number of studies have demonstrated population-level variation in Threespine Stickleback behavior, including aggression (Huntingford 1982; Scotti & Foster 2007), courtship (Foster 1995a; Foster *et al.* 1996; Shaw, Scotti & Foster 2007), diversionary displays (Foster 1988, 1994, 1995), anti-predation (Foster & Ploch 1990; Huntingford *et al.* 1994; Rodewald & Foster 1998; Alvarez & Bell 2007; Messler *et al.* 2007; Dingemanse *et al.* 2009; Kozak & Boughman 2012), learning strategies (Odling-Smee & Braithwaite 2003), as well as variation in correlations among behaviors (i.e. behavioral syndromes: Bell 2005; Dingemanse *et al.* 2010, 2012). As *in situ* observations (Chapter 2) and comparisons of lab-reared individuals (this study) suggest, populations vary in expression of alternative reproductive behaviors as well.

A primary goal of this study was to assess whether there is genetic variation in sneaking propensity, and specifically to determine whether sneaking could be elicited in BC freshwater populations for which no sneaking has been observed under natural conditions (Chapter 2). Results of the common garden behavioral assays demonstrate that freshwater BC populations exhibit sneaking in response to similar courtship contexts that elicit sneaking in known sneaking populations. In addition, there is significant variation among populations for the propensity to sneak: Rabbit Slough (AK) and Buccaneer Marina (BC) had significantly more sneakers and Garden Bay Lake (BC) significantly fewer

sneakers than expected. However, populations had comparable percentages of sneakers performing high intensity sneaking behavior (i.e. nest approaches).

Sneaking behavior in the stickleback is an activational behavioral response, in that expression is mediated by complexes of neural and physiological networks and elicited by contextual environmental and social cues (Carroll & Corneli 1999; Zupanc & Lamprecht 2000; Burmeister 2007; Dingemanse *et al.* 2010; Stamps & Groothuis 2010b; Snell-Rood 2013). Differences in frequency of sneaking behavior among populations between the field and the common garden assay may be due to several factors: maintenance of sneaking in the behavioral repertoire of populations despite variation under natural conditions for the presence/absence of proximate cues required for triggering sneaking behavior, or variation in the extent to which sneaking is disadvantageous in specific environments (Foster 2013b). Such unexpressed behavioral traits can persist indefinitely even if they are no longer subject to direct selection, if indirectly selected due to correlation with other traits, particularly if the behavior involves an activational plastic response (Coss 1999; Lahti *et al.* 2009; Moczek 2008; Pfennig *et al.* 2010; Snell-Rood *et al.* 2010; Moczek *et al.* 2011; Foster 2013a; Snell-Rood 2013). It is possible that the neural circuitry underlying sneaking behavior is also used in other categories of stickleback behavior, resulting in the maintenance of the sneaker repertoire (Foster 1995b; Foster 2013b). Components of sneaking behavior, such as freezing and sinking, have also been observed in anti-predator behaviors, especially in response to piscivorous predators (Giles & Huntingford 1984; Foster & Ploch 1990; K Shaw pers. obs.). Many of the BC freshwater lakes included in this study do contain piscine predators that should select for the maintenance of motor patterns shared in the anti-predator response and the sneaking repertoire (e.g. Garden Bay and Crystal Lake; Foster & Ploch 1990).

The maintenance of an unexpressed behavior in populations suggests that they retain the ability to incorporate this behavior if environmental or social cues change. However, depending on certain

factors, such as the length of time the sneaking repertoire has gone unexpressed within a given population, accumulated changes in repertoire expression and elicitation may vary across populations. Lack of sneaking behavior under natural conditions for BC freshwater populations could also be due to differences in cue detection rather than in the cues themselves. Genetic changes in cue responsiveness (i.e. genetic accommodation of the plastic response; Schlichting & Pigliucci 1998; West-Eberhard 2003; Shuster & Wade 2003) may explain the significantly lower frequency of sneaking males in populations such as Garden Bay Lake (BC).

The higher sneaking propensity of Rabbit Slough fish compared to other populations may be due to the age of fish used in trials. 1 yo rather than 2 yo fish from this population were used in trials due to space limitation in assay tanks and higher mortality rates with age under lab conditions. In populations with several age classes, older fish may invest high reproductive effort in the courtship strategy if probability of further reproduction is low (Candolin 1998) and competitive ability for adequate nest sites is high (Dufresne *et al.* 1990). This finding suggests that investigating age-related variation in sneaking propensity using the sneaker assay may prove an interesting prospect, especially since the importance of age to tactic choice could be population-specific.

For populations that diverge in nature, the convergence of sneaker frequencies and other aspects of sneaking behavior in the lab suggests differences in environmental/social conditions affecting sneaking expression under natural conditions. On the other hand, laboratory-based population differences in sneaker frequencies are indicative of genetic differences in cue responsiveness. While behavioral traits of most populations are similar, the significant differences between some populations in aspects of sneaking propensity and tactic characteristics (e.g. nesting latency and tactic-switching), suggest a complex interplay of variation in environmental/social cues and sensory thresholds within and between populations (Carroll & Corneli 1999; Dingemanse *et al.* 2010). For instance, an environmental

or social change that decreases cue reliability may select for decreased threshold sensitivity, such that sneaking behavior is only elicited when the cue is very strong (Getty 1996; Schlichting & Pigliucci 1998).

### *3.5.2 Individual Variation in Sneaking Propensity: Dyadic Relationships*

Males, both within and among populations, exhibit variation in their propensity to utilize the sneaker repertoire. Individual males utilized different combinations of tactic intensity producing a reproductive tactic continuum. Some males never exhibited sneaking, even if the opportunity arose. Such results are consistent with the idea that populations can be composed of individuals demonstrating variation in behavioral plasticity, due to individual differences in past experiences, current physiological state, assessment abilities, genetic makeup, or an interaction of these factors (Carroll & Corneli 1999; Crowley 2000; Plaistow *et al.* 2004; Duckworth 2010; Piersma & van Gils 2011; Dingemanse & Wolfe 2013).

Sneaker-nonsneaker pairs are overrepresented compared to the proportion expected if the courtship and sneaker tactic were genetically fixed behavioral alternatives, supporting the idea that expression of the sneaker tactic is indeed facultative. However, these results do not rule out the possibility of a genetic component underlying propensity to perform the sneaker tactic. In a study investigating the effect of perceived predation risk on exploratory behavior, Dingemanse *et al.* (2012) found significant between-individual variation in behavior in two stickleback populations, with the majority due to plasticity based on previous experience, but a significant proportion explained by genetic variation in plasticity. In this study, variation among males in the sneaking assay may be due to variation in cue threshold for eliciting the behavior and variation in the social and environmental contexts they experienced. Feedback from conspecifics could increase variation between males if individuals adjust their behavior in response to social feedback (Crowley 2000; Dziewczynski & Forrette 2011). Certain social signals may increase or decrease the likelihood that individual males express opportunistic

sneaking behavior, including tankmate expression of high (or low) intensity courtship behavior or aggression/dominance behavior (Cade 1980; Perrill *et al.* 1982; Sullivan 1982; Waltz & Wolf 1984).

Of the variables investigated in this study, tankmate courtship behavior was the most important predictor of sneaking behavior expression (i.e. higher intensity courtship resulted in higher intensity sneaking behavior, Figure 3.3). Trials in which males performed little courtship or less intense courtship had generally less sneaking, although in some cases sneaking behavior occurred without nest showing or spawning ( i.e. during court categories 1 and 2).

For all three dyad types, there was typically one male in each pair that demonstrated a higher propensity to perform courtship behavior (Figure 3.4a-d). This result might be expected, as male threespines are known to increase their courtship effort after gaining a successful fertilization, likely because there is a limited window of time between when a male obtains his first clutch and when he transitions to territorial, parental care behavior (van Iersel 1953; Sevenster-Bol 1962). In sneaker-nonsneaker pairs, the sneaker males typically demonstrated a lower courtship propensity compared to their tankmate. In sneaker-sneaker pairs, both courtship propensity and sneaking propensity were asymmetric (although not necessarily different males). Overall, these results suggest that there is not a fundamental difference in the pair dynamics between the three different types of paired males -- e.g. lack of sneaking behavior in males of nonsneaker-nonsneaker pairs is not due to pairing two males with high propensity to perform courtship. However, it is possible that if two males with high courtship propensity were paired, pair dynamics may have resulted in behavioral suppression of one of the pair. The lack of differences in courtship propensity of courting males in sneaker-nonsneaker vs. nonsneaker-nonsneaker dyads further supports the idea that sneaking propensity is in part based on individual differences in response thresholds to courtship, not differences in the stimulus itself.

Males within dyads may intrinsically vary in the benefits they derive from plasticity (i.e. state-dependent behavioral plasticity). For example, individuals with greater energy reserves may derive a

greater benefit from switching mating tactics (Wolf, van Doorn & Weissing 2008), because they are able to build and maintain the cognitive and sensory systems needed to be responsive (DeWitt *et al.* 1998; reviewed in: Dingemanse & Wolf 2013). Alternatively, individuals deprived of sufficient resources early in life may develop a cheaper, less reactive behavioral type (Groothuis & Carere 2005) and allocate energy to one tactic or another (Waltz & Wolf 1984). LeComber (2003) suggested the possibility that males in good condition may be the most successful sneakers *and* courters. However, he did not present data that high quality males actually demonstrated greater plasticity in tactic expression. Candolin & Vlieger (2013) showed that successfully courting males are also more likely to sneak. It is possible that lower quality males may utilize a less reactive repertoire that either consists of courtship behavior or sneaking behavior and experience lower reproductive success because they are unable to match their response to current opportunities appropriately (Komers 1997). Males that do not demonstrate sneaking or courting could be saving resources and energy for territoriality and future reproductive opportunities (Candolin & Vlieger 2013). However, we found no effect of body condition on tactic expression in our study. While other condition measures deserve investigation, the relationship between behavioral traits and life history decisions (growth trajectories and condition) might be more flexible than presumed, allowing individuals to choose among various options depending on their current social situation (Riebli *et al.* 2012).

Individual behavioral types (reactive vs non-reactive) may also interact with the social environment to affect behavior within and across contexts (e.g. Sih & Watters 2005; Webster *et al.* 2007; Sih & Bell 2008; Schuett *et al.* 2010; Laskowski & Bell 2014). Differences in aggressiveness can select for both non-reactive individuals and individuals that plastically adopt an aggressive or nonaggressive tactic depending on the context (Dall *et al.* 2004; Wolf *et al.* 2011). Our results show that some individuals demonstrated neither sneaking or courtship or demonstrated only sneaking until the removal of the courting tankmate after the assay trials. This may be a direct result of social interaction with a more

aggressive or dominant tankmate (Wolf *et al.* 2011). Even subtle inequalities in dominance and aggression can account for the ease with which noncourting males transitioned to courtship behavior once their tankmate was removed (93% (53/58) of noncourters transitioned to courtship behavior after tankmate removal; 74% of these noncourters exhibited sneaking behavior during trials). It is possible that the courting tankmate represents a dominant, more rigid (less-reactive) behavioral type than the non-courter in these types of dyads (reviewed in Dingemanse & Wolf 2013).

### 3.5.3 Individual Variation in Sneak Consistency

A meta-analysis by Bell *et al.* (2009) suggested that within-individual variation is one of the largest components of behavioral variation. Using the sneaker assay, we investigated individual consistency in tactic use, i.e., the extent to which individuals respond consistently when encountering the same situation (Dzieweczynski & Forrette 2011; individual stability: Stamps & Groothuis 2010a; individual predictability/intra-individual variation: Biro & Adriaenssens 2013). Recent studies show that behaviors are often highly repeatable when assessed in response to an identical stimulus (Dingemanse *et al.* 2002; Schwagmeyer and Mock 2003; Forstmeier & Birkhead 2004). For example, some mosquitofish are more predictable in a given context than others (Biro & Adriaenssens 2013) and individual *Betta splendens* can remain consistent in their decision-making strategies despite recent aggressive interactions (Dzieweczynski *et al.* 2012). Male stickleback have demonstrated consistency in nest-building behaviors (Rushbrook, Dingemanse & Barber 2008) and parental behaviors (Stein & Bell 2012). Stickleback also exhibit consistent behaviors in the presence of predators (Huntingford 1976) and during social interactions (Pike *et al.* 2008; Harcourt *et al.* 2009).

Measuring consistency of tactic use can provide a sense of individual responsiveness to contextual cues – individuals with high cue thresholds (or decreased cue sensitivity) may require



multiple exposures to a specific cue before demonstrating a response (Dingemanse *et al.* 2010; Stamps & Groothuis 2010a,b). However, interpreting the measurement is not without its own difficulties as the presence of other individuals with potentially different behavioral profiles, as well as physiological changes across repeated trials, can affect the behavioral consistency of an individual (e.g. carryover effects: social interactions within a trial or in a previous trial may influence tactic expression; Sih & Bell 2008; Bell 2013).

In this study, individuals with repeated opportunities to perform sneaking behavior varied in the consistency of tactic expression across trials. Some males were highly predictable in their expression of the behavior while others did not always take advantage of the opportunity to sneak (Figure 3.5). Despite small sample sizes, there was evidence of within-population variation in sneaker consistency scores for all populations indicative of individual variation in cue sensitivity or other physiological factors that may affect response consistency. Overall, BC freshwater populations do not have less consistent sneakers, suggesting that the absence of expression under natural conditions is not linked to large-scale patterns of decreased cue sensitivity. We also investigated the relationship between sneak consistency and several other variables that might influence sneaking behavior (e.g. nesting latency, sneaking latency, and sneak fertilization success -- below).

#### **(a) Importance of nesting latency**

Nesting latency (days until nest completion) was compared among populations and individuals to determine if nesting behavior is related to consistency of sneaker tactic use, as well as individual courtship propensity. If sneaker males are primarily nonterritorial or nonnested males, then males with higher sneaking consistency were expected to have a longer nesting latency. On the other hand, males

who are primed for courtship and demonstrate a high courtship propensity are expected to have a shorter nesting latency.

Males with higher sneak consistency scores were not found to have significantly longer nesting latencies (Figure 3.8). In fact, males with highly consistent sneaking behavior were more likely to be nested than non-nested, and field evidence (Chapter 2) suggests that sneaker males are typically nest owners themselves. These findings conflict with the general assumption that many sneaker males are low quality males with insufficient energy to allocate to territoriality and nest-building (Dawkins 1980; Gross 1983; Taborsky 1998, 2008). However, competitive ability may be an important factor for expression of both sneaking and nesting behavior when males vary significantly in size (Dufresene *et al.* 1990).

In terms of nesting behavior and courtship propensity, early nesting males generally performed higher intensity courtship. Although this is a weak relationship, it suggests some males may be primed for courtship, even before their first successful spawning attempt (Jamieson & Colgan 1989).

Within dyads, one male typically nested significantly later than the other male (Figure 3.7). These results support Jamieson & Colgan's (1992) study demonstrating consecutive nesting and courting success (i.e. there is typically one male who nests and courts first). They suggested that propensity to perform sneaking behavior may stem from a predisposition towards late nest-building. Sneaker males in our assay demonstrated longer nesting latencies than nonsneakers in sneaker-nonsneaker pairs. There were also significant differences among populations – males in Crystal Lake (BC) and Stepan Lake (AK) took significantly longer to complete nests (Figure 3.6), although these populations did not have a significantly higher proportion of sneaker males (Figure 3.11). Thus, other factors may account for the delay in nest completion within these populations. For instance, LeComber *et al.* (2003) found that the

presence of rivals decreased nesting activity in territorial males – such social interactions may have prolonged nest-building behavior in several study populations.

### **(b) Importance of sneak latency**

Sneak latency scores can reveal individual variation indicative of underlying differences in cue thresholds that trigger sneaking behavior. Latencies to sneak are variable among sneaker males experiencing comparable numbers of courtship events -- some males displayed sneaking behavior early, while others demonstrated a longer latency period (e.g. multiple trials) before attempting sneaking behavior. While males with high sneak consistency scores have low sneak latencies by definition, a negative relationship (rather than no relationship) between sneak consistency score and sneak latency was expected if low sneak propensity reflects a higher cue threshold (and thus higher sneak latency score). No relationship between sneak latency and sneak consistency may suggest factors other than cue detection and response, such as male-male interactions or sneaker courtship propensity, affect individual sneaking propensity. A negative relationship was observed between sneak consistency and latency – males with low sneak consistency scores generally demonstrated sneaking behavior after several tankmate courtship trials rather than sneaking only in early trials (Figure 3.9). This suggests that males exhibiting fairly inconsistent sneaking behavior may have high cue thresholds, but it is also possible that these individual differences stem from variability in reproductive condition (physiological state) or variation in the ability to collect necessary information, such as nest location and reproductive status of the neighboring male (e.g. variability in cognitive capacities or sensory integration, Komers 1997; Crowley 2000; Stamps *et al.* 2012; Biro & Adriaenssens 2013).

### **(c) Sneak Fertilization Success**

Sneakers did have fertilization success, but neither sneaker body condition nor latency to perform sneaking behavior across trials had a strong effect on sneak fertilization success. The most important factor was the consistency of males sneaking *across trials* – more consistent sneakers were more likely to obtain sneak fertilization success at least once, and many of these highly consistent males were also successful on more than one occasion (Figure 3.10). These results also demonstrate that low consistency males are not highly successful sneakers that are quickly transitioning from the sneaking to courtship tactic across trials.

The proportions of successful and unsuccessful sneaker males did not differ significantly among populations. However, Rabbit Slough (AK) had a significantly higher number of successful sneakers than expected. The majority of Rabbit Slough males included in the consistency analyses also had high consistency scores. Although this relationship is speculative due to small sample sizes, it does highlight Rabbit Slough as an interesting population to investigate in future behavioral assays – particularly whether high sneaker success is driven by aspects of sneaker behavior, lack of territorial vigilance or courtship aggression by sneak victims or a combination of these factors.

Sneakers typically attempt to steal eggs after a successful sneak fertilization (Van den Assem 1967; Goldschmidt & Bakker 1990; Jamieson & Colgan 1992) – 42% of all sneak fertilizations in the assay concluded with successful egg-stealing. One suggested function of egg stealing is to attract females, who show a preference for nests containing eggs (Rohwer 1978; Ridley & Rechten 1981; Belles-Isles *et al.* 1990; Goldschmidt & Bakker 1990; Goldschmidt *et al.* 1993; Kraak 1996). The presence of eggs in the nest is also suggested to have a priming effect on courtship behavior (Jamieson & Colgan 1989). Thus males might be expected to increase their courtship behavior after eggstealing more than after sneak fertilizations in which eggstealing is unsuccessful. Out of the 29 males who demonstrated sneak fertilization behavior, 13 males performed a sneak fertilization without eggstealing before the final trial

– of these, 10 (77%) performed high intensity courtships of which 9 were successful at gaining fertilizations. Of the 16 males that performed sneak fertilizations followed by eggstealing, 10 performed a sneak fertilization with eggstealing before the final trial and 3 (30%) performed high intensity courtships and all three were successful at gaining fertilizations. Contrary to expectation, the proportion of sneaker males courting with high intensity after eggstealing was significantly lower than males who were only able to steal a fertilization (30% vs 77%: Fisher's Exact test,  $p = 0.04$ ). 7 of 10 eggstealing sneak fertilizers continued sneaking even after successful eggstealing.

#### **(d) Causes and Consequences of Sneak Consistency**

One important question regarding sneaking consistency is whether the consistent use of the repertoire is short-term or long term (Dingemanse & Wolf 2010). For some species, some behaviors are only repeatable over short time spans (Sih *et al.* 2003; Bell & Stamps 2004). Field and lab observations suggest sneaking behavior is absent in the behavioral repertoire of males once they end courtship and transition to parental care (e.g. rearing eggs and fry; K Shaw, pers. obs). This behavioral shift is likely due to changes in hormonal profiles that result in decreased sexual motivation as males in the parental stage dedicate their time and energy to offspring care (van Iersel 1953; Sevenster-Bol 1962). However, it is unknown whether males with a high propensity to sneak during one nesting cycle have a similar propensity to sneak during another nesting cycle.

Other important questions regarding behavioral consistency is how consistent tactic use might develop and whether tactic consistency may be beneficial or detrimental to fitness. For instance, consistent use of habitat type in a species of migratory swan, *Cygnus columbianus bewickii*, is linked to reproductive success and individuals vary in their consistency of habitat choice and thus vary in their reproductive success (Hoye *et al.* 2012). In terms of how consistent tactic use might develop, one

possibility is that individuals are forced into a strategy of trial and error before behavioral routines are developed (Danchin et al 2004). This would suggest that males would increase their sneaking propensity if successful and decrease their sneaking propensity if unsuccessful across consecutive trials. There is the possibility that males with low consistency may have increased their consistency, given a greater number of assay trials, if this trial and error mechanism is important to tactic development. Another, not mutually exclusive, possibility is that a positive feedback mechanism can give rise to high levels of repeatability in tactic use (Wolf *et al.* 2008; Dubois *et al.* 2012; Biro & Adriaenssens 2013). Plastic individuals may increase their experience with a given tactic, thus increasing their performance with a tactic and the benefits of plasticity (Wolf *et al.* 2011; Dingemanse & Wolf 2013). The higher sneak fertilization success rate of consistent sneakers demonstrates the importance of routine building to tactic performance.

Conversely, inconsistent behavior may increase tactic success in some contexts. Territorial males typically become more vigilant as the number of territory intrusions increase and they may decrease courtship behavior (van den Assem 1967; LeComber *et al.* 2003; Dziewczynski & Rowland 2004; Foster *et al.* 2008). Physiological mechanisms that result in controlled variability in behavior may reduce overall behavioral efficiency and increase unpredictability (Brembs 2011). Variation among individuals in such variability-generating physiological mechanisms may produce a fairly unpredictable population-level pattern of expression for this alternative reproductive tactic, which could be advantageous for both inconsistent and consistent sneakers.

#### *3.5.4 Individual Variation in Tactic Use Flexibility*

Males are predicted to use both the courtship and sneaker tactic opportunistically if the fitness gain of using both tactics outweighs the fitness loss of tactic-switching (Candolin & Vlieger 2013). Several studies

have noted the use of sneak spawning by courting males as a facultative tactic but did not assess the extent of individual tactic flexibility (i.e. switching between courtship and sneaking; Jamieson & Colgan 1992; Candolin & Vlieger 2013). The design of behavioral trials in this assay allowed males to choose between alternative reproductive tactics in the presence of a female. What might be considered a single context (courtship trial) to an observer may be multiple contexts for the individual fish, resulting in tactic switches (plasticity) within a single trial when the context changes. The costs and benefits for tactic-switching may vary among individuals causing individuals to differ in their behavioral responses even in identical situations (Dzieweczynski & Forrette 2011). Individuals can also be very responsive but unpredictable in their response – as individuals may choose among different behavioral options even in the absence of contextual differences (Brembs 2011).

Results demonstrate that males can make very rapid changes in mating behaviors -- switching back and forth between sneaking and courting behavior within a 10-min behavioral trial. Males were also observed to switch back and forth between sneaking and courting across trials, with behavioral sequences that may include sneaking to successfully courting then reverting to sneaking behavior again across a number of trials.

For some populations, multiple males not only switched tactics within a single trial (Figure 3.17a), but repeated this tactic-switching in 3 or more trials (Figure 3.17b). Populations varied somewhat in terms of the proportions of tactic switching males and extent of tactic switching by these males. Once again, despite the small sample sizes, these results suggest further investigation into inter- and intra-population level variation in tactic flexibility may be fruitful.

The behavioral plasticity associated with mating tactics in Threespine Stickleback can be categorized as behavioral flexibility or contextual plasticity – as the tactics are labile or reversible, rapid switches between tactics are possible, and behavior generally varies as a function of time or variation in

external stimuli (Piersma & Drent 2003; Dingemanse *et al.* 2010; Stamps & Groothuis 2010a,b; Dziewczynski & Forrette 2011; Taborsky & Oliveira 2012).

Behavioral flexibility can be indicative of behavioral or social competency (i.e. individual ability to respond optimally to social conditions/signals; Oliveira 2009; Taborsky & Oliveira 2012). Evidence of a link between flexibility and competence has been observed in a number of species, including stickleback: male sage brush lizards vary consistently in courtship but can alter their courtship intensity based on female receptivity (Ruiz *et al.* 2008); male guppies can switch from conspicuous courtship to sneak copulation attempts when female cues suggest predation risk is high (Evans *et al.* 2002), and Threespine Stickleback can adjust their level of exploration in relation to the level of boldness expressed by their social partner (Harcourt *et al.* 2009). Ideally, all males would be optimally flexible in their response to the immediate situation – courting when the gravid female is responsive and sneaking when another male is attempting a fertilization. In addition, the ability to switch repeatedly between the two reproductive tactics in response to nest or egg loss could be beneficial in populations where nest cannibalism is common.

Not all males demonstrated comparable levels of tactic flexibility. In terms of constraints on competency, it is becoming increasingly evident that there might be costs to maintaining or rewiring the neural circuitry and sensory systems involved in decision-making, memory, and motor control (Oliveira 2009; Taborsky & Oliveira 2012). The nervous system is under selective pressure to generate adaptive behavior but it is also subject to costs related to the amount of energy it consumes. Regardless of the size and complexity of a sensory system, the more reliable the information it can extract from the environment, the more accurate the decision making and motor control it facilitates (Niven & Laughlin 2008). Construction/maintenance costs of neural and sensory systems, environmental sampling costs, and signal reliability costs can all apply to plastic behavioral traits and will help determine the optimal



degree of plasticity (DeWitt *et al.* 1998). A recent transcriptomics study on sailfin mollies (*Poecilia latipinna*), a species which demonstrates variation among individuals in mating tactic plasticity (Seda *et al.* 2012), showed there was only a partial overlap of transcription profiles for individuals with genetically fixed sneaker expression versus those with facultative sneaker expression (Fraser *et al.* 2014). This study also suggests a substantial physiological cost for behavioral flexibility as a large number of genes involving cognitively demanding functions (e.g. learning, memory, and locomotory functions) were upregulated in plastic individuals performing sneak copulation versus courtship behavior (Fraser *et al.* 2014). Similar upregulation of genes involved in learning and memory has been reported in sneakers of a species with developmentally fixed reproductive tactics, the Atlantic salmon (*Salmo salar*, Aubin-Horth *et al.* 2005b).

### 3.5.5 Evidence of Intrasexual Competition and Relevant Behaviors

Although not directly related to the sneaking repertoire, non-nested males were observed to utilize courtship behaviors during assay behavioral trials. 71% (29/41) of non-nested males expressed low intensity courtship behavior (courtship categories 1 & 2) during an average of 3 trials, the majority of which involved courtship of the female interspersed with low intensity nestbuilding. 34% (10/29) were Crystal Lake males, but each population had at least one non-nested male exhibiting low intensity courtship behavior. These males would fan empty substrate or clumps of nesting material to elicit a response from the female but would often stop this behavior once the female was separated from the non-nested male's tankmate. This decline in courtship behavior by the non-nested male after separating a female from a courting tankmate is especially interesting and suggests the possibility that these behaviors may represent attempts to distract gravid females and decrease a rival male's reproductive success (*sensu* interference competition, van den Assem 1967; Li & Owings 1978a,b; Arak 1984; Hyatt &

Ringler 1989; Raeymaekers *et al.* 2010). Such use of courtship behavior has been observed under natural conditions but is not common (K Shaw, pers. obs). Such behavior could reflect increasing sexual motivation in males during the nest-building process but the majority of these non-nested males performed little nest-building behavior and did not complete a nest during the duration of the assay.

### *3.5.6 Variable Components of the Sneaker Repertoire*

Males used variable motor patterns during the sneaker process. In addition to the typical sneaker repertoire that involves freezing, sinking and slowing moving towards a nest entrance, males were observed to freeze or hide and rush the nest entrance from varying distances, particularly from the midline vegetation. 51% of sneaker males rush from hiding in vegetation to the nest at least once during tankmate showing or spawning behavior. 31% of these males used this behavior during multiple trials with sneak opportunities. A preliminary comparison of the proportion of sneaker males successfully gaining a sneak fertilization with the typical sneaker repertoire (12/29) versus with rushing behavior (17/29), suggests these are fairly equivalent techniques that males can use to approach a rival's nest (exact binomial test, expected proportions 50%,  $p = 0.458$ ). Further fine-scale analyses of male approaches to the nest may reveal some combinations of the motor patterns are more successful than other for completing a sneak fertilization. It would also be interesting to determine whether males consistently use the same motor tactics when they sneak. A lack of consistency in nest approach may indicate that the use of motor patterns to approach a rival's nest is context-, or rather situation-dependent. These types of analyses might best be done with a larger sample size of one of the high sneak propensity test populations used in this study.

### 3.5.7 Conclusions and Future Prospects

This study clearly demonstrates BC freshwater populations retain the ability to express sneaking behavior. Our behavior assay of lab-reared males allows us to rule out purely genetic differences underlying population variation in sneaking propensity. While samples sizes were large enough to reveal the maintenance of the sneaker repertoire in BC freshwater fish, performing this behavioral assay on a larger pool of lab-reared individuals from these populations will provide more powerful insights regarding population-level differences. With the current population samples, it is difficult to discern between the remaining explanations regarding variable expression of sneaking behavior. It is possible the sneaker assay represents a strong stimulus, capable of eliciting sneaking behavior even in populations with high cue thresholds. Consequently, we are left with the possibility that variation in tactic propensity under natural conditions is either due to purely environmental/social differences across populations or to interactions between variable cue thresholds and variable environmental/social factors across populations. An important next step for investigating variation in cue threshold is to test wild-caught individuals from populations in which sneaking behavior is seemingly absent in the sneak behavioral assay. If there are differences in response latencies between wild-caught and lab-reared individuals this will provide a basis to further investigating environmental components of this variation (Carroll & Corneli 1999; Bell *et al.* 2009; Hedrick & Bunting 2014). Results of this study highlight populations most likely to demonstrate tangible differences.

The expression of sneaking behavior in lab-reared fish from freshwater BC populations demonstrates that care must be taken when using lab assays to collect observations of sneak propensities then extrapolating those results to estimations of cuckoldry in the wild. Ideally, a comparison of lab estimations and field estimations of cuckoldry from a subset of populations that differ greatly in their potential for cuckoldry should be compared to determine the extent to which laboratory-

based sneaking propensities mirror sneaking propensities under natural conditions. This study distinguishes potential study populations for such an endeavor.

Behavioral plasticity is expected to evolve when it provides plastic individuals with a fitness advantage over less plastic individuals. Natural selection may also favor individual variation in behavioral plasticity under a variety of circumstances, for example via negative frequency-dependent mechanisms (i.e. the benefits of expressing behavioral alternatives is advantageous when rare and disadvantageous when common) or positive feedback mechanisms (i.e. behavioral plasticity can be less costly for individuals that have demonstrated the flexible behavior before: Wolf *et al.* 2008; Dubois *et al.* 2012). The structure of the stickleback mating system favors negative frequency-dependent effects on plastic mating behavior. Sneaking fertilizations cannot be the dominant reproductive tactic because it requires the presence of nested males employing the courtship tactic. With increased sneaking frequency, nested males may correspondingly adjust their courtship behavior or aggressiveness to decrease their susceptibility to territorial intrusions (LeComber *et al.* 2003; Dziewczynski & Rowland 2004; Foster *et al.* 2008) and the success of the sneaker tactic would likely decrease as its frequency increased. It is possible that positive feedback mechanisms are also important for sneaker success, as we found highly consistent males to have a high rate of sneak fertilization success.

Individual variation in plasticity may also arise due to sensory system constraints. Decreased cue reliability can result in the evolution of a mixture of adaptive behavioral plasticity and diversification bet-hedging (e.g. some genotypes may produce a single behavioral phenotype that utilizes a tactic that is neither optimal nor a failure across all environments or contexts). When both predictable and unpredictable environmental/social variables influence fitness, a combination of plasticity and bet-hedging is expected to evolve (Halkett *et al.* 2004; Wong & Ackerly 2005). Sneak-related cues that can be unpredictable include local female encounter rates or the courtship success of neighboring males on the nesting grounds. Even though individuals commonly experience temporally varying environments

and behavior may be influenced by a wide variety of environmental stimuli over different time scales (Taborsky, Oliveira & Brockmann 2008; Taborsky & Brockmann 2010), it is unlikely that a population harbors genetic variation in the sensitivity of behavioral expression for all possible combinations of environments and social contexts. Thus, the lack of genetic variation for complex behavioral reaction norms may be an important constraint on the evolution of adaptive behavioral plasticity (Gomulkiewicz & Kirkpatrick 1992; Thompson 1999). Even so, there are a number of environmental (territory size and quality) and social cues (dominance status, resource competition), as well as cues relevant to body condition and energetic state that might result in individual differences in stickleback mating tactic plasticity (reviewed in Chapter 1). When the behavior of an individual is dependent on several environmental factors, the corresponding plasticity often takes the form of a multi-dimensional reaction norm (Westneat *et al.* 2009, 2011; reviewed in: Dingemanse & Wolf 2013). Add to this the high likelihood of genetic variation in tactic switching thresholds for these cues (Dominey 1984; Shuster & Wade 2003) and the extent of individual expression of sneaking behavior becomes increasingly difficult to predict.

As suggested by this study, tankmate behavior can play an important role in influencing an individual's sneaking propensity. Differences in sneaking success rates may be due to phenotypic and behavioral attributes of the sneaking males themselves, as well as the behavioral attributes of the sneak victim. The extent of social competency (individual ability to respond optimally to social conditions/signals; Oliveira 2009; Taborsky & Oliveira 2012) may influence an individual's reproductive success with either reproductive tactic. Males in dyads have the opportunity to collect information on their neighbor's territory and breeding status and choose a tactic accordingly (Dall *et al.* 2004; Wolf *et al.* 2011). For sneak victims, strategically switching between courtship and aggressive territorial defense behaviors may be vital to the prevention of cuckoldry. Behaviorally flexible individuals may be able to build up a particular physiological machinery that can be reused, thus decreasing the costs of plasticity (Dingemanse & Wolf 2013). However, there is also a tradeoff in time and energy associated with

behaviors that cannot be performed simultaneously (time-budgeting trade-offs: Dziewczynski & Forrette 2011; Candolin & Vlieger 2013) and there is the possibility that use of the sneaking tactic can jeopardize success via the conventional courtship tactic under certain conditions, such as when there is high potential value in current offspring or low probability of future reproduction (Candolin & Vlieger 2013). Behavioral flexibility may also be constrained by arrival order in a social situation (e.g. arrival on the nesting grounds) and the weight of individual decisions will vary with the actions and decisions of the other individuals present (Dziewczynski & Forrette 2011; Dubois *et al.* 2012). If tactic flexibility is expressed in a consistent manner, it is possible for selection to act on this plasticity (Pigliucci 2001), even if selection acting on the alternative behavioral traits themselves is weak due to low repeatability of expression across individuals (Brodie & Russell 1999). Directly measuring territorial male response (e.g. aggression level, response consistency) to sneaking intruders will provide a better idea of the extent of flexibility in sneak victim behavior (expression of courtship versus territorial behavior) and the potential costs to reproductive success. The effect of tankmate aggression and behavioral flexibility on sneaker male behavior and tactic success can be investigated concurrently.

Based on population-level and individual variation in sneaking propensity, consistency, and flexibility exhibited in the common-garden assay, we can categorize stickleback sneaking behavior as a flexible, opportunistic behavioral tactic that exhibits evidence of individual variation in behavioral plasticity (e.g. genotype x environmental/social interactions influence tactic determination). Results regarding individual variation in consistency and tactic flexibility encourage further investigations into the predictability of and contextual influences on sneaker expression. Such information will shed light on physiological and neural mechanisms underlying within- and between-individual variation in sneaking propensity. The observation that males fall along a behavioral spectrum offers the very exciting prospect of comparing characteristics of such different behavioral types – intermediate males, with highly flexible mating tactics, can be compared to individuals on the more extreme ends of the behavior spectrum to

investigate potential behavioral trade-offs that occur with high behavioral consistency and high tactic flexibility.

### 3.6 ACKNOWLEDGEMENTS

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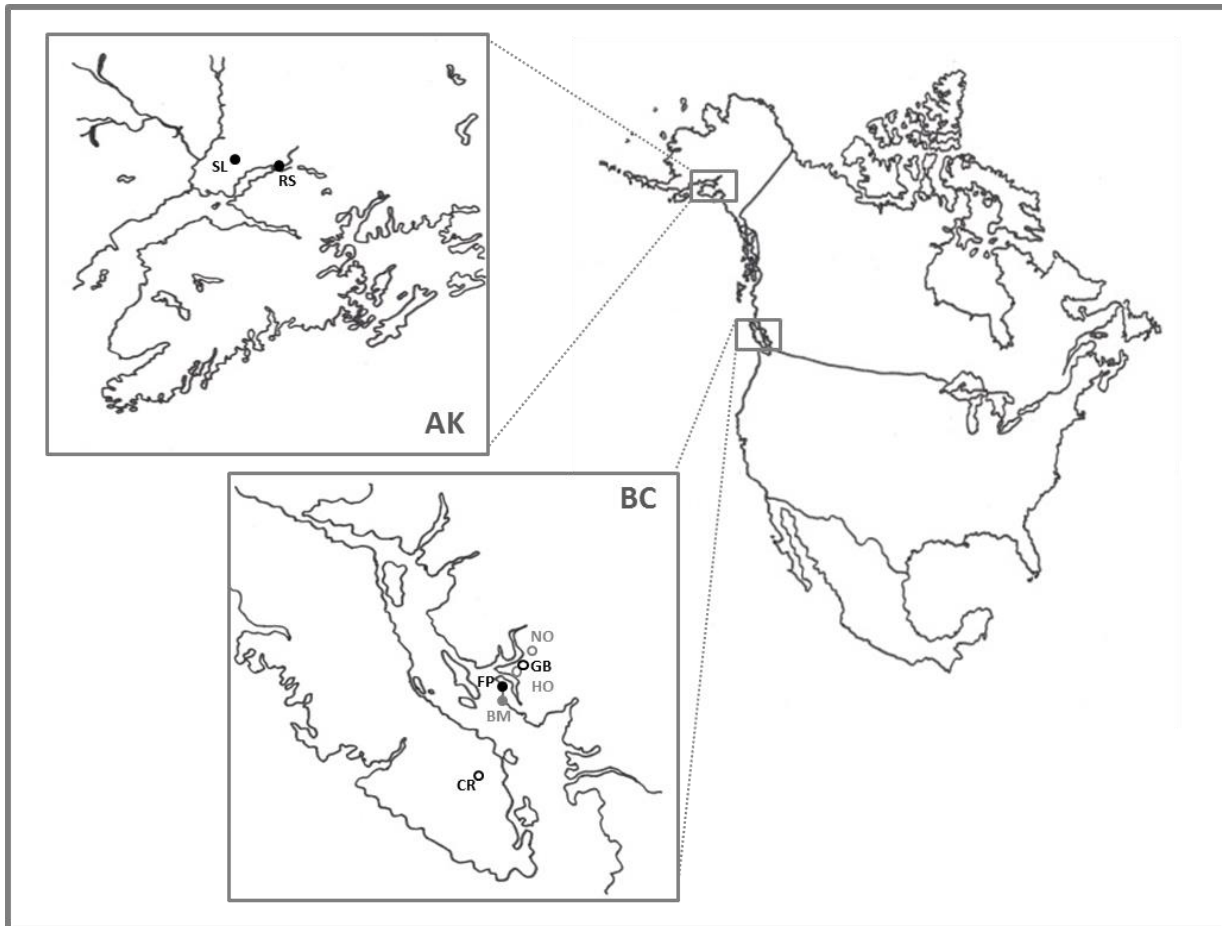
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**Table 3.1.** Information on populations tested in the sneaker assay. Includes the total number of males tested for each population (**N Tested**), number of families (**# Families**), and the year crosses were made (**Cross Year**) and crosses were tested in the assay (**Test Year**).

Region	Type	Population	N Tested	# Families	Cross Year	Test Year
BC	FW	<b>CRYSTAL LAKE</b> 49° 2'42.38"N, 123°57'29.58"W	32	12	2009	2011
		<b>GARDEN BAY LAKE</b> 49°38'52.55"N, 124° 1'2.67"W	32	12	2008	2010
		<b>HOTEL LAKE</b> 49°38'18.98"N, 124° 2'43.10"W	30	12	2008	2010
		<b>NORTH LAKE</b> 49°44'48.87"N, 123°58'3.38"W	24	13	2009	2011
BC	O	<b>BUCCANEER MARINA</b> 49°31'58.49"N, 123°57'22.04"W	26	8	2008	2010
		<b>FRANCIS PENINSULA LAG.</b> 49°36'42.55"N, 124° 1'53.88"W	32	10	2008	2010
AK	FW	<b>STEPAN LAKE</b> 61°34'23.52"N, 149°49'30.63"W	30	10	2009	2011
AK	O	<b>RABBIT SLOUGH</b> 61°32'13.79"N, 149°15'16.14"W	14	5	2009	2010*
			16	10	2010	2011*
		<b>TOTAL NUMBER OF INDIVIDUALS</b>	236			

\* Individuals from Rabbit Slough, AK were tested in the assay at 1 yr of age rather than 2 yrs of age due to faster growth rate and shorter life span under laboratory conditions.



**Figure 3.1.** Geographic distribution of populations used in the behavioral assay. Symbols indicate presence/absence of sneak repertoire in field observations -- sneaking observed ( • ) versus sneaking not observed ( ◦ ). Grayscale symbols and population codes used to differentiate populations in close proximity to others. British Columbia freshwater populations (BCFW) include: Crystal Lake (CR), Garden Bay Lake (GB), Hotel Lake (HO), and North Lake (NO). British Columbia oceanic populations (BCO) include: Buccaneer Marina (BM) and Francis Peninsula Lagoon (FP). AK freshwater populations (AKFW) include: Stepan Lake (SL). Alaskan oceanic populations (AKO) include: Rabbit Slough (RS).

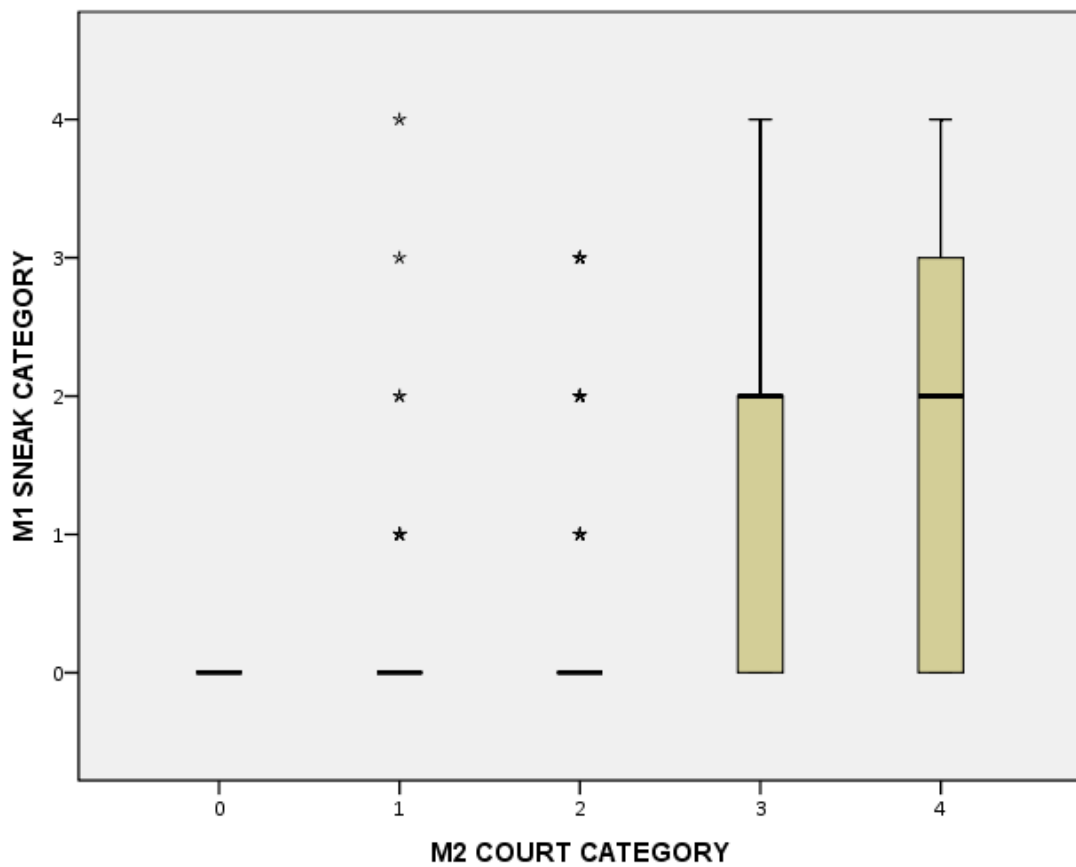


**Figure 3.2.** Sneaker assay tank set-up. Nesting dishes with equal amounts of sand and nesting material were placed on opposite ends of the tank. Rocks and plastic vegetation of various sizes and colors were added to provide cover for sneaking males. Cards with color standards were added for a separate analysis of body color plasticity associated with sneaker tactic expression.

**Table 3.2.** Step-wise AIC scoring of the general additive model representing the effects of tankmate courtship behavior and sneaker courtship behavior on sneaker behavior for sneaker-nonsneaker pairs (n = 73 pairs). The original model (M1S = POP + M1C + M2C + TRIAL (random) + PAIR (random)) was reduced to the model with the lowest AIC score (M1S = M2C), although models retaining sneaker courtship behavior (M1C) and the random effect of male pair (PAIR) were within 2  $\Delta$ AIC values of this simplified model. Models (with log-likelihood values) were calculated with the glmmadmb R package and step-wise AIC scoring was performed using the bbmle R package.

DROPPED TERM	LOG-LIKELIHOOD	$\Delta$ AIC	d.f.
M1C	-572.813	0	3
PAIR (RANDOM)	-572.116	0.6	4
TRIAL (RANDOM)	-572.116	2.6	5
POP	-572.116	4.6	6
NONE	-570.479	15.3	13





**Figure 3.3.** Relationship between M1 sneak category and M2 courtship category across trials for sneaker-nonsneaker pairs ( $n = 73$ ). Outliers (asterisks) may represent several individuals. Category values correspond to courtship and sneak behaviors as outlined in methods section 3.3.4 (0 = low intensity sneaking/courting; 4 = high intensity sneaking/courting).

**Figure 3.4.** Relationship between courtship propensities and sneak propensities for paired males. Propensities measured as the number of trials in which males performed categories of courting behavior or sneaking behavior; only relationships for high intensity courtship (courtship categories 3 & 4) are shown: **(a)** comparison of sneaker and nonsneaker courtship propensities for sneaker-nonsneaker pairs (n = 73); **(b)** comparison of courtship propensities for nonsneaker-nonsneaker pairs (n = 34) – the paired males with lower propensities are designated as Nonsneaker 1 for a more direct comparison of the differences; **(c)** comparison of courtship propensities for sneaker-sneaker pairs (n = 11) – the paired males with lower propensities are designated as Sneaker 1 for a more direct comparison of the differences; **(d)** comparison of sneaking propensities for sneaker-sneaker pairs (n = 11) – the paired males with lower courtship propensities remain designated as Sneaker 1 to provide comparison with **(c)**. The number of lines on the diagram may not match the sample size if some relationships between paired males overlap. Some lines show equivalent propensities (horizontal lines) between paired individuals.

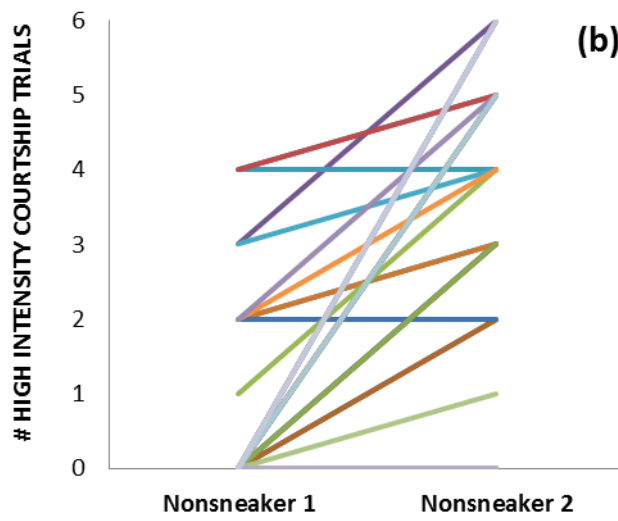
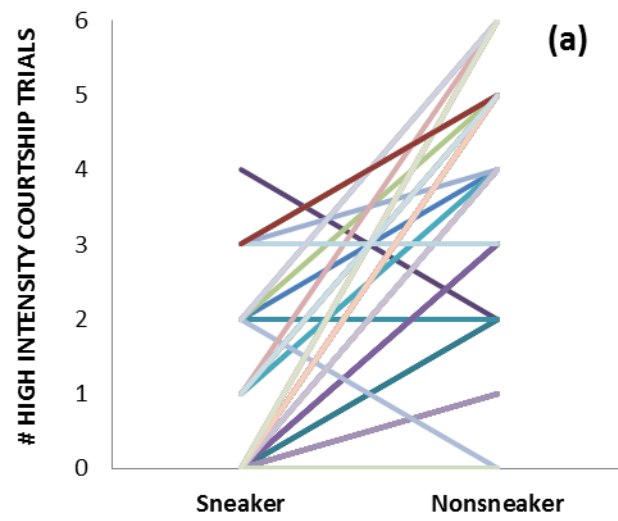


Figure 3.4.

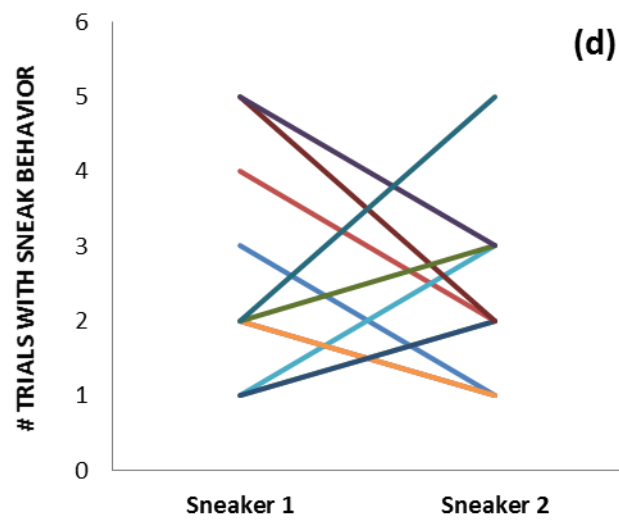
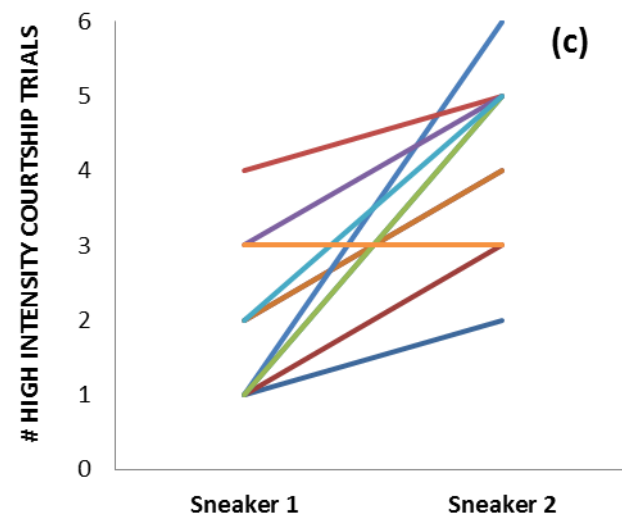
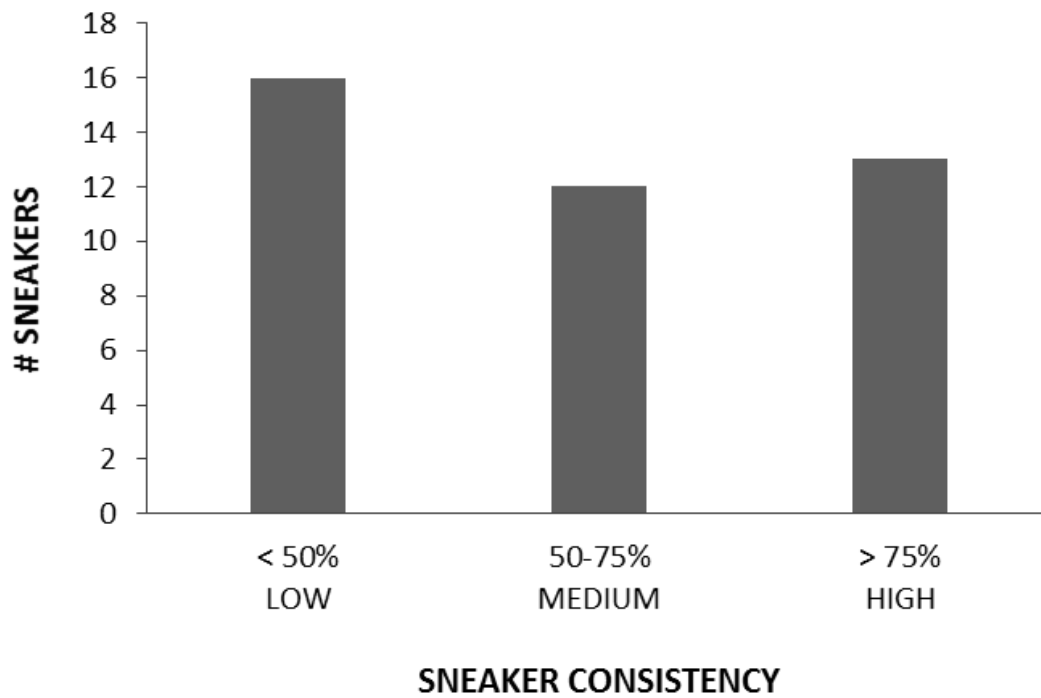


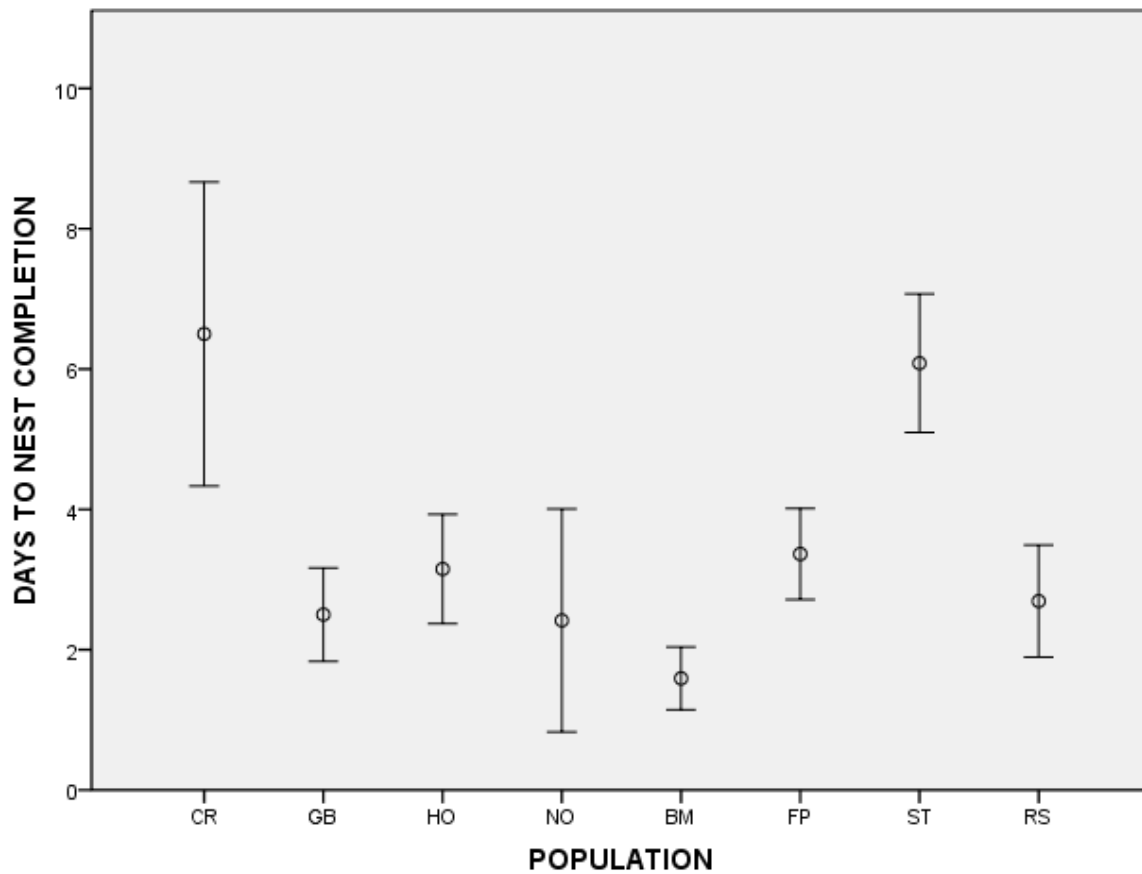
Figure 3.4. (cont.)



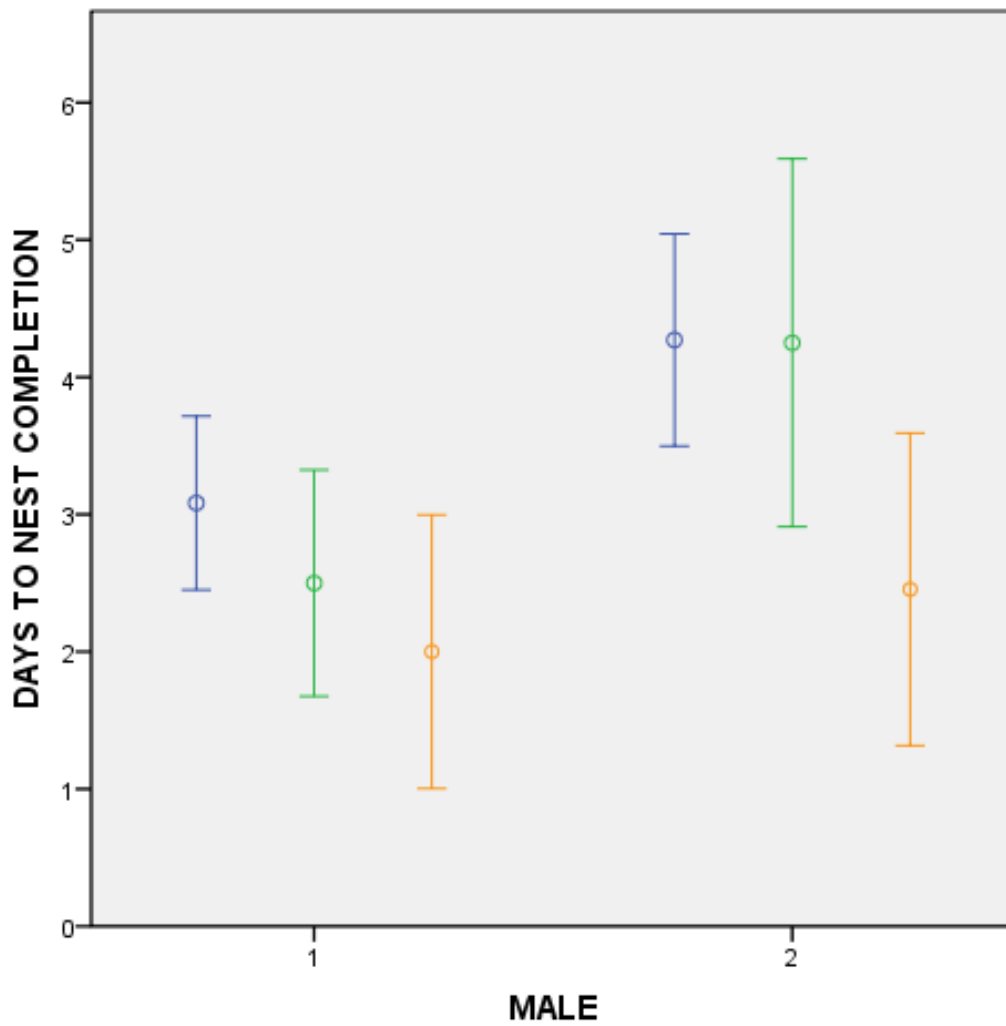
**Figure 3.5.** Distribution of sneaker consistency scores for a subset of sneakers ( $n = 41$ ) in which tankmates performed 5-6 high intensity courtships (courtship categories 3 & 4). Sneaker consistency denotes the percentage of high intensity courtship trials in which a male performed any category of sneaking behavior.

**Table 3.3.** Results of the linear mixed model (with repeated measures) for nesting latency (days to nesting, square-root transformed).

EFFECT	Numerator d.f.	Denominator d.f.	F	Sig.
Intercept	1	147	1479.90	< 0.001
MALE	1	147	20.26	< 0.001
PAIRCODE	2	147	1.07	0.346
POP	7	147	16.73	< 0.001

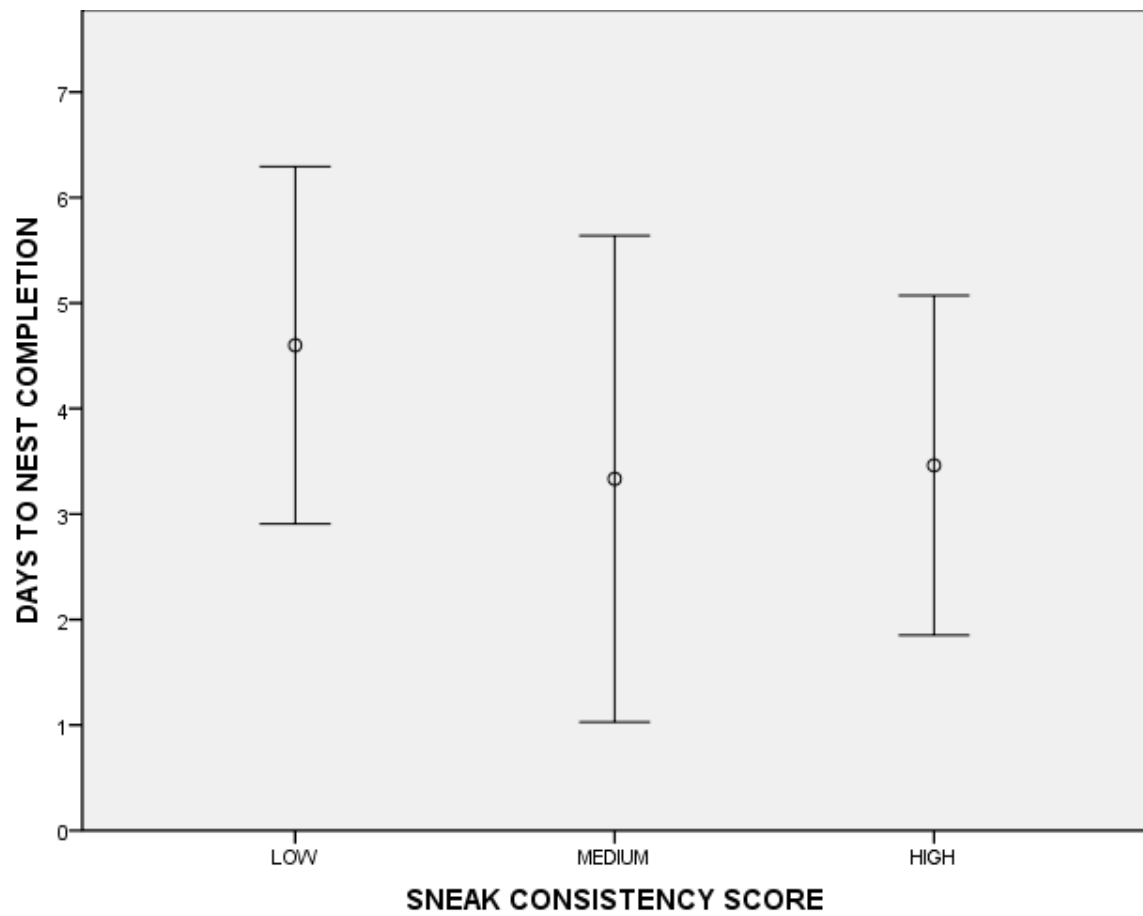


**Figure 3.6.** Means and 95% CIs for population nesting latency (days to nest completion). The sample sizes for populations are as follows: Crystal Lake, BCFW (CR, n = 10); Garden Bay, BCFW (GB, n = 22); Hotel Lake, BCFW (HO, n = 20); North Lake, BCFW (NO, n = 12); Buccaneer Marina, BCO (BM, n = 22); Francis Peninsula Lagoon, BCO (FP, n = 22); Stepan Lake, AKFW (ST, n = 24); Rabbit Slough, AKO (RS, n = 26).

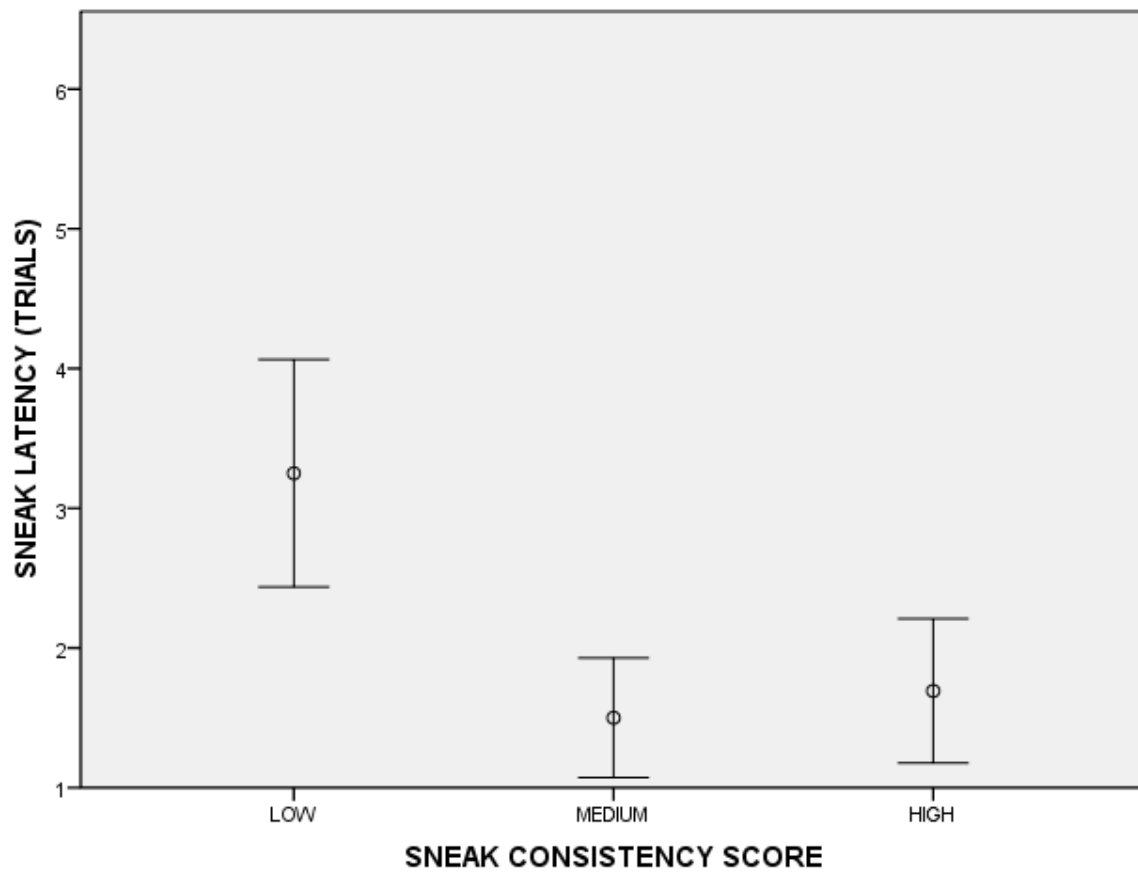


**Figure 3.7.** Means and 95% CIs for M1 and M2 nesting latency (days to nest completion). The bar colors represent pair types: blue bars = sneaker-nonsneaker pairs, where nonsneaker males are represented by M1 and sneaker males are represented by M2. Green bars = nonsneaker-nonsneaker pairs and yellow bars = sneaker-sneaker pairs.

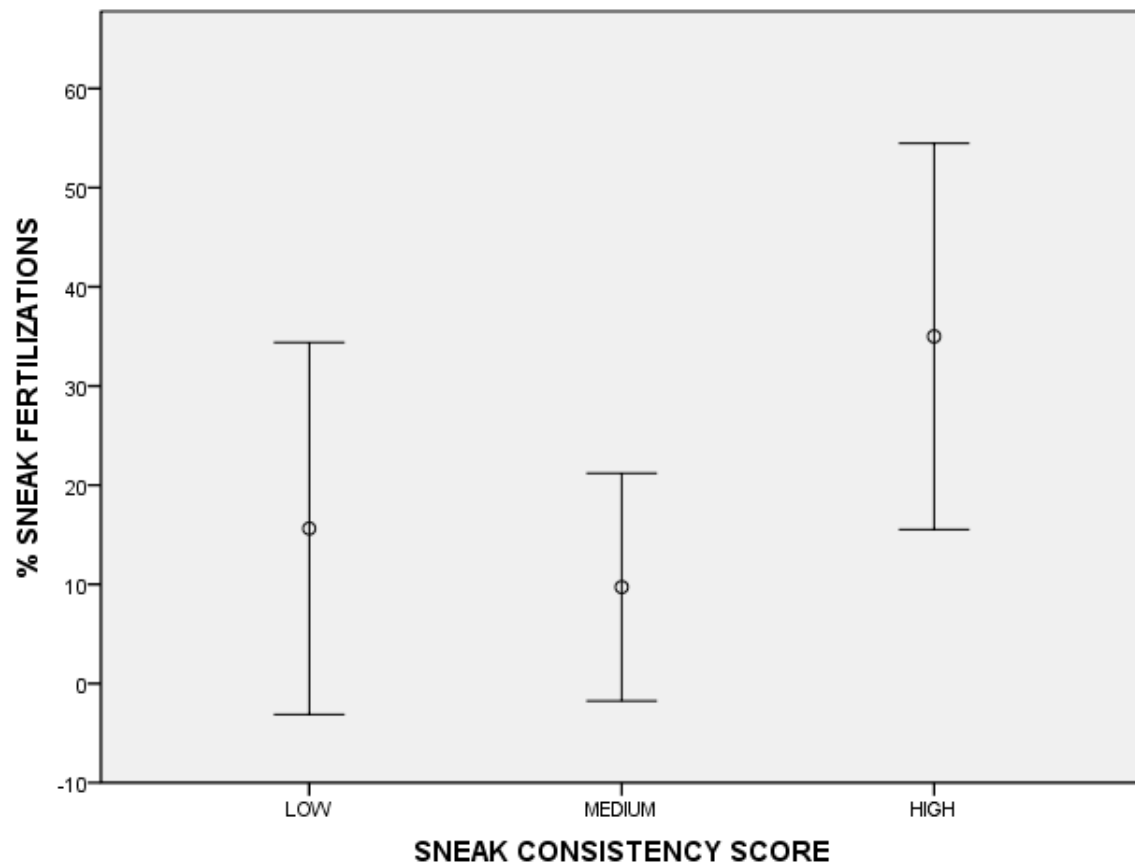




**Figure 3.8.** Means and 95% CIs for nesting latency (days to nest completion) for males with low, medium, and highly consistent sneaking behavior across trials.



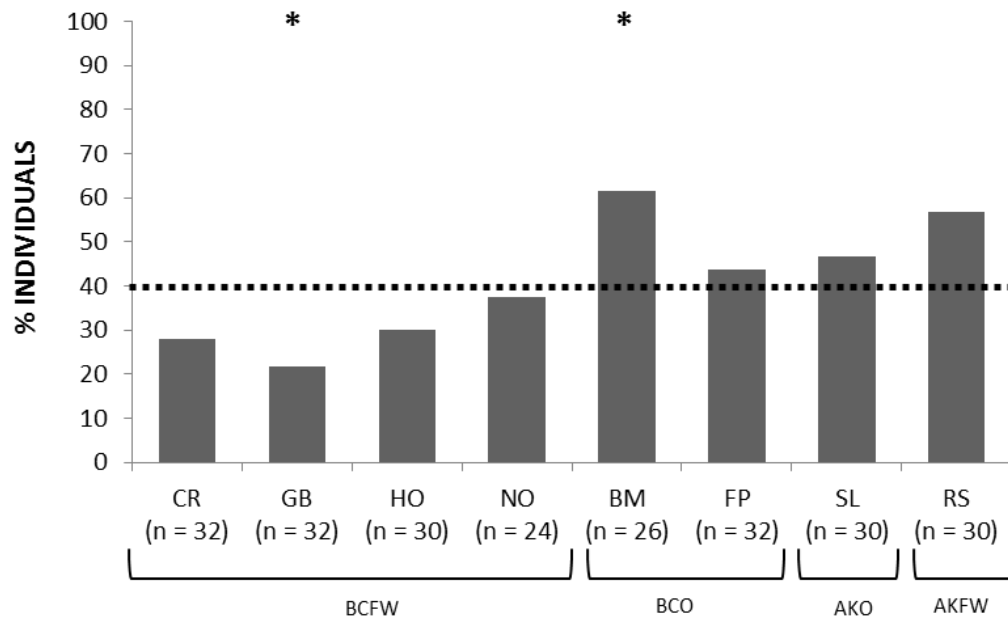
**Figure 3.9.** Means and 95% CIs for sneak latency (number of tankmate courtship trials before sneaking behavior elicited) for males with low ( $\bar{x} = 3.25 \pm 1.53$ ,  $n = 16$ ), medium ( $\bar{x} = 1.50 \pm 0.67$ ,  $n = 12$ ), and highly ( $\bar{x} = 1.69 \pm 0.86$ ,  $n = 13$ ) consistent sneaking behavior across trials.



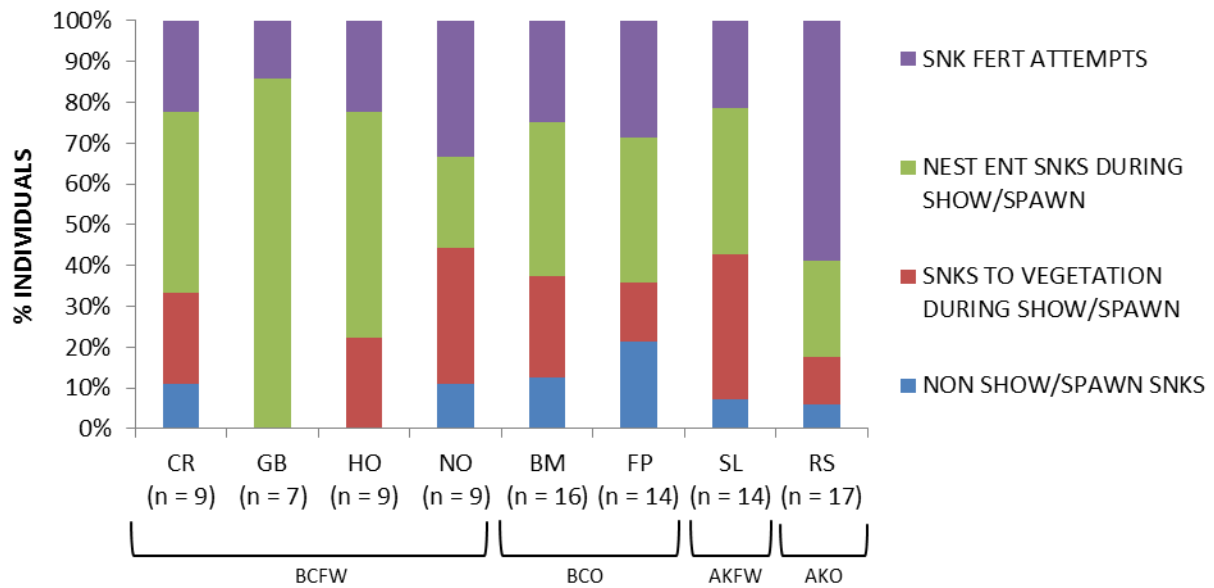
**Figure 3.10.** Means and 95% CIs for the frequency of successful sneak fertilizations for males with low ( $\bar{x} = 15.63 \pm 35.21\%$ ,  $n = 16$ ), medium ( $\bar{x} = 9.72 \pm 18.06\%$ ,  $n = 12$ ), and highly ( $\bar{x} = 35 \pm 32.21\%$ ,  $n = 13$ ) consistent sneaking behavior across trials.

**Table 3.4.** Population-specific results of the sneaker assay. Includes information on the total number of males tested for each population (**N Tested**), as well as the percentage of males expressing sneaking behavior (**% Sneakers, raw sample size in parentheses**). The percentage of males performing successful sneak fertilizations is also presented (**% Success**).

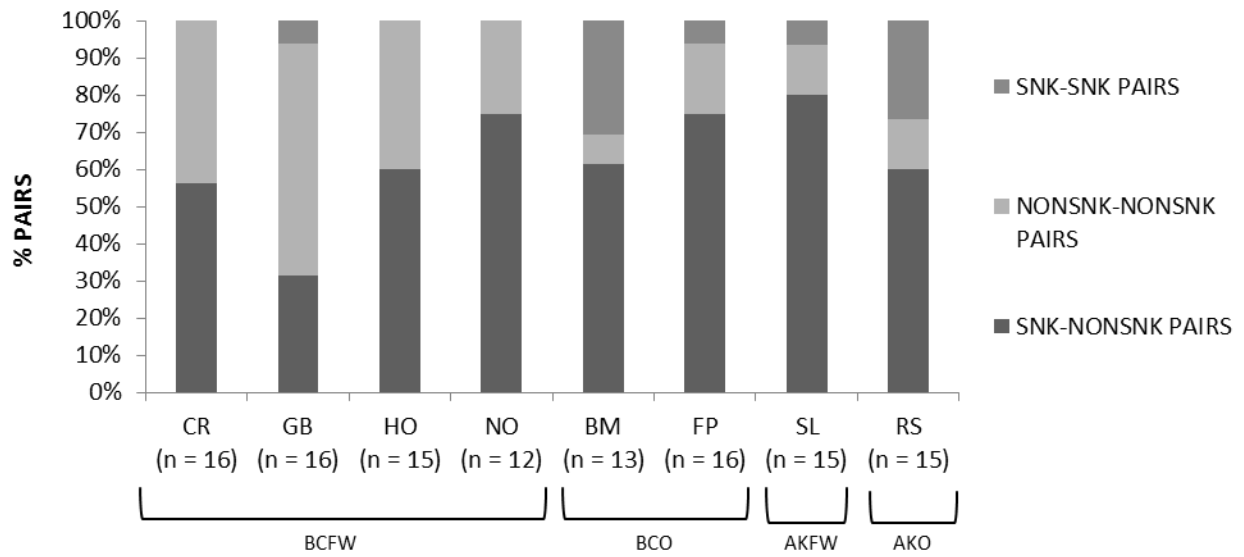
Region	Type	Population	N Tested	% Sneakers	% Success
BC	FW	CRYSTAL LAKE (CR)	32	28% (9)	6%
		GARDEN BAY LAKE (GB)	32	22% (7)	3%
		HOTEL LAKE (HO)	30	30% (9)	7%
		NORTH LAKE (NO)	24	38% (9)	13%
BC	AN	BUCCANEER MARINA (BM)	26	62% (16)	15%
		FRANCIS PENINSULA LAGOON (FP)	32	44% (14)	13%
AK	FW	STEPAN LAKE (SL)	30	47% (14)	10%
AK	AN	RABBIT SLOUGH (RS)	30	57% (17)	33%



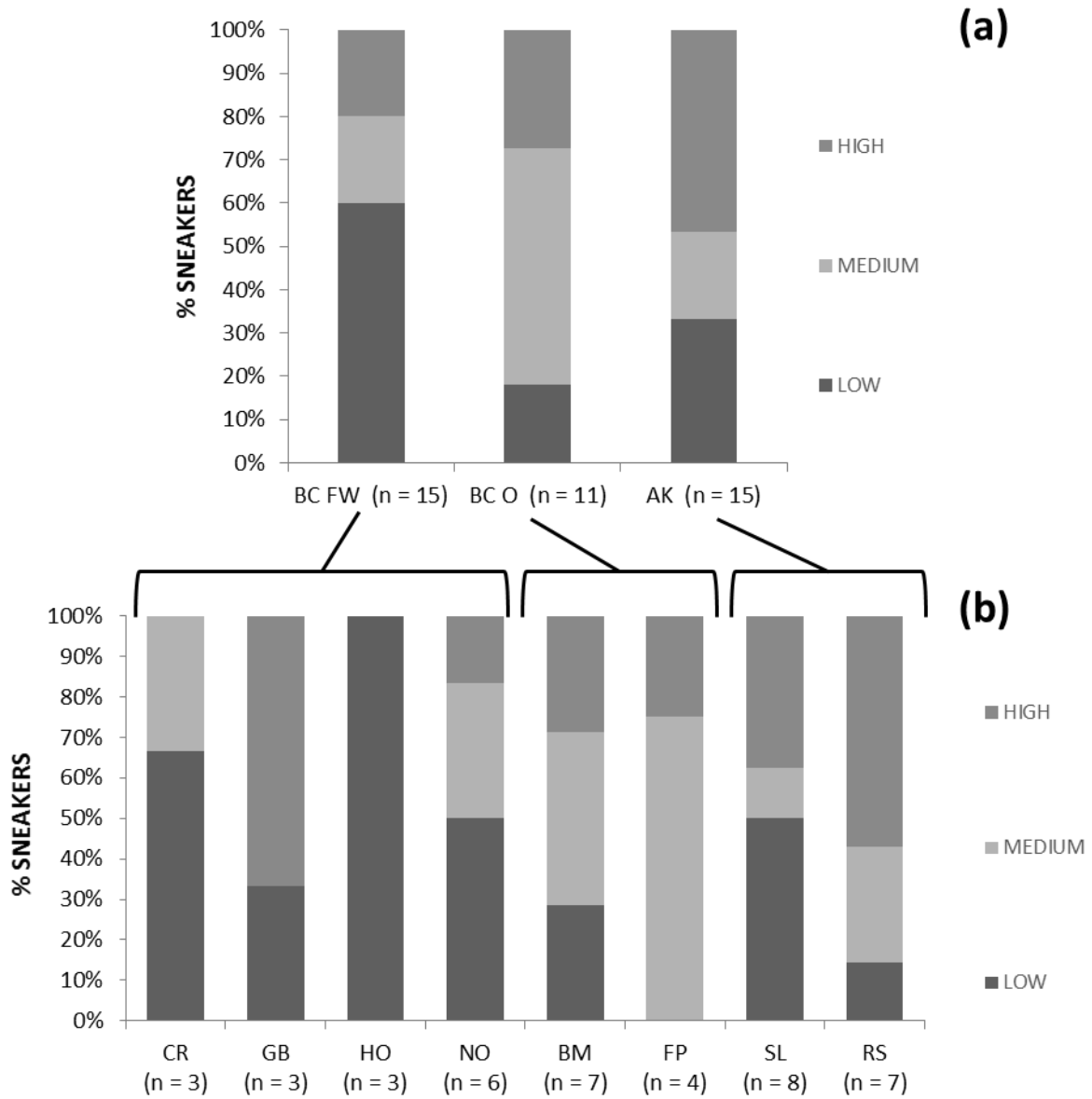
**Figure 3.11.** Population-level comparison of the proportion of males expressing sneaking behavior during at least one behavioral trial. Assays were performed on British Columbia freshwater (BCFW) and oceanic (BCO) populations, as well as Alaskan freshwater (AKFW) and oceanic (AKO) populations. Population codes correspond to Table 3.4. The overall percentage (40%) of individuals performing sneak behavior in the pooled data set is represented by the dashed line. An exact binomial test was performed on the proportions of sneaker and nonsneakers in each populations against an expected ratio of 40% sneakers to 60% nonsneakers. Two-tailed tests were used to determine if the population sneaker proportions deviated significantly from 40% (\* $p \leq 0.05$ ).



**Figure 3.12.** Population-level comparison of male sneaking intensity. Assays were performed on British Columbia freshwater (BCFW) and oceanic (BCO) populations, as well as Alaskan freshwater (AKFW) and oceanic (AKO) populations. Population codes correspond to Table 3.4. The column for each population shows the percentage of sneaker males displaying each category of sneak behavior. These categories represent different levels of sneaking intensity. Each male was counted once and grouped based on the most intense category of sneaking behavior performed across six trials. See section on *Sneaker Assay Protocol* for a more elaborate description of the levels of sneak behavior.

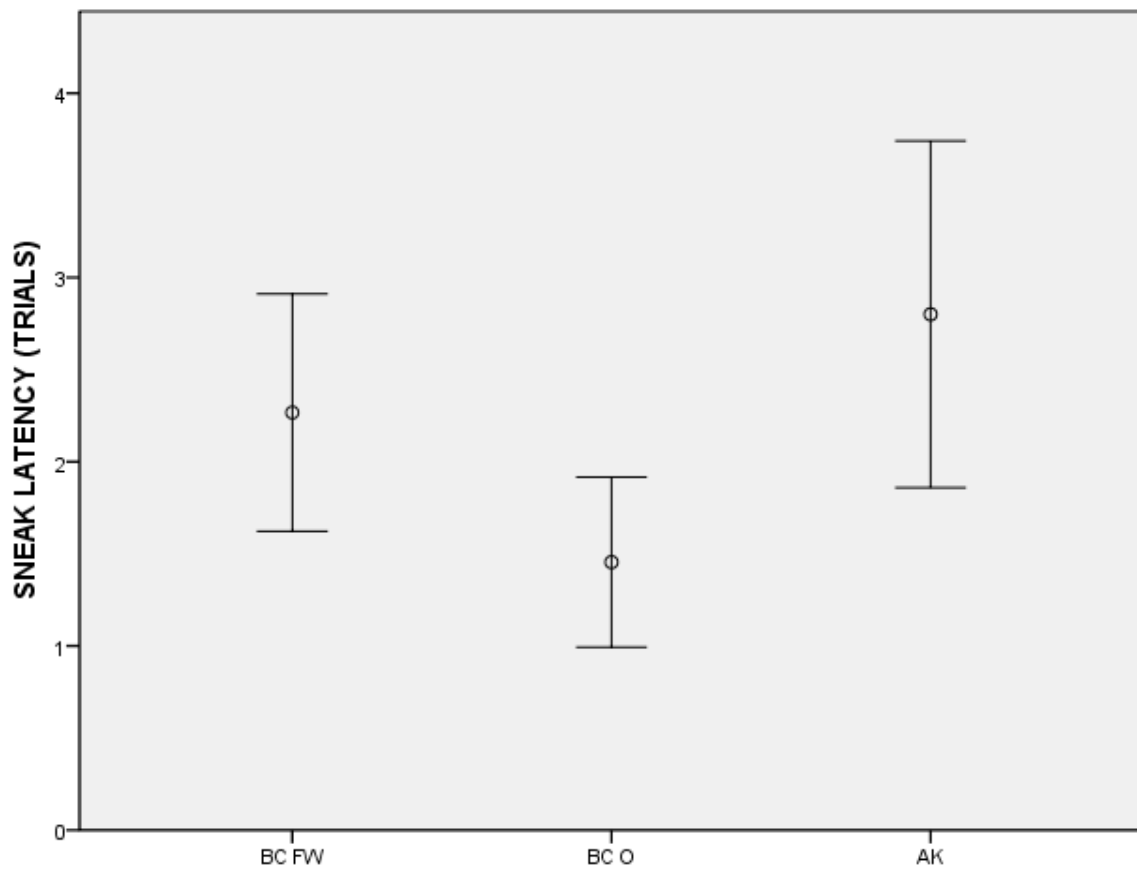


**Figure 3.13.** Population-level comparison of male dyad types. Population codes correspond to Table 3.4. Sample sizes are based on the number of pairs tested for each population.

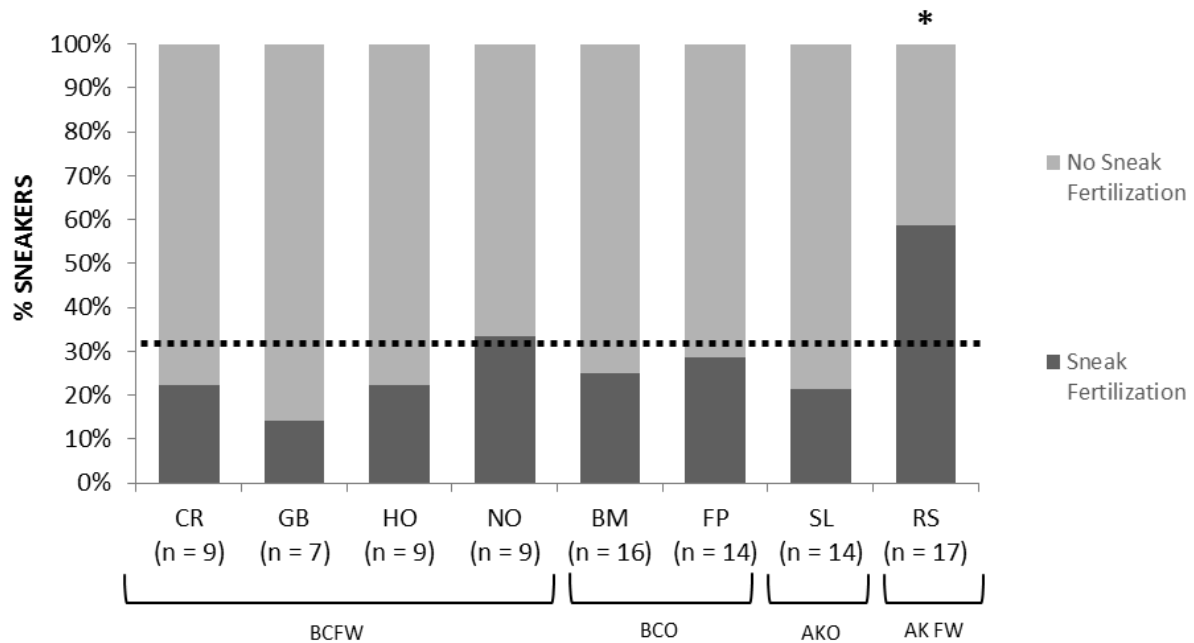


**Figure 3.14.** Population-level comparison of consistency scores – percentage of trials in which sneaker males demonstrated sneaking behavior categorized as low (< 50% of trials), medium (50-75% of trials), and high (> 75% of trials) consistency. Consistency scores were only determined for a subset of sneaker males for which there were 5-6 sneak opportunities available (n = 41). **(a)** grouped population proportions and **(b)** individual population proportions. Population codes correspond to Table 3.4.



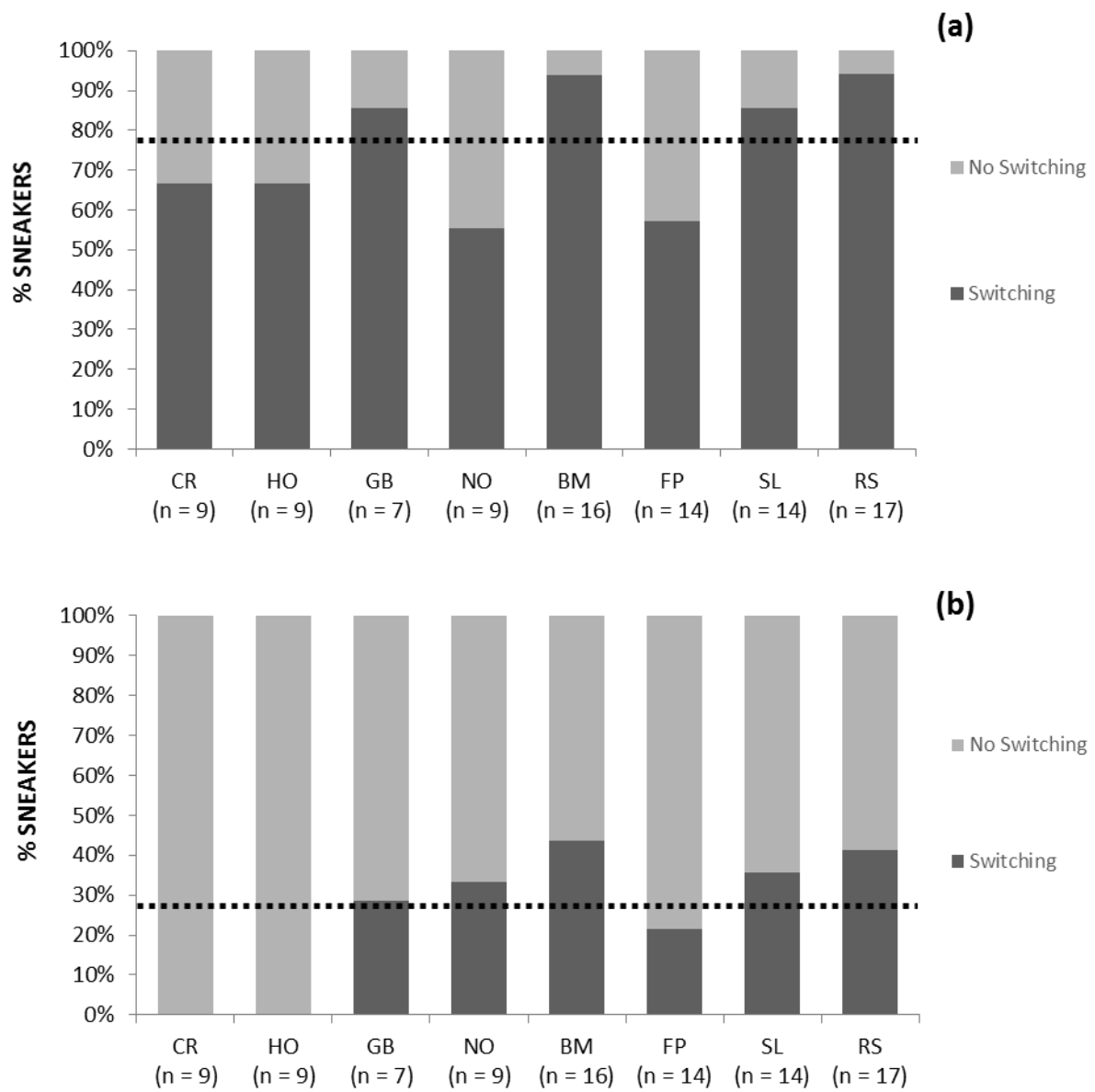


**Figure 3.15.** Means and 95% CIs for sneak latency (number of tankmate courtship trials before sneaking behavior was elicited) for BC freshwater (BC FW) males ( $\bar{x} = 2.27 \pm 1.16$ ,  $n = 15$ ), BC Oceanic (BC O) males ( $\bar{x} = 1.45 \pm 0.69$ ,  $n = 11$ ), and Alaskan (AK) males ( $\bar{x} = 2.80 \pm 1.70$ ,  $n = 15$ ).



**Figure 3.16.** Population-level comparison of sneaker success – percentage of sneaker males in each population demonstrating at least one successful sneak fertilization. The overall percentage (30%) of sneaker males performing at least one successful sneak fertilization in the pool data set is represented by the dashed line. An exact binomial test was performed on the proportions of successful and unsuccessful sneakers in each populations against an expected ratio of 31% successful sneakers to 69% unsuccessful sneakers. A two-tailed test was used to determine if the population success proportions below the 31% line were significantly less than 31% and vice versa for those populations with sneaker proportions above the 31% line (\* $p \leq 0.05$ ).

**Figure 3.17.** Population-level comparison of sneakers demonstrating within-trial tactic switching (i.e. behavioral switches between courtship and sneaking) for sneaker-nonsneaker pairs and sneaker-sneaker pairs combined: **(a)** the percentage of sneakers switching tactics in at least 1 of 6 trials, with  $n$  representing the total number of sneaker males. The overall percentage of sneaker males performing at least one successful sneak fertilization in the pool data set (78%) is represented by the dashed line. **(b)** the percentage of males demonstrating plasticity in at least 50% of trials (3+ trials), with  $n$  representing the total number of sneaker males. The overall percentage of sneaker males performing at least one successful sneak fertilization in the pool data set (28%) is represented by the dashed line. A two-tailed exact binomial test was used to determine if the population proportions were significantly higher or lower than the pooled percentage lines ( $*p \leq 0.05$ ). Population codes correspond to Table 3.4.



**Figure 3.17.**

## CHAPTER 4

# CHARACTERIZING PHYSICAL ATTRIBUTES OF WILD-CAUGHT THREESPINE STICKLEBACK SNEAKERS: SIZE, CONDITION, AND BODY COLOR/PATTERNING

### 4.1 ABSTRACT

Sneaker males of many fish species exhibit relatively smaller body size, lower condition, and cryptic body coloration compared to males adopting conventional mating tactics. Sneaking is an alternative reproductive tactic in a number of Threespine Stickleback populations and, although primarily facultative in nature, some males demonstrate tactic specialization -- highly consistent use of this tactic or lack of expression even when opportunities for cuckoldry are presented. This presents the possibility that certain phenotypic characteristics are associated with the use of the sneaking repertoire and can be predictive of tactic use. We characterize the sneaker tactic in an Alaskan population for size, condition, and/or body coloration/patterning. Sneaker males did not differ from males of other breeding behavior categories (e.g. nest-building, courting, parental with eggs, parental with fry) in either body size (SL) or condition. Using digital image assessment we were unable to detect any significant body coloration and patterning differences between males expressing sneaking behavior and males engaged in nest-building behaviors at the time of collection. Interestingly, the prominent disruptive flank patterning often described in actively sneaking males did not factor significantly into the separation of any of the breeding behavior categories and was observable in images of courting and parental males, which typically did not exhibit this prominent body patterning during *in situ* observation prior to collection. Due to the potential for rapid color plasticity in our study population, using drab body color

and prominent flank patterning to indicate mating tactic use is not a reliable method and should be coupled with behavioral observations.

**KEYWORDS:** Threespine Stickleback, *Gasterosteus aculeatus*, alternative reproductive tactics, crypsis, female mimicry

## 4.2 INTRODUCTION

A number of fish species demonstrate reproductive tactics as alternatives to courtship in the form of sneaking fertilizations or copulations (Taborsky 2008). In some, sneaker males represent alternative developmental trajectories and in others the sneaker tactic is a condition-dependent behavioral strategy that may be sequential (e.g. age-dependent) or context-dependent (opportunistic and reversible). Regardless of the mechanism determining tactic expression, sneaker tactic use is often associated with relatively small body size, dull coloration and disruptive body patterning. Such traits may be due to a number of co-occurring selective pressures, including selection for retention of juvenile characteristics (e.g. small body size at maturation; Dellefors & Faremo 1988; Taborsky 2001; Aubin-Horth & Dodson 2004; Neff 2004; Paez, Bernatchez & Dodson 2011) and crypsis (Arak 1984; Lee & Bass 2006). In some species, male tactic use can be used to categorize males upon collection without direct behavioral observations based on body size and coloration (Gross 1982; Farr *et al.* 1986; Myers *et al.* 1986; De Jonge & Videler 1989; Zimmerer & Kallman 1989). Although viable for species demonstrating discrete alternative morphs or age/size-dependent sequential tactics, the extent to which this identification strategy can be applied to species expressing highly plastic alternative tactics is unclear. Poor condition is a common explanation for why some males may not be able to effectively compete for mates using the typical reproductive tactic (Dawkins 1980; Hazel *et al.* 1990; Taborsky

2008). However, if males demonstrate high tactic flexibility and predominately express sneaking behavior as an opportunistic tactic, we expect no relationship between expression of sneaking behavior and body size, condition and body coloration/patterning. Here we test whether such categorization is possible for sneaker males of the Threespine Stickleback (*Gasterosteus aculeatus*, L.), a species in which males have been observed to employ both consistent use of either the sneaker or courter tactic, as well as opportunistic tactic-switching.

In populations that display the typical nuptial coloration, Threespine Stickleback males begin the breeding season with drab body coloration. Nest building males commonly display golden or brown flank, ventral and dorsal coloration, golden or brown irises, and golden or white coloration on the operculum, throat, and mouth regions. Males usually progress through the breeding cycle to brighter nuptial coloration (Wootton 1976; McLennan & McPhail 1989; Hulslander 2003; McLennan 2006), e.g., the carotenoid-based (astaxanthin/lutein; Wedekind *et al.* 1998) red coloration on the operculum/throat/mouth regions (reviews: Bakker 1994; Foster 1994; Rowland 1994; Bolyard & Rowland 1996). The red throat signal is known to play an important role in male-male interactions during territory acquisition and defense (e.g. Rowland *et al.* 1995; Bolyard & Rowland 1996; Kim & Velando 2014), parental care territorial defense (e.g. Candolin 2000), and female mate choice (e.g. Bakker & Mundwiler 1994). Males also typically develop blue-green flank coloration and bright blue eyes (McLennan 2006; Foster *et al.* 2008). Blue eye coloration is linked to body condition in some populations (Frischknecht 1993), and may serve as an important courtship signal, as females prefer males with the highest contrast between eye and throat (Flamarique *et al.* 2013). Lightening of the dorsum is often observed in actively courting males and is likely related to the activation of iridophores and contraction of melanophores (McLennan 1996). Red throat, blue eye, and bright blue-green body coloration is also characteristic of parental males in both egg-rearing and fry-rearing stages in many populations (Foster *et al.* 2008; Laurin & Scott 2009).

Males of many freshwater and oceanic populations exhibit sneaking behavior -- attempts to fertilize newly spawned eggs in the nests of other males (van den Assem 1967). Such 'sneakers' are typically drab nonterritorial males, but may also be newly territorial or nest building males (van den Assem 1967; Jamieson & Colgan 1992; de Fraipont, FitzGerald & Guderley 1993; Mori 1995; K. Shaw pers. obs.). Males in the courtship stage of breeding can also express sneaking behavior. In some instances, sneaking males have been observed to switch from bright nuptial coloration to drab, cryptic coloration (van den Assem 1967; K Shaw, Chapter 3; K. Shaw pers. obs.).

Not all males adopt sneaking behavior as part of the natural progression of breeding behavioral stages nor do all sneaker males readily switch to nesting and courtship behavior (Chapter 3). This raises the question of whether sneaking behavior can be considered a separate behavioral category characterized by specific body size, condition or color and patterning characteristics.

In this study, *in situ* observations were made in Stepan Lake, a freshwater Alaskan population with a relatively high frequency of sneaking behavior (sneaking during 33% of all complete courtships vs. Alaskan freshwater average of 13%; Chapter 2). For each individual, current behavior stage in the reproductive cycle, digital images of body coloration and patterning, weight, and standard length (SL) were recorded. We used this information to explore associations between expression of sneaking behavior, body size, condition and coloration/patterning within this population.

The drab coloration of sneaker males is often assumed to provide crypsis as it typically involves pronounced disruptive patterning in the form of dark flank barring (van den Assem 1967; DeFraipont, FitzGerald & Guderley 1993; Greenwood *et al.* 2012). However, descriptions of threespine sneaker coloration often liken it to the golden coloration and barring patterning expressed by gravid, courting females (van den Assem 1967; Jamieson & Colgan 1992; K Shaw, pers. obs.). In freshwater populations, gravid females ready to spawn typically demonstrate an increase in melanization – in particular,



darkening of dorsal and flank barring pattern (Wunder 1934; Williams & Delbeek 1989; Rowland *et al.* 1991; McLennan 1996) – along with their increased responsiveness to courting males. To investigate the possibility that threespine sneaker males exhibit female mimicry to allow sneaker males close access to nesting males without experiencing much aggression (Morris 1952; Willmott & Foster 1995), gravid, courting females were also assayed for comparisons with sneaker size and coloration/patterning.

## 4.3 METHODS

### 4.3.1 Study Site & Field Methods

Fish were observed and collected for digital photographs in 2009 (June 27-July 7) from Stepan Lake (61°34'23.52"N, 149°49'30.63"W) in the Matanuska-Susitna Borough of south central Alaska. Males were observed until behavioral stage was determined (nestbuilding, courting, parental with eggs, parental with fry) based on characteristic breeding stage behaviors (Foster *et al.* 2008). The observation period lasted until males and female displayed activities specific to a particular behavioral stage (minimum 10-min. observation periods). Males demonstrating sneaking behavior were collected during expression of the sneaker repertoire. Gravid females were collected when they were observed to show spawning readiness to nested males (i.e. head-up posture) and courting males were collected when exhibiting courtship behaviors. Individuals were captured with hand nets and brought to shore immediately for measurement.

After capture, digital photographs were taken of all individuals (sample sizes for behavioral categories are shown in Table 4.1). Individuals were held on a wet sponge and digital images of the lateral profile were taken in a light box with the standard flash used as the primary lighting source (Olympus Stylus 850 SW; modified from Candolin 1998). After photography, individuals were briefly

placed in a container of water before performing any weight measurements and to minimize stress before release. Attempts were made to complete all procedures within 5 min from capture, to minimize potential color change (see Laurin & Scott 2009). A second set of digital photographs were taken on a subset of individuals held for 20 minutes post-capture to determine the rapidity and magnitude of color change for individuals of this population (**Appendix 4A**).

#### *4.3.2 Ethical Note*

All protocols for observation, collection, and measurement of wild-caught individuals followed the University of Connecticut IACUC No. A08-028 protocol (2009). We held permits from the Alaska Department of Fish and Game for collections (2009).

#### *4.3.3 General Note About Analyses*

Parametric tests were used whenever the data fit the assumptions. Analyses were performed in PASW (SPSS Inc., v 18) and R (R Development Core Team, v 2.15.0).

#### *4.3.4 Comparison of SL and Condition Across Behavioral Categories*

Weight and standard length (SL) data were collected from 120 photographed individuals representing specific breeding behaviors to determine size and individual body condition. Individual weights were recorded in the field and individual standard length (SL) was determined with ImageJ (Rasband 1997-2014, v1.44p) using the standardized digital photographs. The condition measure is commonly used in stickleback studies (Frischknecht 1993; Kurtz *et al.* 2006; Kalbe *et al.* 2009):

$$\text{Condition Factor (CF)} = 100 \times W/L^b$$

where  $W$  is weight (g),  $L$  is length (cm) and  $b$  is the regression coefficient calculated from the log-transformed values of length and weight (Bolger & Connolly 1989).

To identify any growth characteristics related to breeding behaviors or sex, general linear models were applied to determine the relationship between the specific breeding behavior categories (nestbuilding, sneaker, courting, etc.), SL (cm) and condition factor (CF). Only male breeding behavior categories were compared in the analysis of body condition.

#### 4.3.5 Visual Color/Patterning Assessment Methods

Observer-based qualitative methods of color scoring are commonly used in stickleback studies (Bakker & Sevenster 1983; Milinski & Bakker 1990; Goldschmidt, Bakker, Feuth-deBruijn 1993; Candolin 1998; Patriquin-Meldrum & Godin 1998; Wedekind *et al.* 1998; Cubillos & Guderley 2000; Nilsson & Nilsson 2000; Boughman 2001, 2007; Lewandowski & Boughman 2008; Cote *et al.* 2009; Kozak, Head & Boughman 2011). A scale of intensity for a single color (e.g. red or blue intensity or brightness) is often used for scoring and comparing individuals within a particular breeding stage (e.g., color comparisons between courting males or between parental males – e.g. Lewandowski & Boughman 2008; Cote *et al.* 2009; Kozak, Head & Boughman 2011). However because my goal was to compare body coloration across a range of breeding behavior categories, I used a range of color (hue) categories to code all individuals – including gravid females, which typically exhibit drab coloration. Ordinal color categories, ranging from drab (1) to bright coloration (6), were assigned to score flank, dorsum, ventral, eye and throat coloration (Table 4.2). This scale is based on the natural range of colors expressed in this population. Categories can include common combinations of colors that are expressed simultaneously,

often in a layered fashion, either because of the close proximity of different types of chromatophores on certain areas of the body or the effects of iridescence (e.g. greenish-gold dorsum color).

Individuals from many freshwater populations, including Stepan Lake, exhibit flank patterning. Stripes form along the sides as an individual develops, often resulting in either a striped or checkered pattern (Greenwood *et al.* 2012). Aspects of flank patterning (stripe width, regularity, gap size, and contrast) were scored using a 3-pt scale (Table 4.2).

In the field photographs, a Munsell color card (Mini Color Checker, X-Rite, Inc.) was included in digital photographs to check camera white balance and minimize variation in flash lighting ('Levels' feature in Adobe Photoshop Elements v 8.0). Individuals were scored blindly by a single observer sorting the images into groups for each color and patterning variable. A single image was chosen as the representative image for each category to simplify groupings.

Scoring repeatability for body color and flank patterning was determined by rescored categories for a random subset of individuals. The sample size needed to detect an effect size of 0.5, with power  $(1-\beta) = 0.8$  and  $\alpha = 0.05$  was calculated for a paired t-test (using the R package pwr: <http://cran.r-project.org/web/packages/pwr/index.html>). Although the suggested sample size to detect this effect size was  $n = 30$ , 50 repeat measurements were done so that nonparametric analyses, which are generally believed to have slightly lower power than their parametric counterparts, could be used. First, each individual was randomly assigned one of the 10 color/patterning categories. Then a random subset of 50 individuals (of the 121 total individuals) was chosen and the images were rescored by the original observer for their randomly assigned color/patterning category two weeks after the original scoring. To test for repeatability between the initial categorization and the rescored, a related-sample Wilcoxon signed ranks test was performed in PASW. The Wilcoxon signed ranks analysis suggested no significant difference in the median between the original score and the repeat score for the random subset of 50

measurements ( $R$ ;  $Z = -1.633$ ,  $p = 0.102$ ). In total, 94% (47/50) of the images were correctly rescored, suggesting that the scoring categories and methodology is accurate in effectively discriminating the body color/patterning of individuals.

#### *4.3.6 Nonmetric Multidimensional Scaling (NMDS) of Visually Scored Color/Patterning Traits*

Nonmetric multidimensional scaling (NMDS) was used to determine a measure of dissimilarity (or distance) between individuals based on their scores for the set of color/patterning variables (Table 4.2). Multidimensional Scaling uses similarities among objects to derive a reduced set of new variables (axes) from the original variables (Cox & Cox 1994; Quinn & Keough 2002). NMDS calculates a matrix of similarities between individuals based on user specified similarity measurements. Individuals are arranged in either a random or pre-specified starting configuration, and then shuffled around in  $k$ -dimensional space iteratively so that at each step, the match between the inter-individual distances in the configuration and the actual dissimilarities improves (Kruskal 1964a,b). The final configuration is achieved when further movement of individuals no longer improves the match between inter-individual distances in the configuration and the actual dissimilarities (Quinn & Keough 2002). The fit of the data is described in terms of 'stress' – the lower this inverse measure, the better the match between inter-individual configuration distances and actual dissimilarities. Randomized data are then used as a null model for comparison (McCune & Grace 2002). An optimal solution is a dimensionality that produces a result significantly different from random (Monte Carlo test: final stress must be lower than that of 95% of the randomized runs,  $p \leq 0.05$ ). If multiple solutions are significant, additional dimensions are considered if they reduce final stress  $\geq 5$  (on a scale of 0-100, McCune & Grace 2002). The NMDS analysis was performed in PC-ORD (V 4.20, McCune & Mefford 1999) according to the procedures outlined by McCune & Grace (2002). Specifications similar to the 'slow and thorough' autopilot settings were used

(6 axes, 400 iterations, 50 data runs, 50 random runs) with the Euclidean dissimilarity measure. The final instability criterion was set at 0.00001 s.d. in stress values over the last 15 iterations to avoid an unstable solution. Unlike PCA, NMDS often generates axes that are weakly correlated. Moderately correlated axes can cause problems in interpretation and rotation of ordinations (McCune & Mefford 1999) and we take care to note whenever color/patterning variables have fairly strong relationships with both axes.

After determination of appropriate dimensionality by NMDS, a multi-response permutation procedure (MRPP) was conducted to determine how well individuals from the pre-defined breeding behavior categories grouped in body color/patterning space. MRPP is a nonparametric approach for testing the null hypothesis of no difference between two or more groups (McCune & Grace 2002). It is similar to discriminant analysis and multivariate analysis of variance (MANOVA), but does not require distributional assumptions (e.g. normality and variance homogeneity). The MRPP analysis was conducted in PC-ORD (V 4.2, McCune & Mefford 1999). Individuals in the distance matrix of color/pattern variables were grouped according to the 6 pre-defined breeding behavior categories (G = gravid females; S = sneaker males; NB = nestbuilding males; C = courting males; P = parental males w/ eggs; PF = parental males w/ fry) and the default weighting method was used for calculating  $\delta$  (the weighted mean within-group distance) for each group (McCune & Grace 2002). The rank-transformed Euclidean distance measure was used to determine the distance matrix because it results in a nonmetric MRPP and allows for better correspondence between the MRPP results and the NMDS results (McCune & Grace 2002). Following the MRPP analysis for all breeding behavior categories, all pair-wise MRPP comparisons were conducted to determine groups that differ significantly in body color/patterning using the MRPP test statistic,  $T$ , which describes separation between groups—the more negative the value, the stronger the separation (McCune & Grace 2002). In addition, the MRPP analysis provides a measure of effect size that describes within-group homogeneity for pair-wise comparisons, the chance-correlated within-group agreement ( $A$ ):

$$A = 1 - (\text{observed } \delta / \text{expected } \delta)$$

with delta ( $\delta$ ) as the weighted mean within-group distance. If all individuals within groups are identical and groups are well-defined, then  $A = 1$  and if heterogeneity within groups equals the random expectation, then  $A = 0$ . If there is less agreement within groups than expected by chance and high overlap between groups, then  $A < 0$ . For data sets with high heterogeneity, such as community ecology data,  $A > 0.3$  is a fairly high effect size (McCune & Grace 2002).

## 4.4 RESULTS

### 4.4.1 Comparison of SL and Condition Across Behavioral Categories

Differences in SL (cm) across breeding behavior categories were marginally significant (Table 4.3). Examination of parameter estimates shows that this result is not due to differences among male categories (Table 4.4, Figure 4.1); instead gravid females are smaller than courting males (Tukey's HSD: mean SL difference = 0.287 cm, S.E.= 0.092,  $p = 0.026$ ). The effect sizes of SL and the parameters, as measured by partial eta squared ( $\eta_p^2$ ), suggest that the proportion of variance in SL explained by the breeding behavior categories is fairly small ( $\max \eta_p^2 = 1$ ,  $\eta_p^2 \leq 0.1$  = weak effect: Cohen 1988; Tables 4.3 and 4.4).

There was a highly significant relationship between the log-transformed values of length and weight ( $b = 2.653$ ;  $t_{118} = 12.529$ ,  $p < 0.001$ ; Figure 4.2). There were no significant differences in condition factor (CF) across male breeding behavior categories (Table 4.5, Figure 4.3). Removal of one CF outlier improved variance homogeneity among the breeding behavior categories but did not change the overall results of the GLM analysis. Sneaker male body condition did not differ significantly from that of males

in other breeding behavior categories as would be expected if low body condition is a trigger for adopting the sneaker tactic.

#### *4.4.2 Nonmetric Multidimensional Scaling (NMDS) of Visually Scored Color/Patterning Traits*

Although the final stresses of all dimensionalities were significantly different from randomly produced final stresses, the two-dimensional solution was chosen as the appropriate solution because additional dimensions did not reduce final stress by  $\geq 5$  (Table 4.6, Figure 4.4, McCune & Grace 2002). For the two dimension solution, final instability was low (final instability = 0.00001, number of iterations = 71) and final minimum stress, which serves as an inverse measure of fit to the data, suggests little risk that the ordination will result in false inferences (Table 4.6,  $S = 6.64\%$ , Kruskal 1964a; McCune & Mefford 1999; McCune & Grace 2002).

The axes of the two dimension solution combined accounted for 97.5% of the variation in the original matrix space (Axis 1 = 64.8%, Axis 2 = 32.7%; Table 4.7). Correlations between dimensions and the original variables from the main matrix are shown in Table 4.8. Flank color has a strong positive relationship with Axis 1, as well as a fairly strong positive correlation with Axis 2. Dorsal coloration is another variable that has a moderately strong positive correlation with both axes. Ventral coloration, eye coloration, and throat coloration are all highly correlated with Axis 2. The relationship between the ordination axes and the flank patterning variables were found to be negligible, suggesting more inter-individual variation in flank patterning combinations than in body coloration combinations. Individuals were plotted in the two dimensional space based on their dissimilarity scores and the correlated variables were incorporated along the axes for ease of interpretation (Figure 4.5). Despite the placement of bright dorsum and bright flank along Axis 2, males dispersed along upper left side of the plot typically have drab bodies but bright throats and eyes.



The results of the MRPP analysis show high congruency with the groupings visualized in Figure 4.5. A cluster analysis performed in PC-ORD produced similar groupings (Appendix 4B). For the MRPP analysis, the average within-group distances (Table 4.9) show that gravid females (G) and parental males w/ fry (PF) have the tightest dispersions in body color space, followed by sneakers and nest-building males, while courting males (C) and parental males (P) have relatively high dispersions (Figure 4.5). The MRPP comparison involving all groups suggests that there is an average within-group homogeneity -- some pre-defined groups demonstrate more dispersion than others ( $A = 0.468$ ; Table 4.10). All possible pairwise MRPP comparisons were made ( $n = 15$ ) and a Bonferroni correction ( $\alpha = 0.003$ ) was applied to the final p-values (McCune & Grace 2002). Sneaker males and nest-building males overlap greatly in body color space ( $A = -0.013$ ,  $p = 0.866$ ; Table 4.10). Courting males and parental males w/ eggs also have high overlap in body color space ( $A = 0.019$ ,  $p = 0.098$ ; Table 4.10). Finally, despite statistical significance of group separation, the poorly delimited color space of courting males and parental males w/ eggs overlaps greatly with the well-defined color space of parental males w/ fry (Figure 4.5, Tables 4.9, 4.10). Examples of color variation within and across breeding behavior categories are shown in Figure 4.6.

## 4.5 DISCUSSION

### *4.5.1 Size, Condition, and Breeding Behavior Categories*

Sneaker males were neither more similar to females in size, nor did these males have higher or lower condition than males in other stages of breeding behavior. Lower condition in males is often predicted to be a signal to males that they are not likely to be competitive if they adopt the typical mating tactics (Dawkins 1980; Hazel *et al.* 1990; Taborsky 2008). The lack of association between size or condition and

sneaking behavior indicates that opportunistic – rather than size-, age- or intrinsic condition-dependent – use of the sneak tactic is common in this study population.

#### 4.5.2 Associations between Body Color/Patterning and Sneaking Behavior

Sneaker males cannot be readily distinguished from males engaged in nest-building behavior using visual assessment. Laboratory behavioral assays suggest that there is a spectrum of sneaking propensity for males in this population – some males perform sneaking behaviors regardless of opportunities to court and spawn in their own nests, and others readily switch between courtship and sneaking behaviors within short (10 min) time periods (K Shaw, Chapter 3). Given the plastic nature of the sneaking repertoire for some individuals, it is not possible to determine whether field males collected as nest-builders or courters did not also have a propensity to perform sneaking behavior.

Gravid females and sneaker males show separation in color space, suggesting a lack of female mimicry based on color traits. Physical and behavioral resemblances to females have been observed in many species of fish with alternative reproductive tactics (Blenniids: Goncalves *et al.* 1996; Centrarchids: Dominey 1981, Gross 1982; Cichlids: Chan & Ribbink 1990, Oliveira & Almada 1998; Cyprinodontids: Kodric-Brown 1986; Labrids: Taborsky *et al.* 1987; Pinguipedids: Ohnishi *et al.* 1997). Female mimicry in sneaker color patterning and behavior has been described in both the fourspine stickleback (*Apeltes quadracus*, Willmott & Foster 1995) and the ninespine stickleback (*Pungitius pungitius*, Morris 1952). The defining attribute often compared between threespine sneakers and gravid, courting females is distinct flank patterning, a characteristic which was not significantly correlated with either ordination axis. Despite the high inter-individual variation in flank patterning combinations, many sneakers and gravid females do exhibit flank patterning, but so do other individuals of other breeding behavior categories. However, female mimicry is not the only explanation for similarity of flank patterning. Rowe

*et al.* (2004) found similarities between the spectral reflectance of near-shore substrate (estimated based on ambient light measurements) and nested male flanks, suggesting background matching on the nesting grounds is an important function of flank color/patterning. Interestingly, dark barring coloration is conspicuous when viewed in the water column, where gravid females typically approach males, and thus may be a conspicuous signal advertising sexual receptivity (Rowland, Baube & Horan 1991). It is possible that similar body patterning in males and females serve different functions, as they are typically viewed in microhabitats with different ambient lighting characteristics.

#### *4.5.3 Body Color/Patterning Variation within Breeding Behavior Categories*

Although nuptial color is stereotyped as red throat and bright body and eye coloration, courting males exhibited wide dispersion in body color combinations. McLennan & McPhail (1989) observed similar individual variation in courting male coloration in an anadromous British Columbia population. Given that many aspects of nuptial coloration are condition-dependent (Milinski & Bakker 1990; Frischknecht 1993; Candolin 1999; Barber *et al.* 2000; Rush *et al.* 2003), high variability may be expected in such sexually selected characters (McPhail 1984; Rowland 1984; McLennan & McPhail 1989; Bakker 1993; Folstad *et al.* 1994; Bakker & Pomiankowski 1995; Boughman 2001), and different color combinations may have similar success (Flamarique *et al.* 2013). In some populations, including Stepan, bright nuptial coloration is more indicative of the paternal care stage rather than the courtship stage (Hulslander 2003; Foster *et al.* 2008). The conspicuous coloration associated with actively courting males in this population involves rapid changes in chromatophores (activation of iridiphores and contraction of melanophores; McLennan 1996), which may be reversible in the time period between capture during courtship and digital photographs. It is possible that the diverse combinations of color characteristic of courting males is related to the propensity of individuals to facultatively switch reproductive tactics -- such delayed

color change may be beneficial to courting males engaging in opportunistic sneaking although the lack of bright nuptial color may result in fewer courtship successes for such males, depending the strength of female preference for nuptial color traits (Boughman 2007). However, this would not explain the similar dispersion in color space observed for early parental (egg-rearing) males: sneaking has only been observed in parental males when they lose their nest and/or eggs to cannibalistic females/groups or to sneaking/egg-stealing males (K. Shaw & S. Foster, pers. obs.).

#### 4.5.4 Tactic Flexibility & Color/Patterning Plasticity

As flank patterning may serve as a form of crypsis (Rowe *et al.* 2004; Greenwood *et al.* 2012), this variable was included in the analysis to investigate the extent of individual variation. No aspects of flank patterning differed between breeding behavior categories. Disruptive flank pattern development in freshwater stickleback typically begins between the hatching and juvenile stages and a set number and set width of stripes containing melanophores (forming dark bars) versus iridophores (forming light bars) forms by the time an individual matures (Greenwood *et al.* 2012). Even though constitutive flank patterning (e.g. stripe number and width) may be similar across individuals, individuals can rapidly manipulate stripe contrast – melanophore contraction and expansion can produce qualitatively lighter or darker chromatic responses over a span of 1-2 mins (Burton 1975), i.e., between the time of capture and photograph.

Interestingly, flank patterning was more prominent in nest-building and sneaking males than later breeding stage males (e.g. courting and parental males) during *in situ* observations made prior to individual captures. For many males in which flank patterning was seemingly absent during *in situ* observation, this patterning become more apparent in digital images taken under standardized lighting compared to during *in situ* observations. Body color/pattern expression can be affected by ambient

lighting conditions (Reimchen 1989; Boughman 2001; Scott 2001; Laurin 2006; Laurin & Scott 2009; Clarke & Schluter 2011). Interactions between ambient lighting and coloration components (in particular, color contrast -- dark brown stripes against golden flank vs. light gray stripes against bright blue-gray flank) may influence visibility of this signal (or crypsis) component of color patterning, depending on the microhabitat in which it is viewed (e.g. littoral zone/nesting grounds vs open water). Comparison of first and second images for a random subset of individuals from each breeding category found no change in stripe number or gaps, but did suggest changes in stripe contrast (due to either darkening of stripes, lightening of the flank or a combination of these factors; Appendix 4A), highlighting the flexibility of this aspect of body coloration/patterning over time. Further experimentation with more precise methods of color measurement during *in situ* behavioral observations may be necessary to capture the rapidity and magnitude of flank patterning changes in sneaker males, as well as the extent to which this signal is expressed in males with bright flank coloration.

While rapid changes in melanophore contraction or relaxation may result in lightening or darkening of body regions, more constitutive colors do not change as quickly (Frischknecht 1993; Rush *et al.* 2003; K Shaw, pers. obs.) allowing for much of the variation in hue (versus brightness or intensity) to be captured in the color analyses. Drab males re-photographed after 20-mins demonstrated little color change, but many conspicuously colored males converged on aspects of drab coloration (dorsum, eyes, and throat; Appendix 4A). Such increases in drab coloration may be related to cortisol-mediated stress responses to holding (Hoglund, Balm & Winberg 2000).

#### 4.5.5 Conclusions and Future Directions

The goal of the current study was to determine whether specific phenotypic characteristics (body size, condition, coloration/patterning) can be associated with the sneaker tactic for easy identification and

sampling of individuals performing alternative tactics in stickleback populations. Results indicate that such characteristics are not good indicators and their use would produce skewed estimates of sneaker male abundance.

The population used in this study comprises a mixture of individuals that express facultative or specialized use of the sneaker or courtship tactics. High behavioral plasticity suggests a lack of constitutive morphological specialization for the sneaker tactic commonly observed in species exhibiting developmentally divergent switchpoints or age/size-dependent sequential tactics. However, this study cannot rule out the presence of temporary reversible color changes that facilitate either increased crypsis or female mimicry. It is possible that such rapid color changes are shorter-lived than the processing time used for digital image collection in this study. Capture of such immediate responses will require real-time acquisition of color measurements during the expression of sneaking behavior either *in situ* or under laboratory settings (e.g., noninvasive spectrophotometry; Rush *et al.* 2003). Similar methods may be used to measure sneaker coloration changes during sneaker repertoire expression.

Plastic aspects of body color/patterning specific to sneaking behavior may be identifiable under laboratory conditions more so than field conditions for several reasons. *In situ*, it is often difficult to distinguish whether a sneaker male was also a territorial male or nest-tending male (or even a courting male sneaking opportunistically), because males were collected immediately upon expression of the sneaker repertoire. Use of behavioral assays to elicit sneaking behavior and longer observation periods under laboratory conditions allow for easier categorization of individual breeding behavior and the extent of flexible tactic switching versus specialization. Also, color intensity of breeding males under lab conditions is generally greater so that the magnitude of the difference between drab and bright color patterns may be more easily assessed (McLennan & McPhail 1989; Barber *et al.* 2000; Hulslander 2003). However, care must be taken when extrapolating laboratory results to coloration under natural

conditions, particularly if lab-reared fish are used. Prominence of flank patterning and male color signals is dependent on the environment in which fish are reared and can be markedly different between lab and field (Hulslander 2003; K Shaw, pers. obs.). Further investigations into flexible aspects of body coloration will provide exciting insights into the evolution of concomitant behavioral and morphological/physiological plasticity during alternative tactic evolution in threespines. However, it is unlikely that body coloration and patterning alone will be a viable proxy for identifying breeding behavior categories and sneaker tactic expression in male stickleback due to the high inter-individual variation in color combinations expressed by males within breeding categories and the presence of rapid intra-individual color/patterning plasticity.

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**Table 4.1.** Sample sizes for fish photographed in Stepan Lake, AK (2009).

Behavioral Category	Total
<b>Females</b>	
Gravid Females	21
<b>Males</b>	
Nestbuilding	20*
Sneaking	20
Courting	20
Parental (with eggs)	21
Parental (with fry)	19
	<b>121</b>

\* Sample sizes for SL and CF (condition factor) analyses include n = 19 nest-building males, one male photographed without weight measurement.

**Table 4.2.** Variable list and coding scheme descriptions used for the visual color and body patterning comparison.

Variable	Score
<b>Flank Color</b>	
Light Gold/White-Silver	1
Light Brown/Gold	2
Brown/Greenish-Gold	3
Blue/Greenish-Gold	4
Blue/Silver-Gray	5
Light Blue/White	6
<b>Flank Patterning</b>	
<b>Stripe Width</b>	
Thin stripes (on average)	1
Medium stripes (on average)	2
Thick stripes (on average)	3
<b>Stripe Regularity</b>	
Low (random widths)	1
Medium (large to small or uniform size with uneven gaps)	2
High (stripes uniform width across flank)	3
<b>Stripe Gaps</b>	
Small gaps (on average)	1
Medium gaps (on average)	2
Large gaps (on average)	3
<b>Stripe Contrast</b>	
Faint stripes (dark on dark or light on light)	1
Medium stripes	2
Dark stripes (dark on light drab or bright)	3



**Table 4.2 (cont.)**

<b>Variable</b>	<b>Score</b>
<b>Dorsum Color</b>	
Light Brown/White-Silver	1
Light Brown/Gold	2
Brown/Greenish-Gold	3
Blue/Greenish-Gold	4
Blue/Silver-Gray	5
Light Blue/White	6
<b>Ventral Color</b>	
Light Brown/White-Silver	1
Light Brown/Gold	2
Brown/Greenish-Gold	3
Blue/Greenish-Gold	4
Blue/Silver-Gray	5
Light Blue/White	6
<b>Eye Color</b>	
Light Gold/White	1
Brown/Brown-Gold Mixed	2
Mixed Brown/Gold/Blue	3
Mixed Dark Blue/Bright Blue	4
Bright Blue	5
Light Blue/White	6
<b>Throat Color</b>	
White	1
Gold-White	2
Light Pink	3
Pink-Red	4
Red/Yellow-Orange	5
Bright Red/Orange	6
<b>Throat Color Extent</b>	
Mouth/Chin only	1
Spotty coverage under Eye and on Operculum	2
High coverage under Eye and on Operculum	3

**Table 4.3.** Results of the GLM analysis investigating the relationship between breeding behavior category and SL (cm) for the 2009 data.

Source	SS	d.f.	MS	F	P	$\eta p^2$ <sup>a</sup>
Intercept	2271.561	1	2271.561	26451.138	0.000**	0.996
Breeding Behavior Category	0.981	5	0.196	2.284	0.051	0.091
Error	9.790	114	0.086			
Total	2284.002	120				
Corrected Total	10.771	119				

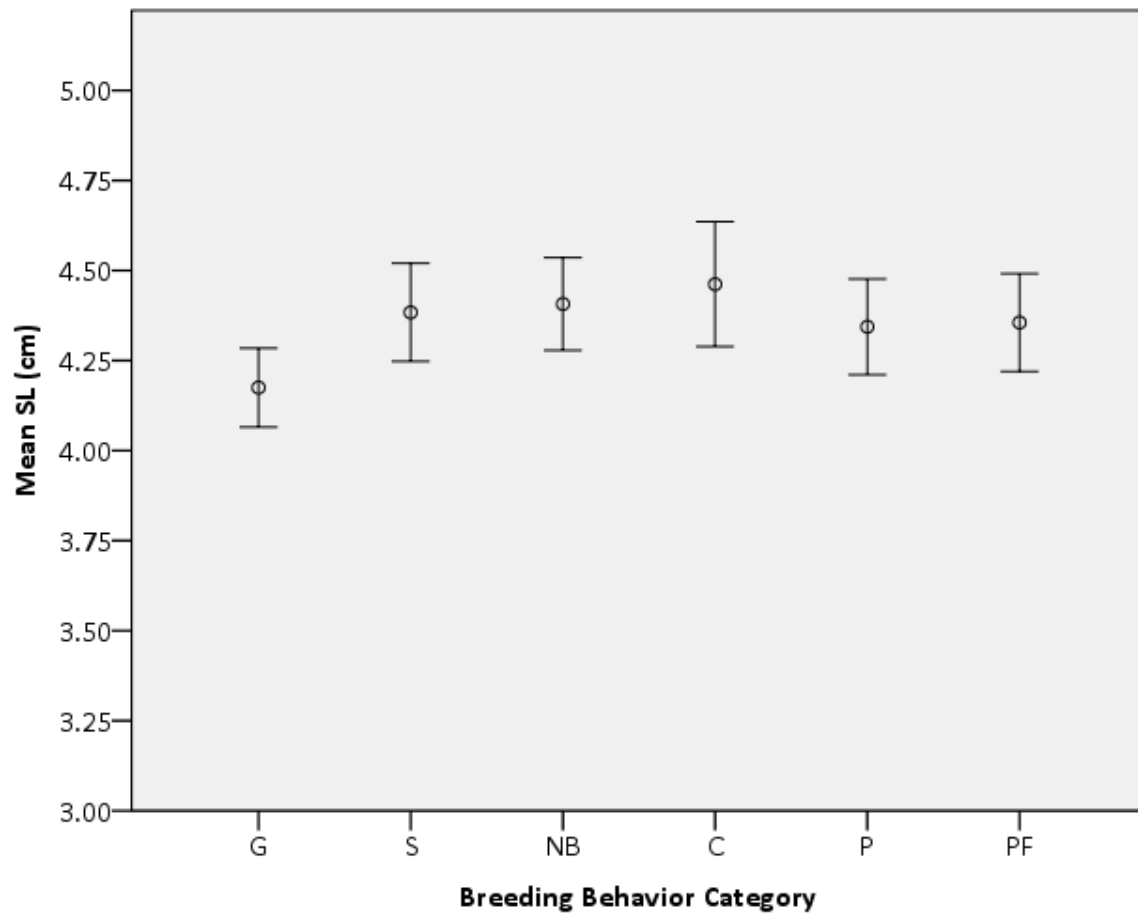
<sup>a</sup> values closer to 1 indicate a greater amount of variation accounted for by the model term  
 \* and \*\* = significant at  $p \leq 0.05$  and  $0.0001$ , respectively.

**Table 4.4.** Parameter estimates for the GLM investigating the relationship between breeding behavior category and SL (cm) for the 2009 data. Mean and standard deviation (S.D.) for SL included for each breeding behavior category.

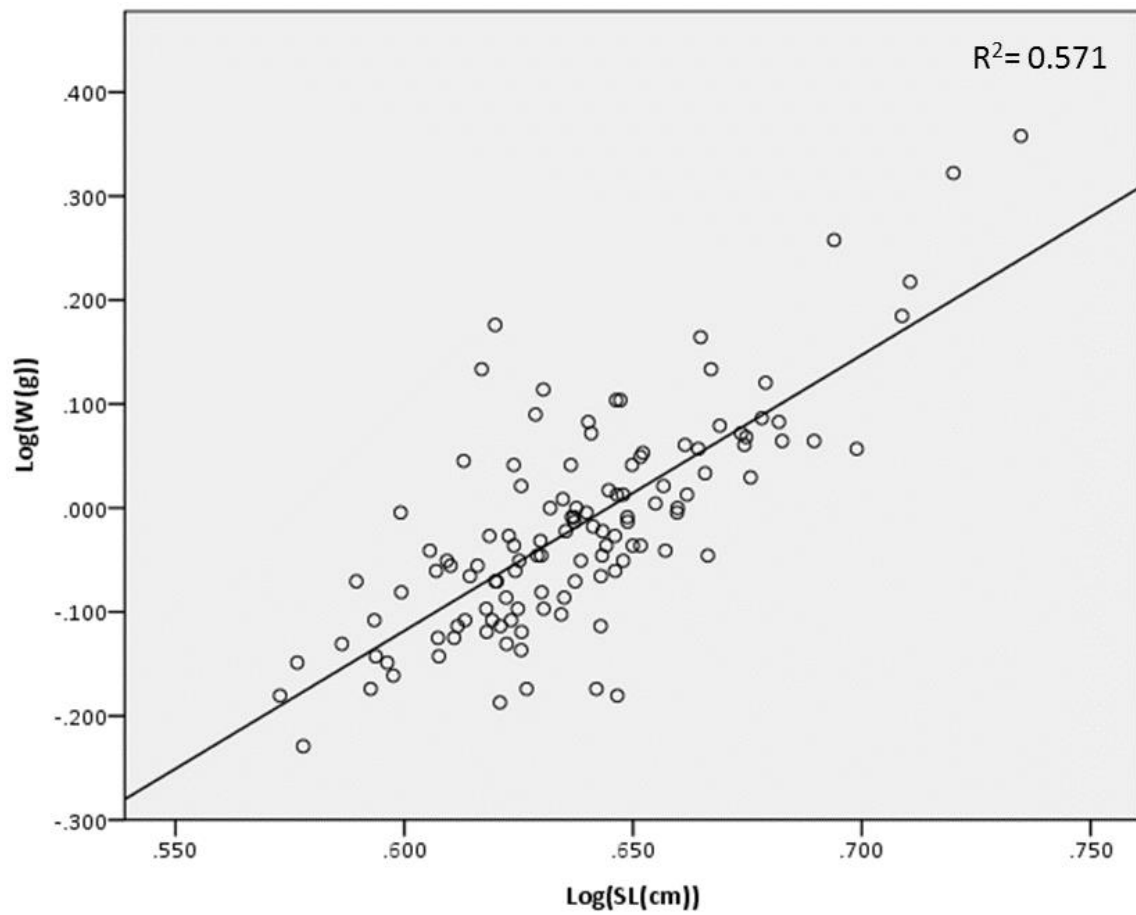
Parameter	Mean SL	S.D.	b	S.E.	t	P	95% CI		$\eta^2$ <sup>a</sup>
							Lower Bound	Upper Bound	
Intercept	.	.	4.384	0.066	66.900	0.000**	4.254	4.514	0.975
Gravid Females	4.175	0.240	-0.209	0.092	-2.283	0.024*	-0.390	-0.028	0.044
Sneaker Males	4.384	0.291	0	.	.	.	.	.	.
Nest-building Males	4.407	0.267	0.023	0.094	0.248	0.805	-0.163	0.209	0.001
Courting Males	4.462	0.371	0.078	0.093	0.843	0.401	-0.105	0.262	0.006
Parental Males w/ Eggs	4.344	0.292	-0.040	0.092	-0.438	0.662	-0.222	0.141	0.002
Parental Males w/ Fry	4.356	0.282	-0.028	0.094	-0.301	0.764	-0.214	0.158	0.001

<sup>a</sup> values closer to 1 indicate a greater amount of variation accounted for by the model term

\* and \*\* = significant at  $p \leq 0.05$  and  $0.0001$ , respectively



**Figure 4.1.** Relationship between breeding behavioral categories and mean SL (cm). Error bar = 95% CI. Breeding behavior abbreviations: G = gravid females; S = sneaker males; NB = nestbuilding males; C = courting males; P = parental males w/ eggs; PF = parental males w/ fry. Significant pair-wise differences based on Tukey's HSD indicated (\*).

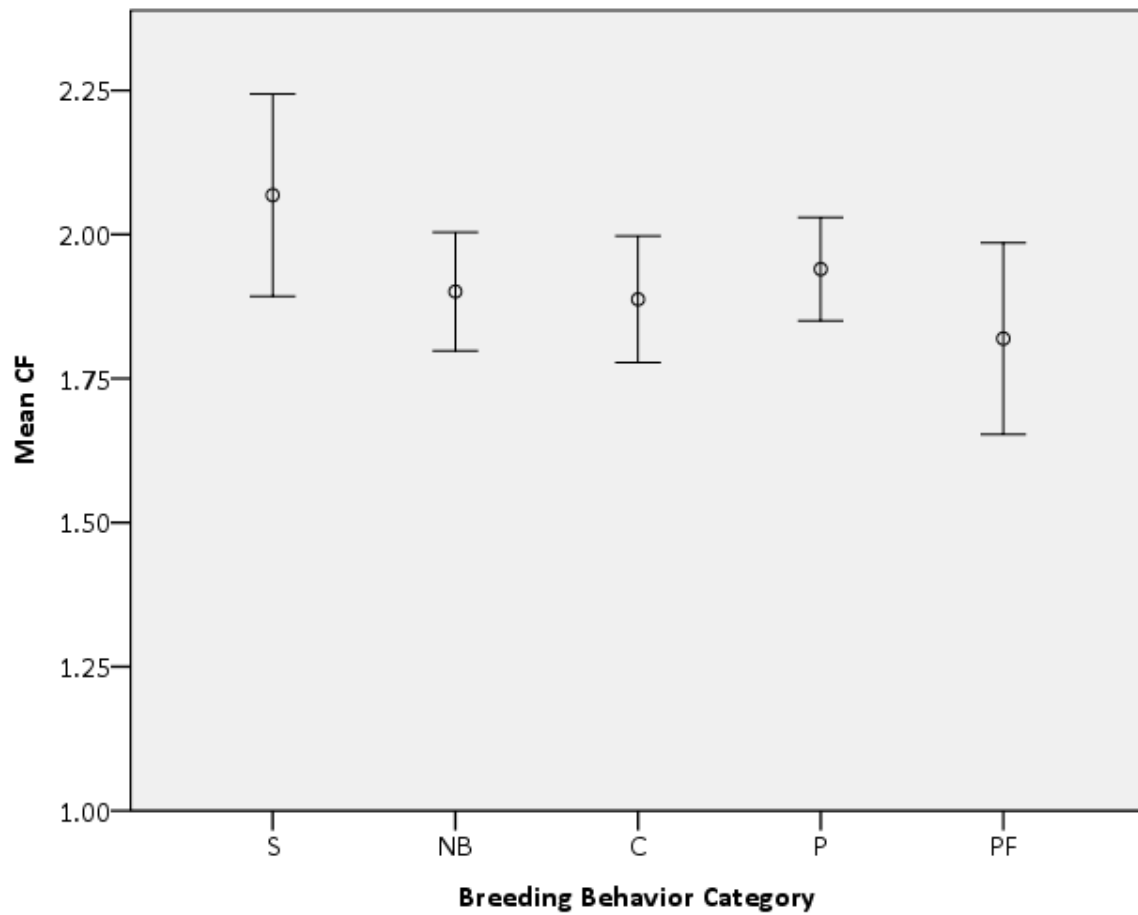


**Figure 4.2.** Relationship between log-transformed SL (cm) and weight (g). The regression coefficient from this relationship ( $b = 2.653$ ) was used to determine individual condition factor (CF).

**Table 4.5.** Results of the GLM analysis investigating the relationship between male breeding behavior category and condition factor (CF) for the 2009 data.

Source	SS	d.f.	MS	F	P
Intercept	365.655	1	365.655	4610.917	0.000**
Breeding Behavior Category	0.667	4	0.167	2.102	0.087
Error	7.454	94	0.079		
Total	374.842	99			
Corrected Total	8.121	98			

\* and \*\* = significant at  $p \leq 0.05$  and 0.0001, respectively.



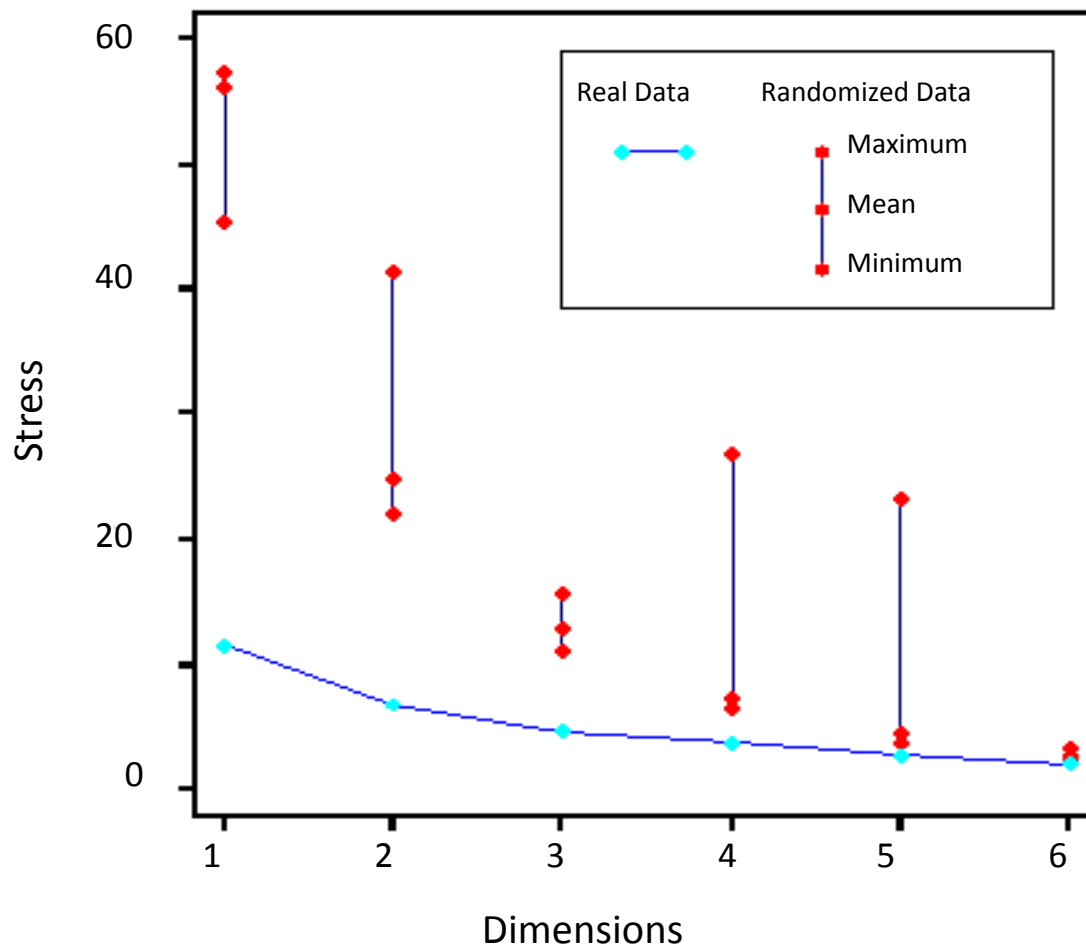
**Figure 4.3.** Relationship between male breeding behavioral categories and mean condition factor (CF). Error bar = 95% CI. Breeding behavior abbreviations: S = sneaker males; NB = nestbuilding males; C = courting males; P = parental males w/ eggs; PF = parental males w/ fry.

**Table 4.6.** The best solution for each dimensionality (1-6 axes) tested in the NMDS analysis. PC-ORD settings for each dimensionality tested: Euclidean distance measure, 400 iterations, 50 data runs, 50 random (Monte Carlo) runs, and a stability criterion of 0.00001 over the last 15 iterations. The best solution for each dimensionality is the data run with the lowest final stress. Although the final stress values for all dimensionality solutions were lower than that for 95% of the randomized runs (i.e.  $p \leq 0.05$  for the Monte Carlo test), the 2-dimensional solution (in bold) was chosen as the most appropriate solution, as the reduction in final stress was  $< 5$  for higher dimensionality (3+ axes) solutions. Results are also shown graphically in **Figure 4.4**.

Stress in real data (50 runs)				Stress in randomized data (Monte Carlo test, 50 runs)			
Axes	Minimum	Mean	Maximum	Minimum	Mean	Maximum	p <sup>a</sup>
1	11.439	46.623	57.256	45.419	56.256	57.300	< 0.05*
2	<b>6.640</b>	<b>7.601</b>	<b>8.106</b>	<b>21.980</b>	<b>24.545</b>	<b>41.367</b>	< 0.05*
3	4.822	5.005	5.481	11.031	12.971	15.683	< 0.05*
4	3.652	3.754	4.050	6.493	7.302	26.780	< 0.05*
5	2.795	2.817	3.008	3.827	4.429	23.227	< 0.05*
6	2.100	2.176	2.400	2.686	2.896	3.229	< 0.05*

<sup>a</sup>  $p$  = proportion of randomized runs with stress  $\leq$  observed stress, i.e.,  $p = (1 + \text{number of randomized runs} \leq \text{observed minimum stress}) / (1 + \text{number of randomized runs})$ . \* = significant at  $p \leq 0.05$





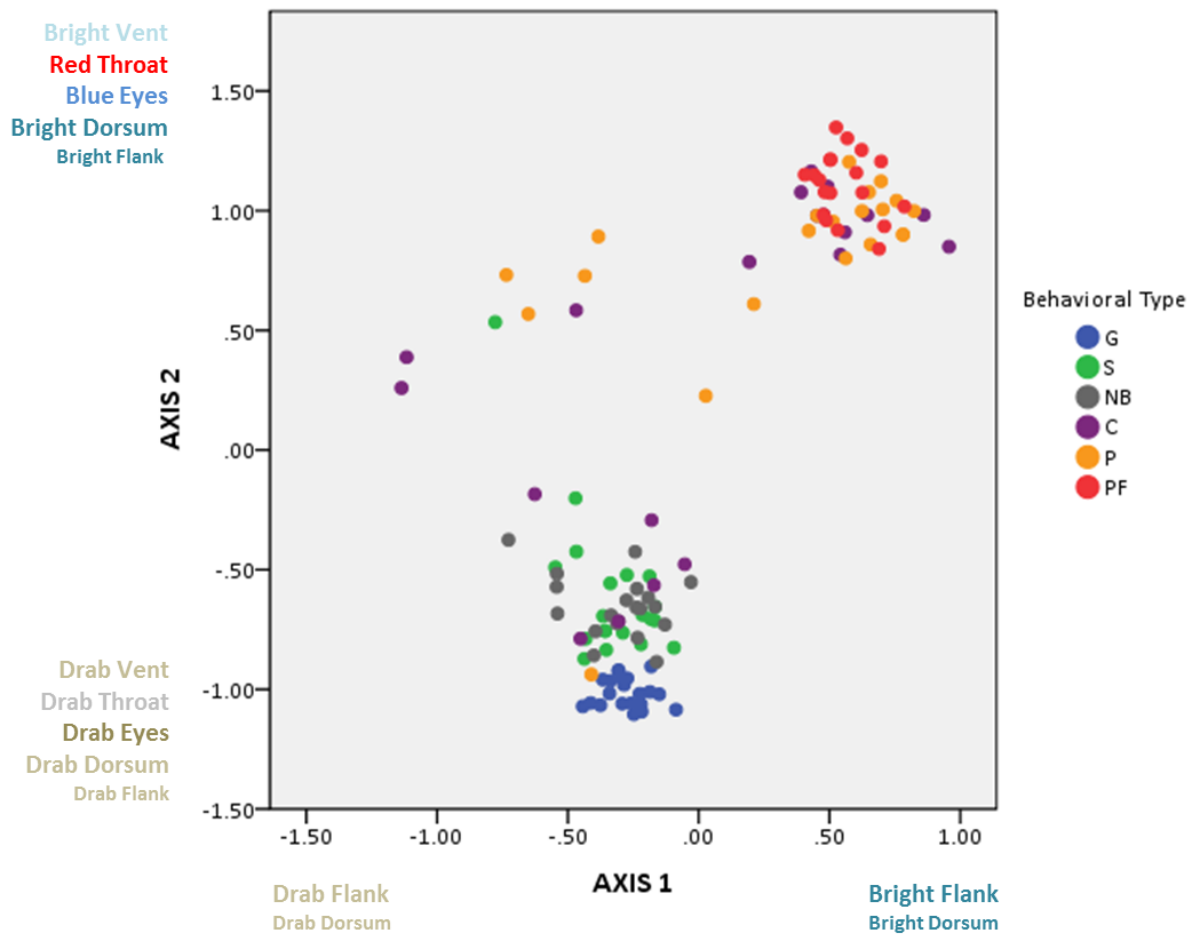
**Figure 4.4.** A scree plot showing stress as a function of dimensionality. Plotted data corresponds to minimum stress values in the real data compared to the mean, minimum, and maximum stress values in the randomized data for each dimensionality in **Table 4.6**.

**Table 4.7.** Coefficients of determination ( $r^2$ ) for the correlations between ordination distances and distances in the original matrix (n = 7260 pairs), which serves to demonstrate the quality of data reduction. The 'increment' to  $r^2$  is added as axes are added. The increment and cumulative  $r^2$  values were adjusted for any lack of orthogonality (independence) of axes.

Axis	$r^2$	
	Increment	Cumulative
1	0.648	0.648
2	0.327	0.975

**Table 4.8.** The Pearson's correlation coefficients ( $r$ ) between color/patterning variables and ordination axes. Descriptions of color/patterning variables are listed in **Table 4.2**. Variables with the highest correlation coefficients for each axis are in bold. \* denotes the variables that are secondarily correlated to the other axis.

Matrix Variable	Axis 1 $r$	Axis 2 $r$
Flank Color	<b>0.941</b>	0.873*
Flank Patterning		
Stripe Width	0.077	-0.059
Stripe Regularity	-0.084	0.004
Stripe Gaps	-0.026	-0.143
Stripe Contrast	0.166	0.396
Dorsal Color	0.850*	<b>0.859</b>
Ventral Color	0.730	<b>0.973</b>
Eye Color	0.760	<b>0.877</b>
Throat Color	0.690	<b>0.969</b>
Throat Extent	0.138	-0.278



**Figure 4.5.** The final configuration for the 2-dimensional NMDS solution specified in **Table 4.6**. The color/patterning variables that have the highest correlations with the ordination axes (**Table 4.8**) are specified along the appropriate axis. NMDS often generates weakly correlated axes – the variables that are secondarily correlated to the other axis are noted in smaller font along the ordination axes. Behavioral types: G = gravid females; S = sneaker males; NB = nestbuilding males; C = courting males; P = parental males w/ eggs; PF = parental males w/ fry.

**Table 4.9.** Average within-group distance and sample size for each breeding behavior category (group) included in the MRPP analysis. The average within-group distances are calculated from a rank-transformed Euclidean distance matrix. Abbreviations used for the breeding behavior categories (G = gravid females; S = sneaker males; NB = nest-building males; C = courting males; P = parental males w/ eggs; PF = parental males w/ fry).

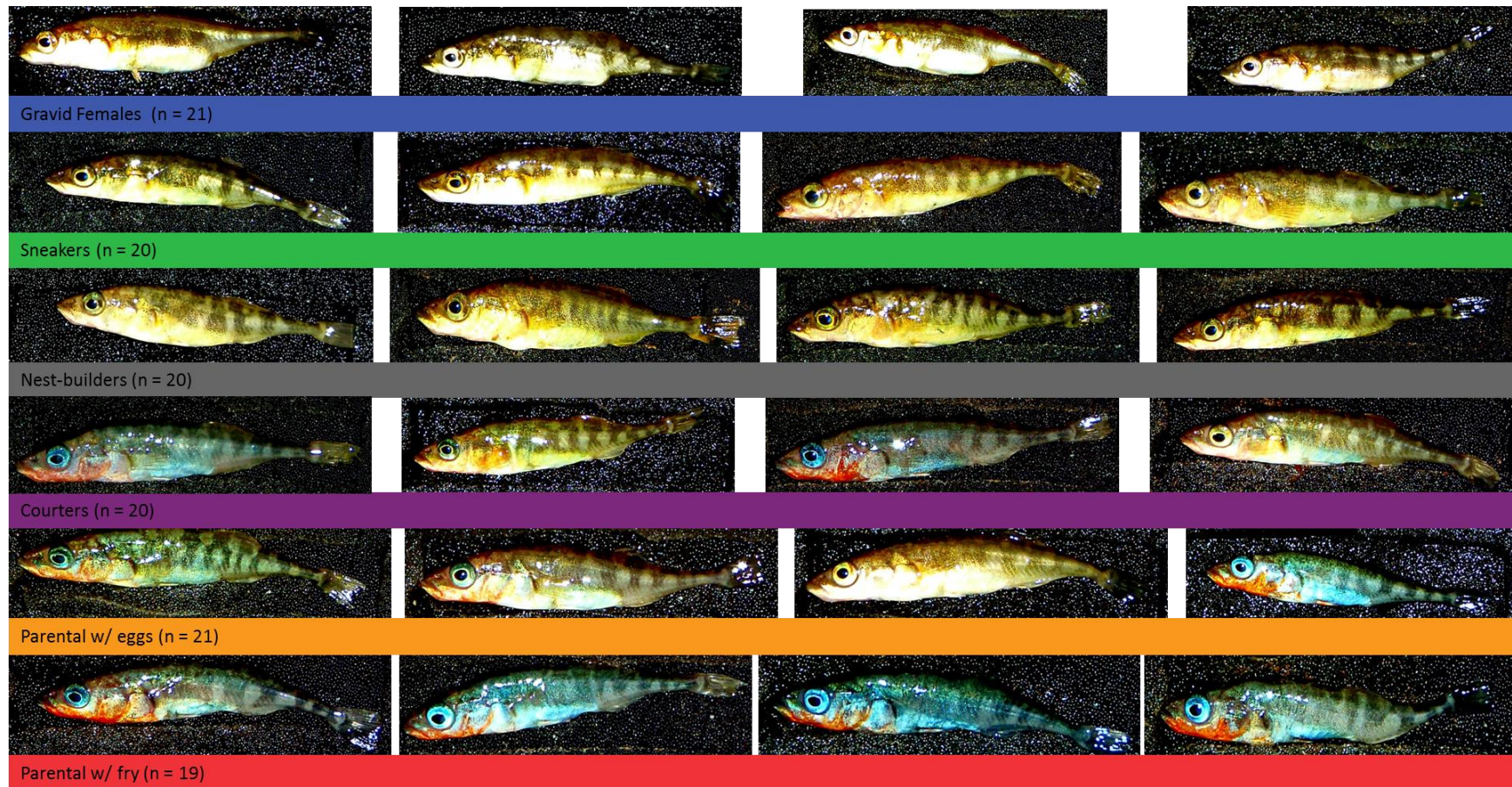
Group	n	Ranked Euclidean Distance
G	21	0.119
S	20	0.248
NB	20	0.206
C	20	0.469
P	21	0.378
PF	19	0.172

**Table 4.10.** Summary statistics for the MRPP analysis. The comparison combining all groups and the pairwise comparisons were conducted using a rank-transformed Euclidean distance matrix. Delta ( $\delta$ ) is the weighted mean within-group distance. The test statistic,  $T$ , describes the separation between groups – the more negative the value, the stronger the separation. The chance-correlated within-group agreement ( $A$ ) serves as a description of effect size ( $1 - (\text{observed } \delta / \text{expected } \delta)$ ). Abbreviations used for multiple comparisons of breeding behavior categories (G = gravid females; S = sneaker males; NB = nest-building males; C = courting males; P = parental males w/ eggs; PF = parental males w/ fry).

Comparisons	Observed $\delta$	$\delta$ under null hypothesis			$T$	$p^a$	$A^b$
		Expected	Variance	Skewness			
All groups	0.266	0.499	0.0001	-1.097	-32.035	0.0000	0.468
G vs S	0.407	0.499	0.0000	-0.947	-14.434	0.0000*	0.184
G vs NB	0.386	0.499	0.0000	-1.157	-16.239	0.0000*	0.227
G vs C	0.331	0.499	0.0001	-2.443	-20.366	0.0000*	0.338
G vs P	0.279	0.499	0.0001	-2.521	-23.896	0.0000*	0.440
G vs PF	0.257	0.499	0.0001	-2.400	-24.847	0.0000*	0.485
S vs NB	0.505	0.499	0.0000	-1.018	1.023	0.8659	-0.013
S vs C	0.390	0.499	0.0001	-2.355	-12.534	0.0000*	0.220
S vs P	0.300	0.499	0.0001	-2.504	-20.413	0.0000*	0.399
S vs PF	0.254	0.499	0.0001	-2.481	-23.702	0.0000*	0.490
NB vs C	0.386	0.499	0.0001	-2.323	-13.424	0.0000*	0.227
NB vs P	0.295	0.499	0.0001	-2.510	-21.392	0.0000*	0.410
NB vs PF	0.255	0.499	0.0001	-2.442	-24.158	0.0000*	0.489
C vs P	0.489	0.499	0.0001	-2.033	-1.323	0.0977	0.019
C vs PF	0.444	0.499	0.0000	-1.578	-8.193	0.0000*	0.109
P vs PF	0.463	0.499	0.0000	-1.191	-5.683	0.0003*	0.071

<sup>a</sup> p-value is based on numerical integration of the Pearson type III distribution of possible  $\delta$  values (which accounts for the fact that the underlying permutation distribution may be substantially skewed). A Bonferroni correction was applied to correct the error rate because of multiple comparisons ( $n = 15$  pairwise comparisons), \* =  $p \leq 0.003$ .

<sup>b</sup>  $A$  = within group homogeneity: for group comparisons,  $A = 1$  suggests groups are well-defined,  $A = 0$  suggests overlap between group, and  $A < 0$  suggests high overlap between groups



**Figure 4.6.** Examples of color variation within and across breeding behavior categories.

## APPENDIX 2A

### Descriptions of Study Populations from Alaska (AK) and British Columbia (BC)

This study includes observational data from seven oceanic and seventeen freshwater populations of Threespine Stickleback in northwestern North America -- 15 populations from the Cook Inlet region of Alaska (between 59 and 63 degrees north latitude) and 9 from southern British Columbia (between 48 and 50 degrees north latitude). GIS coordinates for study populations are provided in Table 2A.1.

Four of the Alaskan populations are oceanic, anadromous populations. The Anchor River and Beluga Slough populations are on the western side of the Kenai Peninsula near Kachemak Bay, which forms the southeastern edge of Cook Inlet, whereas the Resurrection Bay population is on the southern side of the Kenai Peninsula near Seward. Rabbit Slough is in the Matanuska-Susitna (Mat-Su) Valley north of the Knik arm of Cook Inlet. Anchor River, Beluga Slough, and Resurrection Bay individuals were observed in salt marsh tides near the inlets whereas Rabbit Slough fish were observed approximately 12 km upstream of the connection to Cook Inlet.

Ten of the freshwater Alaskan sites are in the Mat-Su Valley. Y Lake is in a separate drainage to the north of Mat-Su Valley in the Talkeetna Mountains but this lake drains into the Susitna River. Willow and Lynne lakes are located near the confluence of Willow Creek and the Susitna River, approximately 65 km inland from the river's junction with Cook Inlet. Willow Lake is connected to Willow Creek via a short, direct tributary. Lynne Lake is located in the upper portion of a different Willow Creek tributary. At least 25 stream km and seven intervening lakes separate these populations. Big Beaver Lake is located on a tributary to the Meadow Creek system approximately 22 km (straight line) southeast of Lynne Lake. Meadow Creek is part of the Big Lake – Fish Creek system which enters Cook Inlet separately from the Susitna River. Stepan Lake is located on a different tributary to Meadow Creek and is separated from



Big Beaver by several km of stream length. Meadow Creek drains into Big Lake. Observations were collected from both Big Lake and the Fish Creek region of Big Lake (referred to as the Big Lake Creek site in this study), which is upstream from the connection to the Knik Arm by approximately 18 km. Stephan Lake is located approximately 2.5 km south of Big Lake and is separated by several lakes and streams. Whale Lake is 4 km east of Big Lake and has no inlet or outlet. About 20 stream km and six lakes separate Big Beaver Lake and the last two study sites found to the east in the Meadow Lakes region -- Bruce Lake and Beverly Lake. Like Whale Lake, Bruce Lake has no inlet or outlet. Beverly Lake has no inlet and only a temporary outlet to Meadow Creek under high flood conditions. Nine of the lakes range in size from 8-113 ha, while Big Lake is 1,009 ha in surface area.

Two of the British Columbia oceanic populations, Buccaneer Marina (near Secret Cove) and Francis Peninsula Lagoon (near Madeira Park), are located on the Sechelt Peninsula -- part of the mainland east of Vancouver Island. These sites are separated by 10 km. The third oceanic site, Swy-A-Lana, is found on the east side of Vancouver Island near Nanaimo, which is across the Strait of Georgia from the Sechelt Peninsula. All are located in shallow lagoons with significant exchange of ocean water.

Sproat (3,775 ha), Cowichan (6,204 ha), and Crystal (21 ha) Lakes are on Vancouver Island in disjunct river drainages. Hotel, Garden Bay and North Lakes are on the Sechelt Peninsula across the Strait of Georgia. Hotel and Garden Bay Lakes are connected by a stream approximately 0.33 km in length but are divergent in behavior and morphology (Foster 1994a,b, 1995; Foster *et al.* 1998). They both are connected to North Lake through creeks and lakes draining a higher elevation lake (Klein Lake) in two directions. These lakes range from 8-23 ha in surface area.

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**Table 2A.1.** Approximate GIS coordinates for study populations (shown in decimal degrees; Google Earth v4.3.7).

<b>Populations</b>	<b>Latitude</b>	<b>Longitude</b>
<b>Oceanic, Alaska</b>		
Anchor River	59.780217	-151.836984
Beluga Slough	59.639292	-151.522173
Rabbit Slough	61.535528	-149.244251
Resurrection Bay	60.128074	-149.423625
<b>Freshwater, Alaska</b>		
Beverly Lake	61.614044	-149.572928
Big Beaver Lake	61.586595	-149.824333
Big Lake	51.541908	-149.825556
Big Lake Creek	61.535178	-149.825765
Bruce Lake	61.608924	-149.547687
Lynne Lake	61.712441	-150.041318
Stepan Lake	61.574679	-149.827130
Stephan Lake	61.486272	-149.937330
Whale Lake	61.529426	-149.750326
Willow Lake	61.740250	-150.059232
Y Lake	62.303577	-150.066160
<b>Oceanic, Canada</b>		
Buccaneer Marina	49.533540	-123.953225
Francis Peninsula Lag.	49.612478	-124.030517
Swy-A-Lana	49.165042	-123.936066
<b>Freshwater, Canada</b>		
Cowichan Lake	48.826577	-124.072971
Crystal Lake	49.055908	-123.949294
Garden Bay Lake	49.645012	-123.999479
Hotel Lake	49.637006	-124.042136
North Lake	49.751211	-123.971510
Sproat Lake	49.266044	-124.980118

**APPENDIX 2B.** All categories of sneaking observation frequencies for all populations and years. Range of observation dates and estimated hours of observation for each year included (total obs hrs = number of observers x average number of hours per observer x number of observation days). Values in the courtship columns show the number of courtships in which sneaking did, and did not occur. Value in parentheses is the proportion of observations involving sneaking. "**All Complete Courtships**" shows all courtships in which the male showed the nest entrance to the female. "**1 Complete Courtship/Male**" is a subset of the data in which a single courtship was selected at random for any males for whom we had multiple observations. The column headed "**All Incomplete Courtship Attempts**" includes the frequency of sneaking that occurred during courtship attempts in which either the male or female aborted an active courtship before nest showing. The column headed "All Courtship Attempts" includes the frequency of sneaking that occurred during all courtship attempts (i.e. complete courtships + incomplete courtships). "**In/Outside Courtship**" gives the total number of sneaker observations during courtship versus outside courtship (i.e. sneaks on other territorial male behaviors).

APPENDIX 2B.

Populations	Obs Dates	Total Obs Hrs	All Complete Courtships	1 Complete Courtship/Male	All Incomplete Courtships	All Courtship Attempts	# Sneak Attempts (In/Outside Courtship)
<b>Oceanic, Alaska</b>							
<b>Anchor River</b>							
1992	7/3	8	0/11 (0)	0/9 (0)	0/4 (0)	0/15 (0)	0/1
1995	5/20-5/22	24	4/12 (0.33)	4/11 (0.36)	0/7 (0)	4/19 (0.21)	4/1
<b>Beluga Slough</b>							
1994	7/3	12	1/9 (0.11)	1/7 (0.14)	N/A	N/A	N/A
<b>Rabbit Slough</b>							
1992	6/23	12	1/7 (0.14)	1/7 (0.14)	N/A	N/A	N/A
2006	6/6	2	0/1 (0)	0/1 (0)	2/3 (0.67)	2/4 (0.50)	2/0
2009	6/29-7/6	16	0/6 (0)	0/6 (0)	3/24 (0.13)	3/30 (0.10)	3/6
<b>Resurrection Bay</b>							
2009	6/30	8	3/5 (0.60)	2/3 (0.67)	0/26 (0)	3/31 (0.10)	3/1
<b>Freshwater, Alaska</b>							
<b>Beverly Lake</b>							
2008	6/19-7/8	88	2/12 (0.17)	1/7 (0.14)	6/28 (0.21)	8/40 (0.20)	8/41
<b>Big Beaver Lake</b>							
1994	6/24-6/27	12	2/24 (0.08)	2/21 (10)	N/A	N/A	N/A
2009	6/18-7/9	32	3/24 (0.13)	2/13 (0.15)	5/43 (0.12)	8/67 (0.12)	8/24

APPENDIX 2B (cont.)

Populations	Obs Dates	Total Obs Hrs	All Complete Courtships	1 Complete Courtship/Male	All Incomplete Courtships	All Courtship Attempts	# Sneak Attempts (In/Outside Courtship)
<b>Freshwater, Alaska</b>							
<b>Big Lake</b>							
1992	6/11-6/30	30	5/16 (0.31)	4/14 (0.29)	2/8 (0.25)	7/24 (0.29)	7/4
<b>Big Lake Creek</b>							
2008	6/13-6/20	8	0/1 (0)	0/1 (0)	0/6 (0)	0/7 (0)	0/2
2009	6/18-7/3	44	1/17 (0.06)	1/10 (0.10)	3/18 (0.17)	4/35 (0.11)	4/26
<b>Bruce Lake</b>							
1993	5/22-7/2	200	1/29 (0.03)	1/24 (0.04)	N/A	N/A	N/A
<b>Lynne Lake</b>							
1994	6/15-6/17	18	1/17 (0.06)	1/16 (0.06)	0/6 (0)	1/23 (0.04)	1/1
<b>Stepan Lake</b>							
1992	6/1-6/22	220	18/54 (0.33)	14/40 (0.35)	5/24 (0.21)	23/78 (0.29)	23/77
2006	6/7-6/14	4	2/6 (0.33)	2/5 (0.40)	0/2 (0)	2/8 (0.25)	2/4
2008	6/20-7/7	72	4/12 (0.33)	4/11 (0.36)	1/3 (0.33)	5/15 (0.33)	5/81
2009	6/21/7/7	56	2/4 (0.50)	2/4 (0.50)	3/9 (0.33)	5/13 (0.38)	5/80
<b>Stephan Lake</b>							
1992	5/21-5/31	40	4/46 (0.09)	4/34 (0.12)	4/14 (0.29)	8/60 (0.13)	8/4
<b>Whale Lake</b>							
1993	5/22-7/2	200	0/35 (0)	0/18 (0)	N/A	N/A	N/A

APPENDIX 2B (cont.)

Populations	Obs Dates	Total Obs Hrs	All Complete Courtships	1 Complete Courtship/Male	All Incomplete Courtships	All Courtship Attempts	# Sneak Attempts (In/Outside Courtship)
<b>Freshwater, Alaska</b>							
<b>Willow Lake</b>							
1992	5/20-7/1	200	1/24 (0.04)	1/20 (0.05)	N/A	N/A	N/A
2009	6/17-6/19	8	0/1 (0)	0/1 (0)	0/11 (0)	0/12 (0)	0/2
<b>Y Lake</b>							
1992	6/12-7/8	40	3/18 (0.17)	3/17 (0.18)	7/8 (0.88)	10/26 (0.38)	10/17
2009	7/8	5	0/1 (0)	0/1 (0)	0/5 (0)	0/6 (0)	0/8
<b>Oceanic, Canada</b>							
<b>Buccaneer Marina</b>							
1993	6/12-6/15	12	2/21 (0.1)	2/20 (0.10)	N/A	N/A	2/0
<b>Francis Peninsula Lag.</b>							
1997	6/12	12	1/13 (0.08)	1/13 (0.08)	N/A	N/A	1/0
2008	6/1-6/4	16	3/8 (0.38)	3/8 (0.38)	1/10 (0.10)	4/18 (0.22)	4/3
2009	5/24-6/9	90	20/102 (0.20)	11/30 (0.37)	17/142 (0.12)	37/244 (0.15)	37/61
<b>Swy-A-Lana</b>							
1996	6/17-6/18	12	0/14 (0)	0/14 (0)	N/A	N/A	N/A

APPENDIX 2B (cont.)

Populations	Obs Dates	Total Obs Hrs	All Complete Courtships	1 Complete Courtship/Male	All Incomplete Courtships	All Courtship Attempts	# Sneak Attempts (In/Outside Courtship)
<b>Freshwater, Canada</b>							
<b>Cowichan Lake</b>							
1989	5/14-6/26	12+	0/20 (0)	0/19 (0)	N/A	N/A	0/0
<b>Crystal Lake</b>							
1985	5/15-8/1	700	0/72 (0)	0/24 (0)	N/A	N/A	0/0
<b>Garden Bay Lake</b>							
1986	5/15-7/8	400	0/25 (0)	0/21 (0)	N/A	N/A	0/0
<b>Hotel Lake</b>							
1989	5/14-6/26	12+	0/27 (0)	0/16 (0)	N/A	N/A	0/0
2008	6/4-6/10	8	0/0 (0)	0/0 (0)	0/6 (0)	0/6 (0)	0/0
2009	5/28-6/8	56	0/25 (0)	0/18 (0)	0/67 (0)	0/92 (0)	0/0
<b>North Lake</b>							
1989	5/14-6/26	12+	0/19 (0)	0/16 (0)	N/A	N/A	0/0
2009	6/3-6/7	24	0/6 (0)	0/5 (0)	0/6 (0)	0/12 (0)	0/0
<b>Sproat Lake</b>							
1989	5/14-6/26	12+	0/161 (0)	0/60 (0)	N/A	N/A	0/0



## APPENDIX 2C

### Stickleback Behavior: Have We Seen It All?

**Objectives:** Describe the use of behavioral discovery curves (e.g. cumulative new behaviors observed per minute of observation time) to compare the timing of specific behaviors across stickleback populations. Such comparisons can be especially useful for investigating the effect of sampling effort on the observation of potentially rare or absent behaviors, such as sneaking.

When investigating the presence or absence of a behavior within a population it is important to discern how much observation time is sufficient to confidently assess whether a behavior is absent or at least rare enough to be an insignificant component of a population's behavioral repertoire. This dilemma is even harder to reconcile when the behavior of interest is putatively rare, such that attempting to verify absence evokes a Popper-esque conundrum (Popper 1963). Considering we cannot spend an infinite number of observation hours with a study organism, there may be several different approaches that can be combined to lend support to the conclusion that a behavior is sufficiently rare enough to be biologically insignificant, at least in terms of affecting the behavior of other individuals or being subject to selective pressures on an evolutionary timescale.

Why study seemingly biologically insignificant behaviors in populations? Generally speaking, spending many observation hours to study a rare behavior in a repertoire may not be a useful allocation of time and effort. However, when behaviors vary in their presence and perceived absence across

populations it may be worth investigating the extent of this geographic variation in presence/absence – especially if the behavior of interest may have a viable impact on survival, mate choice, or reproductive success (e.g. Foster 1994b, 2013). Sneaking behavior is an alternative reproductive tactic performed by male stickleback that may have important consequences for population dynamics, mate choice, and reproductive success (Sugg & Chesser 1994; Magurran 1996, 1998; Jones *et al.* 2001; Lurgiader *et al.* 2001; Le Comber 2003; Shuster & Wade 2003; Juanes *et al.* 2007; Cornwallis & Uller 2010; Morris *et al.* 2010). Geographic variation in the presence/absence and propensity to perform sneaking behavior has been documented in Pacific Northwest stickleback populations (Foster *et al.* 1996; Chapter 2). In populations in which sneaking is rare or seemingly absent, it is important to be confident that such findings are unlikely to be artifacts of insufficient sampling effort. Here I discuss methodological approaches based on behavioral discovery curves (Jule, Lea & Leaver 2009), which are utilized to assess the relationship between the presence/absence of sneaking and sampling effort, and to provide general information regarding geographic variation in the territorial male behavioral repertoire of Pacific Northwest Threespine Stickleback.

Tracking the accumulation of new behaviors across timed observations intervals can be useful in determining the extent of the behavioral repertoire for a species, as it indicates the probability of observing new behaviors with increased observation time (Jule, Lea & Leaver 2009). Other studies have employed the idea of species accumulation curves (Gotelli & Colwell 2001) to determine how observations of new behaviors relate to observation effort by using a cumulative measure of observation effort (i.e. behavioral accumulation curves, Dias *et al.* 2009) and similar techniques that standardize sample sizes (i.e. rarefaction techniques, Peshek & Blumstein 2011).

While these behavioral curves are invaluable to the formation of ethograms for new study organisms, they can also be used for interpopulation comparisons in species for which detailed

ethograms or descriptions of behavioral repertoires already exist. For example, Figure 2C.1a compares two hypothetical populations with different saturation points (i.e. high vs low number of behaviors in repertoire), suggesting that within the given period of observation time, a certain set of behaviors are lacking in the population with the lower saturation point. Depending on the extent of observation effort, this pattern may imply that these behaviors are rarer, if not absent, in the population with the lower saturation point. Figure 2C.1b shows behavior curves with different slopes, suggesting that the number of behaviors accumulates faster in one population compared to the other. If data are collected in a systematically similar way across populations (which might not always be possible, see below), differences in behavior accumulation may suggest differences in general activity levels between populations or a large effect of individual variation in behavior. In all of the above scenarios, further investigation would be necessary to discern specific population-level differences but behavior accumulation curves can provide a visual representation of the behavioral repertoire that may lead to investigations of population-level patterns of variation in behavioral repertoires. For instance, a lower saturation point for a population may be indicative of individuals performing less aggressive behaviors in a given population.

To investigate the extent to which the presence/absence of sneaking alone or of multiple behaviors within the general male stickleback repertoire varied across populations, behavioral discovery curves were calculated and compared across several sneaking/nonsneaking populations. Timing of sneaking and several sneaking-related behaviors (e.g. courtship) during the observation periods was compared across populations in an effort to determine whether sampling effort was satisfactory based on population activity levels.

## METHODS

Published descriptions (e.g. Wootton 1976, Rowland 1994 for reviews; also Foster 1994; Foster *et al.* 2008), plus *in situ* behavioral observations of courtship behavioral patterns and the general behavioral repertoire of breeding threespine males allowed for the creation of a basic ethogram of typical breeding male behaviors (reactions to conspecific and intruder encounters, territorial and nest activities, courting, and sneaking; n = 48 behaviors). Field observations on groups of males were collected in a similar fashion in several populations during the 2009 breeding season – Francis Peninsula Lagoon (an anadromous British Columbia population with 90 total observation hrs – 45 hrs (2700 mins) per observer), Hotel Lake (a freshwater British Columbia population with 56 total observation hrs – 28 hrs (1680 mins) per observer), North Lake (a freshwater British Columbia population with 24 total observation hrs – 12 hrs (720 mins) per observer), Rabbit Slough (an anadromous Alaskan population with 16 total observation hrs – 8 hrs (480 mins) per observer), and Stepan Lake (a freshwater Alaskan population with 56 total observation hrs – 28 hrs (1680 mins) per observer). Francis Peninsula, Rabbit Slough, and Stepan Lake represent populations in which sneaking behavior is present (observed in 15%, 10%, and 38% of all courtship attempts, respectively; Appendix 2B). Hotel Lake and North Lake represent populations in which sneaking behavior has not been observed (despite multiple years of *in situ* behavioral observations, Appendix 2B).

Since stickleback males often nest in groups, behavioral observations can be made on several individuals simultaneously. This can result in quicker approximation of the complete behavioral repertoire, given that the number of behaviors observed during an observation periods can increase more rapidly when several individuals are observed simultaneously (Jule, Lea & Leaver 2009). In each population, 3-min spot observations were made on several territorial males simultaneously (average group size of 2-3 individuals per spot observation). These spot observations on male groups were made

in consecutive rounds during each daily observation period, such that a single male was observed an average of 9 mins each observation day. For each population, two observers made observations in separate areas during the same daily observation period, each observing a range of 4-8 groups of males in consecutive rounds of 3-min spot observations on any given observation day. For a visual comparison of intra-population variability in rates of behavioral discovery, data from the two observers are considered separately.

For each observer, the first occurrence of a behavior in the ethogram was recorded starting at the first 3-min spot observations and, continuing at three minute intervals, first occurrences were counted until no new behaviors were identified. Behaviors not initially incorporated in the ethogram were also considered but the resulting accumulation curves demonstrated that even the behavior discovery curves with the greatest behavior repertoire (Francis Peninsula Lagoon,  $n = 47$  behaviors) was still less than the number of potential territorial male behaviors included in the initial ethogram ( $n = 48$  behaviors). The last new behavior for any of the behavioral curves was observed at 774 mins (~13 hrs), although the majority of behavioral curves saturated at much earlier times. Observations in all populations discussed in this paper were made during the 2009 breeding season, which typically begins mid-May in British Columbia populations (Francis Peninsula Lagoon 5/24-6/9, Hotel Lake 5/28-6/8, North Lake 6/3-6/7) and early to mid-June in Alaskan populations (Rabbit Slough 6/29-7/6; Stepan Lake 6/21-7/7). Most populations were well into the breeding season during observations, such that males of different behavioral stages (e.g. nest-building, courting, or parental care) were often observed simultaneously during the 3-min periods. However, observations at Francis Peninsula Lagoon began early enough in the breeding season that males were just arriving at the spawning grounds and were highly synchronous in their behavioral stage. Potential effects of these temporal differences in data collection are discussed below.

In order to provide a statistical test of the similarities between the behavioral discovery curves within populations (observer 1 vs observer 2) and among populations, Kolmogorov-Smirnov two-sample tests were applied. This nonparametric test assesses differences between two distributions under the null hypothesis that the two distributions are identical. The maximum vertical deviation between the relative cumulative frequency distributions is used as the test statistic (D). This test can be useful in a comparison between curves because it is sensitive to differences in location, dispersion, and skewness (Sokal & Rohlf 1995).

## RESULTS

Table 2C.1 lists the time of the first observation for several behaviors of interest, including complete courtship repertoires (involves nest showing), incomplete courtship repertoires (involves participation by both male and female but aborted before nest showing), and sneaker attempts on various territorial male activities for each population. Sneak fertilization attempts may occur during both types of courtships (Appendix 2B), thus the earliest instance of either type of courtship provides a general idea of how early in the total observation time evidence of the sneaker repertoire is possible. The time of first observation of sneaking behavior outside of courtship (e.g. in response to nest activity or territorial behavior by males) is also shown because the sneaker repertoire directed towards territorial males is often observed outside of courtship in many populations exhibiting this behavior (Chapter 2; Appendix 2B). Comparison of the time to first courtship observation (either complete or incomplete) across the populations suggests that such events are easily observable in the British Columbia freshwater populations (Table 2C.1, 9 mins in Hotel Lake and 3 mins in North Lake). Courtship events took slightly longer to observe in the other populations (Table 2C.1, 24 mins in Francis Peninsula Lagoon, 39 mins in

Rabbit Slough, and 54 mins in Stepan Lake). Time to observation of the sneaker repertoire ranged from 6 mins to 543 mins for the populations in which sneaking was observed – the earliest observation of sneaking behavior was 39 mins in Francis Peninsula, 66 minutes in Rabbit Slough, and 6 mins in Stepan Lake (Table 2C.1). No observations of behavioral components associated with the sneaker repertoire were observed in Hotel Lake or North Lake within the total observation time for each observer.

Table 2C.1 also provides info on the time of the first successful sneak fertilization attempt. This behavior occurred during only one observational period for a single observer in Francis Peninsula Lagoon and the first observation of this behavior did not occur until 522 mins (ca. 9 hrs) into the observation time period. The difference in timing between observation of sneaking behavior and successful attempts suggests that success of this alternative reproductive tactic may be rare relative to the number of attempts made. Assuming a constant frequency for the success of this tactic within the Francis Peninsula Lagoon population, a total of 10 sneak fertilization successes are expected to be observed within a 90-hr total observation period, however, the actual data suggest an occurrence of only 3 sneak fertilization successes within that total observational time, suggesting a success rate lower than expected.

Figure 2C.2 provides a visual comparison of the behavioral discovery curves. The first observation of both courting (white arrows) and sneaking (black arrows) are denoted on each curve. For courtship, this behavior is observed early in the observation period across all populations, typically before the behaviors accumulate to the point of diminishing returns. The same is true of sneaking behavior in both Francis Peninsula and Stepan Lake. Sneaking in Rabbit Slough is observed closer to the point of behavior saturation (Figure 2C.2). The curves begin to level off between 200 and 400 mins for Hotel Lake, North Lake, Rabbit Slough, and Stepan Lake but not until between 500 and 700 mins for Francis Peninsula Lagoon. Differences in the inflection points between observer curves (observer 1 vs observer 2) within populations are mostly due to variation in the observation of infrequent territorial

activities (e.g. circle fight) and slight variations in observer interpretation of behavior and choice of descriptive terms (e.g. push at nest vs dig in nest).

Figure 2C.2 also demonstrates that the curves for the populations level out at different cumulative behaviors. For the most part, the differences in total observed behaviors between populations are due to the absence of either all the categories, as is the case with Hotel Lake and North Lake, or some of the categories, as is the case with Rabbit Slough and Stepan Lake, associated with the sneaker repertoire. Otherwise, very few missing behaviors from the original ethogram overlap across populations and include various interaction behaviors or courtship and nest activity behaviors.

Results of the Kolmogorov-Smirnov two-sample test (Sokal & Rolf 1995) revealed no significant difference between observer curves for all populations (Francis Peninsula Lagoon:  $D = 0.006 < D_{0.05} = 0.285$ ; Hotel Lake:  $D = 0.009 < D_{0.05} = 0.316$ ; North Lake:  $D = 0.042 < D_{0.05} = 0.345$ ; Rabbit Slough:  $D = 0.044 < D_{0.05} = 0.340$ ; Stepan Lake:  $D = 0.016 < D_{0.05} = 0.314$ ). This suggests multiple observers using the same data collection techniques experience similar saturation points and rates of behavioral accumulation despite the potential for spatial heterogeneity in behavior or observer visibility.

## DISCUSSION

Given the overall similarity in behavioral discovery curves (e.g. cumulative new behaviors observed per minute of observation time) for populations in which sneaking has and has not been observed, territorial male behavioral repertoires are very similar in terms of the types of territorial male behaviors expressed. The slopes of the behavior accumulation curves in Figure 2C.2 are visually similar, indicating that the general activity rates are similar across populations that did or did not exhibit sneaking during the observation periods. Similarities in rates of behavior accumulation between the sneaker and non-



sneaker populations suggest that it is not a lack of activity in general that is likely to prevent sneaking expression in Hotel Lake or North Lake or a lack of activity that may increase the likelihood of sneaking in the other populations exhibiting sneak behaviors.

The absence of sneaking expression in Hotel and North are also unlikely to be due to a lack of opportunity – the presence of courtship behavior occurs early in observations for both populations. In addition to this, the sneaker and nonsneaker populations included in this analysis generally demonstrate no bias or clear pattern in the total number of all courtship behaviors (i.e. complete and incomplete) observed across the observation periods (Appendix 2B; estimated frequencies for courtship behaviors in sneaking populations: Francis Peninsula Lagoon = 2.7/hr, Rabbit Slough = 1.9/hr, and Stepan Lake = 0.2/hr; estimated frequencies for courtship behaviors in nonsneaking populations: Hotel Lake = 1.6/hr, North Lake = 0.5/hr).

The timing of initial sneaker observations is generally early in the cumulative behavioral discovery curves for sneaking populations (Figure 2C.2), suggesting that with the sampling effort used in the BC freshwater populations, sneaking would likely have been observed if it were a typical part of the territorial male behavioral repertoire in these populations. Hotel Lake, in particular, was subject to the same total observational time period as Stepan Lake. Given the early occurrence of sneaking outside of courtship in Stepan Lake, a similar occurrence might be predicted in Hotel Lake. The lack of such observations – along with the fact that the territorial male behavioral repertoire levels out between 200-400 mins despite 28 total observational hours per observer – suggests that the sneaker repertoire is very unlikely to be a significant component of the male behavioral repertoire in Hotel Lake.

Important considerations must be made regarding the potential limitations of using behavioral discovery curves in comparative studies. Consistency in data collection methods is very important. It is best to compare populations for which similar observation periods were used because there is a greater

chance that individual variation in behavioral repertoires will affect the rate of behavioral accumulation when observation time per individual is longer. A similar argument can be made for group size – the number of individuals observed simultaneously during a given observation period will affect the rate of behavioral accumulation (Jule, Lea & Leaver 2009). It may be difficult to compare behavioral accumulation rates among stickleback populations that exhibit large differences in inter-nest distances, as greater inter-nest distances typically limits how many individuals can be observed simultaneously. For stickleback in particular, group composition may also affect the rate of behavior discovery. In Figure 2C.2, the rate of behavior accumulation and the point of saturation are slightly drawn out in Francis Peninsula Lagoon compared to the other populations. This may be due to the large number of newly territorial males in transects of both observers at the beginning of the observation time. Observations on these homogenous groups of males may have slowed the rate of behavioral accumulation as they synchronously moved between the breeding behavioral stages (i.e. territorial nesting, courting, and parental) and exhibited different categories of territorial male behavior. Generally, quick saturation times for breeding behavioral curves may be expected for stickleback populations in which individual male breeding cycles are typically asynchronous (e.g. newly nested males nesting close to parental or courting males).

It is also important that the types of behaviors recorded are consistent and the terminology uniform during observations across populations so that the resulting curves are not biased by inclusion or exclusion of specific behaviors for different populations. The choice to be specific or general in the behavioral categories utilized may simply be a matter of the comparison sought (the specificity of the behavioral categories used will affect the slope of the discovery curve: broad categories, e.g. chase, can potentially result in a faster rate of accumulation in the curve than narrower categories, e.g. chase neighbor vs chase female vs chase juvenile).

If the environment in which observations are performed is highly heterogeneous, multiple observers in different areas may be useful to increase the probability that the best estimate of the total repertoire is obtained for a given population. For instance, the same methods of field data collection and behavioral discovery curve data collection were performed in Francis Peninsula Lagoon and Rabbit Slough as those for the freshwater populations, however, total observed behaviors for observer 1 saturate at a lower number than those for observer 2. In the case of Rabbit Slough, observer 1 collected shoreline observations in an area with high algae cover. Males in this area were highly aggregated but nesting under clumps of floating algae, such that many courtship and nest-related behaviors were obstructed. For the most part, the clearer observational area of observer 2 resulted in observation of a number of behaviors missed by observer 1. Similar trends in Francis Peninsula Lagoon may have been due to differences in rock cover for nests in the areas assigned to observer 1 and 2.

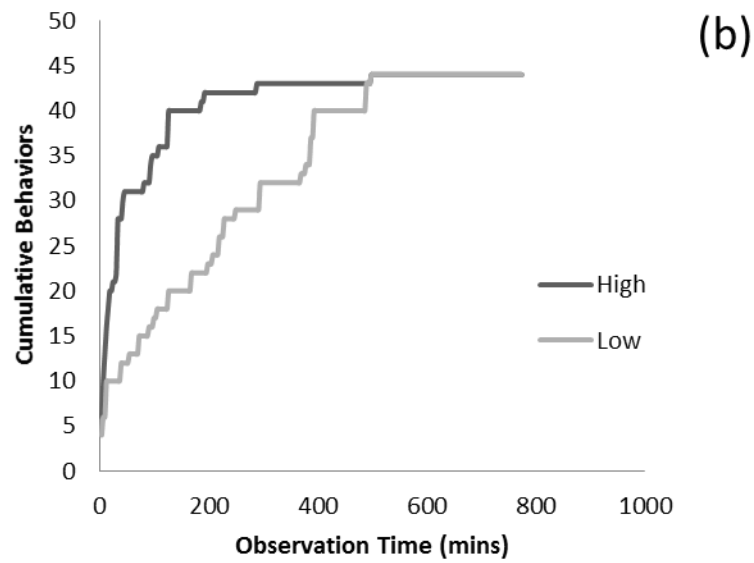
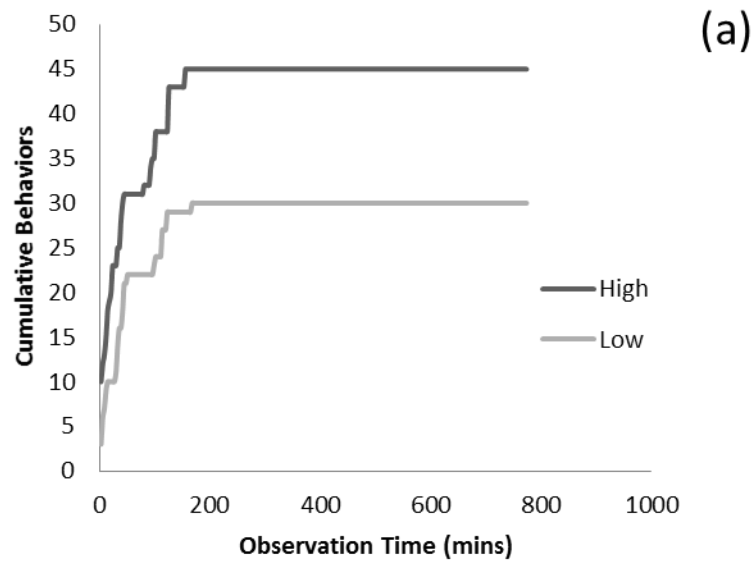
A significant point to stress is that the rate of accumulation cannot always discern rarity. For instance, a rare behavior may happen within 1 hr and not again within the next 200 hrs. However, these behavioral discovery curves do provide evidence that this lack of sneaker repertoire observations, especially in the Hotel Lake population, is not due to the lack of observational effort, differences in overall repertoire between populations, or a low rate of general activity but likely represents a real biological differences in the expression of this behavior. Based on the low potential incidence of sneak fertilization success demonstrated within this small subsample of populations, it might be assumed that sneaking is an inconsequential behavior for many populations because it may rarely result in increased reproductive success. However, the frequent number of sneaking attempts by territorial males and nonterritorial males alike – on both courting and noncourting males (Chapter 2; Appendix 2B) – may have significant effects on male time budget allocation and energy allocation, possibly resulting in significant indirect or even direct effects on male reproductive fitness. The comparison shown here does provide some leverage to suggest population-level differences in behavioral repertoires can be specific

to sneak-related behaviors rather than simply broad differences in territorial male behavior. In general, such comparative studies utilizing behavioral discovery curves can lead to more focused investigations of the variation in social and environmental conditions that promote population-level differences in a behavior of interest.

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**Figure 2C.1.** Comparison of hypothetical behavior discovery curves for populations with (a) different behavior saturation points and (b) different rates of behavior accumulation.

**Table 2C.2.** Time of first observation (mins) for territorial male behaviors related to the sneaker repertoire. Includes male courtships ('complete courtship' involves nest showing, 'incomplete courtship' includes male-female interactions aborted before nest showing) and several scenarios in which the sneaker repertoire may be observed. The category 'outside courtship' refers to observation of the sneaker repertoire in response to territorial male behavior other than courtship, e.g. nest activity. Two time estimates are shown for each category to represent observer 1/observer 2. Absence of a behavior during the entire observation period is denoted by (--). Populations are oceanic British Columbia (BCO), freshwater British Columbia (BCFW), oceanic Alaska (AKO), and freshwater Alaska (AKFW).

Population	1st complete courtship	1st incomplete courtship	1st sneak on complete courtship	1st sneak on incomplete courtship	1st sneak outside courtship	1st sneak fertilization
Francis Peninsula Lagoon (BCO)	39/138	24/141	39/318	156/543	87/258	522/--
Hotel Lake (BCFW)	162/9	30/57	--/--	--/--	--/--	--/--
North Lake (BCFW)	36/93	78/3	--/--	--/--	--/--	--/--
Rabbit Slough (AKO)	78/45	39/186	--/--	66/--	--/99	--/--
Stepan Lake (AKFW)	108/54	78/78	--/54	78/--	6/66	--/--

**Figure 2C.3.** Behavior discovery curves of territorial male repertoires in threespine stickleback populations – (a) Francis Peninsula Lagoon, BC (anadromous), (b) Hotel Lake, BC (freshwater), (c) North Lake, BC (freshwater), (d) Rabbit Slough, AK (anadromous), and (e) Stepan Lake, AK (freshwater). Combining the observations from observer 1 and 2, white arrows denote the first instance of courtship and black arrows denote the first instance of sneaking behavior for each population.



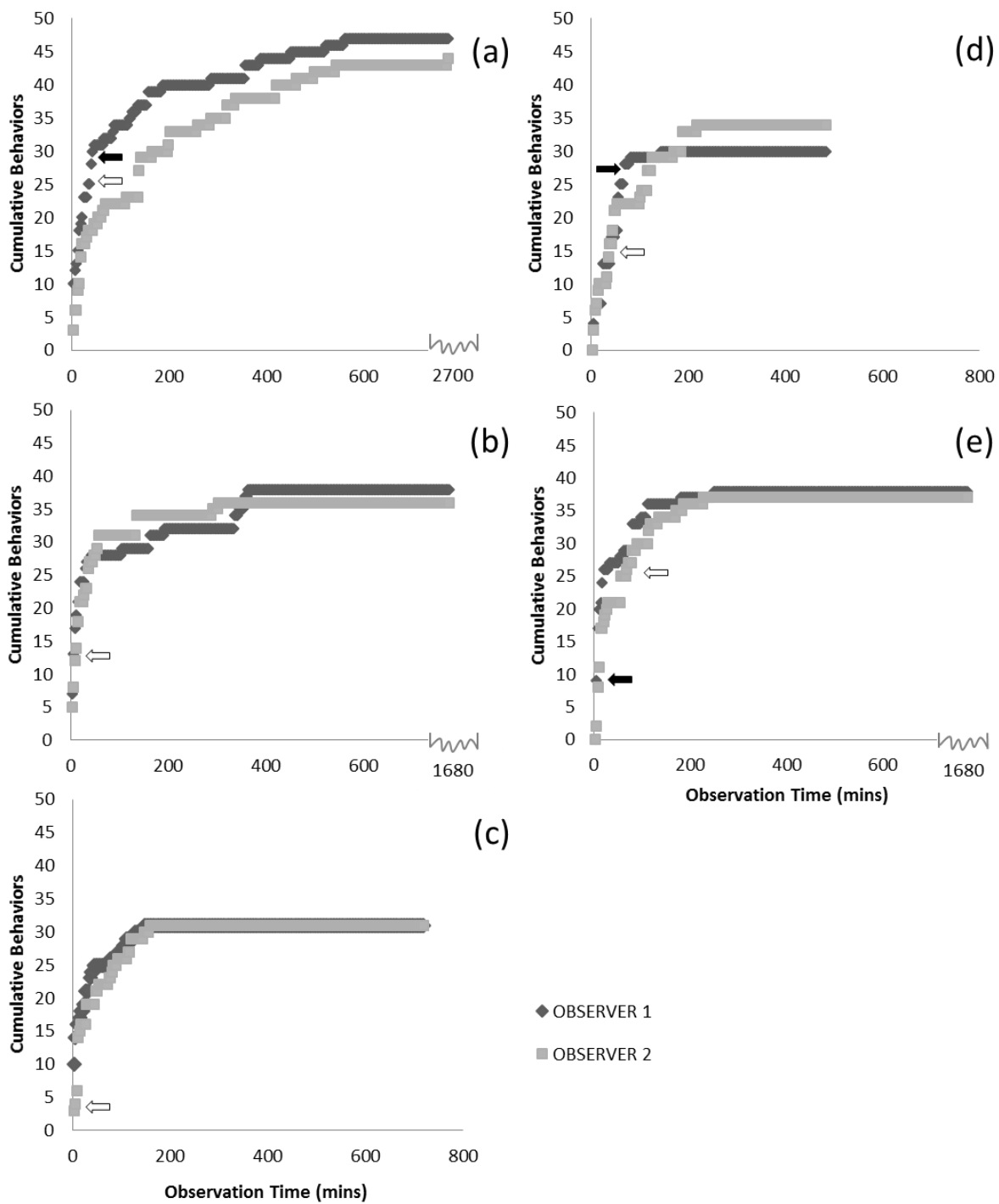


Figure 2C.3.

## APPENDIX 2D

### Is There A Relationship Between TNPC and Sneaking Behavior?

Objective: To investigate the potential relationship between frequency of sneaking behavior, the Trans-North Pacific Clade (TNPC), and Johnson & Taylor's (2004) accessibility hypothesis.

Johnson & Taylor (2004) investigated the importance of historical contingency in present-day stickleback populations by testing the hypothesis that the Northeastern Pacific post-glacial colonization has been influenced by lake elevation and dispersal ability. In particular, the presence of Trans-North Pacific Clade (TNPC, aka Japanese Clade) mtDNA haplotypes was significantly more likely to be detected in lower elevation populations compared to higher elevation populations. Clade identity was of particular interest in this study because the TNPC and the ENAC (Euro-North America Clade) may be responsible for some of the discontinuous morphological variation observed across regions -- including differences in armor reduction and melanism (Haglund *et al.* 1992; Lavin & McPhail 1993; Orti *et al.* 1994). It may be possible that haplotypes associated with these clades differ in behavioral traits, such as sneaking propensity.

The TNPC is found in freshwater and marine habitats of the western Pacific but is rare relative to the ENAC in the eastern Pacific. Prior to Johnson & Taylor (2004), the TNPC haplotypes had not been observed in lakes or streams south of northern Vancouver Island, BC (Orti *et al.* 1994; Deagle *et al.* 1996; Thompson *et al.* 1997; Taylor & McPhail 1999). Phylogeographic data suggests the TNPC haplotypes may have spread to the eastern Pacific via nearshore and offshore migration after originating 2 mya when low sea levels isolated the Sea of Japan from the rest of the Pacific Ocean (Higuchi & Goto 1996; Quinn & Light 1989). Taylor & Johnson (2004) sampled both southern Alaskan populations and BC populations

extending further south and east than previously attempted to provide a more complete picture of the distribution of the TNPC haplotypes. Their results also supported previous observations that TNPC haplotypes seem to be restricted to low elevation populations (Deagle *et al.* 1996; Thompson *et al.* 1997). The historical scenario proposed to explain this pattern involves the timing of TNPC migration during isostatic rebound. It has been suggested that the TNPC haplotypes likely arrived in the western Pacific during an intermediate point of isostatic rebound, resulting in mixing of ENAC and TNPC in lower elevation lakes more so than high elevation lakes (Awise *et al.* 1984; Deagle *et al.* 1996; Hewitt 1996).

Using elevation differences as a proxy for the presence/absence of the TNPC in sneaking study populations, there is no support for a relationship between elevation and population sneak frequency (1 courtship per male: Spearman's  $\rho = -0.348$ , d.f. = 22;  $p = 0.0953$ ; all courtships: Spearman's  $\rho = -0.306$ ; d.f. = 22;  $p = 0.1454$ , Figure 2D.2, Table 2D.1). This lack of relationship holds for comparison within AK populations only, although the relationship between sneak frequencies based on 1 courtship/male and elevation approaches significance in the direction predicted by the Taylor & Johnson (2004) study (Spearman's  $\rho = -0.496$ , d.f. = 13;  $p = 0.059$ ). Based on the current data, there is no evidence to suggest that elevation (as it may serve as a proxy for the presence of TNPC haplotypes) is related to the variation observed in the frequency of sneaking behavior in the Pacific Northwest.

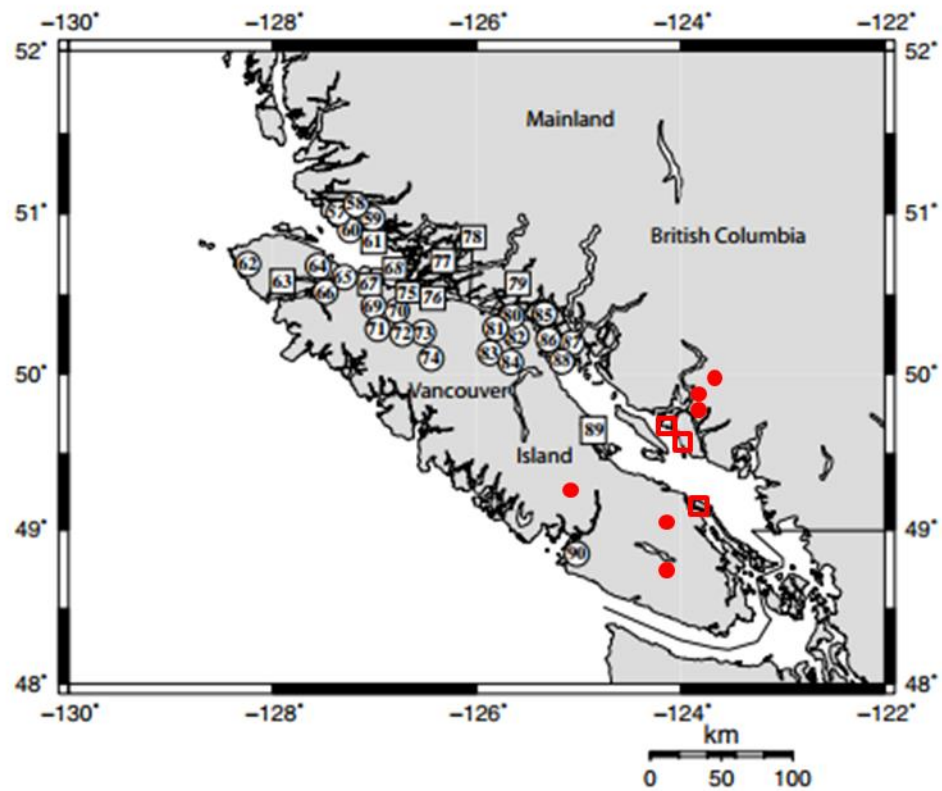
Simple presence or absence of TNPC haplotypes may not be related to the frequency of sneaking behavior in populations but rather the frequency with which these haplotypes occur in populations may influence population-level sneaking frequencies. It is likely that individuals with either TNPC or ENAC haplotypes retain the ability to sneak, as sneaking behavior has been observed across populations from Japan, the Pacific Northwest, and the Atlantic basin (reviewed in Chapter 1). However, individuals may express different cue thresholds or propensities due to genetic differences among the haplotypes. Mixing of these haplotypes in different proportions across Pacific Northwest populations could influence

population-level patterns of sneaking frequency. Investigating these associations, however, will require haplotyping samples from the AK and BC populations used in the sneaker study (Table 2D.1).

Unlike the freshwater BC populations, the oceanic BC populations included in the sneaking analyses do show evidence of sneaking behavior. Of particular interest, Francis Peninsula Lagoon has shown an increased frequency in sneaking observations over the decades of behavioral observation collection (Chapter 2, Appendix 2B). It is possible that this perceived increase in sneaker frequency is due to random year to year variation in sneaker frequency not captured because of the duration of time between observation years or it may simply be due to an increase in sampling effort in more recent years. However, as shown in Figure 2D.1, the study populations for sneaker behavioral analyses are southern BC populations in relation to populations sampled by Taylor & Johnson (2004). The current haplotype distributions suggest that northern Vancouver Island may represent the southernmost extent of TNPC dispersal in the western Pacific Ocean, as samples from the two most southern populations lacked TNPC haplotypes (Johnson & Taylor 2004). There is the possibility that haplotype-dependent differences in sneaker propensity exist and, rather than physical barriers, there is a temporal component affecting the presence of TNPC haplotypes in the surveyed regions of BC. Francis Peninsula Lagoon may be experiencing increase frequencies of sneaking expression in recent years as TNPC haplotypes continue to move southerly through the Strait of Georgia. The TNPC haplotype may be in the process of migrating further south along the coast of the BC mainland and testing for the presence of this haplotype in in oceanic and freshwater populations across a range of elevations in the southern region of Vancouver Island and the mainland of BC may help reveal this migratory pattern if it exists.

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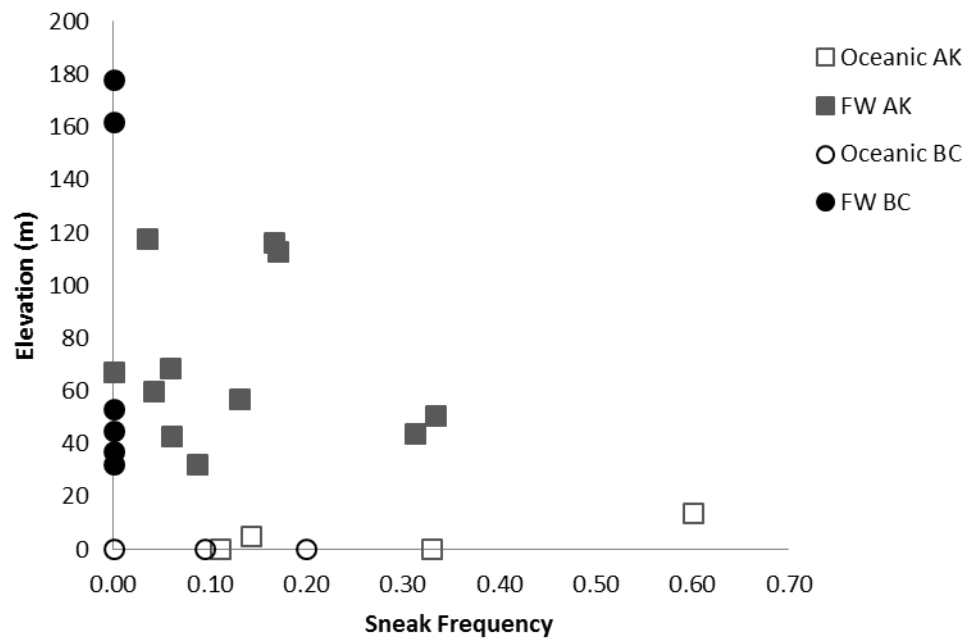


**Figure 2D.1.** Populations in the Vancouver Island area utilized in Taylor & Johnson (2004) study. Populations included in the sneaker study (listed in Table 2D.1) are indicated in red (squares = oceanic; circles = freshwater). Original map from Taylor & Johnson (2004).

**Table 2D.2.** Data used to determine the relationship between sneaking propensity and population elevation (m). 'All Complete Courtships' includes the number of complete courtships (defined as a courtship in which the male showed the nest entrance to the female) in which sneaking did occur out of the total number of complete courtships. Each population is represented by either the single year in which observations were obtained or the year with the greatest number of observation hours (see codes below for years). Population Elevation is given in meters and obtained either directly from Johnson & Taylor (2004) or from Google Earth (Google, Inc., v 4.3.7).

Populations <sup>1</sup>	All Complete Courtships	Population Elevation (m)
<b>Oceanic, Alaska</b>		
Anchor River <sup>g</sup>	4/12 (0.33)	0
Beluga Slough <sup>f</sup>	1/9 (0.11)	0
Rabbit Slough <sup>d</sup>	1/7 (0.14)	5
Resurrection Bay <sup>l</sup>	3/5 (0.60)	14
<b>Freshwater, Alaska</b>		
Beverly Lake <sup>k</sup>	2/12 (0.17)	113
Big Beaver Lake <sup>l</sup>	3/24 (0.13)	57
Big Lake <sup>d</sup>	5/16 (0.31)	44
Big Lake Creek <sup>l</sup>	1/17 (0.06)	43
Bruce Lake <sup>e</sup>	1/29 (0.03)	118
Lynne Lake <sup>f</sup>	1/17 (0.06)	69
Stepan Lake <sup>d</sup>	18/54 (0.33)	51
Stephan Lake <sup>d</sup>	4/46 (0.09)	32
Whale Lake <sup>e</sup>	0/35 (0)	67
Willow Lake <sup>d</sup>	1/24 (0.04)	60
Y Lake <sup>d</sup>	3/18 (0.17)	116
<b>Oceanic, Canada</b>		
Buccaneer Marina <sup>e</sup>	2/21 (0.1)	0
Francis Peninsula Lag. <sup>l</sup>	20/102 (0.20)	0
Swy-A-Lana <sup>h</sup>	0/14 (0)	0
<b>Freshwater, Canada</b>		
Cowichan Lake <sup>c</sup>	0/20 (0)	162
Crystal Lake <sup>a</sup>	0/72 (0)	178
Garden Bay Lake <sup>b</sup>	0/25 (0)	37
Hotel Lake <sup>l</sup>	0/25 (0)	53
North Lake <sup>c</sup>	0/19 (0)	45
Sproat Lake <sup>c</sup>	0/161 (0)	32

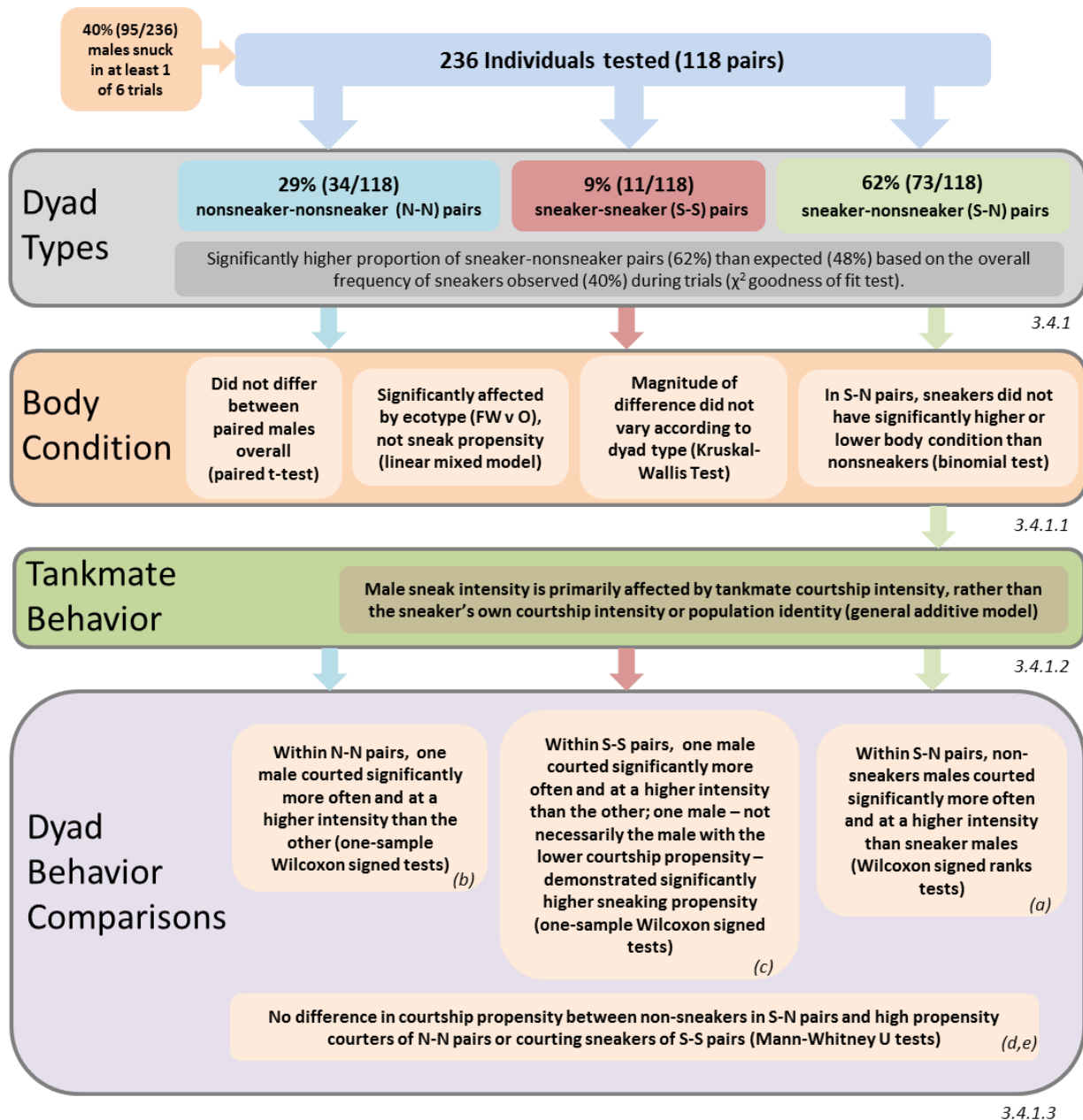
<sup>1</sup> Sample years = (a) 1985; (b) 1986; (c) 1989; (d) 1992; (e) 1993; (f) 1994; (g) 1995; (h) 1996; (i) 1997; (j) 2006; (k) 2008; (l) 2009



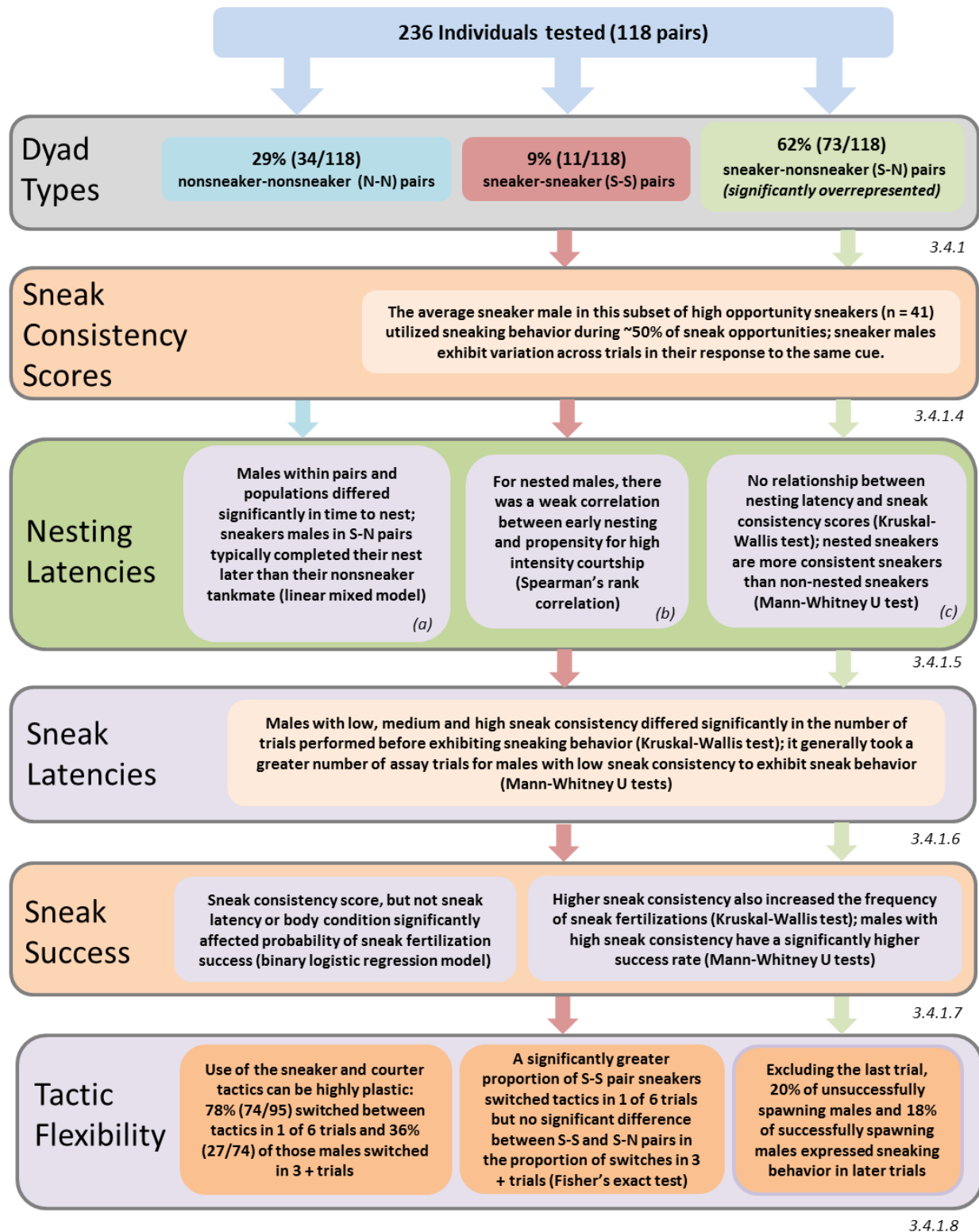
**Figure 2D.3.** The relationship between population sneaking frequencies and elevation (m) is not statistically significant (Table 2D.2; all courtships: Spearman's rho = -0.306; d.f. = 22; p = 0.1454).



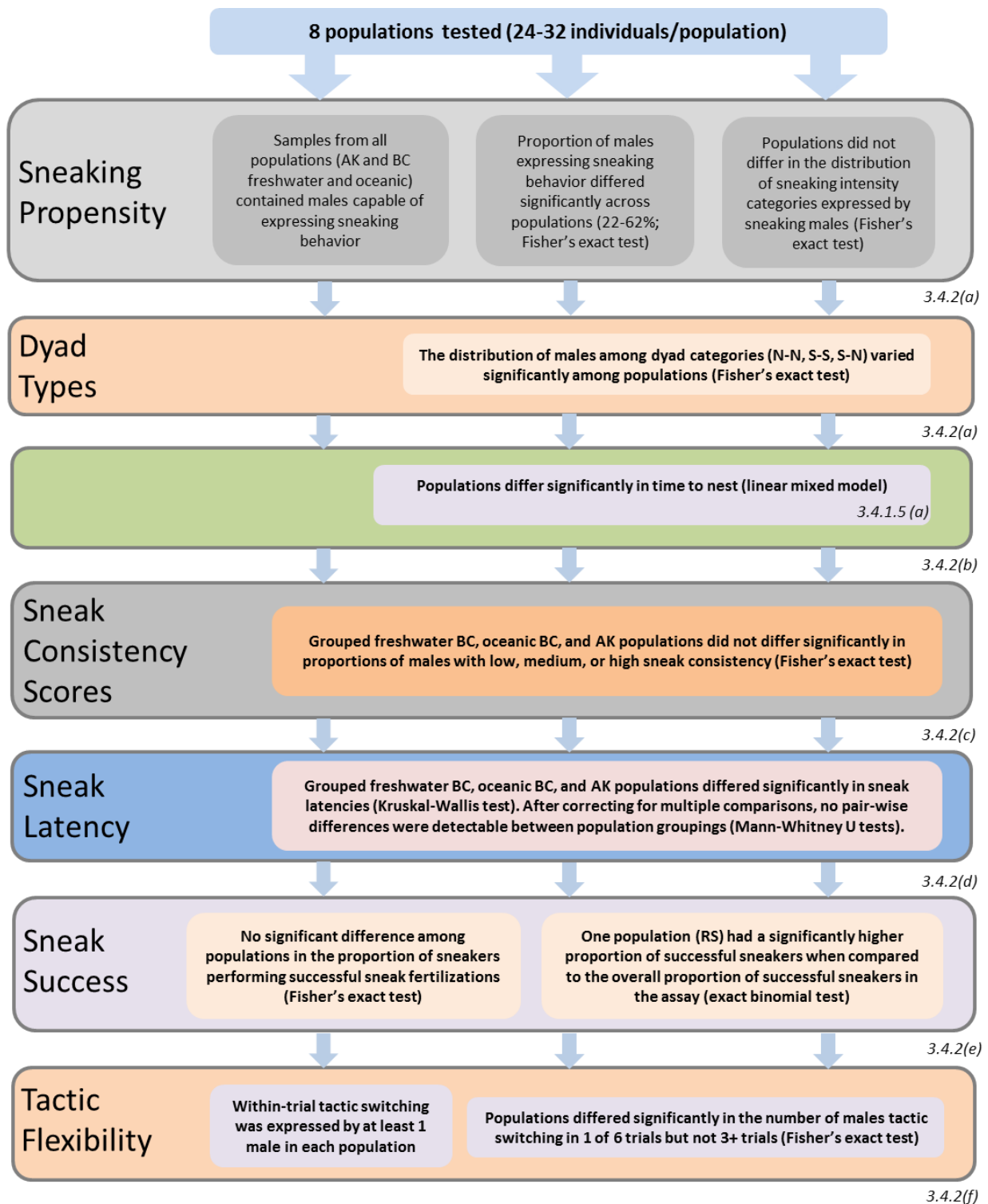
**Appendix 3A.** Results summary for the combined population analyses. Locations for the corresponding methods/results in the text are provided in *italics*.



Appendix 3A. continued



**Appendix 3B.** Results summary for the population-level analyses. Locations for the corresponding methods/results in the text are provided in *italics*.



## APPENDIX 4A

### Rapidity of color/patterning change within Stepan Lake

Threespine Stickleback can exhibit fairly quick changes in body coloration. Melanophore-based body lightening and darkening can occur over a time span of several minutes (Burton 1975), while more drastic changes in body coloration are noticeable over a span of 20 minutes, with the extent and location of body color change dependent on breeding behavior category (e.g. courting versus parental male; Laurin & Scott 2009). We investigated whether any large magnitude color changes were observable in our study population 20-minutes post-capture. Comparing male color/patterning scores between the 5-minute post-capture image and the 20-minute post-capture image may reveal that some color/patterning variables are more flexible than others or display a greater magnitude of change along a drab-bright continuum. It is also possible that color changes may more readily occur in some breeding behavior categories compared to others.

In the field, images were taken of a random subset of individuals 20-minutes post-capture ( $n = 28$ : sneakers = 11; nestbuilding males = 2; courting males = 7; parental males w/ eggs = 4; parental males w/ fry = 4) to determine the extent of plastic color change that may occur within a time span that extends beyond that of the original handling time for data collection. These secondary images were scored for the color/patterning variables using the same methods used to score the original images. By using the NMDS scores procedure in PC-ORD (V 4.20, McCune & Mefford 1999), the original dataset based on color/patterning variables and the individual scores from the original two dimensional NMDS solution were used to calibrate and calculate scores for the new data points. This method is valuable because it provides a predictive algorithm for the new data points without altering the positions of the original data points (McCune & Mefford 1999). This way, the direction and magnitude of color/patterning

change for the subset of individuals with repeat measurements could be visualized in the same ordination space as the original NMDS analysis.

The same distance measure used in the original NMDS analysis -- the Euclidean distance measure -- was used for the NMDS scores analysis. The option of finding scores for the new data points based on positioning along simultaneous axes was chosen because the goal of the analysis was to optimize the overall position of the new data points in the ordination space, rather than focusing on fit with respect to a single axis (McCune & Mefford 1999). Individual data points were flagged for poor fit based on several criteria. For this analysis, individual data points were flagged if their stress value in the final configurations was  $\pm 2$  s.d. that of the mean stress score. Individual points were also flagged if their final score fell beyond the extrapolation limit, which was set at  $\pm 5\%$  of each axis length.

The mean stress ( $\bar{x} = 6.710$ . s.d. = 0.098) of fitting the new data points to the chosen 2-axis ordination solution fell within the range of stress scores obtained for the original two dimensional solution (see Table 4.6) and, like the final stress value of the original ordination, this low stress value can be interpreted as a good ordination solution (McCune & Grace 2002). None of the data points fell outside of the extrapolation limit. The final stress score of a single individual was flagged for exceeding the final stress criterion, however, even this comparably higher final stress score (7.0717) falls well within the range of stress scores for the original 2-axis solution (Table 4.6) and would still be interpreted as a good fit to the data (McCune & Grace 2002). The scores for the subset of individuals with repeated measures were overlain on the original ordination and the magnitude and direction of color/patterning changes were determined and emphasized (Figure 4A).

While this comparison does not provide a quantitative assessment of body color changes, it does provide some indication of the direction and magnitude of plastic changes in body coloration over a short time scale (20 mins post-capture). Comparing the breeding behavior groups with the largest

sample sizes for the repeated measurements, courting males ( $n = 7$ ) and parental males ( $n = 4$ ) demonstrated the greatest changes over 20 mins -- in particular shifting towards drabber dorsal coloration and drabber throat and eye coloration (Figure 4A). Parental males w/ fry ( $n = 4$ ) exhibited little body color changes, shifting positions only within the area in color space that category occupies. Sneaker males ( $n = 11$ ) also demonstrated little color change -- only the largest magnitude changes are shown in Figure 4A. Thus, the majority of sneaker males expressed either no shift away from their original color space or very small shifts. A few sneaker males did exhibit demonstrable shifts, increasing throat and eye conspicuousness during their holding period. The single courting male that shifted into the empty portion of color space, shifted towards a color combination slightly different than other individuals expressing losses in color -- this individual originally expressed bright body coloration overall but shifted towards drabber flank and ventral coloration while retaining bright dorsum, throat, and eye coloration. This shift seems more likely to be related to flank coloration as it influences Axis 2 rather than Axis 1. It is important to mention that all of the individuals were held in white buckets, such that divergent background matching should not account for the difference in magnitude and direction of color changes in individuals.

61% of individuals demonstrated a change in eye coloration by 20 mins post-capture. This is an interesting result given that this color trait is generally believed to have low flexibility once the coloration appears in breeding males. Blue eye coloration has been correlated with growth and body size of breeding males in several populations (Frischknecht 1993; Bakker & Rowland 1995) and is thought of as a long-term indicator of condition in breeding males (Frischknecht 1993). However, Rush *et al.* (2003) also observed the potential for eye coloration to be labile depending on an interaction between individual condition and social context. In the current study, 47% of the individuals showing eye color change showed a decrease in eye coloration. All of these individuals were courting males or parental males w/ eggs. The other 53% of individuals exhibiting eye color change demonstrated an increase in

eye color and all of these males were originally drab sneakers or nest-builders. Similar patterns were observed for throat color change but only 39% of those remeasured exhibited either an increase or decrease – the 36% that exhibited an increase were sneaker males. 39% of individuals also expressed change in flank color – 82% of these individuals were courting or parental males w/ eggs showing a decrease in bright flank color. 43% of males exhibited changes in stripe contrast (due to either darkening of stripes, lightening of the flank or a combination of these factors) – 83% of these males were courting or parental males decreasing stripe contrast over time. Only 21% of individuals showed changes in dorsum color, all decreasing bright coloration – the majority of these being courting or parental males w/ eggs. 50% of remeasured individuals expressed changes in ventral coloration – 93% of these individuals, a mixture of males across breeding behavior categories, showed a decrease in bright coloration.

The decrease in coloration due to handling and holding over a period of time is similar to previous findings. Decreases in bright nuptial coloration have been observed in parental males of another population in which the effects of handling time on body coloration was measured with spectrophotometry (Laurin & Scott 2009). Shared hormonal and neuronal pathways of stress responses and breeding behaviors may be responsible for such contextual color changes (e.g. increased cortisol levels: Morgan et al. 1999; Overli et al. 1999; Hoglund, Balm & Winberg 2000). The increase in conspicuous coloration (e.g. eye and throat color) observed in a subset of sneaker males seems counterintuitive but it is possible this sample includes facultative sneakers that subdued their nuptial coloration during sneaking acts via temporary physiological changes and the holding period allowed time for their body coloration to return to pre-sneaking levels of expression.

The large shifts expressed by individuals after holding combined with the generally well-defined groupings that arose based on the original NMDS and MRPP analyses suggest that much of the original

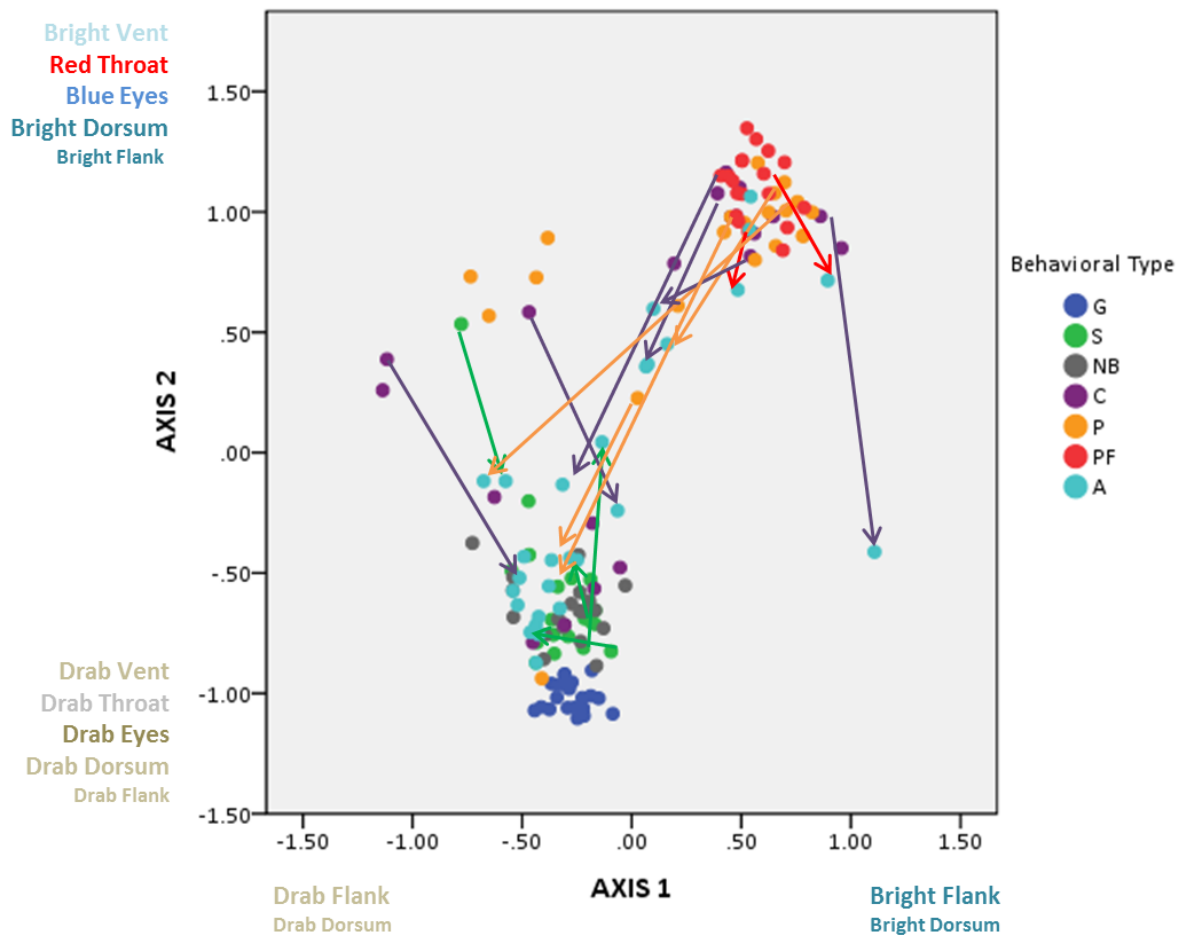
coloration was captured in the initial digital images. However, there are likely aspect of color variation that fluctuate too quickly to catch even within the short time period in which the measurements were taken. In general, discerning which color/patterning variables are most likely to change over a short period of time sets the foundation for further investigations into the rapidity of such changes. The patterns from this analysis seem to suggest that loss of bright coloration is more easily attainable on a short time scale compared to gain of bright, conspicuous coloration. Whether those individuals with greater magnitude changes can also quickly reverse those changes from drab to bright, remains to be investigated.

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**Figure 4A.1.** The final configuration for the 2-dimensional NMDS solution for the original data set ( $n = 121$ ) overlain by the scores of the subset of individuals rescored for the color/patterning variables 20 mins post-capture ( $n = 28$ ). Behavioral types: G = gravid females; S = sneaker males; NB = nestbuilding males; C = courting males; P = parental males w/ eggs; PF = parental males w/ fry; A = Subset of individuals with secondary photo take 20 mins post-capture. The arrows connect individuals with noticeable differences between the original score and the secondary score.

## APPENDIX 4B

### Use of hierarchical clustering to confirm dimensionality choice for NMDS analysis

To determine if the 2-dimensional solution was a good choice for interpretation of the color/patterning variables, a different grouping method was employed on the same individuals ( $n = 121$ ) using the same color patterning variables ( $n = 10$ ) described in Table 4.2. The goal is to determine if these two grouping methods produce similar results.

Cluster analysis defines groups of items based on their similarities (McCune & Mefford 1999). A cluster analysis was performed in PC-ORD (v 4.20, McCune & Mefford 1999) using the Euclidean distance measure and Ward's method as a linkage (sorting) method. This linkage method was chosen based on its compatibility with the Euclidean distance measure (e.g. combinatorial matrix reduction; McCune & Grace 2002). Wishart's (1969) objective function was used to scale the dendrogram. This function measures the amount of information remaining as groups are combined until no information remains. The results of the two methods are compared in **Figure 4B**. A number of the groups specified by the cluster analysis correspond well with the grouping in color space for the two dimensional NMDS solution. However, a few of the clusters do overlap in ways that are not particularly intuitive based on the positions in color space (e.g. overlap between the purple and blue polygons). In general, the results of the cluster analysis demonstrate that the 2 dimensional solution used to visualize the individuals in color space was a suitable choice.

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**Figure 4B.1.** The dendrogram based on hierarchical clustering of the color/patterning variables used in the NMDS analysis. Correspondence between groupings in the dendrogram and NMDS analysis are color-coded. Behavioral types colored coded in the NMDS figure: G = gravid females; S = sneaker males; NB = nestbuilding males; C = courting males; P = parental males w/ eggs; PF = parental males w/ fry. Scaling is based on the objective function (see Appendix 4B).

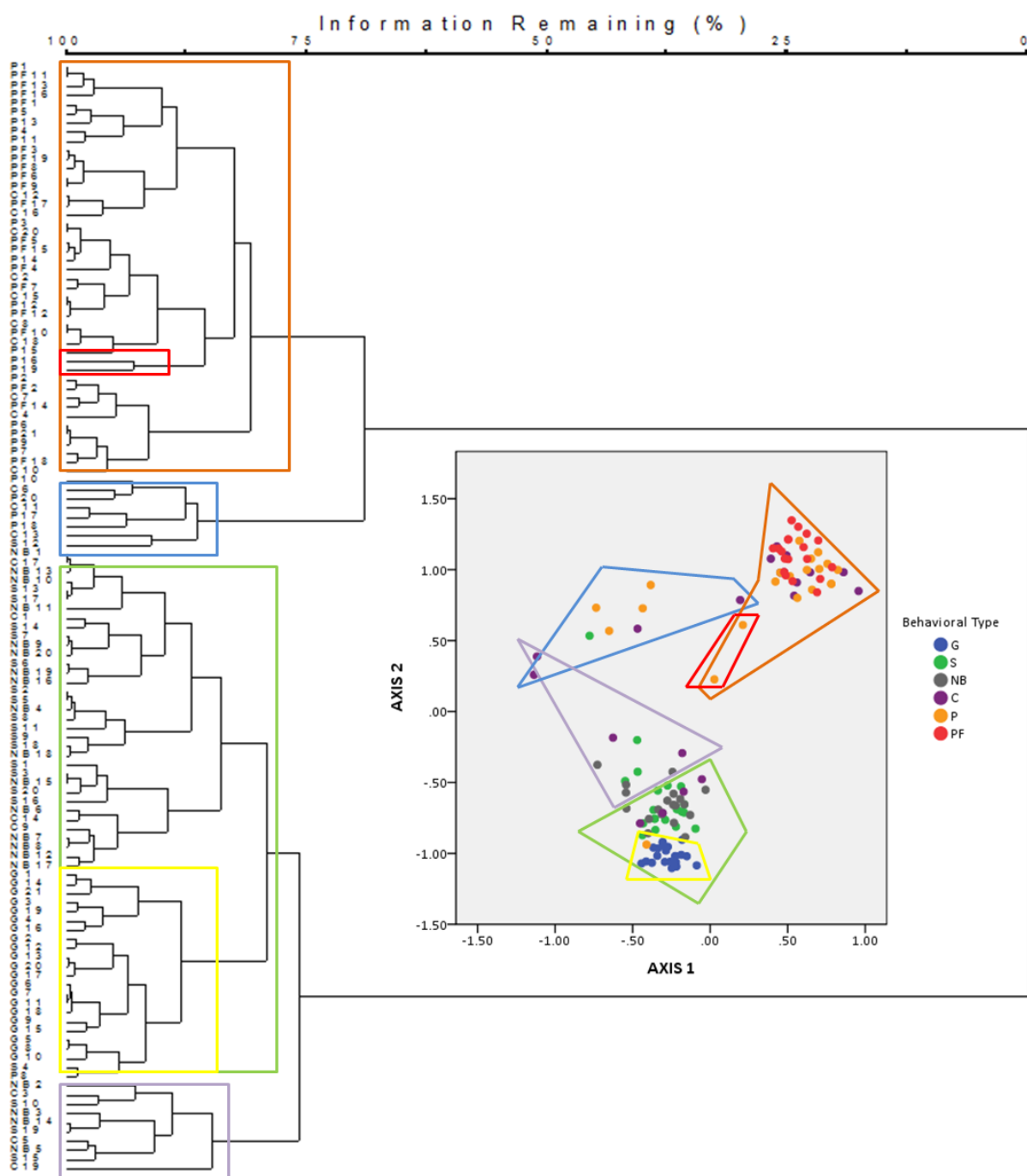


Figure 4B.1.