Characterizing Phenotypic Diversity in Marine Populations of Threespine Sticklebacks

by

Ainsley Fraser
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Supervisory Committee

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Supervisory Committee

Dr. Rana El-Sabaawi (Department of Biology)
Supervisor

Dr. Francis Juanes (Department of Biology)
Departmental Member

Dr. John Taylor (Department of Biology)
Departmental Member

Dr. Tom Reimchen (Department of Biology)
Additional Member

Abstract

The threespine stickleback (*Gasterosteus aculeatus*) is an important model for studying evolution. Sticklebacks are widely distributed in the northern hemisphere and inhabit freshwater, brackish, and marine waters. Anadromous and marine populations (hereafter marine) are assumed to be homogenous in space and invariant in time in their phenotypic characteristics, despite marine environments varying on regional and local scales. Recent studies suggest there is in fact genetic and phenotypic structure in marine sticklebacks, yet the ecological causes remain unclear. My goal was to assess trait variation in marine stickleback populations around Southern coastal British Columbia (BC), and to determine whether or not oceanographic and habitat characteristics explained this variation. The area around Vancouver Island was ideal because four distinct oceanographic regions surround the island with varying coastal habitat types. Between May-July 2019, I sampled ~600 sticklebacks from 15 sites. I then characterized trait variation using two-dimensional (2D) geometric morphometric analysis to compare individuals between oceanographic regions and coastal habitats. I focused on five traits: armour phenotype, head size, body size, head shape, and body shape. I chose these traits because they are ecologically important and well-studied in freshwater populations, where their ecological drivers are known. I found that marine sticklebacks did vary morphologically among and between regions and habitats, but the variation was not immediately related to environmental variation, nor obviously comparable to variation in freshwater populations. Sexual dimorphism was the largest source of variation in the data, a well-established finding. But oceanographic and habitat variables influenced differences between males and females. I concluded that marine sticklebacks offer abundant opportunities for expanding our knowledge of drivers of morphology in nature.

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Dedication

I dedicate this thesis to Marion Wardell Fraser, my grandmother who passed away during the second year of my master’s degree. She loved disruptive women who took up space. I am including this dedication because she never let me feel incapable, but my main motivation is that she strongly opposed receiving attention like this.

Introduction

The threespine stickleback (*Gasterosteus aculeatus*) is one of the most important models for studying the evolution of vertebrate morphology. Marine, anadromous, and freshwater stickleback populations are widely distributed across temperate and boreal regions in the northern hemisphere (Bell and Foster, 1994). Based on both geographic and genetic evidence, the accepted theory is that freshwater sticklebacks evolved from an ancestral marine population (Schluter and Nagel, 1995; McKinnon and Rundle, 2002; Colosimo, 2005; Fang *et al.*, 2018), during the most recent glacial retreat ~10,000 - 20,000 years ago (Wootton, 1976; Bell and Foster, 1994). Marine sticklebacks colonized postglacial lakes repeatedly across the northern hemisphere, resulting in thousands of genetically and phenotypically distinct freshwater populations (Bell, 1976; Aguirre and Bell, 2012). Among freshwater populations, there are phenotypically diverse sympatric, allopatric, and parapatric forms (McKinnon *et al.*, 2004; Schluter *et al.*, 2004; Vines and Schluter, 2006; Hendry *et al.*, 2009). The presence of so many distinct stickleback populations gave researchers an excellent opportunity to observe the effects of differing environments on phenotype, life history, and behaviour. In current studies, marine populations are assumed to be homogenous in space and invariant in time, and they are often used to approximate ancestral stickleback morphology, despite occurring in environments that are geographically and oceanographically isolated (Bell, 1976). Recent studies suggest that this assumption is invalid and that there is genetic and phenotypic structure in marine stickleback populations, but the scale and ecological causes of this variation are unclear (DeFaveri, Jonsson, *et al.*, 2013; Morris *et al.*, 2018).

To date, most large-scale studies of morphological variation in sticklebacks examine freshwater populations (Hagen and Gilbertson, 1972; Walker, 1997; Berner *et al.*, 2009; McGee *et al.*, 2013; Smith, Zięba, Spence, Klepaker, *et al.*, 2020). Consequently, the ecological factors driving morphological evolution in freshwater ecosystems have been well-studied, while relatively little is known about the ecological factors structuring marine populations (Lavin and McPhail, 1985; Matthews *et al.*, 2010; Lefébure *et al.*, 2011; Aguirre and Bell, 2012). Until now, evidence of local adaptation and small-scale genetic structuring in marine sticklebacks has primarily been characterized in either a single small basin (*i.e.* the Baltic Sea) (DeFaveri and Merilä, 2014; Bergström *et al.*, 2015; Jakubavičiūtė *et al.*, 2018), or across very large basins (*e.g.* the Atlantic vs. Pacific Oceans) (Haglund *et al.*, 1992; Ortí *et al.*, 1994; Mäkinen and Merilä, 2008; Fang *et al.*, 2018). Relatively speaking there are currently no studies on an intermediate scale, where a variety of oceanographic features and habitat types can be found. Oceanographic variation and habitat availability on intermediate scales may have an important structuring effect on marine stickleback populations (Emmett *et al.*, 2000; Dallimore and Jmieff, 2010; Schoch *et al.*, 2014). Factors including temperature and salinity, which correlate with morphological variation in freshwater sticklebacks (Barrett *et al.*, 2009; Lefébure *et al.*, 2011), do not vary randomly in the ocean but are governed by underlying oceanographic variation (Seckel, 1968; Roden, 1975; Rudnick and Ferrari, 1999). Other oceanographic factors, including currents, habitat availability, and chlorophyll concentration have been shown to drive genetic and morphological divergence in a number of vertebrate and invertebrate marine species (Juan-Jordá *et al.*, 2009; Zhan *et al.*, 2009; Hammami *et al.*, 2013). Habitat characteristics, such as benthic availability and predator presence, also correlate with morphological variation in freshwater sticklebacks (Walker, 1997; Matthews *et al.*, 2010; Schluter *et al.*, 2010). However, it is currently unknown whether marine sticklebacks vary in response to these environmental features, or if the ecological factors driving morphological variation in freshwater sticklebacks also drive variation in marine sticklebacks.

Research Summary

The goal of my thesis is to survey and quantify morphological variation in marine threespine sticklebacks on an intermediate scale (*i.e.* Southern British Columbia (BC) and Vancouver Island). Very broadly, I have characterized marine stickleback morphology from numerous stickleback populations and have tested whether they vary between and among oceanographic regions, or between and among different marine habitats. My study is the most comprehensive characterization of the phenotypic heterogeneity of marine sticklebacks to date and continue to relate phenotypic variation in marine sticklebacks to their oceanographic environments as a few studies have done previously (Spoljaric and Reimchen, 2008; Morris *et al.*, 2018). Thus, my research will allow future researchers to refine their assumptions about using marine sticklebacks as proxies for ancestral stickleback morphology, and will set the stage for future studies on the genetic structure of marine stickleback populations.

The coast of Vancouver Island and the coast of Southern mainland BC are optimal locations to conduct this research because sticklebacks are widely distributed in this region. This is also where researchers have found a lot of freshwater stickleback diversity in lakes and streams. Specifically, I focused on oceanographic regimes which drive temperature and salinity, and habitat characteristics, because these are known to correlate with freshwater stickleback phenotypes and vary greatly in this region. The stickleback populations I sampled are likely from a mixture of anadromous (migratory) and sea-spawning marine populations. Distinguishing the two life histories was difficult because I did not track migration patterns throughout the year (*i.e.*

using tagging or catch-and-release). Thus, I will henceforth refer to all populations as marine sticklebacks.

Stickleback morphology

My thesis examines three aspects of stickleback morphology: armour, head shape, and body shape. I have chosen to focus on the bony armour lateral plates because they have received more attention from evolutionary biologists than any other morphological trait and have been shown to vary among and within populations. Specifically, I analyzed the proportion of bony plate coverage on an individual (*i.e.* complete, partial, and low plated). I also used two-dimensional (2D) geometric morphometric techniques to analyze the morphological differences (*i.e.* head and body shape) between stickleback populations. Two-dimensional (2D) morphometric techniques have been widely used in studies describing morphological variation within freshwater populations, and between freshwater and marine sticklebacks (Albert *et al.*, 2008; Kimmel *et al.*, 2008; Sharpe *et al.*, 2008; Spoljaric and Reimchen, 2011), and are also commonly used to study fish morphology (Andersson, 2003; Marques, 2019), lizards (Gray *et al.*, 2019), and squirrels (Swiderski, 2003). I chose the landmarks for 2D morphometrics in order to hopefully capture variation in head and body shape associated with environment. My thesis also compares stickleback morphology between males and females, because, like freshwater populations, marine sticklebacks are also sexually dimorphic (Kitano *et al.*, 2007; Aguirre *et al.*, 2008).

Armour

Sticklebacks have bony armour that protects their scaleless bodies (Bell and Foster, 1994). The components of this armour include lateral bony armour plates, three dorsal spines, a pelvic girdle, and pelvic spines (Bell and Foster, 1994). This armour is thought to have evolved to support the dorsal and pelvic spines while predators attempt to eat them, and to reduce injuries during escape (Reimchen, 1983, 1992b). Sticklebacks are generally divided into three major plate phenotypes: complete, partial, and low plated morphs (Bell and Foster, 1994). Complete stickleback morphs have bony plates that form a single row of at least 30 plates along each side of the body, partial morphs have between 10-30 plates, and low morphs have less than 10 (Hagen and Gilbertson, 1972; Jamniczky *et al.*, 2018). Sticklebacks vary in bony armour plate phenotypes between freshwater populations and between freshwater and marine/anadromous populations (Barrett, 2010; Reimchen *et al.*, 2013; Maciejewski *et al.*, 2019). Generally marine sticklebacks are complete plate morphs, while freshwater populations typically possess fewer plates covering only the anterior portion of the body (Barrett, 2010). This reduction of lateral plates in freshwater populations follows colonization by a marine population and can happen within a few generations (Bell and Foster, 1994).

The adaptation of armour and plates to a novel freshwater environment is influenced by genetic factors which are reinforced by ecological factors present in the environment (Baumgartner, 1994). Genetically, this rapid plate evolution has been explained by allelic variation in *Ectodysplasin* (*Eda*), a heavily studied gene which is largely responsible for the formation of body armour in freshwater sticklebacks (Colosimo, 2005). Freshwater sticklebacks usually possess two homozygous recessive low armour *Eda* alleles, while marine sticklebacks are generally homozygous dominant for the high armour *Eda* (Bell and Foster, 1994). Sticklebacks that are heterozygous for the *Eda* alleles are usually fully plated or have an intermediate number of plates because the dominant allele is not fully dominant in controlling plate number expression (Cano *et al.*, 2006; Schluter *et al.*, 2010).

Interestingly, complete morphs are sometimes found in freshwater environments (Bańbura, 1994; Kitano *et al.*, 2008; Yamasaki *et al.*, 2019), and heterozygotes are present in both freshwater and marine environments at low frequencies (Hagen and Gilbertson, 1972; Wiig *et al.*, 2016). This can be explained by the genetic and ecological factors present in a freshwater habitat acting on an ancient low plated allele, which in turn was brought repeatedly into freshwater environments by founding populations of marine sticklebacks (Barrett *et al.*, 2008). This ancient low plated allele originated ~two million years ago, so it is much older than the freshwater low plated morphs inhabiting the postglacial lakes (Colosimo, 2005). Thus, the frequency of low plated alleles increased in freshwater populations due to the selective advantage of reduced plates in the local environment (Myhre and Klepaker, 2009).

The precise characteristics present in freshwater habitats that favour plate reduction are unclear, but several factors have been suggested. One hypothesis is that the low plated allele could be favoured in freshwater environments because lower salinities have been shown to increase growth rate in stickleback populations (Marchinko and Schluter, 2007; Barrett *et al.*, 2008). In other words, differences in salinity create a trade-off between lateral plate phenotype and growth rate (Barrett *et al.*, 2008). Freshwater sticklebacks carrying complete plate *Eda* alleles have complete armour but reduced growth rate when switched from high to low salinity environments, and low plated morphs in freshwater conditions have higher growth rates (Barrett *et al.*, 2009; Spence *et al.*, 2012). Additionally, Schluter *et al.* (2021) found that females who were homozygous for the low plate *Eda* alleles had double the amount of surviving offspring compared to females who were homozygous for the complete plate *Eda* alleles. On the other hand, the fitness advantage of having fewer bony lateral plates in freshwater habitats has been related to the differences in predator presence and intensity at the habitat level. In freshwater, spine length, plate phenotype, and head depth of sticklebacks change significantly when exposed to habitats with different prey type and predators (Reimchen, 1995, 2000; Day and McPhail, 1996; Kristjánsson *et al.,* 2002; Wund *et al.*, 2008). When large piscivorous fish dominate marine and freshwater habitats, selection favours large spines and complete plated morphs (Reimchen, 1994; Kitano *et al.*, 2008). In four BC lakes, Rogers *et al.* (2012) observed that freshwater sticklebacks in lakes with a predator presence of the prickly sculpin (*Cottus asper*) were genetically and phenotypically more similar to marine sticklebacks than freshwater populations without prickly sculpin. Paccard *et al.* (2018) observed similar patterns in marine stickleback populations in bar-built estuaries along the central California coast. They found that populations with sculpins present had more streamlined bodies, longer dorsal spines, and more lateral plated than the populations with sculpins absent. Marine sticklebacks are often in pelagic environments and thus can experience higher predation pressure (Reimchen, 2000; Marchinko, 2009b). For marine sticklebacks, variation in armour phenotype could also be driven by differences in predator presence and diversity between habitats (*i.e.* salt marsh vs. tidal flat supporting eelgrass beds) (Callaway *et al.*, 2012; Huang *et al.*, 2015), but there is a knowledge gap on how predators affect marine stickleback armour and lateral plates.

Head and body shape

Evidence suggests that variation in stickleback head and body shape are influenced by ecological factors (Walker, 1997; Sharpe *et al.*, 2008; Ramler *et al.*, 2014; Mazzarella *et al.,* 2015). In terms of head morphological traits, previous studies have found that the habitat characteristics (*i.e.* niche and predation pressure) affect key trophic morphological traits such as snout length, orbit size, jaw length, and number of gill rakers (Lavin and McPhail, 1985; McPhail, 1994; Caldecutt and Adams, 1998; Kimmel *et al.*, 2008, 2012; Seebacher *et al.*, 2016;

Østbye *et al.*, 2016). Gill rakers are present on the anterior side of the gill arch and prevent prey from escaping out through the gill slits of fish (McGee *et al.*, 2013). Head size and shape differ between local freshwater populations based on variation in habitat availability (Matthews *et al.*, 2010). For example, limnetic sticklebacks have longer and more numerous gill rakers due to feeding on zooplankton prey (Matthews *et al.*, 2010), while benthic sticklebacks have shorter and less numerous gill rakers (Østbye *et al.*, 2016). In contrast, we know little about marine stickleback head morphology. Svanbäck and Schluter (2012) found low variation in gill raker length and gape width in anadromous populations. However, they were interested in the overall variation and plasticity of trophic traits and did not research the ecological factors possibly driving the variation (Wund *et al.*, 2008; Svanbäck and Schluter, 2012). Although, Rogers *et al.* (2012) and Jamniczky *et al.* (2015) found the trophic apparatus to be heritable and associated with adaptive peak shifts in freshwater environments.

Many studies have found that sticklebacks have a large capacity for phenotypic plasticity in body shape in response to salinity, temperature, and habitat characteristics (Day *et al.*, 1994; Spoljaric and Reimchen, 2007, 2008, 2011; Wund *et al.*, 2008; Garduño-Paz *et al.*, 2010; McCairns and Bernatchez, 2012; Morris *et al.*, 2014; Ramler *et al.*, 2014; Mazzarella *et al.*, 2015). Leaver and Reimchen (2012) transferred a highly derived freshwater population from Mayer Lake, Haida Gwaii, BC, made up of melanistic males and exceptionally large body sizes (*i.e.* gigantism) to a small, eutrophic pond nearby that lacked resident fish or any substantial piscivorous predators. Within eight generations, the Mayer Lake colonizing population had significantly changed morphologically. Armour phenotype was reduced in the pond population, including plate count, pelvic and dorsal spine length. While trophic traits also shifted to longer and more numerous gill rakers, longer jaws and increased eye diameter (Leaver and Reimchen, 2012). Mazzarella *et al.* (2015) collected freshwater sticklebacks in Norway, crossed them in the lab, and split the surviving larvae into a freshwater group or saltwater group. After only one generation, sticklebacks in the saltwater treatments had longer jaws and shallower bodies than sticklebacks in the freshwater treatment (Mazzarella *et al.*, 2015). McCairns and Bernatchez (2012) sampled sticklebacks from the various location in the St. Lawrence River estuary in Canada. They found that stickleback populations varied significantly in body shape between the two local populations in the freshwater upper estuary and more saline lower estuary (McCairns and Bernatchez, 2012). However, body shape has not been compared among marine stickleback populations inhabiting high salinity environments, and it is unknown if small variations in salinity could also influence body shape variation (Walker and Bell, 2000; Leinonen *et al.*, 2006). Ramler *et al.* (2014) found that wild caught marine sticklebacks raised in three different, novel temperatures (13°C, 17°C, 21°C) differed significantly in head and body shape. Stickleback morphology is impacted by growth rate, which is significantly lower at colder temperatures; maximum growth rate occurs at warmer temperatures of 17°C - 21°C (Lefébure *et al.*, 2011; Ramler *et al.*, 2014).

On a larger scale, temperature across latitudes may also affect the maximum body size of adult sticklebacks (Bergmann, 1847; James, 1970; Ahti *et al.*, 2020). Bergmann's rule states that species at higher latitudes, experiencing colder temperatures, will be larger than the same species found at lower latitudes because of lower resting metabolic rates caused by low surface area to volume ratios (Bergmann, 1847). Morris *et al.* (2017) found that the standard length of sticklebacks along the west coast of North America increased with latitude (supporting Bergmann's rule), and that the number of vertebrae increases with latitude (supporting Jordan's rule). The selection of large offspring body size at colder average temperatures may also explain this pattern (Pettersen *et al.*, 2019). For example, female sticklebacks that developed at lower average temperatures have fewer but larger offspring (Shama, 2015). Kim *et al.* (2017) also found that males bred in environments with warmer average winter temperatures were smaller as adults.

On a smaller scale, stickleback morphology can vary among stickleback populations from different habitats. This pattern can be explained as genetic and plastic responses to differences in habitat characteristics (Day *et al.*, 1994; Day and McPhail, 1996; Bell and Sih, 2007; Scotti and Foster, 2007; Wund *et al.*, 2008). Famous examples of habitat-related morphological divergence are the benthic-limnetic species pairs found in a few lakes in Southern BC (*i.e.* Paxton, Hadley, and Little Quarry Lakes) (McPhail, 1984, 1993; Gow *et al.*, 2008). Previous studies have found that variation in body shape is associated with the trophic niche of an individual and the swimming habits associated with that niche (Matthews *et al.*, 2010). Sticklebacks found in a shallow habitat with higher benthic habitat availability have deep bodies, large heads, and large caudal fins. These populations feed on large benthic invertebrates which are mostly sedentary, and therefore relatively easy to capture (Matthews *et al.*, 2010). Thus, benthic sticklebacks do not expend as much energy in prey capture and are better adapted for burst swimming with their large caudal fins and deep muscular bodies (Taylor and McPhail, 1986). Sticklebacks from deeper, limnetic habitats generally have elongated, shallow body shapes, small heads, and smaller caudal fins (Walker, 1997; Kimmel *et al.*, 2008). Limnetic sticklebacks prey on mobile zooplankton, so they must maintain steady swimming speeds to catch prey and avoid predators (Matthews *et al.*, 2010). Thus, limnetic sticklebacks have elongated bodies and small caudal fins that are better suited for steady, prolonged swimming (Taylor and McPhail, 1986).

These highly derived, benthic-limnetic body shapes were compared to anadromous stickleback populations by Svanbäck and Schluter (2012), who found that the anadromous populations exhibited an intermediate body shape, between average benthic and limnetic shapes. Aguirre *et al.* (2008) also found that an anadromous population in Rabbit Slough, Alaska exhibited intermediate body shape characteristics, while Taylor and McPhail (1986) found that anadromous sticklebacks from the Salmon River estuary in BC exhibited more limnetic traits when compared to resident freshwater populations in the river (*i.e.* narrower body, narrower caudal peduncle).

Sexual dimorphism

Sticklebacks are sexually dimorphic in both behavioural and morphological traits (Wootton, 1984). Males build nests, court females, and are the primary parental caregiver, while females primarily produce eggs (Wootton, 1984). In addition, multiple morphological structures are important for mate choice. Males express red, nuptial colouration to attract females during the breeding season, although males in populations on the west coast of North America and the Scottish Hebrides express a melanic phenotype instead of red (Hagen and Moodie, 1979; Kimmel *et al.*, 2008; Smith, Zięba, Spence, and Przybylski, 2020). Males and females also differ in their somatic traits. Females are generally larger than males, and have larger pelvic girdles, while males generally have larger heads and mouths than females (Aguirre and Akinpelu, 2010). Multiple hypotheses exist to explain the evolution of this prominent sexual dimorphism in stickleback morphology including: natural selection for greater egg production (leading to larger females), sexual selection from male preference for large females, and even that larger males are preferred by females in some populations (Tinbergen, 1951; Moodie, 1982; Rowland, 1994; Candolin, 1998; Schluter, 2001). Large males could succeed in nesting territory contests, while increased fecundity is suggested to occur with increased female body size (Stanley and Wootton, 1986; Kitano *et al.*, 2007). Males could have larger heads in order to facilitate specific behaviours, such as collecting benthic debris with their jaws to build nests or to bite other stickleback males and egg predators when defending the nest (Reimchen and Nosil, 2004; McGee and Wainwright, 2013). Differences between sexes are often more pronounced than morphological differences between populations of sticklebacks, especially in the head region (Caldecutt *et al.*, 2001; Aguirre *et al.*, 2008; Cooper *et al.*, 2011). Additionally, Spoljaric and Reimchen (2008) noted that sexual dimorphism was highest in larger, clear lakes than in populations inhabiting shallow, small, stained ponds around northern Haida Gwaii, BC. The larger lakes are thought to allow males and females to occupy different niches in the water column (Nosil and Reimchen, 2005).

In marine populations, females are larger than males and males have larger heads than females, as seen in freshwater populations (Kitano *et al.*, 2007; Aguirre *et al.*, 2008; Spoljaric and Reimchen, 2008; Yershov and Sukhotin, 2015). Males also display sexual colouration in marine populations (R. Saimoto, 1993; Golovin *et al.*, 2019). Spoljaric and Reimchen (2008) observed six marine populations around northern Haida Gwaii, BC and found great variability in body shape dimorphism among their marine sites. However, they did note that dimorphism was less expressed in their marine habitats compared to the 99 lake populations in their study. And body shape dimorphism patterns were the same in their freshwater lake, stream, and marine populations (*e.g.* males with larger heads and gapes, while females were larger overall) (Spoljaric and Reimchen, 2008). A few studies other have looked specifically at sex ratio variation among marine populations as it relates to male-biased predation, but otherwise we know far less about sexual dimorphism in and among marine populations than in freshwater populations (Mäkinen *et al.*, 2006; Rollins *et al.*, 2017; Golovin *et al.*, 2019).

The current state of marine stickleback research

Because marine sticklebacks can potentially migrate between open water and coastal regions, appear to be unhindered by physical barriers in the coastal environment that might impede gene flow, and possess a relatively unaltered morphology compared to the fossil of a marine stickleback, the idea that marine sticklebacks have remained unchanged over thousands of years has been treated as a fundamental assumption in stickleback research (Foster, 1992; Bell and Foster, 1994; Taylor and McPhail, 1999; Hohenlohe *et al.*, 2012). This assumption was validated by early genetic studies looking at population differentiation among sticklebacks using a variety of methods (*i.e.* mtDNA, microsatellites, or a focus on allozymes), which uncovered little genetic structuring among marine stickleback populations (Haglund *et al.*, 1992; Ortí *et al.*, 1994; Colosimo, 2005; Mäkinen *et al.*, 2006; Mäkinen and Merilä, 2008). As a result, many studies have used different marine populations across the northern hemisphere to represent the ancestral stickleback phenotype, and assume that contemporary marine populations are homogenous (Walker and Bell, 2000; Kristjánsson *et al.*, 2002; Shaw *et al.*, 2007; Wund *et al.*, 2008; Arif *et al.*, 2009; Terekhanova *et al.*, 2014; Ab Ghani *et al.*, 2016; Miller *et al.*, 2019). This presumed ancestral morphology includes a complete row of bony armour plates, with longer dorsal and pelvic spines, a larger average body size, a more streamlined body shape, and a smaller head than freshwater sticklebacks (Klepaker, 1996; Leinonen *et al.*, 2006; Bjærke *et al.*, 2010; Taugbøl *et al.*, 2014; Pedersen *et al.*, 2017).

Nevertheless, the common ancestor may display more variation than previously detected (DeFaveri, Jonsson, *et al.*, 2013; DeFaveri, Shikano, *et al.*, 2013; Morris *et al.*, 2018). For instance, researchers have identified two or three distinct coastal marine stickleback ecotypes (*i.e.* anadromous, marine, estuarine) (Snyder and Dingle, 1989; Baker, 1994; McPhail, 1994). Anadromous populations migrate to freshwater habitats as adults to breed in summer months and to raise juveniles in fresh water, while marine populations spend their entire life cycle in full-salinity environments (King, 2016; Ahnelt, 2018). Thus, anadromous sticklebacks likely pay a larger metabolic cost than marine sticklebacks due to their rapid shift in habitats, from fresh water to full-salinity open ocean, after the breeding season (Dufresne *et al.*, 1990; Picard *et al.*, 1990; King, 2016). Marine populations also have a strong homing ability, or the ability to return to a familiar site after a displacement to an unfamiliar location (Gerking, 1959), as seen in the White Sea populations, and in eastern Pacific populations including ones in BC (R. Saimoto, 1993; Ivanova *et al.*, 2019; Lajus *et al.*, 2020). Two types of homing responses exist in marine sticklebacks: the natal homing response, which occurs when an adult fish returns to the location of their birth, and the reproductive homing response, which occurs when an adult fish returns to a breeding site after displacement (R. Saimoto, 1993). Sticklebacks that exhibit natal and reproductive homing responses among marine populations show researchers that gene flow is likely lower than currently assumed, meaning that marine populations could be genetically isolated from one another (Ward *et al.*, 2013; Pedersen *et al.*, 2017; Lajus *et al.*, 2020). Morris *et al.* (2019) specifically found *Eda* allele expression at various frequencies between local populations of marine sticklebacks along the west coast of North America. This variation in *Eda* genotypes across marine populations may have caused different freshwater adaptation rates in the past, a factor that studies do not often consider (Hagen and McPhail, 1970; Taylor and

McPhail, 1999; Barrett *et al.*, 2008; Schluter *et al.*, 2010; Haenel *et al.*, 2019; Miller *et al.*, 2019).

The third possible coastal marine ecotype is estuarine sticklebacks, which inhabit brackish and/or high salinity marine habitats without spending any time in fresh water to breed. Because few estuarine populations have been identified, I will focus only on the anadromous and marine ecotypes (Taugbøl *et al.*, 2014; Arai *et al.*, 2020; Wasserman *et al.*, 2020).

The diversity of sticklebacks in Southern British Columbia and Vancouver Island

The Canadian Pacific coast, in BC, has long been a hub for stickleback research in freshwater lakes and streams, coastal estuaries, marine bays and lagoons. Genetically and phenotypically divergent freshwater populations can be found throughout the Haida Gwaii archipelago (Spoljaric and Reimchen, 2011; Reimchen *et al.*, 2013), Vancouver Island (Raeymaekers *et al.*, 2009; Di Poi *et al.*, 2016; Weber *et al.*, 2017), and Southern mainland BC (Barrett *et al.*, 2009; Jamniczky *et al.*, 2015; Miller *et al.*, 2019).

Parapatric freshwater sticklebacks are not only found in the same lake, but also found across transitions between lake and stream habitats in BC freshwater watersheds, including the Sangan River watershed, Misty, Pye, Drizzle, and Mayer Lake-stream systems (Reimchen *et al.*, 1985; Berner *et al.*, 2009; Oke *et al.*, 2016; Rennison *et al.*, 2019). Lake-stream sticklebacks differ in morphology, sensory systems, and behaviours despite continuous possibility for gene flow between lake and stream populations (Deagle *et al.*, 1996; Sharpe *et al.*, 2008; Berner *et al.*, 2009; Oke *et al.*, 2016; Weber *et al.*, 2017). The morphological differences between lake-stream populations are very similar to those of the benthic-limnetic pairs found in a single lake. Stream sticklebacks have deeper, large bodies for burst swimming, and shorter gill rakers (Lavin and

McPhail, 1986; Taylor and McPhail, 1986; Berner *et al.*, 2009). Conversely, lake sticklebacks have shallow, streamlined bodies for sustained swimming, and longer gill rakers (Lavin and McPhail, 1986; Hendry *et al.*, 2002). These foraging morphological differences coincide with the prey resource availability in streams and lakes, suggesting lake-stream pairs maintain partial ecologically based reproductive barriers despite inhabiting physically connected habitats (Rundle and Schluter, 2004; Rundle and Nosil, 2005; Berner *et al.*, 2009).

Other projects in BC have compared freshwater populations with at least one marine population, such as in the Little Campbell River Estuary south of Vancouver, BC (Hagen and Moodie, 1979; Withler *et al.*, 1986; Dalziel *et al.*, 2012; Jamniczky *et al.*, 2015; Miller *et al.*, 2015). The marine populations adjacent to freshwater populations are used to represent the ancestral morphology to provide context among freshwater population comparisons (Ingram *et al.*, 2012). Hagen (1967) found there were two genetically diverse stickleback populations inhabiting Little Campbell River: one freshwater population upstream, and an anadromous population entering the brackish downstream waters to breed in the summer (Hagen, 1967). Many researchers have collected an anadromous population to cross with the Little Campbell River and other freshwater populations by *in vitro* fertilization in the lab (Hagen, 1967; Kitano *et al.*, 2007; Dalziel *et al.*, 2012; Rogers *et al.*, 2012).

Researchers have crossed marine and freshwater populations from BC for several reasons. For example, researchers have tested whether cold tolerance is heritable, have investigated genetic contributions to sexual dimorphism, and analyzed *Eda* allele expression (Withler *et al.*, 1986; Marchinko, 2009b; Barrett *et al.*, 2011).

Perhaps the most commonly studied marine population in BC is from Oyster Lagoon, on the Sechelt Peninsula north of Vancouver (R. K. Saimoto, 1993; Marchinko, 2009a; Barrett, 2010; Barrett *et al.*, 2011; Di Poi *et al.*, 2016; Barry, 2019). The Oyster Lagoon population is purely marine, and has also been used in lab-reared crosses with freshwater sticklebacks to investigate genetic variation in stickleback stress response, ontogeny of phenotypic variation, and analysis of genetic variation at certain loci (Withler and McPhail, 1985; Di Poi *et al.*, 2016; Pistore, 2018). Despite the abundance of research on BC stickleback populations, little is known about variation in the marine populations along the coast of Vancouver Island and the coast of Southern mainland BC.

Oceanographic variation in Southern British Columbia and Vancouver Island

With a mesothermic maritime climate and one of the longest fjord coastlines in the world, BC has a unique coastline (Thomson, 1981). My sampling area covered distinct oceanographic regions around Southern BC: The Strait of Georgia, the Juan de Fuca Strait (both part of the Salish Sea), the northern coast of Vancouver Island, and the west coast of Vancouver Island. These four regions are dominated by different oceanographic forces. The Strait of Georgia is estuarine dominated (Thomson, 1981; Masson and Cummins, 2004). The Juan de Fuca Strait is dominated by tidal mixing (Davenne and Masson, 2001). The west coast of Vancouver Island is influenced by upwelling and multiple fjords providing freshwater runoff (Pickard and McLeod, 1953; Dallimore and Jmieff, 2010). Lastly, the northern coast is a transition zone between the upwelling and downwelling domains of the eastern Pacific (Irvine and Crawford, 2011).

Oceanographic regimes may profoundly influence marine stickleback morphology for multiple reasons. Current patterns govern the covariation between temperature and salinity, which control seawater density, and the ability of distinct bodies of water to mix (Strub *et al.*, 1987; Smith, 1995; Hickey and Banas, 2003). Therefore, oceanographic features can act as barriers to gene flow (White *et al.*, 2010; Pascual *et al.*, 2017) via habitat fragmentation, geographic distance, currents, oceanic fronts, and differences in salinity and temperature (Bernardi, 2000; Hemmer-Hansen *et al.*, 2007; Schunter *et al.*, 2011; Poortvliet *et al.*, 2013; Milano *et al.*, 2014; Tanner *et al.*, 2014). Differences in current patterns and coastal ecosystems can create physical separation between regimes, which can keep population structures distinct (White *et al.*, 2010). This is especially true for populations that breed in coastal systems, and whose larvae/juveniles have limited swimming ability (Gilg and Hilbish, 2003; Mitarai *et al.*, 2009; Weersing and Toonen, 2009; Ross *et al.*, 2012). Current patterns often create distinct oceanographic regions (*e.g.* estuaries, upwelling zones) that vary in their biological and physical properties (Townsend, 2010). If oceanography is indeed a barrier, I would expect sticklebacks to vary among these regions. Indeed, work by Morris *et al.* (2018, 2019) shows that genetic and morphological differences in eastern Pacific sticklebacks overlay major oceanographic features of the Pacific ocean, such as the Alaska current gyre, the California current upwelling system, and the transitional zone between them (although they did not consider oceanographic variation as a driver of the patterns).

Because oceanographic regimes also govern the covariation of temperature and salinity, they can influence organismal traits because of physiological responses within each regime. The Northeast Pacific is located within the California Current System, where temperature and salinity increase southward along the coastline (Hickey, 1998). Local populations of multiple marine species vary genetically and phenotypically along temperature and salinity gradients where temperature affects metabolic rate and body size, and salinity affects ionic regulation and growth rate (Bœuf and Payan, 2001; Sampaio and Bianchini, 2002; Juan-Jordá *et al.*, 2009; Nielsen *et al.*, 2009; Teacher *et al.*, 2013; Árnason *et al.*, 2013; Milano *et al.*, 2014). The tidepool copepod

(*Tigriopus californicus*) for example, shows pronounced adaptive divergence to local temperature and salinities, with significant latitudinal clines found along the west coast of North America (Leong *et al.*, 2018). Morphological and genetic divergence across temperature and salinity gradients have been noted in Atlantic herring (*Clupea harengus*) populations (Bekkevold *et al.*, 2005; Jørgensen *et al.*, 2008), as well as two genetically distinct local populations of Atlantic cod (*Gadus morhua*) which adapted to local salinity differences between the North Sea and Baltic Sea (Larsen *et al.*, 2012; Árnason *et al.*, 2013).

The Baltic Sea marine sticklebacks show substantial genetic and morphological differentiation along steep temperature and salinity gradients in both lateral plate numbers and body shape (Guo *et al.*, 2015; Jakubavičiūtė *et al.*, 2018). All the more reason to predict that oceanographic regimes to help explain morphological variation across regions in marine sticklebacks. After all, when a species occupies a range spanning a heterogenous environment, a single phenotype is unlikely to yield high fitness throughout the range (Via *et al.*, 1995).

The four regions surrounding Vancouver Island noticeably differ in average temperature and salinity. I compared data from seven BC lighthouses in May, June, and July between 1956 – 2019 (Table A.2), and found that average sea-surface temperatures are highest in the Strait of Georgia and lowest along the northern coast of Vancouver Island (DFO, 2020). An estuarine dominated system is fed by warm, freshwater runoff from the nearby rivers, while tidal mixing zones are created from tidal currents which circulate from the surface to the bottom of continental shelf waters (Masson and Cummins, 2004). Upwelling brings cold, saline water up to the surface from the deep waters, while downwelling is the reverse process (Pickard and McLeod, 1953). The tidally mixed Juan de Fuca Strait and upwelling zone on the west coast recorded intermediate temperature and salinity averages (DFO, 2020). On a regional scale, the

Strait of Georgia is the least saline due to runoff from the Fraser River (Davenne and Masson, 2001). For example, snow melt leads to enormous freshwater pulses from the Fraser. The northern coast of Vancouver Island is more saline because it is a transition area between upwelling and downwelling zones, and has the fast currents which funnel into the Queen Charlotte Strait (Irvine and Crawford, 2011; Marine Planning Partnership for the North Pacific Coast (B.C.) *et al.*, 2015). Based on this, I expect stickleback populations in the Strait of Georgia to experience the highest temperatures and lowest salinities, while populations in the northern coast of Vancouver Island and the Juan de Fuca Strait to experience the lowest temperatures and highest salinities. Populations on the west coast of Vancouver Island will likely experience intermediate temperatures. However, departures from this trend at individual sites can be caused by local variation in tidal cycles, freshwater inputs, topography, or urban development.

Habitat variation in Southern British Columbia and Vancouver Island

In addition to large-scale differences in oceanography, the coastal habitats where sticklebacks breed vary in their physical, chemical, and biological structures (Palumbi, 1994; Roff and Taylor, 2000). Along the Southern coast of BC, marine sticklebacks have been noted in different types of habitats including tidal flats, salt marshes, and lagoons. These habitats exist in estuaries as well as fully marine, coastal environments (Flynn *et al.*, 2006). Most of my sites were in estuaries, except for three: Oyster Lagoon, Salt Lagoon, and Coles Bay, Saanich (Appendix A, Table 1). The first and second are coastal lagoons. The third is a tidal flat with eelgrass beds.

Inhabiting different coastal habitats could potentially lead to morphological variation in sticklebacks because of biological and physical differences between habitats. The presence of tidal currents and the proximity to fresh water determine variation of biological and chemical properties in coastal habitats (Griffin and LeBlond, 1990; Simpson *et al.*, 1990; Vaz *et al.*, 2005). Webster *et al.* (2011) sampled sticklebacks from different locations in the Great Eau estuary in eastern England, where they found significant variation in body depth and pelvic/dorsal spine length between stickleback populations sampled in four different habitats (*i.e.* salt marsh, estuary channel, man-made ditch in the estuary, and upstream river sites). Additionally, the complexity of coastal habitats differs, leading to differences in stickleback morphology. Garduño-Paz *et al.* (2010) found that freshwater stickleback body shapes differed between simple vs. complex habitats, potentially influenced by the swimming needs of each habitat. Sticklebacks in the complex habitats (*i.e.* included large rocks and macrophytes) had shallow, elongated bodies better suited for swimming between interstitial spaces to catch prey, while sticklebacks in the simple habitat were deeper bodied and shorter in length (Garduño-Paz *et al.*, 2010). We currently do not know whether habitat complexity affects stickleback morphology in the ocean.

In the coastal ocean, microhabitats such as lagoons and estuaries vary in their tidal regimes and can therefore vary in temperature and salinity. When comparing coastal habitats with stickleback populations, it is important to remember that anadromous sticklebacks do not stay in estuaries and coastal habitats year-round. The coastal habitats serve as nursery grounds for anadromous sticklebacks before migrating back to the sea for the winter months. Nevertheless, temperature and salinity gradients between coastal microhabitats impacts growth rate and food availability for other fish breeding in those habitats (Fry, 1969; Brett and Groves, 1979; Miller and Dunn, 1980; Lankford and Targett, 1994). For example, the growth rate of local populations of weakfish (*Cynoscion regalis*) differed significantly along temperature and salinity gradients within a Delaware estuary (Lankford and Targett, 1994). In the Campbell River estuary on Vancouver Island, Macdonald *et al.* (1987) found that temperature, salinity, and water velocity were the main driving factors determining which microhabitats juvenile salmonids occupied before migrating out to sea. Overall, if temperature and salinity create barriers between stickleback populations between regions (on a large scale), then I would expect them to also create barriers among different habitats within each region (on a smaller scale).

Tidal flats are exposed to more wave action, and have greater pelagic habitat availability than other habitats (Weinmann *et al.*, 1984; Rybkina *et al.*, 2017). Tidal flats can support vegetative beds such as eelgrass and have either cobble, sand, or gravel substrate (Weinmann *et al.*, 1984; Levings *et al.*, 1997; Semeniuk, 2005; Zacharias *et al.*, 2008). Even at the lowest low tidal mark, eelgrass beds are generally submerged and exposed to the surrounding sea (Dallimore and Jmieff, 2010; Seebacher *et al.*, 2016). Tidal flats are therefore heavily influenced by the regional oceanographic regimes they border, thus the salinity and temperature gradients on a tidal flat generally follow the neighbouring open ocean (Hickey and Banas, 2003). In estuaries, tidal flats border salt marshes at the lower intertidal zone (Flynn *et al.*, 2006). Tidal flats that do not border estuaries are locally affected by small tidal creeks or by tidal processes (Fagherazzi *et al.*, 2004). Temperature and salinity on tidal flats are also influenced by tidal advection and flood and ebb currents (McGurk, 1989; Hickey and Banas, 2003). Primary productivity is high on tidal flats with eelgrass beds because eelgrass blades suspend detritus and planktonic organisms. This in turn provides prey for suspension-feeding invertebrates and fish species which feed throughout the water column (Kelly *et al.*, 2008).

Salt marshes are found between the mean sea level of a region and the mean highest high water mark (Fagherazzi *et al.*, 2004). In my study area, salt marsh habitats are only found in estuaries (*e.g.* delta fronts of large rivers, the heads of protected inlets) (Pritchard, 1967;

Hutchinson, 1982). Salt marshes are semi-enclosed, coastal habitats with organic substrate, and connected to the open ocean by a network of tidal channels which carry water and sediment during daily tidal events (Fagherazzi *et al.*, 2004). Sea water entering salt marshes with the tides is considerably diluted with the freshwater in the estuary (Chapman, 1974; Campbell, 1986). Because the flood and ebb tides typically occur twice daily along the coast of Vancouver Island and the coast of Southern mainland BC (*i.e.* semidiurnal), salinity and temperature fluctuate significantly in salt marshes (Campbell, 1986). However, salt marshes are very productive ecosystems due to the presence of salt marsh vegetation which provides detritus for invertebrates, which in turn feed fish and other vertebrates (Armonies, 1986; Campbell, 1986).

Lagoons have restricted access to the neighbouring ocean, usually with a small channel which connects a lagoon to the open coast or an estuary (Mace and Mackie, 1970; Robinson and Brown, 1983; Kjerfve, 1986). Lagoons are shallow, sheltered habitats with organic substrate and increased benthic availability (Kjerfve, 1994; Emmett *et al.*, 2000). Due to the shallow depth, temperatures in a lagoon are associated with the weather, where intense solar radiation keeps temperatures warm in the summer months while surface cooling occurs in the winter months (Kjerfve, 1986). Salinity is influenced by seepage of saline water from underneath the lagoon, fresh water inflow from rain, and evaporation (Mace and Mackie, 1970). Thus, high temperatures and low rainfall experienced during the summer months in BC would certainly increase salinities in my lagoon sites. Green and brown filamentous algae blooms are common blooms in the summer months, with prey availability dominated by deposit-feeding benthic invertebrates (Peterson, 1979; R. K. Saimoto, 1993; Barnes and Villiers, 2000).

Hypotheses and study description

My project broadly aims to assess morphological variation among marine sticklebacks across the small geographic scale of Vancouver Island and Southern BC and had three main objectives. My first objective is to survey many coastal sites around Vancouver Island and Southern BC to locate viable marine stickleback populations (*i.e.* populations from which a sufficient number of sticklebacks can be collected). My second objective is to characterize morphological variation among and within these populations: between sexes, and among regions and habitats. My third objective is to assess whether this variation can be attributed to specific physical factors (*i.e.* oceanographic regime, habitat characteristics). I will examine the following hypotheses:

1) I hypothesize that stickleback morphology will vary between oceanographic regions (Strait of Georgia, the Juan de Fuca Strait, west coast of Vancouver Island, and north coast of Vancouver Island), either because currents act as a barrier between the regions, or because of variation in temperature or salinity among oceanographic regimes. If temperature and salinity are driving these differences, I would expect that oceanographic regions with higher salinity will have more armoured sticklebacks, while those from lower temperatures will have larger bodies.
2) I hypothesize that there will be habitat differences in head and body morphology, as well as armour phenotype. If they are driven by temperature and salinity variation, I would expect that habitats with higher salinities would have more heavily armoured sticklebacks, while those from lower temperatures would have larger bodies. If these are driven by physical structure, I would expect that habitats with greater benthic availability to have sticklebacks with large heads and a head shape which suits a benthic-dominant

niche. Conversely, I would expect pelagic dominant habitats to have sticklebacks with smaller heads and a head shape to suit a pelagic niche.

3) I expect to see body shape and head shape differ between male and female sticklebacks due to sexual dimorphism, and I will test whether sexual dimorphism patterns are consistent among regions or habitats. Characterizing sexual dimorphism will allow me to assess the relative importance of sexual dimorphism (*i.e.* within population variation) compared to oceanographic and ecological factors (*i.e*. among population variation).

Methods

Between May – July 2019, a total of 534 fish were collected from 15 sites around Vancouver Island and Southern BC, Canada (Appendix A, Table A.1). Over 40 sites were scouted, but sticklebacks were only caught in high numbers at 20 of those sites. But 5 sites were excluded due to methodology that was inconsistent with the other 15 that I analyzed in this study. At each site, sticklebacks were caught with beach seines at 1-2m depth and 2-3m offshore, or with un-baited minnow traps. Sticklebacks were collected and euthanized following a University of Victoria (UVic) animal use protocol (#2019-008) and Fisheries and Oceans (DFO) collection permits (XE-17-2019, XE-75-2019). Each fish was individually preserved in 95% ethanol. Three points of salinity and temperature were recorded at ~1 meter depth at each site using a hand-held YSI (YSI Inc., Yellow Springs, OH, USA) during daylight hours. Sites were not revisited throughout the sampling season due to lack of time and the nature of our exploratory field season, and these salinity and temperature readings were not taken at similar points in the daily tidal cycle. I sampled in all four oceanographic regions of Vancouver Island: the Strait of Georgia, the Juan de Fuca Strait, and the western and northern coasts of Vancouver

Island (Fig. A.5). Within each region I sampled from three different coastal habitat types: tidal flats, salt marshes, and lagoons.

Laboratory processing and fish imaging

In the lab, each fish was sexed by inspection of internal gonads, and fin clips were collected (stored in 95% ethanol) for archiving purposes. 14 specimens were excluded if I could not properly identify the sex, if there were < 3cm in length (as those were likely juveniles), or if the specimen had been severely injured and were missing body parts. Each specimen was categorized into one of three plate morphs: low (10 or fewer plates with no keel), partial (11-25 plates, missing plates only on the middle of the body), or complete (26-35 plates) (Bell and Foster, 1994). The head and body of each fish were then photographed for geometric morphometric analysis. The head of each specimen was photographed first using a SPOT Flex camera (SPOT Imaging, Diagnostic Instrument, Inc., Stirling Heights, MN, USA) mounted on a Wild Leica- M420 dissecting microscope at 10.5x magnification (Leica Biosystems, Wetzlar, Germany). Each specimen was placed in a homemade apparatus to ensure each was photographed at the same position in the field of view. This apparatus was a square metal cookie cutter glued onto a Styrofoam block, and every specimen was then pinned to the Styrofoam (Fig. A.2). Photographing conditions remained constant between all specimens to ensure shape analysis was accurate (Zelditch *et al.*, 2012). Each headshot was calibrated and scaled to include a 1mm scale bar to ensure that measurements could be extracted from the landmark data (Marques, 2019). After completion of all headshots, the 534 specimens were photographed again to capture the body (referred to as body shots from here on). The Styrofoam apparatus was secured to a table, where an individual was placed right side down and pinned to the Styrofoam to keep the body secure. A Nikon D3500 (Nikon Corp., Tokyo, Japan) was mounted above the Styrofoam apparatus, thus each image was captured from 12cm above a specimen. A ruler was placed in each photo as a scale bar for later calibration.

Geometric morphometrics

The original images were converted into *tps* format using the software *tps.Util* version 1.61, and then organized into files for superimposition (Rohlf, 2015). The *tps* images were uploaded into *tps.Dig* version 2.05 to digitize landmarks on the head and body shots (Rohlf, 2005). I chose landmarks that provided adequate coverage of morphology and were on the same plane. It was also important that each landmark was easy to find consistently on an image (Zelditch *et al.*, 2012). Leinonen *et al.* (2006) warned that too few landmarks might not capture morphological differences, while too many could lead to less clear geometric morphometrics. For headshots, 13 anatomical landmarks were placed around the left side of the head (Fig. 1), which were adapted from landmarks used in previous studies (Caldecutt and Adams, 1998; Aguirre and Akinpelu, 2010). For body shots, 15 landmarks were placed on the left side of the body (Fig. 2). The body landmarks were also based on previous studies (Schluter *et al.*, 2004; Leinonen *et al.*, 2006; Seebacher *et al.*, 2016; Jakubavičiūtė *et al.*, 2018).

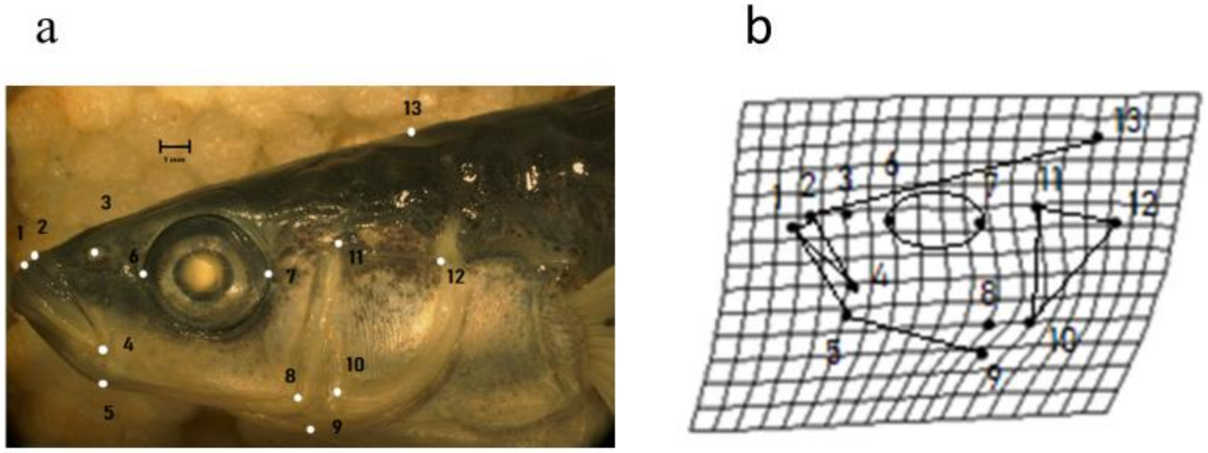

Figure 1. Analysis of stickleback head shape. Panel (a) shows an image of the left side of a female stickleback head sampled from the Black Creek Estuary site with anatomical landmarks (1-13) used for geometric morphometric analysis (see methods). Panel (b) is a thin-plate spline deformation grid which represents variation in the shape of the specimen in panel (a). The deformation grid is based on the Procrustes shape coordinates, generated from a generalized Procrustes analysis using the shape landmarks shows in panel (a) as described in the methods.

The landmarks used for geometric morphometric analysis of head shape were: 1. Anterior tip of the upper lip; 2. Anterior tips of the snout; 3. The nostril; 4. The axis of the jaws; 5. Posterioventral edge of angular; 6. Anterior edge of the eye; 7. Posterior edge of the eye; 8. Posterioventral edge of the third suborbital; 9. Anterioventral edge of the interoperculum; 10. Anterioventral edge of the operculum; 11. Anteriodorsal edge of the operculum; 12. Posteriodorsal edge of the operculum; 13. Posterior extant of the supraoccipital. The bone description of landmarks was based on (Bowne, 1994; Wund *et al.,* 2008; Aguirre and Akinpelu, 2010) (Fig. 1).

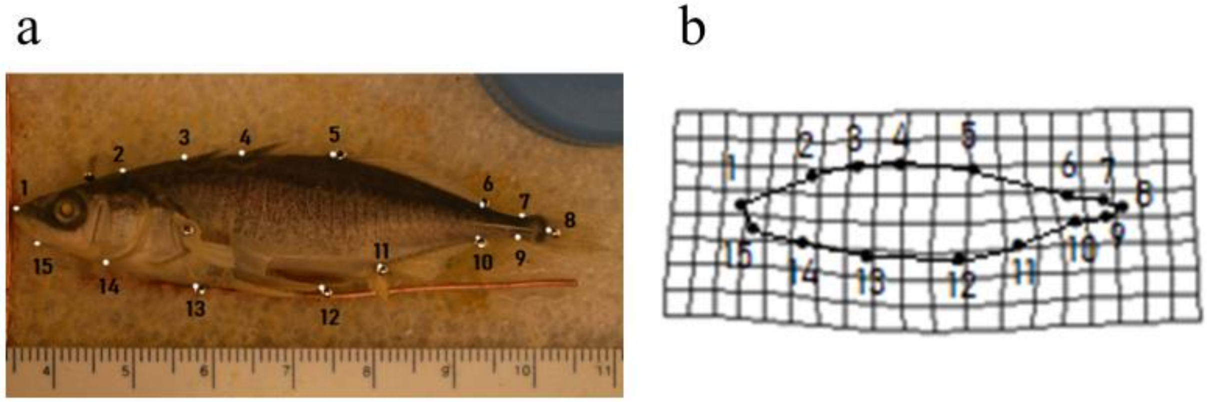

Figure 2. Analysis of stickleback body shape. Panel (a) shows an image of the left side of a female stickleback body sampled from the Englishman River site, with anatomical landmarks (1-15) used for geometric morphometric analysis (see methods). Panel (b) is a thin-plate spline deformation grid which represents variation in the shape of the specimen in panel (a). The deformation grid is based on the Procrustes shape coordinates, generated from a generalized Procrustes analysis using the shape landmarks shows in panel (a) as described in the methods.

The landmarks used for geometric morphometric analysis of body shape were: 1. Anterior tip of the upper lip; 2. Posterior extant of the supraoccipital; 3. Anterior base of the first dorsal spine; 4. Anterior base of the second dorsal spine; and 5. Anterior base of the third dorsal spine; 6. Posterior edge of the dorsal fin; 7. Anteriodorsal edge of the caudal fin; 8. Caudal end of the caudal keel; 9. Anterioventral edge of caudal fin base; 10. Posterior edge of ventral fin; 11. Anterior edge of anal fin; 12. Posterior process tip of the pelvic girdle; 13. Anterior process tip of the pelvic girdle; 14. Ventral tip of pectoral girdle; 15. Posterior edge of angular (Aguirre *et al.*, 2008; Webster *et al.*, 2011) (Fig. 2).

After the landmarks were digitized, landmark coordinates (X and Y positions) were uploaded into R (R Core Team, 2020). I extracted linear distances from each image using the “linear.dist” function in the *landvR* package for R (Guillerme *et al.*, 2019). Head length (mm) was extracted from head shots (landmarks 1 and 12) as a proxy for head size (Fig. 1). Standard length (cm) was extracted from body shots (landmarks 1 and 8) as a proxy for body size (Fig. 2).

Generalized Procrustes analysis

The head and body shape coordinates were analyzed separately. In the *geomorph* package, the “gpagen” function was used to perform a Generalized Procrustes Analysis (GPA), which is the most common approach for separating shape from size (Mitteroecker *et al.*, 2013; Adams *et al.*, 2020). During GPA, every individual (set of landmarks) was superimposed on top of one another by centering, scaling, and rotating landmark coordinates (Zelditch *et al.*, 2012; Jojić *et al.*, 2014). Centering took the X and Y coordinates of the centroid (the distance of all landmark coordinates of one individual to the center of the shape), and subtracted it from the corresponding coordinates of each landmark (Zelditch *et al.*, 2012; Marques, 2019). Scaling divided the landmark coordinates by the centroid size (CS) of the individual (Kimmel *et al.*, 2008). The rotation process consisted of spinning each specimen to reduce the distance between homologous landmarks. Following GPA, 26 vectors of shape were produced for the head dataset (X and Y coordinates for 13 landmarks), while 30 vectors of shape were produced for the body dataset (X and Y coordinates for 15 landmarks) (Appendix B) (Jojić *et al.*, 2014). Additionally, an extra vector was produced for each dataset which described the geometric size of each specimen’s head or body (*i.e.* CS) (Zelditch *et al.*, 2012). This separation between shape and size measurements allowed me to test for the possible correlation between the shape and size of an individual (Mitteroecker *et al.*, 2013; Klingenberg, 2016).

Linear mixed-effects models of head and body size

First, I assessed how sex, region, and habitat were associated with variation in head and body size. Centroid size (CS) of the head and head size (mm) were highly correlated (Partial Least Squared correlation coefficient = 0.958, $p = 0.001$) (Fig. C.1), as were CS and body size (cm), (Partial Least Squared correlation coefficient = 0.991, $p = 0.001$) (Fig. C.2). Therefore, the analysis of head and body size was conducted on head length (mm) and standard length (cm) using linear mixed-effects models (LMMs), as they are clearer and easier to interpret than Procrustes linear models.

Linear mixed effect models were built using either head size (mm) or body size (cm) as the response variable. After exploratory analyses I chose not to log-transform either head size or body size variables because both were normal data and there was no significant difference in the models when the variables were log-transformed (I checked for normality and homogeneity of the residuals using histograms and qq-plots).

The predictor variables included in a global model were oceanographic region (region), coastal habitat type (habitat), sex, the interactions between sex and region, the interaction between sex and habitat, and the sampling site as a random factor. Site was included as a random effect after assessment with the “exactRLRT” function from the *RLRsim* package in R (Bartoń, 2020). The global model for body size was subjected to model selection based on corrected Akaike’s information criterion for small sample sizes (AICc) (Galipaud *et al.*, 2014). The best model fit was selected as the one with the lowest AICc score (Burnham and Anderson, 2002; Garamszegi, 2011). A delta AICc (ΔAICc) was calculated as the difference between the best-fit model and alternative models (Burnham and Anderson, 2002; Posada and Buckley, 2004; Grueber *et al.*, 2011; Symonds and Moussalli, 2011). A general rule of thumb used in ecological studies assumes that models with Δ-values between 4 -7 are equally valid (Burnham and Anderson, 2002; Richards, 2008; Galipaud *et al.*, 2014). After model selection, a decision was made on whether or not to average top models with ΔAICc < 4, based on recommendation of Nakagawa and Freckleton (2011) and Cade (2015).

Although there might have been a temporal dependence in the data, I did not include dates in the models because time-series analyses require data intensive data sets, and my sites and dates were confounded. I think any temporal variation will likely be included in the random Site factor.

All models were fitted with the *lme4* package for R, model selection was performed with the *MuMIn* package (Robinson, 2019; Bartoń, 2020). Multicollinearity was assessed between levels of each fixed effect of the global model using the “vif.mer” function for R (Frank, 2011). This function calculated VIF values (Variance Inflation Factor) specifically for mixed-effect models fit with the *lme4* package. VIF values ~3 suggested low to moderate collinearity, while values above indicated significant collinearity (Allison, 2012). Because region was a categorical factor with more than three levels, the reference category was set to the level with the largest sample size (*i.e.* Strait of Georgia (SoG)). This prevented over-estimated VIF values (Burnham and Anderson, 2002; Symonds and Moussalli, 2011). Variance explained by the model was calculated by both fixed and random effects (R^2_c , conditional R^2), and for the total variance explained by only fixed effects (R^2_m , marginal R^2) using the “r.squaredGLMM” function of the *MuMIn* package (Burnham and Anderson, 2002; Posada and Buckley, 2004). To further confirm the reliability of our top models, I checked for normality and homogeneity of the residuals across the regression line using histograms and qq-plots.

Principal components analysis

Principal components analysis (PCA) was used to analyze variation in the Procrustes shape coordinates. The “plotTangentSpace” function in the *geomorph* package for R performed PCA on the covariance matrix of each dataset to extract loadings and scores along each principal component axis (Wickham, 2016). To help visualize the shape differences, thin-plate spline deformation grids were generated for the two most extreme shape differences (*i.e.* minimum and maximum PC scores) using the “plotRefTarget” function from the *geomorph* package in R (Adams *et al.*, 2020).

To assess the relationship between size (*i.e.* Log CS) and body shape (*i.e.* Procrustes shape variables), a two-block partial least squares (PLS) analysis was performed using the “two.b.pls” function from the *geomorph* package (Zelditch *et al.*, 2012). A block refers to one of two blocks of variables I assessed (*i.e.* Procrustes shape coordinates and Log CS). Partial least squares find linear vectors that express the most covariance between blocks to describe correlation (Mosimann, 1970; Klingenberg, 2016).

Procrustes linear models (LMs) were built to test for differences in head and body shape (Procrustes shape variables) in relation to Log CS, sex, region, and habitat. Site was nested into oceanographic region as a random effect. Procrustes ANOVAs were performed using the “procD.lm” function in *geomorph* (Adams *et al.*, 2020). Procrustes distances were used as a measure of the sum of squares, which were then evaluated through permutation to obtain *p*-values for the Procrustes LM variables (Goodall, 1991; Anderson, 2001; Scrucca *et al.*, 2016).

Bayesian cluster analysis

While linear models test for differences based on a proposed hypothesis, Bayesian cluster analysis checks to see if there are patterns of clustering in the data without *a priori* assumptions. Bayesian cluster analysis was performed on the PC scores, generated by the shape analysis, to assess whether there was clustering of specimens within the head and body datasets. Cluster analysis assumed that the multivariate observations (*i.e.* PC scores) were independent and the datasets consisted of a certain number of clusters (Fraley and Raftery, 2007; Khan, 2009). Unlike traditional cluster analysis, Bayesian cluster analysis fits the Principal Component data with more than one model with variable number of clusters and different cluster shapes (Fraley and Raftery, 2002, 2007).

In the Bayesian cluster analysis, the first two PC scores from both the head and body datasets were analyzed using fourteen multivariate Gaussian mixture models from the *mclust* package for R (Fraley and Raftery, 2007; Scrucca *et al.*, 2016). The distribution structure, orientation, volume, and shape of a cluster was determined by the covariance matrix of a Gaussian model (Fraley and Raftery, 2002; Willcox, 2011). I found the optimal number of clusters in each dataset and the best fit Gaussian mixture model, with a Bayesian Information Criterion (BIC) plot produced in *mclust* (Schwarz, 1978; Scrucca *et al.*, 2016). The highest (*i.e.* most negative) BIC value indicated the best fit Gaussian mixture model (Fraley and Raftery, 2002, 2007).

Results

Environmental variation

My spot samples of average temperature and salinity at each site, recorded during sampling did not follow my predictions, which I based on the oceanographic characteristics of each region, as well as DFO lighthouse data (DFO, 2020). First, based on the lighthouse data I predicted that the estuarine dominated SoG region would have the warmest temperature and lowest salinity. However, at the time of sampling, my SoG sites recorded the highest temperatures (average 19.95°C) (Fig. 3a) and the second-lowest salinity (average 25.03 PSU) (Fig. 3b). Second, I predicted the North region, as a transition zone between west coast upwelling and downwelling areas of the Alaska current, would have the coldest temperatures and highest salinities. The North region did record the highest salinities (average 30.83 PSU) (Fig. 3b), but only the second-coldest temperatures (average 18.93°C) (Fig. 3a). Third, as a transition zone between regions, I had expected to find intermediate characteristics in the JdF region. However, at the time of sampling, the tidally mixed JdF region exhibited the lowest temperature (average 18.80°C) as well as the lowest salinity (average 22.10 PSU) (Fig. 3a, 3b). The West region had intermediate temperatures (average 19.09°C) and salinities (30.06 PSU) (Fig. 3a, 3b), consistent with a coastline where an upwelling zone meets multiple prominent estuaries.

Despite my sites being further inland and often in estuaries (Table A.1), there were similarities between my data and the BC lighthouse dataset (DFO, 2020). The SoG sites and the SoG lighthouses recorded the warmest average temperatures among the regions. The North sites and the North lighthouse recorded the highest average salinities. The BC lighthouse dataset also showed JdF and the North lighthouses had very similar average temperatures (Table A.2). I observed similar, cold temperatures between the JdF and North sites (Fig. 3a). The West sites recorded intermediate temperature and salinities, like the West region lighthouses (Table A.2).

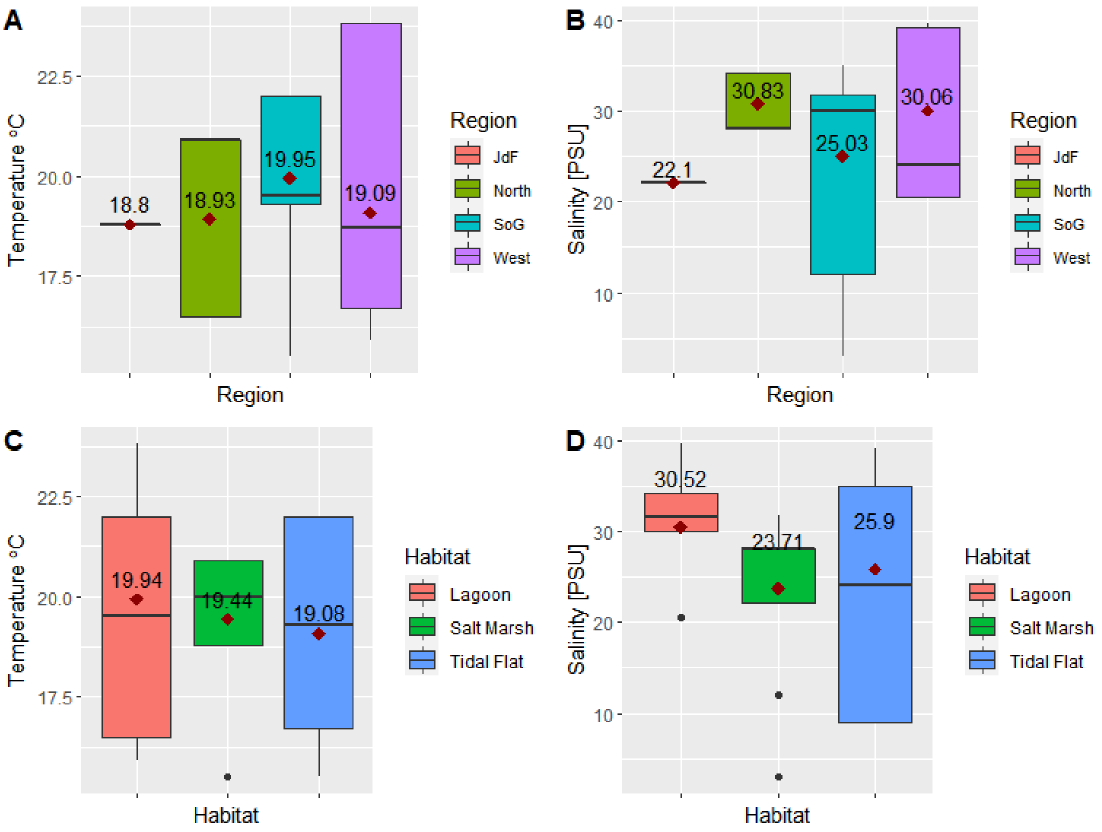

Figure 3. Box and whisker plots showing (a) regional variation in temperature (°C) sea surface temperatures (SST) at the time of sampling (spot samples) between the Juan de Fuca Strait (JdF), the Strat of Georgia (SoG), and the West and North coasts of Vancouver Island, and b) regional variation in sea surface salinity (PSU), (c) habitat variation in SST, and (d) habitat variation in sea surface salinity (PSU). The data are averages of three sampling points collected during the survey.

The temperature and salinity patterns I observed among habitats largely followed my predictions based on variation in physical characteristics, with a few exceptions. I predicted that lagoons, which are shallow and have poor tidal mixing, would be warmer and more saline than tidal flats. Salt marshes were expected to exhibit intermediate temperatures and salinities because sea water entering with the tides is also diluted by the freshwater supply in the estuary. Indeed, lagoons had the highest temperature (average 19.94°C), while tidal flats recorded the lowest temperature (average 19.08°C), and salt marshes were intermediate (average 19.44°C) (Fig. 3c). As predicted, lagoons also had the highest average salinity of 30.52 PSU. However, salt marshes had the lowest salinity (average 23.71 PSU), while tidal flats had an intermediate salinity (average 25.90 PSU) (Fig. 3d).

Variation in lateral plate morphology

Of all the fish collected, 97% were complete plate morphs, 2.2% were partial morphs, and 0.56% were low morphs. Because of the small number of partial and low morphs samples I combined the two into one category of non-complete plate morphs. Each region had non-complete morphs present in at least one of the sites (Fig. 4). Non-complete morphs were also found in each habitat (Fig. 5). The association between salinity and plate morphs was unclear among regions and habitats. The sites, habitats, and regions with the lowest salinities did not always have the highest proportion of non-complete morphs.

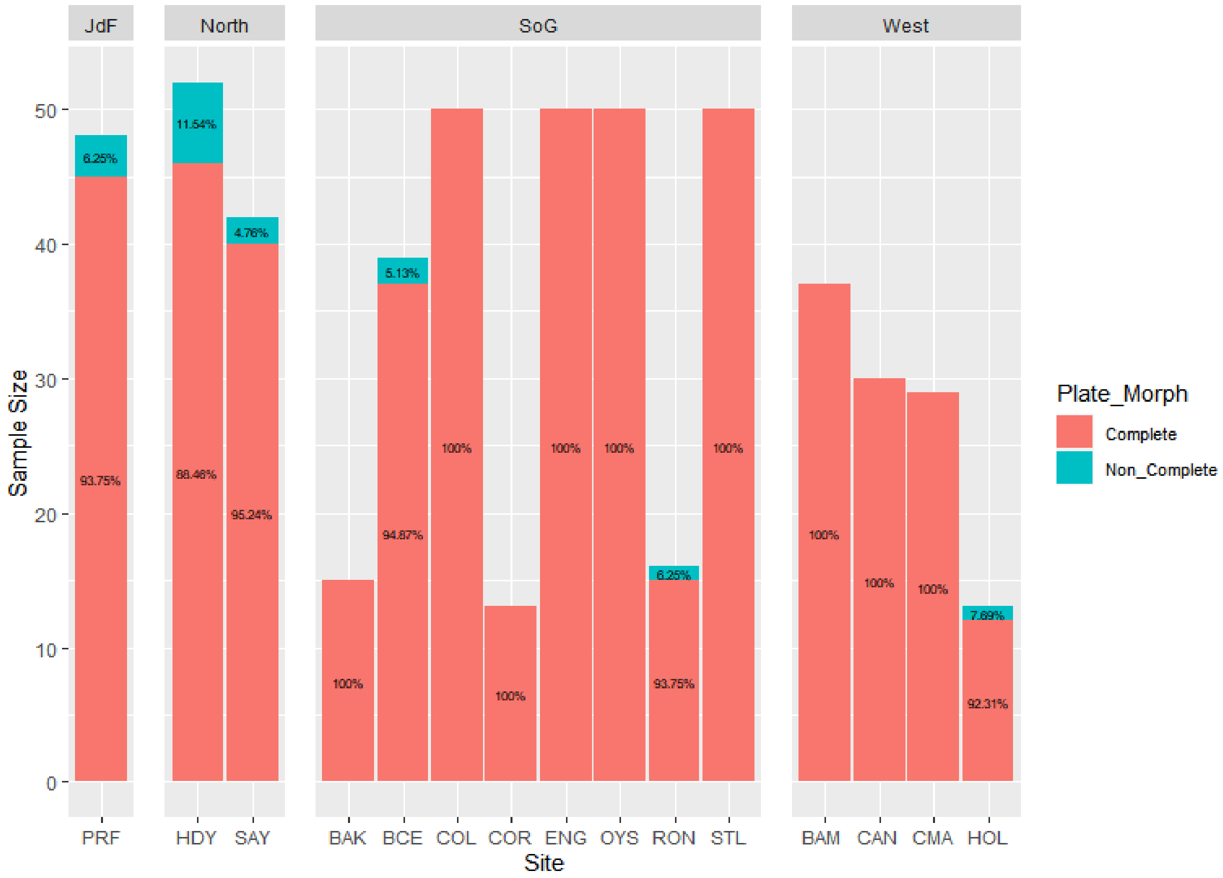

Figure 4. The proportion of complete plate morphs represented by the red portion of the bar vs. non-complete plate morphs in blue (*i.e.* low and partial plate morphs) at each site. Each sampling site is separated into the respective oceanographic region, Juan de Fuca Strait (JdF), Strait of Georgia (SoG), the west and northern coasts of Vancouver Island, BC. Sample size from each site on the y-axis. Percentage values in each bar indicate the total proportion value for that individual site. Site abbreviations are identified in Table A.2.

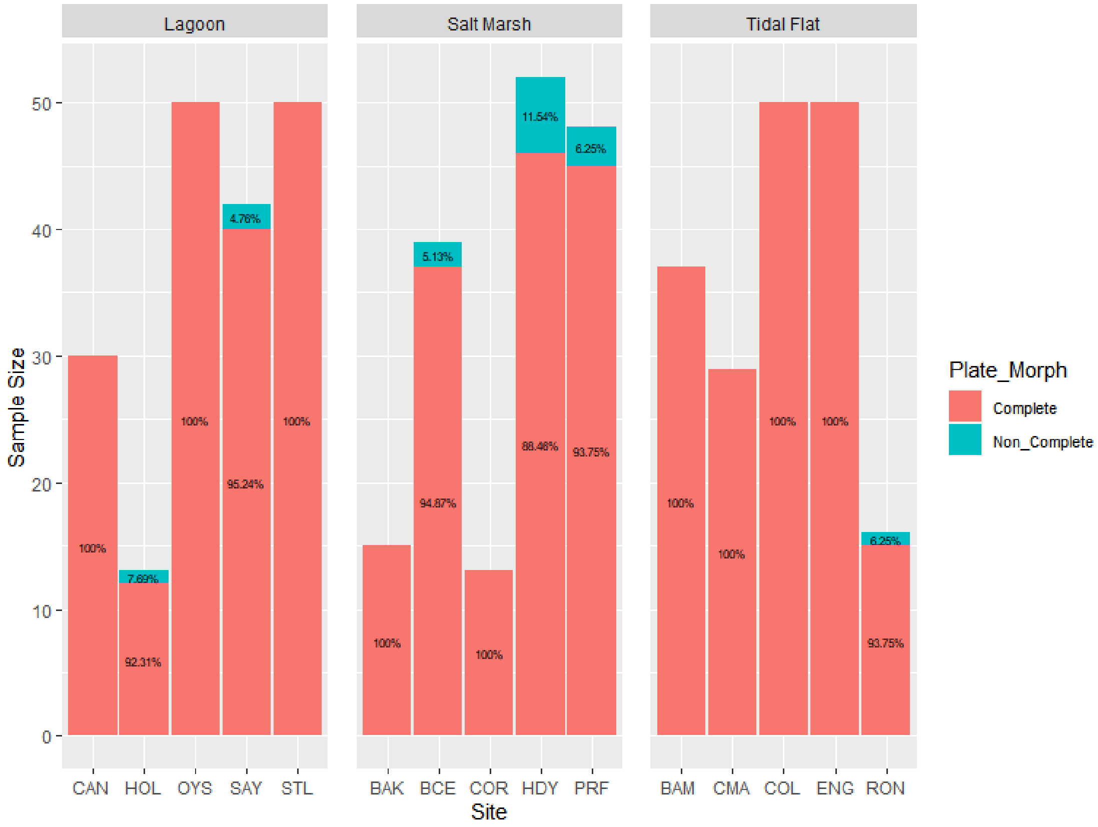

Figure 5. The proportion of complete plate morphs as the red portion of the bar vs. non-complete plate morphs in blue (*i.e.* low and partial plate morphs) at each sampling site. Each site is also separated into the respective habitat type for each site, with total sample size from each site on the y-axis. Percentage values in each bar indicate the total proportion value for that individual site. Site abbreviations are identified in Table A.2.

Head size variation

Head size (*i.e.* head length) varied between 9.65mm and 17.63mm, with an average of 13.43mm. The global linear mixed-effects model of head size variation included the fixed effects of sex, region, habitat, along with the interaction between sex and region, the interaction of sex and habitat, with site included as a random effect (Table D.1). Following model selection, the head dataset had three top models with ΔAICc < 4 (Table D.1), with R^2m values which ranged from 0.221 – 0.287, and R^2c values from 0.502 – 0.654 (Table D.2). In addition, none of the top models included the null (intercept only) model (Table D.1). All top models included sex, region, and the interaction between sex and region (Table D.1). But the models differed on whether they included habitat. The conventional next step would have been to average the top models. However, since some of the top models included interaction terms, I opted not to pursue averaging and to simply go with the model with the lowest AICc value (as per the recommendation of Scheipl *et al.* (2008)).

The final linear mixed effects model included the fixed effects of sex and region, along with the interaction between sex and region, with site included as a random effect (Table 1). VIF values were all lower than 3.0, indicating a lack of collinearity between fixed effects (Table 1).

Table 1. The best linear model explaining variation in head size (*i.e.* head length), based on model selection. Each fixed effect is separated by each level, excluding the reference category (The Strait of Georgia for region, females for sex). The estimate states if the level has a larger or smaller head size than the reference category of that fixed effect. The t-tests were calculated using Satterthwaite approximations to degrees of freedom. Collinearity was estimated between fixed effects in the global model based on VIF values (variance inflation factor). Also reported are standard error (SE), degrees of freedom (df), and the confidence interval (Conf. Interval). The response variable was specimen head size (mm), while sex, region, and the interaction between sex (Male [M] and Female [F]), and region (Rgn) were fixed effects. Site was included as a random effect (not shown). JdF is the Juan de Fuca Strait, SoG is the Strait of Georgia, North is the northern coast of Vancouver Island, and West is the west coast of Vancouver Island.

The linear mixed-effects model							
Fixed Effects	Estimates	SE	df	t-value	Conf. Interval	Pr (|> t|)	VIF
Intercept	13.55	0.316	12.2	42.8	12.93 – 14.17	**< 0.001**	-
Sex (M)	0.51	0.133	522	3.86	0.25 – 0.77	**< 0.001**	1.87
Rgn (JdF) $^{\Delta}$	-1.38	0.921	10.9	-1.50	(-3.19) – (0.42)	0.133	1.07
Rgn (North)	-1.35	0.702	11.9	-1.92	(-2.73) – (0.03)	0.054	1.17
Rgn (West)	-0.42	0.551	12.5	-0.766	(-1.50) – (0.66)	0.444	1.20
Rgn (JdF): Sex (M) $^{\Delta}$	-0.91	0.367	516	-2.48	(-1.63) – (-0.19)	**0.013**	1.17
Rgn (North): Sex (M)	0.02	0.280	523	0.0580	(-0.53) – (0.56)	0.954	1.38
Rgn (West): Sex (M)	0.46	0.243	519	1.91	(-0.01) – (0.94)	0.056	1.52

$^{\Delta}$The reference category was Rgn (SoG)

As predicted, there was a significant difference in head size between sexes (t = 3.86, p = < 0.001) (Table 1). Male head size was, on average, 6% larger in female sticklebacks (Fig. 6) (Welch two-sample *t*-test, $p < 0.05$). There were no significant regional differences in head size (Table 1, Fig. 6), but there was a significant interaction between sex and region, indicating that sexual dimorphism in head size depended on the region (Table 1). In most regions, males had a larger head size than females (Fig. 6, Table 1). However, this pattern was reversed in JdF, where females had larger heads than males (Fig. 6).

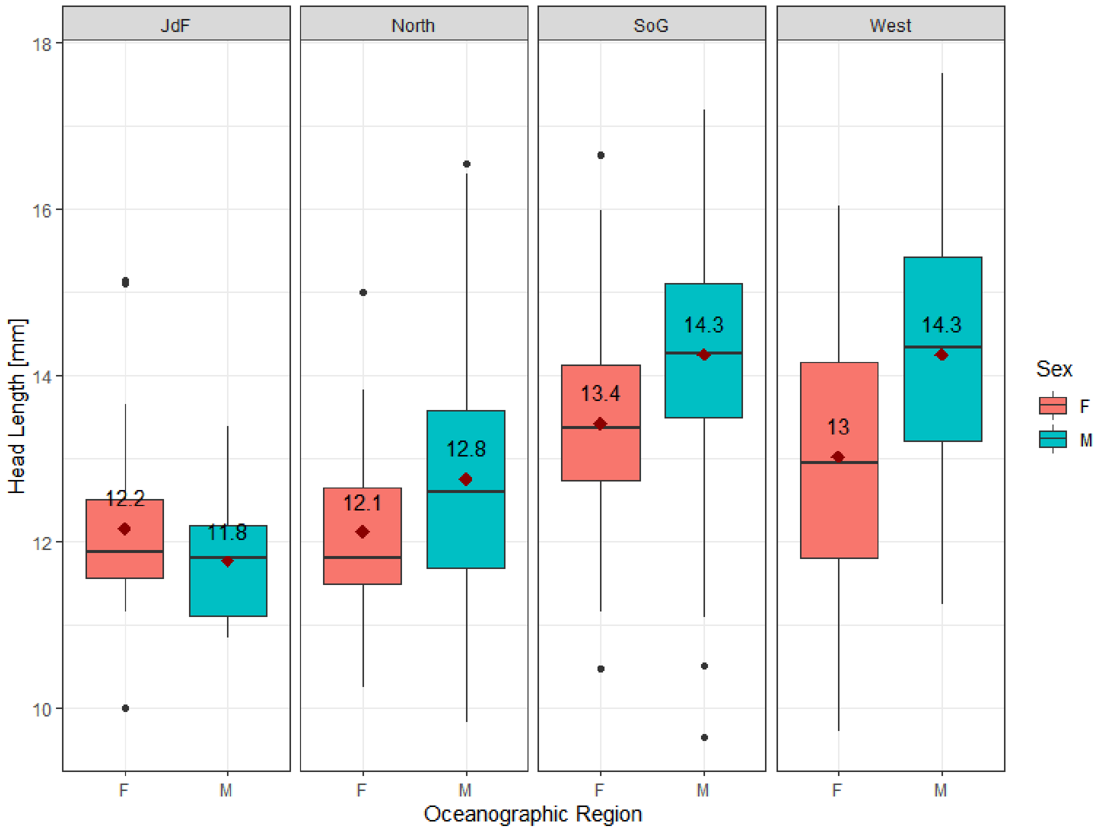

Figure 6. Differences between male (M) and female (F) head size, represented by head length (mm), across oceanographic regions. The regions include the Juan de Fuca Strait (JdF), the northern coast of Vancouver Island (North), Strait of Georgia (SoG), and the west coast of Vancouver Island (West). The solid black lines represent the median of each habitat type, while the red triangles represent average head size for each sex (with the average value in black above each point).

Body size variation

Body size (*i.e.* standard lengths) varied between 3.98cm and 7.95cm, with an average of 5.48cm. The body size variation global linear mixed-effects model included the fixed effects of sex, region, and habitat, along with the interaction between sex and region, and the interaction between sex and habitat, with site included as a random effect (Table D.3). Following model selection, the body dataset had five top models with ΔAICc < 4 (Table D.3), with R^2m values which ranged from 0.385 – 0.464, and R^2c values from 0.636 – 0.650 (Table D.4). The null (intercept only) model was not amongst the top five models (Table D.3). All top models included sex and region (Table D.3), but the models differed on whether they included habitat and the two interaction terms. Again, due to the presence of two interaction terms in some top models, I opted not to pursue model averaging and to simply go with the model with the lowest AICc value (as per the recommendation of Scheipl et al. (2008)). The full model selection for the body dataset was included in the appendix (Table D.3).

The final linear mixed-effects model included the fixed effects of sex, region, and habitat, along with the interaction between sex and region, and the interaction between sex and habitat, with site included as a random effect (Table 2). Most VIF values were lower than 3.0 (Table 2). Yet, the sex (Males) term exhibited collinearity, with a VIF score of 3.92. This was not surprising, according to Allison (2012), VIF scores can become inflated when the term in question is included in an interaction term. Which was the case for my model, where sex was included in two interaction terms (Table 2).

Table 2. The best model explaining body size (*i.e.* standard length) variation, based on model selection. Each fixed effect is separated by each level, excluding the reference category (The Strait of Georgia for region, females for sex, and lagoons for habitat). The estimate states if the level has a larger or smaller body size than the reference category of that fixed effect. The t-tests were calculated using Satterthwaite approximations to degrees of freedom. Collinearity was estimated between fixed effects in the global model based on VIF values (variance inflation factor). Also reported are standard error (SE), degrees of freedom (df), and the confidence interval (Conf. Interval). The response variable was specimen body size (cm), while sex, habitat, region, the interaction between sex (Male [M] and Female [F]), and region (Rgn), and the interaction between sex and habitat (Hbt) were fixed effects. Site was included as a random effect (not shown). JdF is the Juan de Fuca Strait, SoG is the Strait of Georgia, North is the northern coast of Vancouver Island, and West is the west coast of Vancouver Island.

The linear mixed-effects model							
Fixed Factors	Estimates	SE	df	t-value	Conf. Interval	Pr (|> t|)	VIF
Intercept	5.58	0.171	9.65	25.4	5.15 – 6.01	**< 0.001**	-
Sex (M)	-0.259	0.07	517	-3.4	(-0.41) – (-0.11)	**0.001**	3.92
Rgn (JdF) $^{\Delta}$	-1.13	0.344	9.23	-2.54	(-2.0) – (-0.26)	**0.011**	1.26
Rgn (North)	-0.775	0.256	9.94	-2.38	(-1.41) – (-0.14)	**0.017**	1.29
Rgn (West)	-0.161	0.205	9.86	-0.61	(-0.67) – 0.35	0.54	1.36
Hbt (Salt Marsh) *****	0.323	0.225	10	1.14	(-0.23) – 0.89	0.25	1.87
Hbt (Tidal Flat)	0.648	0.202	9.73	2.51	0.14 – 1.15	**0.012**	1.52
Hbt (Salt Marsh): Sex (M)	-0.0476	0.116	520	-0.406	(-0.28) – 0.18	0.685	2.84
Hbt (Tidal Flat): Sex (M)	-0.235	0.094	515	-2.49	(-0.42) – (-0.05)	**0.013**	2.34
Rgn (JdF): Sex (M)	-0.122	0.172	517	-0.701	(-0.46) – 0.22	0.483	1.55
Rgn (North): Sex (M)	0.174	0.125	520	1.37	(-0.07) – 0.42	0.170	1.76
Rgn (West): Sex (M)	0.196	0.0988	516	1.97	0.00 – 0.39	0.049	1.61

$^{\Delta}$ The reference category was Rgn (SoG)
*The reference category was Hbt (Lagoon)

Male and female body size differed significantly (*t* = - 3.4, *p* = 0.001) (Table 2). As predicted, females had a larger body size than males, average 5.5cm vs. 5.4cm (Fig. 7). There was also significant body size difference among regions (Table 2). Sticklebacks from the JdF had the smallest body size (average 4.68cm), while SoG had the largest (average 5.73cm) (Fig. 8). Unlike head size, there was no significant interaction between sex and region, indicating that sexual dimorphism in body size was consistent across regions (Table 2) (Fig. 9).

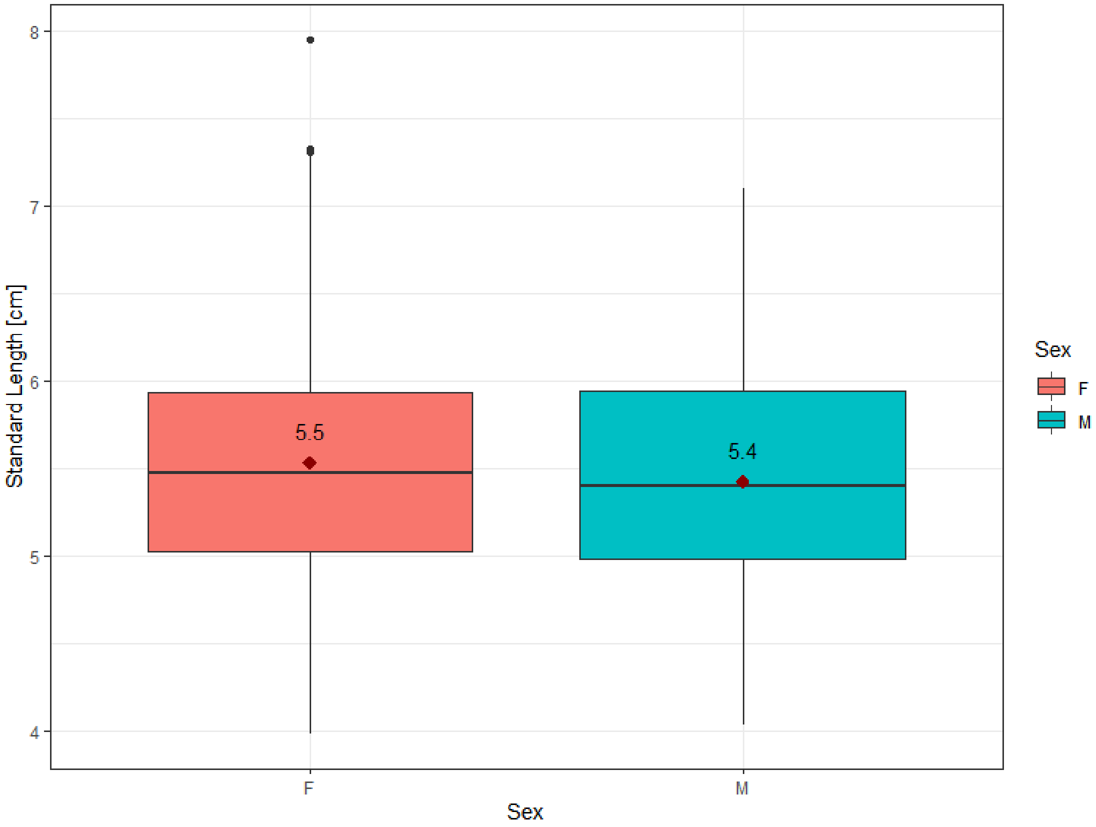

Figure 7. Differences between male (M) and female (F) body size, represented by standard length (cm). The solid black lines represent the median standard lengths. The red triangles represent the average standard length for each sex (with the average value in black above each point).

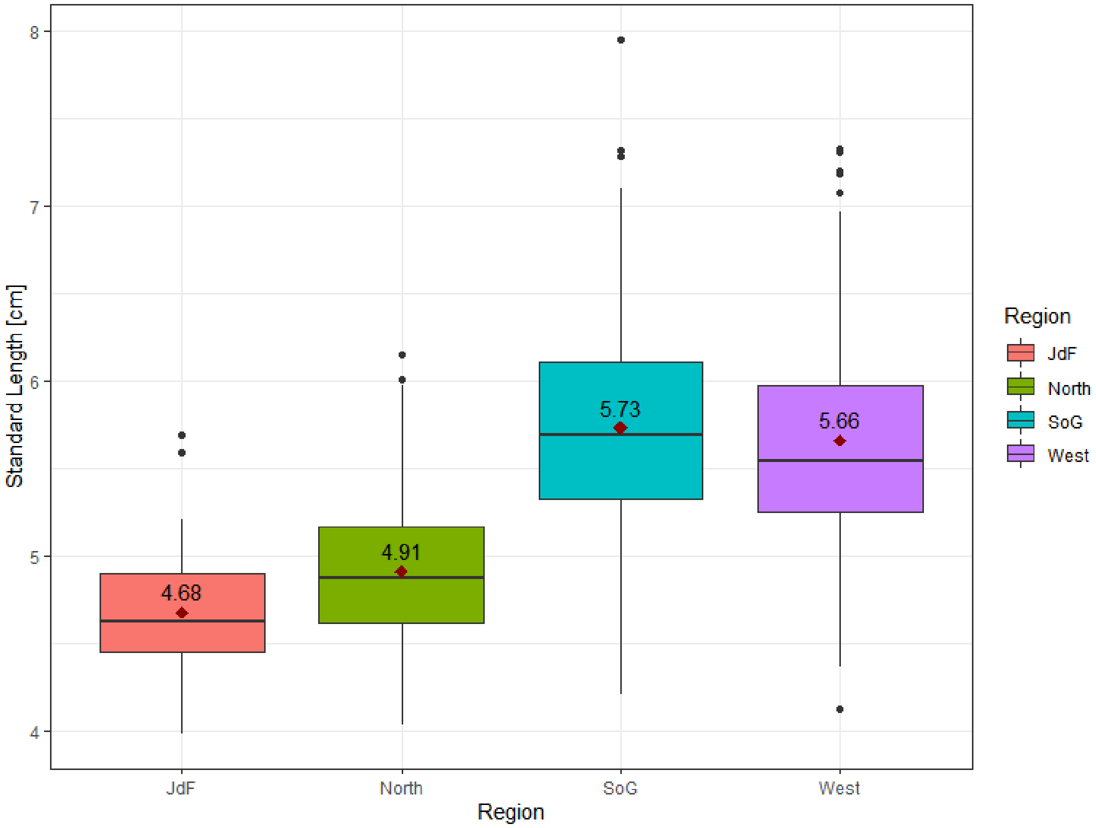

Figure 8. Regional variation between body size, represented by standard length (cm), observed in four oceanographic regions. The regions include the Juan de Fuca Strait (JdF), the northern coast of Vancouver Island (North), Strait of Georgia (SoG), and the west coast of Vancouver Island (West). The solid black lines represent the median standard length. The red triangles represent the average standard length for each region (with the average value in black above each point).

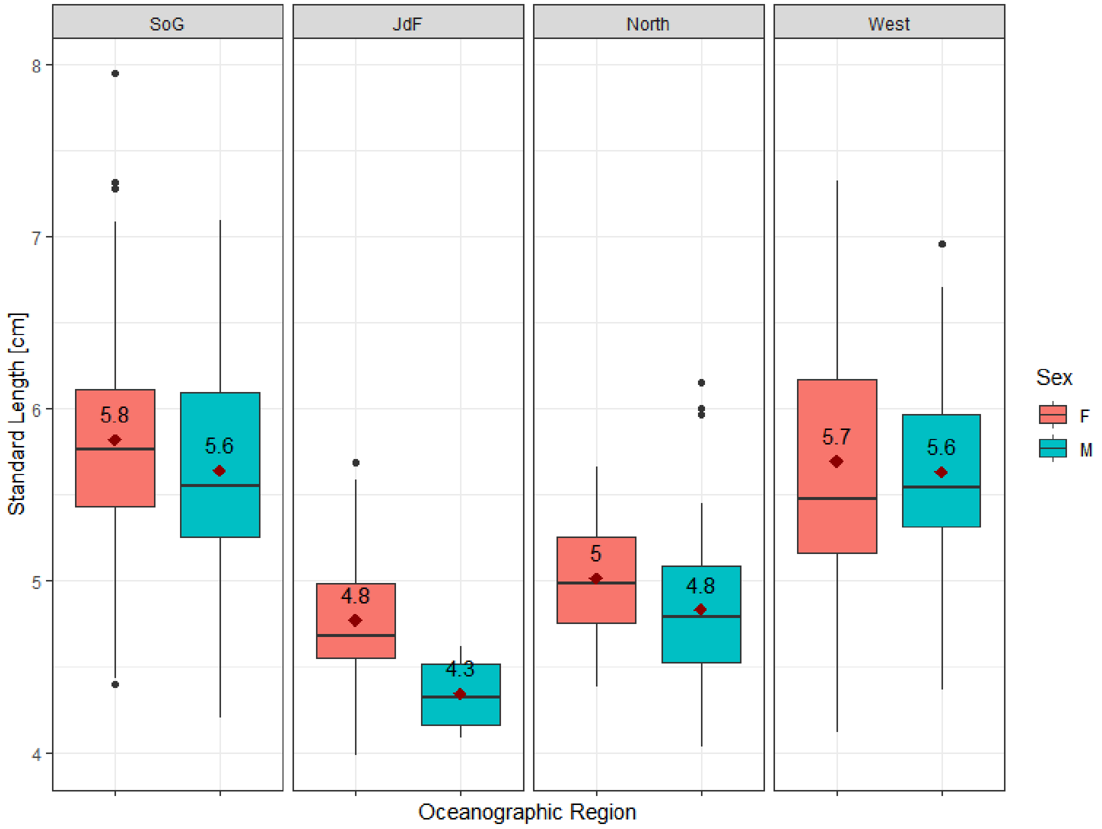

Figure 9. Differences between male and female body size, represented by standard length (cm), observed in each oceanographic region. The regions include the Juan de Fuca Strait (JdF), the northern coast of Vancouver Island (North), Strait of Georgia (SoG), and the west coast of Vancouver Island (West). The solid black lines represent the median standard length, while the red triangles represent the average standard length for each region (with the average value in black above each point).

Body size varied significantly among habitat types ($t = 2.51$, $p = 0.012$ for Tidal Flat level) (Table 2). Tidal flat sticklebacks had the largest standard length (average 5.9 cm) and salt marshes had the smallest (average 5.2cm) (Table 2, Fig. 10). As the intermediate habitat type with shared characteristics from lagoons and tidal flats, I had expected that salt marsh specimens would exhibit intermediate body size characteristics. There was also a significant interaction between sex and habitat, indicating that sexual dimorphism in body size depended on the habitat type (Table 2), because body size differences were more pronounced in some habitats (tidal flats) than others (lagoons) (Fig. 9).

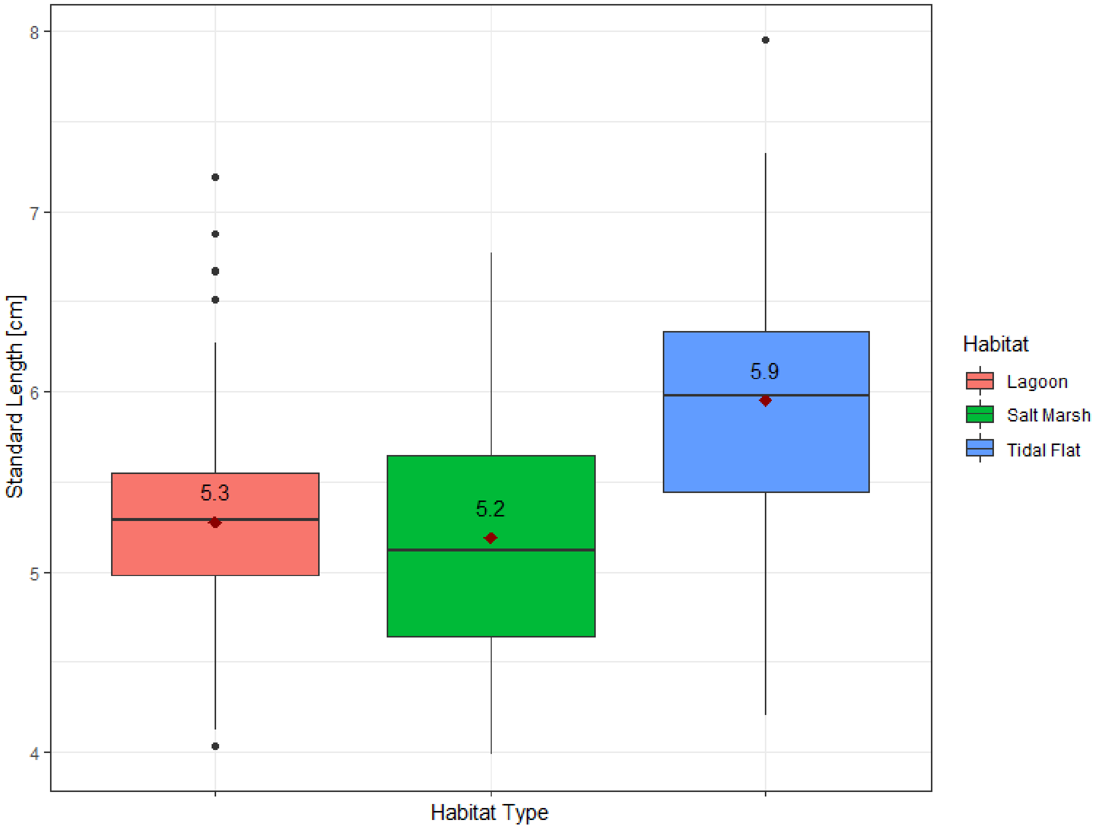

Figure 10. The variation of body size, represented by standard length (cm), observed in each habitat type. The solid black lines represent the median of each habitat. The red triangles represent the average standard length (with the average value in black above each point).

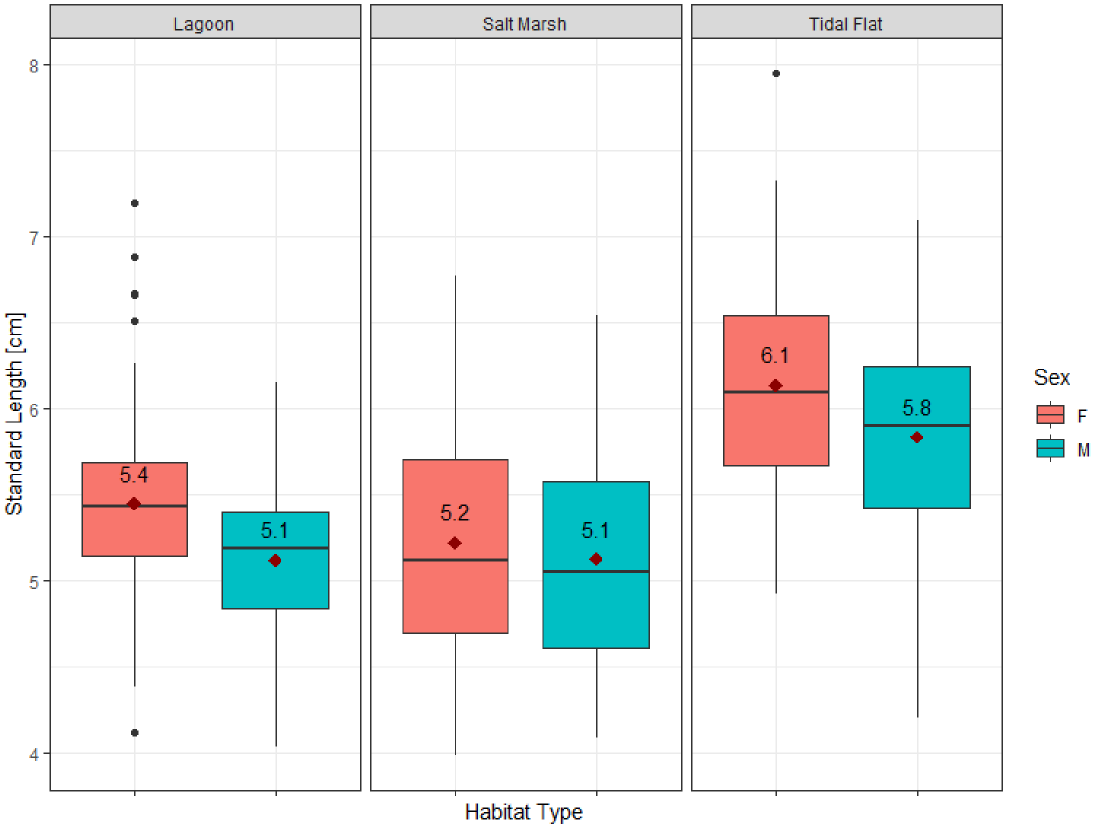

Figure 11. Sexual dimorphism in body size, represented by standard length (cm), observed among different habitat types. Males (M) and females (F) are shown in each habitat type. The solid black lines represent the median standard length. The red triangles represent the average standard length (with the average value in black above each point).

Variation in head shape

A PCA analysis on the head shape data, with the centroid size (*i.e.* CS) excluded, showed that 42.9% of the total head shape variation was explained by the first two Principal Components (PCs) – (PC1 = 24%, PC2 = 18.9%). I included deformation grids along each axis of my PCA plots to help visualize the morphological variation described by each axis. The variation along PC1 axis deformation grids represented variation between deep squat heads vs. shallow elongated heads, while PC2 represented variation between convex and concave heads (Fig. 14).

I conducted a Procrustes model to analyze variation in head shape. The Procrustes shape variables were used as the response, with Log CS, sex, region, and the interaction of sex and region as the fixed factors. I included Log CS as a fixed factor because the relationship between head shape and the geometric head size was significant (Partial Least Squares correlation coefficient = 0.588, p= 0.001) (Fig. E.1). Log CS accounted for 4.49% of total variation (R^2 = 0.0449) (Table 3). Site was included as a nested random effect. Habitat was not included in my Procrustes LM because exploratory analysis indicated that it was not an important factor.

Table 3. Results of the Procrustes linear model (LM) for head shape, which included log centroid size (Log CS), region (Rgn), sex, and the interaction between region and sex. Site was included as a nested random effect (not shown). Df = degrees of freedom, SS = sum of squares, MS= mean square, RSq= R-squared value, and F = F statistic.

Fixed Factors	Model Coefficients					
	df	SS	MS	RSq	F	p-value
Log CS	1	0.0891	0.0891	0.0449	26.84	0.001
Sex	3	0.0770	0.0257	0.0388	7.730	0.001
Rgn	1	0.0631	0.0631	0.0318	19.01	0.001
Rgn : Sex	3	0.0142	0.00474	0.00716	1.420	0.091
Residuals	525	1.740	0.00332	0.877	-	-

There were significant sex differences in head shape ($F_{3,534}$ = 7.73, p = 0.001), which accounted for 3.38% of the variation (R^2= 0.0388) (Table 3). However, males and females did not show clear clustering patterns along either PC axis. Instead, there was substantial overlap between sexes (Fig. 12). The pattern from the PCA analysis suggested that females had relatively compressed heads with a narrower operculum, while male clustering favored a relatively deeper head and broader operculum (Fig. 12). This pattern of clustering suggested that males and females were distributed along a continuum between these two head shapes.

Bayesian cluster analysis confirmed these patterns. The first two PCs of the head shape data were fit with an XXI model, which identified only one cluster suggesting no significant clustering in the PC components (Appendix F, Fig. F.1). The XXI model has a diagonal shape, multivariate volume, and normal orientation, as verified by estimated classification plot (Fig. F.2). The cluster analysis helped support the lack of clustering seen in our two head shape PCA plots (Fig. 12, Fig. E.2).

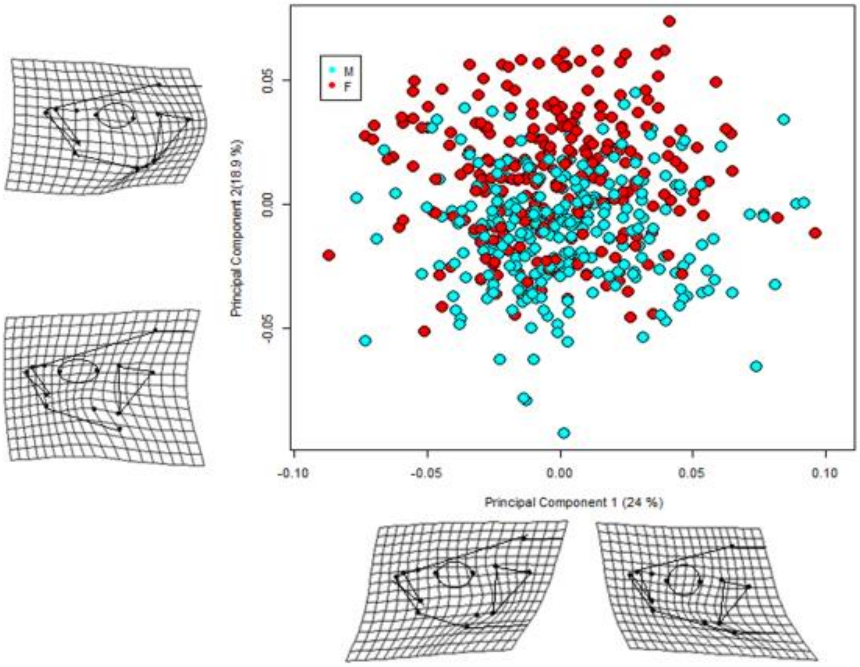

Figure 12. Principal Component Analysis (PCA) of head shape. The head shape for each specimen was obtained from a set of thirteen anatomical landmarks (Fig. 1). The landmarks were converted into 26 shape variables using a Generalized Procrustes Analysis (GPA). These vectors of shape were used to carry out the PCA. Each data point represents data from a female stickleback (red circles) and male stickleback (light blue circles). Deformation grids were plotted with 1.5x magnification to facilitate visualization of head shape differences. The deformation grids represent the difference between the specimen on each end of each shape axis (PC1 and PC2). The effects of body size were removed from this analysis.

There were significant regional differences in head shape ($F_{1,534} = 19.01$, $p = 0.001$), accounting for 3.18% of the variation ($R^2 = 0.0318$). While the interaction between region and sex was not significant ($F_{3,534}$ = 1.42, p = 0.091), accounting for 0.719% of variation (R^2= 0.00716) (Table 3). Overall, the PCA plots showed little clustering between regions (Fig. E.2), suggesting a continuum of head shapes along regions rather than stark clustering pattern.

Variation in body shape

A PCA analysis on the body shape data, with centroid size (*i.e.* CS) excluded, found that 37.6% of the total body shape variation was explained by the first two Principal Components (PCs) – (PC1 = 24%, PC2 = 13.6%). The variation along PC1 was driven by the difference in relative body and snout length, while PC2 separated fish based on head depth and the relative size of the caudal peduncle. PCA analysis on the body dataset revealed slightly stronger clustering patterns than for head shape analysis, despite the first two PCs representing less variation in body shape.

A Procrustes linear model (LM) for body shape included Log CS, sex, region, habitat, the interaction between sex and region, and the interaction of sex and habitat as the fixed factors. I included Log CS as a fixed factor because the relationship between body shape and the geometric head size was significant ($F_{1,534}$ = 36.23, p = 0.001) (Table 4). Log CS accounted for 4.86% of total variation (R^2 = 0.0487). Site was included as a nested random effect.

Table 4. Results of the Procrustes linear model (LM) for body shape, which included log centroid size (Log CS), region (Rgn), habitat (Hbt), and sex. Site was included as a nested random effect (not shown). Df = degrees of freedom, SS = sum of squares, MS = mean square, RSq = R-squared value, and F = F statistic.

Fixed Factors	Model Coefficients					
	df	SS	MS	RSq	F	p-value
Log CS	1	0.051	0.51	0.0487	36.2	0.001
Sex	1	0.13	0.13	0.128	95.4	0.001
Rgn	3	0.070	0.02	0.0666	16.5	0.001
Hbt	2	0.19	0.0097	0.0185	7.0	0.001
Sex : Hbt	3	0.0075	0.004	0.0072	2.7	0.001
Sex : Rgn	2	0.012	0.004	0.0115	2.9	0.001
Residuals	517	0.72	0.001	0.695	-	-

There was a significant difference between male and female body shapes ($F_{1,534}$ = 95.38, p = 0.001), which accounted for 12.8% of variation (R^2= 0.128) (Table 4). Sticklebacks showed a diffuse clustering pattern by sex along PC1 (Fig. 13). Female sticklebacks tended to cluster on the right of PC1, towards a deeper body, broader pelvic girdle, a more compressed snout, with larger spacing between dorsal spines. Male sticklebacks tended to cluster toward the left side of PC1, towards a shallower, more streamline body with an elongated snout, smaller relative pelvic girdle, and closely spaced dorsal spines. Female sticklebacks were loosely clustered together, with more variation in body shape, compared to the tightly clustered male sticklebacks (Fig. 13).

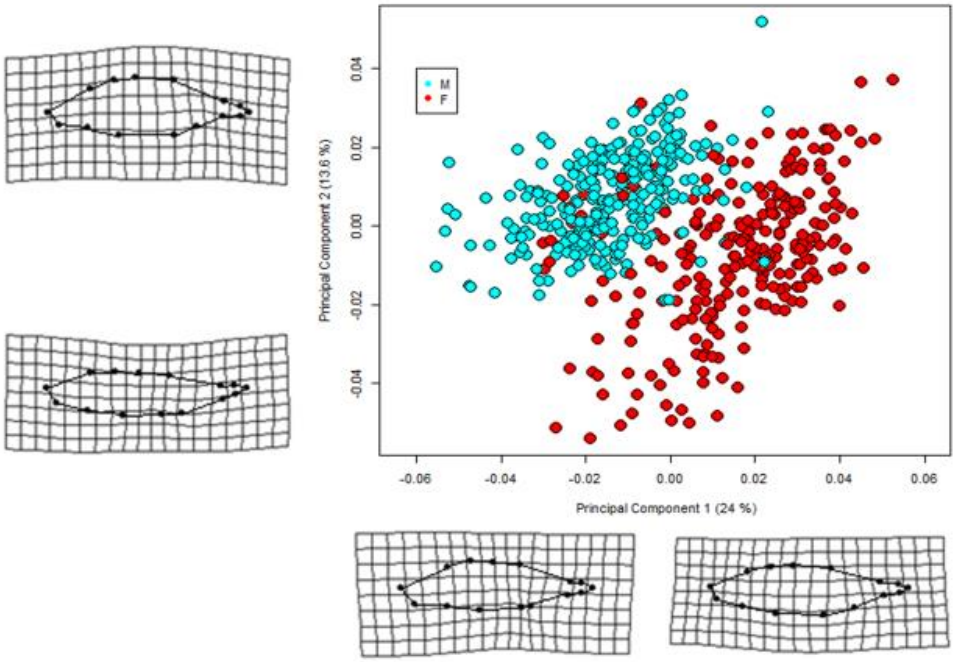

Figure 13. Principal Component Analysis (PCA) of body shape. The body shape for each specimen was obtained from a set of fifteen anatomical landmarks (Fig. 2). The landmarks were converted into 30 shape variables by way of a Generalized Procrustes Analysis (GPA). These vectors of shape were used to carry out the PCA. Each data point represents data from a female stickleback (red circles) and male stickleback (light blue circles). The body shape variation is illustrated with the four deformation grids, which represent the difference between the specimen on each end of each shape axis (PC1 and PC2). The effects of body size were removed from this analysis.

Although there was no noticeable clustering of the body shape data by regions (Fig. E.4), the Procrustes LM showed a significant yet relatively small body shape difference among regions ($F_{3,534}$ = 19.44, p= 0.001), representing 6.66% of the total variation (R^2 = 0.0666). The SoG sticklebacks tended to cluster to the left side of PC1 and upper side of PC2 towards a deeper body shape with widely spaced dorsal spines, and a broader pelvic girdle. The JdF sticklebacks clustered towards the bottom of the PC2 axis, or a shallow, more streamlined body shape with compact dorsal spine spacing and a narrower pelvic girdle. The West sticklebacks had the most extreme distribution along PC1 of the regions (Fig. 13), exhibiting all body shapes from streamlined, elongated bodies to squat, deeper body shapes. The North sticklebacks did not cluster in any way and overlapped broadly with the other regions. As I saw in the body size analysis, the JdF and SoG sticklebacks were the most noticeably distinct regions (Fig. 8, Fig. 9).

The interaction of sex and region was significant ($F_{2,534} = 2.898$, $p = 0.001$) (Table 4), suggesting that sex differences in body shape, much like head shape and size, varied among regions. However, this effect was small, explaining 1.15% of the variation ($R^2 = 0.115$) (Table 4).

Habitat had a small yet significant effect on body shape ($F = 6.89$, $p = 0.001$), accounting for 1.9% of the total variation ($R^2 = 0.019$) (Table 4). There were no noticeable clusters among habitats, but instead a continuum of variation along PC1 that was present in most habitats (Fig. E.5). The only notable pattern was a portion of salt marsh sticklebacks which clustered towards the bottom of the PC2 axis, towards a streamlined body with a narrower peduncle. It was interesting to see the only clustering from sticklebacks found in salt marshes, a harsh estuarine habitat type which experiences large, daily fluctuations in physical characteristics.

The interaction between sex and habitat was significant ($F_{3,\,534} = 2.731$, $p = 0.001$), suggesting that sex differences in body shape varied among habitats. However, the interaction only explained 0.723% of body shape variation, ($R^2 = 0.00723$) (Table 4).

Bayesian cluster analysis supported these patterns, the first two PCs of the body shape data identified two clusters (Fig. F.3), indicating significant clustering of male and female body shape. The dataset was fit best with a VVV model (BIC = -2894.888) (Fig. F.4), The VVV model had an ellipsoidal orientation, varying volume, and varying shape. An estimated classification plot (Fig. F.4) mirrored the cluster pattern seen in the PCA plot of the male and female distribution of body shape variation (Fig. 13).

Discussion

The overall goal of my thesis was to test predictions about oceanographic environments on and within species morphological variation of marine sticklebacks around Vancouver Island and Southern mainland BC. I characterized morphology in and among many previously undescribed populations and assessed whether the variation was attributed to regional or habitat differences. I chose to characterize the three specific morphological traits armour, head size and shape, and body size and shape, because they are ecologically important. Bony armour trait variation relates to defense weaponry and predator evasion (Reimchen, 1983; Bell and Foster, 1994). Head shape variation affects trophic morphology and traits related to prey capture and feeding (Wund *et al.*, 2008; Ingram *et al.*, 2012). And differences in body shape affect maneuverability and sustained swimming abilities in sticklebacks (Garduño-Paz *et al.*, 2010; Matthews *et al.*, 2010; Webster *et al.*, 2011). I also chose these traits because their ecological drivers have been well-studied in freshwater but not marine sticklebacks, providing a point of comparison with my study.

My first objective was to explore morphological variation within and among the four oceanographic regions around Vancouver Island. If oceanography on regional scales acted as a barrier between breeding populations, then I predicted stickleback morphology would vary by regions. This hypothesis was supported for head size, body size, head shape, and body shape, but not by lateral plate phenotypes. My second objective was to explore variation among three micro habitats where marine sticklebacks are found in BC. If oceanography on smaller scales was a barrier between breeding populations, then I predicted stickleback morphology would vary among habitats. This hypothesis was supported for body size and body shape, but in no other morphological traits. My third objective was to assess morphological differences between males and females, and to compare relative importance of sexual dimorphism to other factors (region and habitat). Despite significant differences in morphology across regions and habitats, sexual dimorphism was the biggest driver of morphology in the dataset. However, dimorphism patterns varied among regions and habitats, suggesting that regional or local factors played an important role in driving morphological variation within as well as among populations. The sex ratios found in this study ranged from 1:1 at the Canton Lagoon, Moutcha Bay, to male-biased (*e.g.* Salt Lagoon and Salmon River Estuary), as well as female biased (*e.g.* Port Hardy Estuary and Oyster Lagoon) (Appendix A, Table A.1). This variation in sex ratios is similar to what other researchers have found studying marine stickleback populations during the summer months (Cano *et al.*, 2008; Rollins *et al.*, 2017; Golovin *et al.*, 2019). Visual identification of sex is sometimes difficult, and future studies should confirm visual sex identification with genetic identification (Peichel *et al.*, 2004; Cano *et al.*, 2008; Rogers *et al.*, 2012; DeFaveri and Merilä, 2013a). I did not include fish that we could not sex in the morphological analysis. Below I discuss the variation in more detail and propose hypotheses that can be tested in future studies.

Environmental variation

Temperature and salinity from spot sampling at each site did and did not match the long-term average trends in the DFO lighthouse data, but overall my predictions were close to the observations. The average temperatures I recorded across regions were similar to the DFO lighthouse data (DFO, 2020). I found temperatures to be highest in the SoG, and comparably low in JdF and the North (Fig. 3a). However, the salinities I recorded did not match patterns seen in the DFO lighthouse data (Fig. 3b) (DFO, 2020). This discrepancy could be caused by the nature of the spot sampling. I sampled each site only once throughout the field season and I sampled at various times in the tidal cycles depending on when I was at the site. Additionally, my sampling sites were much further inland than the DFO lighthouses (Appendix A - Fig. A.3). The geographic position of most DFO lighthouses at the mouth of inlets and straits around Vancouver Island may not be able to represent the substantial temperature and salinity gradients which exist along these inlets and estuaries (Pickard, 1961, 1963).

Between coastal habitats, lagoons had the highest average temperatures and highest salinities (Fig. 3c). I expected this because of increased evaporation rates and little rainfall during the summer months in BC (Mace and Mackie, 1970). I observed the lowest average temperatures on tidal flat sites, but lowest average salinities were at salt marsh sites (Fig. 3c, 3d). Tidal flats were expected to be coldest due to their proximity to colder open ocean waters, while salt marshes may have lower average salinities due to freshwater influence in estuaries (Chapman, 1974; Campbell, 1986; Hickey and Banas, 2003). Unlike the data from the BC lighthouses, there are no long-term habitat-specific datasets for temperature and salinity as readily available. Therefore, it is important to note that the temperature and salinity patterns reported in my study represent what I recorded the day I sampled each site, and could have varied depending on tides, time of day, date of sampling, or local effects (*e.g.* coastal land use).

While I believe that average salinities and temperatures of my spot sampling reflect the general physical oceanographic characteristics of each habitat, future studies might benefit from more extensive sampling. For example, a conductivity, temperature, and density (CTD) recorder can be used to collect surface data of the top few meters in the water column from multiple stations in a study area (Abookire *et al.*, 2000; Hickey and Banas, 2003). Continuous records (for example HoBo® or iButton® recorders) can be used to collect temperature readings across many days and tidal cycles.

Variation in lateral plate morphology

Non-complete bony armour plate morphs were observed in each region (Fig. 4) and in each habitat (Fig. 5). My hypothesis that salinity differences would be associated with plate phenotype was not met. This compares to the findings of DeFaveri and Merilä (2013a), who found that there was no correlation between marine population plate heterogeneity and environmental conditions (*i.e.* salinity). The absence of a relationship could have been caused by the nature of spot sampling, different sample sizes among my sites, or because plate morphology was driven by some factor other than salinity. While the older literature suggests that salinity itself drives plate heterogeneity in sticklebacks (Heuts, 1947; Münzing, 1963), more recent literature suggests that predator presence, the species of predator, and intensity of predation (which are sometimes confounded with salinity), are ecological drivers of plate morphology (Reimchen, 1995, 2000; Marchinko, 2009b; Leinonen *et al.*, 2011; Zanella *et al.*, 2015). Wasserman *et al.* (2020) found support for such drivers in their study of 20 estuarine populations of marine sticklebacks. In that study, the presence of predators (*i.e.* steelhead trout (*Oncorhynchus mykiss*) and sculpin (*Leptocottus armatus*, *Cottus asper*, and *Cottus aleuticus*)) was strongly associated with higher *Eda* complete allele frequencies and subsequently more complete morphs. However, the ability of salinity to drive armour differences cannot be ignored because Barrett *et al.* (2008, 2009) observed that salinity had pleiotropic effects on armour and growth of sticklebacks. Therefore, there is likely a combination of environmental and physiological factors which help drive plate heterogeneity among marine populations.

Heterogeneity of plate morphology was also seen by Morris *et al.* (2018), who studied marine populations from Alaska to southern California. They found that there was a higher proportion of low plated sticklebacks in their southern sites (*i.e.* California and Oregon) compared to their northern sites in Alaska and BC. This latitudinal pattern could have been caused by thermal gradients across latitudes on the west coast of North America. After all, complete morphs have been associated with colder winter temperatures, in both freshwater and marine populations, and low plate morphs were associated with regions with milder winter temperatures (Wootton, 1976; Smith, Zięba, Spence, Klepaker, *et al.*, 2020). Jamniczky *et al.* (2018) sampled four marine populations from Pender Harbour on the Sunshine Coast in BC: two lagoons, a tidal flat, and a tidally influenced stream. They found that the three full marine sites contained only complete morphs, while the tidally influenced stream contained non-complete and complete morphs. In contrast, I observed non-complete morphs in creeks and rivers that were tidally influenced (*i.e.* Black Creek Estuary and Port Hardy Estuary), as well as in some lagoon and tidal flat sites (*i.e.* Sayward Estuary Lagoon and Roberts Bank). Perhaps our results differ because I sampled more habitats from a larger geographic area. All in all, my study suggests that the non-complete morph is consistently found in marine populations and in many marine environments. Future studies should explore the genetic information among marine habitats with non-complete morphs and how it relates to the physical oceanographic variation between marine stickleback populations.

I do wish to slightly revise my original hypothesis which sought to reject the assumption that marine stickleback morphology has remained unchanged from ancestral marine populations. I want to specify which aspects of morphology I believe are relatively homogenous in marine populations by separating lateral plate morph from head and body shape. The majority of my specimens were complete morphs (*i.e.* 97%), while only 2.76% were non-complete morphs (Fig.

4, Fig. 5). Thus, in terms of lateral plate morphology in marine sticklebacks, I believe the assumption of relative homogeneity is supported by my study.

Head size variation

Head size differed significantly between sexes, by 0.8mm on average (Table 1). This was expected because males generally have larger heads than females (Kitano *et al.*, 2007; Aguirre and Akinpelu, 2010). Interestingly, the interaction between sex and region was also identified as significant by the linear mixed effects model (Fig. 6, Table 1). My hypothesis that the habitat type and variation in habitat availability would have a stronger effect on head morphology than region was not met. Research that identified regional phenotypic variation across marine populations primarily analyzed body shape and plate phenotype, but not head morphology (DeFaveri and Merilä, 2013a; Jakubavičiūtė *et al.*, 2018). There has not been a study, to my knowledge, that examined head morphology of marine sticklebacks and how it is associated with oceanographic regional variation. Studies that compared head morphology in freshwater and marine populations generally focused on small scale variation in niches and habitats (Kimmel *et al.*, 2008; Svanbäck and Schluter, 2012; Seebacher *et al.*, 2016; Østbye *et al.*, 2016).

Interestingly, the JdF site in Port Renfrew exhibited the opposite pattern to the other three regions, because females had larger heads than males (Fig. 6). Studies on both freshwater and marine sticklebacks have only observed female sticklebacks with smaller/more compressed heads than males (Kristjánsson *et al.*, 2002; Aguirre *et al.*, 2008; Aguirre and Akinpelu, 2010). Sexual dimorphism in head length has been suggested as an ancestral morphological feature, with a genetic basis, because it is present in anadromous populations and freshwater populations (Kitano *et al.*, 2007). In fact, researchers suggest that phenotypic variation in wild sticklebacks has a genetic basis, with a strong potential for environmental factors to contribute to variation in morphology (Albert *et al.*, 2008; Sharpe *et al.*, 2008). For example, Aguirre and Akinpelu (2010) found that males had larger heads than females between each of their sites, but each population had unique growth rates indicating that population-specific factors affected both sexes. However, their sites were quite diverse. They sampled populations from freshwater streams, benthic dominant lakes, limnetic dominant lakes, and one anadromous population from the tidally influenced Rabbit Slough.

Average JdF female and male head size also differed by the smallest amount (0.4mm) compared to the other regions (Fig. 6). Pistore (2018) suggested that populations with lower dimorphism (smaller difference between male and female morphological characteristics) could be under stronger pressure from other drivers of morphology (*i.e*. food availability and predation), so they cannot expend extra energy on sexual dimorphism. My sampling site in Port Renfrew could in fact have unique predating intensity compared to other regions on average, as it is an area well protected from development. Port Renfrew and the San Juan River estuary are situated adjacent to the National Pacific Rim Park Reserve as well as Indigenous reserve lands (*i.e*. Pacheedaht and Gordon River First Nations). Further research in the Juan de Fuca Strait and Port Renfrew must be done to explore the unique sexual dimorphism found in this marine stickleback populations. Evaluating head size according to prey type found at each sampling site, as well as in the bellies of each fish, could help explore population-specific drivers of variation. Gut contents would support the hypothesis (or perhaps not) that varying diets lead to variation in trophic traits (Sambrook, 1990; Jakubavičiūtė *et al.*, 2017).

Body size variation

The linear mixed effects model found that body size differed significantly between males and females (Fig. 7, Table 2). This was unsurprising because females are generally larger than males in freshwater and marine populations (Kitano *et al.*, 2007; Aguirre and Akinpelu, 2010). However, the average body size only differed by 0.1cm (Fig.7), so it is unlikely that these statistically significant findings are biologically important. Body size varied significantly between regions, and sticklebacks were largest at SoG sites and smallest at the JdF site differing by 1.05cm (Fig. 8, Table 2). The temperatures I measured were highest, on average, at SoG sites, and lowest at the JdF site (Fig. 3a). This qualitative association will require more in-depth water property surveys at each sampling site to properly assess how temperature may be associated with body size in marine sticklebacks. Perhaps average temperatures in each region impact stickleback body size. Kim *et al.* (2017), for example, found that male sticklebacks bred in environments with higher average winter temperatures were smaller as adults. But for my thesis I chose not to test for a correlation between temperature/salinity and the shape data because it would not have made sense to do so, given my study design.

Bergmann's rule was not supported by my data, contradictory to Morris *et al.* (2017) who found support for Bergmann's rule and Jordan's rule (*i.e.* the number of vertebrae increase with latitude) along the west coast of North America. However, Vancouver Island only spans 2° latitude. This is a smaller geographic area compared to the studies that found support for Bergmann's rule in stickleback populations, which covered over 30° latitude (Morris *et al.*, 2017; Smith, Zięba, Spence, Klepaker, *et al.*, 2020). It is likely that the intermediate scale of my study area was not large enough to find evidence of Bergmann's rule.

It is also important to note that sticklebacks generally reach adulthood at one-two years, after which they stop growing (Wootton, 1984). But some populations can continue to grow throughout their adulthood and can live for 8-9 years (Reimchen, 1992a; Gambling and Reimchen, 2012; DeFaveri and Merilä, 2013b; Yershov and Sukhotin, 2015). In the White Sea, marine populations of up to 5-year old sticklebacks have been observed (Ivanova *et al.*, 2016; Yurtseva *et al.*, 2019). Thus, although the variation I observed between sexes, regions, and habitats was statistically significant according to the linear model, the variation could simply be the difference in average age between populations. Otoliths can be used to characterize age differences among populations (MacColl *et al.*, 2013; Rollins *et al.*, 2017). I did not analyze otoliths as part of my study, and preservation in 95% ethanol degrades otoliths in sticklebacks (T. Reimchen, *pers. comm*.). The more common method to preserve fish for otolith inspection is freezing below -20°C or preserving the otolith in formalin solution (Rollins *et al.*, 2014; Jakubavičiūtė *et al.*, 2018; Yurtseva *et al.*, 2019). Additionally adding replicate sampling dates throughout the breeding season, and in different years, would add a measure of repeatability to future studies.

Dorgham *et al.* (2018) found that marine populations in the White Sea showed local spatial heterogeneity throughout the spawning season. Males were larger, with larger anterior parts of the body, at higher quality eelgrass spawning grounds. They concluded that this was likely because large males could out-compete smaller males for nesting grounds. Therefore the variation I observed among regions, and within regions between sexes, could have been magnified by local factors related to competition for good spawning grounds (Dorgham *et al.*, 2018).

Body size also varied significantly between habitats (Fig. 10, Table 2), which I predicted to occur because of differences in habitat availability and/or physical characteristics of each habitat. I observed the largest sticklebacks in tidal flats and the smallest in salt marshes, differing by 0.7cm (Fig. 10). My hypothesis that larger sticklebacks would be found in habitats with lower average temperatures was therefore partially supported because tidal flats recorded the lowest average temperatures, but the highest temperatures were observed in lagoons, and not salt marshes (Fig. 3c). Seebacher *et al.* (2016) found that salt marsh sticklebacks in the Great Eau estuary had more slender, less muscular bodies, as well as lower swimming performance relative to other sticklebacks in the estuary. I also did not observe a relationship between habitat salinity and size because sticklebacks in salt marshes and lagoons had comparable body sizes, but different salinity levels (Fig. 3d). This contrasts with the findings of DeFaveri and Merilä (2014), who found that salinity had a significant effect on body size in the Baltic Sea populations. However, the Baltic Sea is an unusual marine ecosystem because there are steep salinity gradients along the basin (HELCOM, 2010). Therefore, the link between salinity and body size remains inconclusive, and more work is needed to explore the ecological factors driving body size variation between and within marine habitats.

Females were universally larger than males across habitats (Fig. 11, Table 2). The greatest difference between average male and female body size was in tidal flat sites (Fig. 11). This was similar to what Spoljaric and Reimchen (2007, 2008) found in Haida Gwaii archipelago lake populations, where sexual dimorphism was greater in freshwater populations from large, deep lakes than in shallower lakes. The larger morphological differences between male and female body size I observed on tidal flats could be caused by the relatively large pelagic area in those habitats. For example, in their study of freshwater sticklebacks in Boulton Lake, BC, Reimchen and Nosil (2004) found that with more limnetic area in the lake, males and females occupied different niches. Females were found in higher densities in the water column, while males lived in the benthic area, where they were likely guarding the nests. Exposure to different niches means males and females could be exposed to different predator regimes as well, also potentially influencing body size between sexes (Nosil and Reimchen, 2005).

Variation in head shape

Sexual dimorphism was an important driver of morphology, as the Procrustes LM showed significant variation in head shape between sexes (Table 3). However, there was no clear clustering pattern in the PCA plot (Fig. 12). Instead, there was substantial overlap between males and females in head shape. Females had relatively compressed heads with a narrower operculum, and the male cluster had a deeper head with a broader operculum (Fig. 12). Kristjánsson *et al.* (2002) looked at two marine populations in an Icelandic fjord and found males with larger jaws, and longer snouts than females, which compared to my results. While the dimorphic differences in head shape were the opposite to what Aguirre and Akinpelu (2010) found in multiple Alaskan freshwater populations. Their study showed that female sticklebacks had wider opercula and a compressed snout and jaw, while males had compressed opercula but matched our results of more elongated snouts and mouths. The landmarks that I used on the head shots for geometric morphometrics were very similar to Aguirre and Akinpelu (2010). Perhaps head shape varies between geographic regions in the northern hemisphere and the patterns I observed could be specific to BC marine populations.

The Procrustes LM also showed significant head shape variation between regions (Table 3). But there was almost no clustering observed between the four regions in the PCA plot

(Appendix E- Fig. E.2). Head shape varied along a continuum, as opposed to a stark clustering pattern. Morris *et al.* (2018) also found the shape of six marine stickleback populations from California to BC were distributed along a continuum, although their study analyzed the morphometric variation of the entire body, not the head shape alone. In the Baltic Sea, Jakubavičiūtė *et al.* (2018) sampled marine populations from four regions and also looked at overall body shape as opposed to just head shape. They did note that sticklebacks from one region (*i.e.* Kalmer Sound) had longer snouts than the other regions, but there was no further mention of head shape variation (Jakubavičiūtė *et al.*, 2018).

Habitat did not have a significant effect on head shape (Table 3), which was surprising. My hypothesis that head shape would vary for a fish that exploits a benthic habitat vs. a fish that is adapted to a pelagic habitat was not supported. I based my hypothesis on the exploitation of benthic vs. limnetic habitats and the associated food availability in each niche, which is a very common diversifying mechanism affecting freshwater fish (Walker, 1997; Spoljaric and Reimchen, 2007; Willacker *et al.*, 2010). However, the lack of clustering among regions and habitats agrees with the results of Svanbäck and Schluter (2012), who found that anadromous populations had intermediate head and body morphologies instead of tending toward a benthic or limnetic shape.

Bayesian cluster analysis found no clustering pattern of head shape (Appendix F - Fig. F.2).

Because little to no research has focused on head shape variation in marine sticklebacks, I was not quite sure what to expect. I found variation in head shape between sexes and regions. The sexual dimorphic differences in head shape appeared to have a stronger influence compared to regional and habitat differences. In addition, the variation I found did not follow, or compare to, patterns observed in freshwater head shape. Marine habitats should therefore be studied more in-depth to fully understand the drivers of marine stickleback trophic morphology across regions and habitats.

Variation in body shape

As with head shape, Procrustes LM found body shape varied significantly between sexes (Table 4). However, unlike head shape the PCA plot had a more pronounced clustering pattern between males and females, which was supported by Bayesian cluster analysis (Fig. 13, Appendix F - Fig. F.3, Fig. F.4). Thus, sexual dimorphism has a strong impact on body shape of marine sticklebacks. Females clustered towards a broader pelvic girdle and more compressed snout, while males tended towards a compressed pelvic girdle with an elongated snout (Fig. 13). The differences I saw matched what other researchers have observed. Females have larger pelvic girdles and more compressed heads and jaws compared to males (Kitano *et al.*, 2007; Aguirre *et al.*, 2008; Spoljaric and Reimchen, 2008). Additionally, Kitano *et al.* (2007) noted that difference in body depth between males and females differed between freshwater populations. This could help explain the variation I observed between male and female body depths along the PC2 axis (Fig. 13). Males clustered tighter towards an intermediate to deep body, while females varied along a continuum of body depths. Overall the lack of pronounced sexual dimorphism in body shape was similar to what Spoljaric and Reimchen (2008) observed among their six marine populations in northern Haida Gwaii, BC, where dimorphism was less pronounced among marine populations compared to freshwater lake populations.

Although there was no noticeable clustering of body shape by region in the PCA plot, the Procrustes LM found significant variation among regions (Table 4, Appendix E – Fig. E.4). The

SoG sticklebacks clustered towards a deeper, broader body shape, while the JdF sticklebacks clustered towards a more streamlined body shape (Fig. E.4). This was comparable to the regional variation Morris *et al.* (2018) observed along the west coast of North America (Alaska to southern California). The California populations in warmer waters had deeper, squatter body shapes than the more streamlined bodies in northern populations (*i.e.* Alaska and BC). I observed deeper, squatter stickleback bodies at the warmer SoG sites, and more streamlined bodies at the cold JdF site. However, my PCA plots showed far less clustering among regions than observed by Morris *et al.* (2018).

Finally, the Procrustes LM found that habitat was a significant factor, while the PCA plot showed no clustering (Table 4, Appendix E - Fig. E.5). The coastal habitats along Vancouver Island can change even throughout the day. For instance, the marine populations I caught might migrate around an estuary, as the tides go in and out, to find shelter, prey, or an ample depth. If marine sticklebacks are occupying more than one habitat type, their morphologies may be adapted for the inconsistent nature of estuaries as opposed to just one habitat type, as I had predicted. As tides rise and fall within coastal ecosystems, the speed of tidal currents change (Harris and Rattray Jr., 1954; Semeniuk, 2005; Townsend, 2010). The difference in flow speed, for example, could impact body morphology suited for different swimming needs among habitats. Swimming ability is proportional to body size of a fish, larger individuals are able to occupy deeper habitats that have less protection from strong water currents (Kennedy and Strange, 1982; Macdonald *et al.*, 1987; Everest and Chapman, 2011). Therefore, while habitat has a significant impact on stickleback morphology, further investigations are needed to know what elements of a habitat are causing this effect.

There was one cluster that stood out in the habitat body shape PCA plot, which was the JdF site in Port Renfrew (Appendix E, Fig. E.4). This site is a salt marsh habitat and the only region where I observed females with larger average heads than males (Fig. 6). Because the Juan de Fuca Strait is tidally mixed, the region has little temperature and salinity stratification throughout the water column (Waldichuck, 1957). Tidal forces facilitate intense mixing of the brackish surface waters and the deep, saline waters. The other three oceanographic regions surrounding Vancouver Island have distinct water column stratification and are dominated by upwelling, downwelling, or Fraser River runoff (Thomson, 1981). Siegle *et al.* (2013) found that the Juan de Fuca Strait acted as a physical boundary between populations of yelloweye rockfish (*Sebastes ruberrimus*) off the east and west coasts of Vancouver Island. These mixing zones in the water column may also act as a barrier to larval dispersal for other marine species (Sköld *et al.*, 2003; Perrin *et al.*, 2004). The life history strategy of marine sticklebacks does not include a larval stage, but this unique oceanographic region is likely contributing to the unique morphological variation I observed. I was only able to sample one site in the Juan de Fuca Strait, so future research should sample more marine populations in this region.

Overall, variation in head and body shape supported my hypothesis that there is regional and habitat variation between marine sticklebacks around Vancouver Island. However, sexual dimorphism was the biggest driver of morphological patterns.

It is important when conducting morphological studies such as this to avoid falling into the adaptationist programme outlined by (Gould and Lewontin, 1979). Many of the morphological traits I observed have a known genetic basis in stickleback research and have been shown to evolve adaptively. However, morphological variation in stickleback can also be plastic, or a product of genetic and environmental interaction. Future studies should evaluate the genetic basis of trait variation in marine sticklebacks as well as their scope for plasticity using in-lab breeding and common garden experiments.

Suggestions for future research

My thesis was the first to look at multiple marine stickleback populations around Vancouver Island and Southern mainland BC. Plenty of opportunity exists for future research endeavours to relate stickleback morphological variation to the marine scape they inhabit. Surveying marine populations on a smaller scale, such as in a single estuary or inlet, could help explain local habitat-specific morphological variation. My results provide preliminary evidence that marine sticklebacks vary morphologically among regions and habitat and that their morphology is influenced strongly by sexual dimorphism. However, more sampling, surveying, and analysis of BC marine populations is needed to properly characterize the breadth and causes of local phenotypic structuring.

My thesis opens an exciting new venue of marine stickleback research. It is unclear whether we can explain marine stickleback variation using the same ecological factors we have found to explain freshwater stickleback variation. Research has confirmed that local marine populations vary genetically and that marine populations across large geographic areas vary phenotypically (DeFaveri and Merilä, 2013a; Morris *et al.*, 2018). The next step is to explain this variation in relation to the ecological conditions which vary between marine environments.

Throughout the discussion I highlighted several analyses that future studies could elaborate on. In addition to these analyses, I suggest several other venues for future research. Namely, to conduct a detailed study at a single location or estuary to disentangle factors that influence stickleback morphology on a smaller scale. This was my plan for a secondary field season, which was derailed because of the COVID-19 Lockdown. The study I presented above focused on an intermediate spatial scale and attempted to capture as many populations as possible. However, there is habitat diversity, temperature and salinity gradients, and physical oceanographic variation found within many estuaries along the coast of Vancouver Island. Conducting a study on stickleback populations within such an environment would be very useful for understanding how morphology relates to environmental conditions. Repeated sampling over the summer breeding months, or even year-round, would provide detailed observations of salinity and temperature changes over the course of a season and daily tidal cycles. In addition, focusing on a single estuary would allow researchers to conduct detailed observations on predators, prey availability, and migration patterns of marine sticklebacks.

In my opinion, a good site for these projects would be Cowichan Bay on Vancouver Island, which is located 55 km north of Victoria, BC. Cowichan Bay is the site of two river outflows, the Cowichan River and Koksilah River (Cowichan Community Land Trust, 2004). Freshwater sticklebacks have been found in both rivers and I sampled marine populations in the estuary (Idyll, 1942; Durston, 2016, *pers. obvs.*). The Cowichan Bay estuary includes large mud flats, salt marshes, tidal flats which support eelgrass beds, and estuarine lagoons (Cowichan Community Land Trust, 2004, *pers. obvs.*). Because of this habitat variation, I believe it would be an optimal location to sample marine stickleback populations from each habitat (*i.e.* tidal flat, salt marsh, lagoon), and from each river outflow, repeatedly over a field season. Through repeated sampling at the same point of each day (*i.e.* high tide and low tide), or the use of automated loggers, the salinity and temperature gradients could be properly characterized. Something that lighthouse data from the DFO cannot properly characterize due to the position of each lighthouse (DFO, 2020) (Appendix A – Fig. A.3). For instance, Pickard (1963) characterized nine major inlets along just the west coast of Vancouver Island. They found that salinity would increase as much as 17%$_0$ from the mouth of an inlet to the head, while surface temperatures increased as much as 5°C. This amount of variation in water column properties could not be captured by one lighthouse at the mouth of each sound, therefore it would be very beneficial to focus on one ecosystem and its unique salinity and temperature variability.

Any future research in Cowichan Bay would benefit greatly from consultation with Cowichan First Nation (Cowichan Tribes), whose traditional territory includes the Cowichan River and Koksilah River water sheds (Hill, 2011). The Cowichan Tribes are the largest First Nations group on Vancouver Island and were the original stewards of the two rivers, a relationship which continues today with their involvement in research and conservation efforts (Hill, 2011; Williams *et al.*, 2013). For example, the Cowichan Tribes have partnered with Fisheries and Oceans Canada and the Cowichan River Hatchery since 1978 to monitor salmon stocks in the rivers (Williams *et al.*, 2013). The Cowichan Tribes members have sat on the Cowichan Watershed Board since 2010; the Board creates and implements sustainable water management strategies to help protect and enhance the Cowichan watershed (Wood, 2011). The main stem of the Cowichan River was even designated as a Canadian Heritage River in 2003 (Williams *et al.*, 2013). The Cowichan Tribes' involvement, as well as any local First Nations groups on Vancouver Island, would greatly enhance future stickleback research.

Cowichan Bay is an ecologically important estuary, providing habitat for over 200 bird species (Great Pacific Engineering & Environment, 2015). These species include a few birds which are known to prey on sticklebacks, including great blue herons, mergansers, and kingfishers (Kelsall and Simpson, 1980; Reimchen and Douglas, 1984; Raven, 1986; Wade, 2014). I also observed sculpin, likely Pacific staghorn sculpin (*Leptocottus armatus*), in my minnow traps while sampling in Cowichan Bay as by-catch. Sculpins are piscivorous fish that feed on sticklebacks (R. K. Saimoto, 1993; Ingram *et al.*, 2012; Rybkina *et al.*, 2017). Thus, Cowichan Bay would be a good site for evaluating the effects of predation on bony armour phenotypes and body shape. Perhaps the most important area for future research on marine populations would be to include analysis on predator presence and intensity where marine sticklebacks are sampled. Body shape is known to be associated with predator species (Spoljaric and Reimchen, 2007). They found that Haida Gwaii freshwater populations subjected to predators similar to ancestral marine predators (*i.e*. toothed piscivores like salmonids), had less derived body shapes (*i.e.* small heads and shallow bodies). While freshwater populations with a predator presence of birds and macroinvertebrates had more derived shapes (*i.e.* large heads and deeper bodies). Ab Ghani *et al.* (2016) found that artificially crossed marine sticklebacks from the Baltic Sea developed longer, narrower caudal peduncles and larger eyes when exposed to chemical cues from a potential predator. And variation in caudal peduncle length is known to strongly influence swimming performance in sticklebacks (Bergström, 2002; Leinonen *et al.*, 2011).

Additionally, my study did not include methodology that classified reproductive status among the populations. For instance, I did not assess whether my specimens were sub-adults, reproductive adults, or a combination of the two. This can be done by comparing standard length with age and nuptial colouration of the sticklebacks (Spoljaric and Reimchen, 2011). As well as including repeated sampling dates, perhaps one per month, to each site throughout a field season. Future studies should assess reproductive status of sticklebacks, because marine stickleback exhibit ontogenetic changes in body and head shape (Spoljaric and Reimchen, 2011). Otherwise there is no way that researchers could confidently declare if the observed morphological variation was due to different ages of sticklebacks which grew throughout a sampling season.

Within an estuary, researchers could evaluate life history strategies and migration patterns of marine sticklebacks. A common method is to evaluate otolith microchemistry and structure. Arai *et al.* (2003) used otolith microchemistry methodology to investigate migration patterns in various Japanese stickleback populations. They observed Sr:Ca ratios in otoliths, which are positively correlated with salinity, and concluded in a later study that stickleback populations with an anadromous phenotype were actually year-round estuarine residents (Arai *et al.*, 2020). This has not been evaluated in BC marine populations yet. Otoliths are also examined for age determination in fish. Annual and daily rings of growth can estimate the life span of the specimen (Jones and Hynes, 1950; Maceina and Sammons, 2006). As I mentioned before, marine and freshwater sticklebacks can continue growing throughout their adulthood and live much longer than the 2 year average life span (Reimchen, 1992a; Yershov and Sukhotin, 2015; Ivanova *et al.*, 2016; Yurtseva *et al.*, 2019). Thus, future studies should also age marine populations in BC to help evaluate the variation in body size.

Other head traits should be evaluated in marine sticklebacks, such as gill rakers. Gill rakers are known to vary between freshwater sticklebacks in different niches based on prey availability (McPhail, 1984, 1992; Schluter and McPhail, 1992; Hart and Gill, 1994). Gill raker length and concentration differs between marine and freshwater populations, but it has not been assessed between marine populations by any researchers I am aware of (Voje *et al.*, 2013; Østbye *et al.*, 2016). Prey availability and prey type differs between BC marine habitats. Eelgrass tidal flats support both planktonic and benthic epifaunal organisms (Kelly *et al.*, 2008; Kennedy, 2016). Salt marshes are the most unstable environment but still support a large diversity of invertebrate species, mostly epifaunal to survive the drought periods of the tide (Armonies, 1986; Callaway *et al.*, 2012). Lagoons are dominated by deposit-feeding invertebrates that are larger (Barnes and Villiers, 2000). Based on what has been observed in freshwater sticklebacks, head shape adapts to the feeding strategy needed for different prey types (*i.e.* striking vs. suction of prey) (Caldecutt and Adams, 1998; Higham *et al.*, 2017).

I suggested the study area of Cowichan Bay, but there are numerous estuaries and inlets which exist along the BC coast where marine sticklebacks could be sampled from, including Barkley Sound, where I sampled marine sticklebacks in Bamfield Inlet (which empties into the Sound) (Appendix A- Fig A.1). Barkley Sound is an expansive, coastal embayment with three channels: Loudon Channel, Imperial Eagle Channel, and Trevor Channel (Taylor and Haigh, 1996). Each channel varies in depth, productivity, and physical oceanographic characteristics. For example, the Trevor Channel is the deepest and heavily influenced by the freshwater outflow from the Alberni Inlet (Taylor and Haigh, 1996). The Eagle Channel is the most exposed to open ocean influences, with an intermediate depth compared to the shallow, productive Loudon Channel (MacIsaac *et al.*, 1991). Environmental gradients vary substantially in Barkley Sound, summer surface temperatures can range from 5°- 20°C and surface salinities can range from 5ppt – 32ppt (Taylor and Haigh, 1996; Whippo and O’Connor, 2013). Additionally, Barkley Sound has a low human population in the watershed and therefore is a relatively unaltered embayment (Keil *et al.*, 2011). As a large ecosystem, with low anthropogenic impacts, Barkley Sound contains diverse habitats and niches suitable for future researchers to observe local marine stickleback populations. Even on Vancouver Island there are many optimal study locations, supporting my view that BC is an ideal place to study morphological variation in marine sticklebacks on numerous geographic and oceanographic scales.

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Appendix A

Table A.1. List of sites including each respective site abbreviation, descriptions of where to park, where to deploy traps or seine net, GPS coordinates, date of collection during my 2019 field season, and total sticklebacks caught at each site with sex ratios between males (M) and females (F). There were five sites where I caught sticklebacks successfully, but I could not analyze the fish because they were preserved incorrectly. These specific sites are denoted by "*Preserve*".

Location	**Abbr.**	**Latitude & Longitude**	**Date of Collection**	**Description of Site**	**Fish Caught**	
					M	**F**
Roberts Bank, off Tsawwassen, BC	RON	49.02945, -123.19034	12-May-19	Sampled with Raincoast Conservation Foundation. I collected by-catch from their team on a crab boat. My contact was Dave Scott (dave@raincoast.org)	10	6
Englishman River Estuary	ENG	49.33161, -124.28803	15-May-19	Walk east around lagoon, and south along the stone fence. eelgrass beds present along the shore.	26	24
Koksilah River Estuary	KOK	48.75259, -123.63851	21-May-19	Drive down Westcan Terminal Rd. On the left there is a newly constructed walking bridge. Drop traps under and around the bridge.	*Preserve*	
Courtenay River Estuary	COR	49.68108, -124.97257	18-May-19	From the Birdwatching Platform, walk out into Courtenay R. The deeper the better, best sampling at 1 hour after low tide.	6	7
Black Creek Estuary	BCE	49.85643, -125.10291	26-May-19	Place traps right in the creek. Park on opposite shore to the Provincial Park.	10	29
Oyster Lagoon	OYS	49.61232, -124.031	26-May-19	Park at these coordinates. Anywhere in the lagoon is good. Locals are familiar with stickleback research.	11	39
Salt Lagoon, Pender Harbour	STL	49.61446, -124.03043	26-May-19	Walk over from Oyster Lagoon. Very easy to catch fish anywhere and anytime of day.	31	19
Cowichan River Estuary	CRE	48.76695, -123.63239	10-Jun-19	Park on the side of road. Walk around the muddy estuary channel at rising tide. Place traps near marshy banks, it is very hard to walk in the muck.	*Preserve*	
Bamfield South Inlet	BAM	48.81331, -125.15667	03-Jun-19	Park in marked lot, walk down to the marked trail (200m), then follow side trail out to the flats. Place traps anywhere near vegetation on the flat.	22	15
Contuma River Estuary, Moutcha Bay	CMA	49.79257, -126.4445	08-Jun-19	I rented kayaks from Moutcha Bay Lodge Outfitters. Healthy eelgrass beds at the coordinates, left traps from low tide for 4 hours. Only access by boat/canoe/kayak.	21	8

Canton Lagoon, Moutcha Bay	CAN	49.79672, -126.47354	10-Jun-19	Can see lagoon from just off the logging road. Park in a truck pull out around the corner, walk along side of road. Left traps in shady areas during rising tide. Be aware of cougars.	15	15
Campbell River, BC	BAK	50.03921, -125.26754	20-Jun-19	Baike Island park. Would not recommend sampling here or anywhere in Campbell River, BC.	13	2
Salmon River, Sayward Estuary	SAY	50.37836, -125.94948	21-Jun-19	Cross active logging road, which runs parallel to the local road. There is parking for an estuary trail. Walk to the end of the estuary trail (600m) to a lagoon. Anywhere there is easy to catch stickleback.	36	6
Holberg Estuary	HOL	50.65184, -128.00274	25-Jun-19	Very Rough logging road. Need a radio with “Holberg Rd. frequencies” from Western Forest Products North Island. Drive halfway down Dock Rd. On the inland side there are two pools which fill with the tide. Place traps here during rising tide.	5	8
Rupert Inlet, Waukwaas Creek	RUP	50.58804, -127.41746	26-Jun-19	Another logging road, “Rupert Main” is the busiest logging road for the North Island. Also need radio and 4x4 truck. Park in truck pullout south of the bridge crossing the creek, there is a rail down to the creek. Place traps in eddies and shaded areas downstream of the bridge. The couple who owns the house nearby are friendly, but do not walk through their property.	*Preserve*	
Port Hardy Estuary	HDY	50.70001, -127.47179	27-Jun-19	At the end of Goodspeed Rd. park in Nature Trail parking. Walk across the bridge for bird viewing, and off to the left where there is a newly dug channel. Plenty of stickleback there.	19	33
Sooke River Campground	SOK	48.38976, -123.70729	02-Jul-19	Ask the staff at Sooke River Campground to use their shoreline, park in guest parking, walk down staircase to shore, and place traps under overhanging trees.	*Preserve*	
Coles Bay Regional Park	COL	48.6298, -123.46732	03-Jul-19	Park in parking lot, walk down to the beach. Walk straight out and you will find large eelgrass beds. Seine anywhere there at low tide.	36	14
Albert Head Lagoon	ABH	48.39598, -123.48949	03-Jul-19	Place traps anywhere along the shore of the lagoon. There is very low inflow, so water levels should not change too much with the tide.	*Preserve*	

San Juan River	PRF	48.5665, -124.39376	09-Jul-19	Walk down flag-taped path at rising tide. Walk toward the River in the channel, place traps there.	11	37

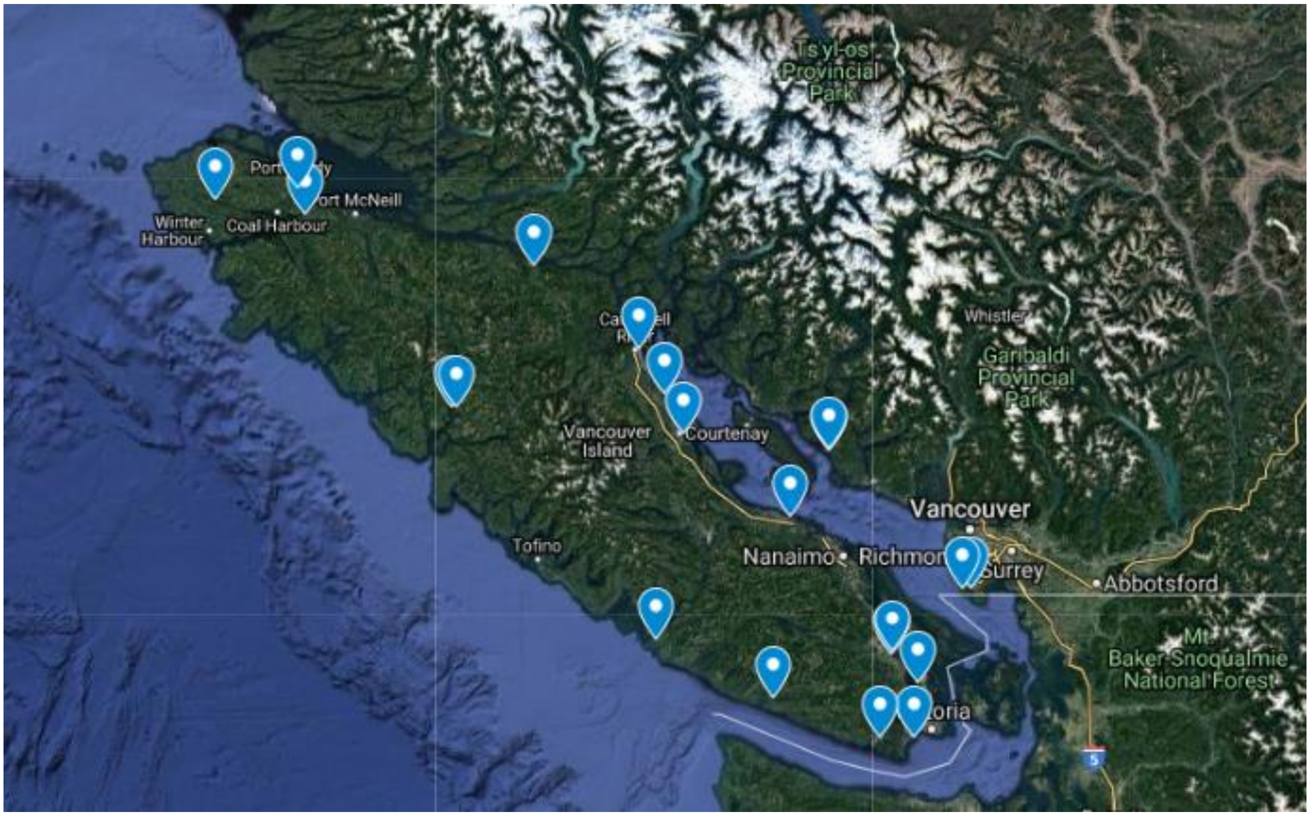

Figure A.1. Map of the locations of my sites sampled on the Southern Mainland coast of BC and around Vancouver Island, BC. Two points on this map are actually four separate sites (Moutcha Bay and the site on the Sunshine Coast). The sites were in such close proximity on the map, they look like a single point on the map. All latitudinal and longitudinal coordinates can be found above in Table A1. Most sites can be accessed by paved road, but 5 sites were accessed along active logging roads (Bamfield South Inlet, Moutcha Bay (2 Sites), Rupert Inlet, and Holberg, BC). Experience driving on logging roads is crucial. Map was constructed using Google Earth.

Figure A.2. The homemade Styrofoam apparatus to take photographs of each specimen under the microscope or camera. The square metal cookie cutter was glued into place and pins helped to secure the body and head of each specimen. All supplies were purchased at a local craft store.

Table A.2. The average sea-surface temperatures (SST) and sea-surface salinities (SS Salinity) obtained from four BC lighthouses. Each lighthouse was chosen to represent average temperature and salinity values found in the four oceanographic regions around Vancouver Island, BC: Juan de Fuca Strait (JdF), the west coast (West), the North coast (North), and the Strait of Georgia (SoG). I reported the average SST and SS salinities in May, June, and July each year. These datasets were collected, daily, from 1956-2019. Data was obtained from the Department of Fisheries and Oceans (DFO, 2020).

BC Lighthouse Name	Coordinates	Average SST (°C)			Average SS Salinity (PSU)			Region
		May	June	July	May	June	July	
Race Rocks	48.298, -123.532	9.84	10.6	11	31.2	31.1	31.1	JdF
Kains Island	50.441, -128.032	10.9	12.1	14.4	30.5	31.4	31.6	West
Amphitrite Point	48.922, -125.541	11.6	11.9	13.4	30	30.6	30.5	West
Nootka Point	49.593, -126.615	13.4	14.7	16.6	29.4	30.1	31.5	West
Pine Island	50.976, -127.728	9.46	9.7	11.3	31.4	31.5	31.5	North
Entrance Island	49.209, -123.808	12.8	15.7	16.7	27.5	24.9	25.2	SoG
Chrome Island	49.209, -124.685	12.9	15.2	17.4	28.3	26.8	26.6	SoG

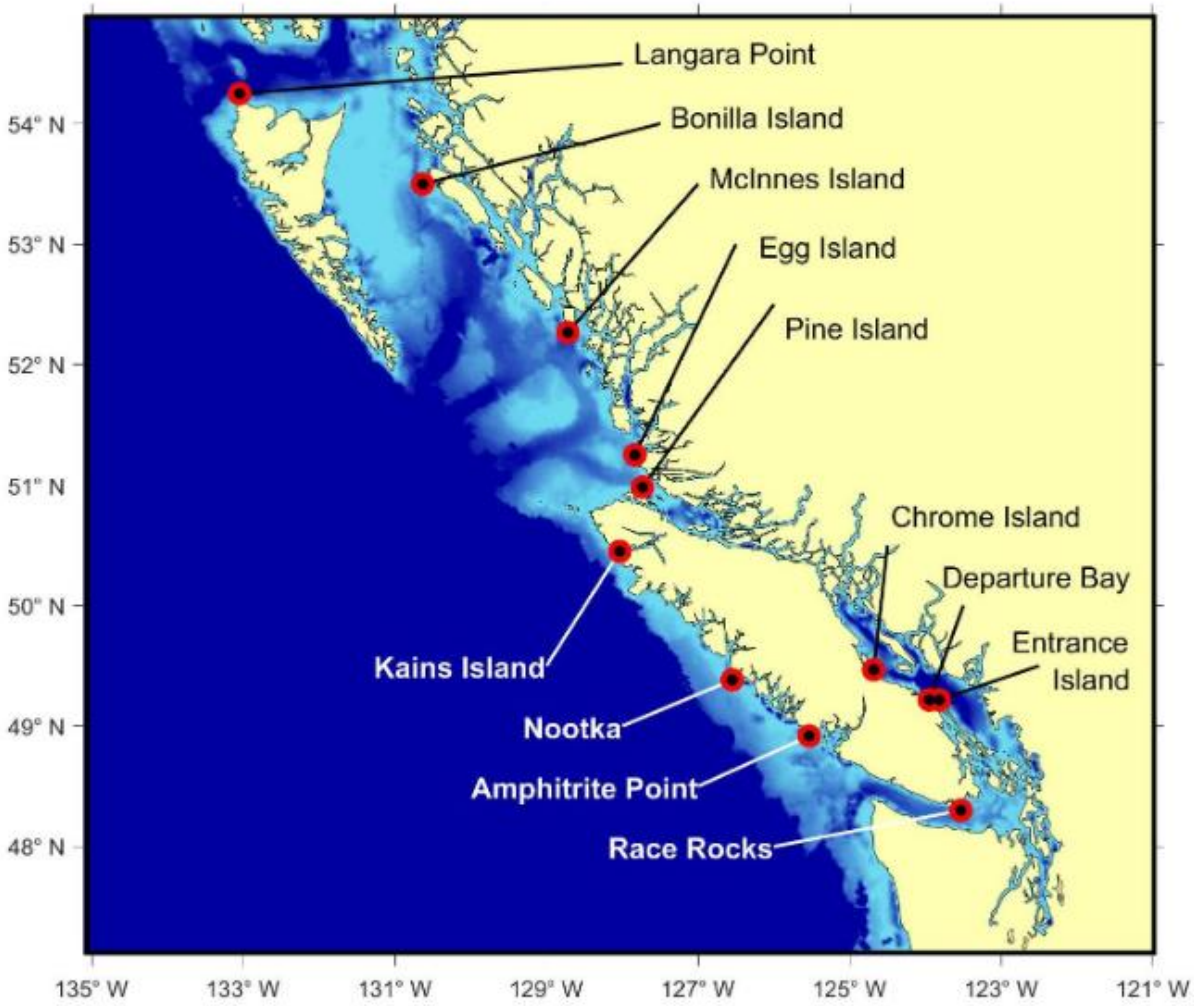

Figure A.3. Map of BC lighthouse positions, including the lighthouses I reported in Table A.2. Map was constructed by the Department of Fisheries and Oceans (DFO, 2020). The map shows the entire BC coastline, while the map in Figure A.1 includes only Vancouver Island and Southern mainland BC.

Table A.3. Total numbers of male and female sticklebacks sampled in each oceanographic region, as well as the total number of males and females sampled for the study.

Region	Abbr.	Females	Males	
Strait of Georgia	SoG	140	143	
Juan de Fuca Strait	JdF	37	11	
West Coast	West	46	63	
North Coast	North	39	55	
		262	272	Total

Table A.4. The number of male and female sticklebacks sampled in each habitat, as well as the total number of males and females sampled for the study.

Habitat	Females	Males	
Lagoon	87	98	
Salt Marsh	108	59	
Tidal Flat	67	115	
	262	272	Total

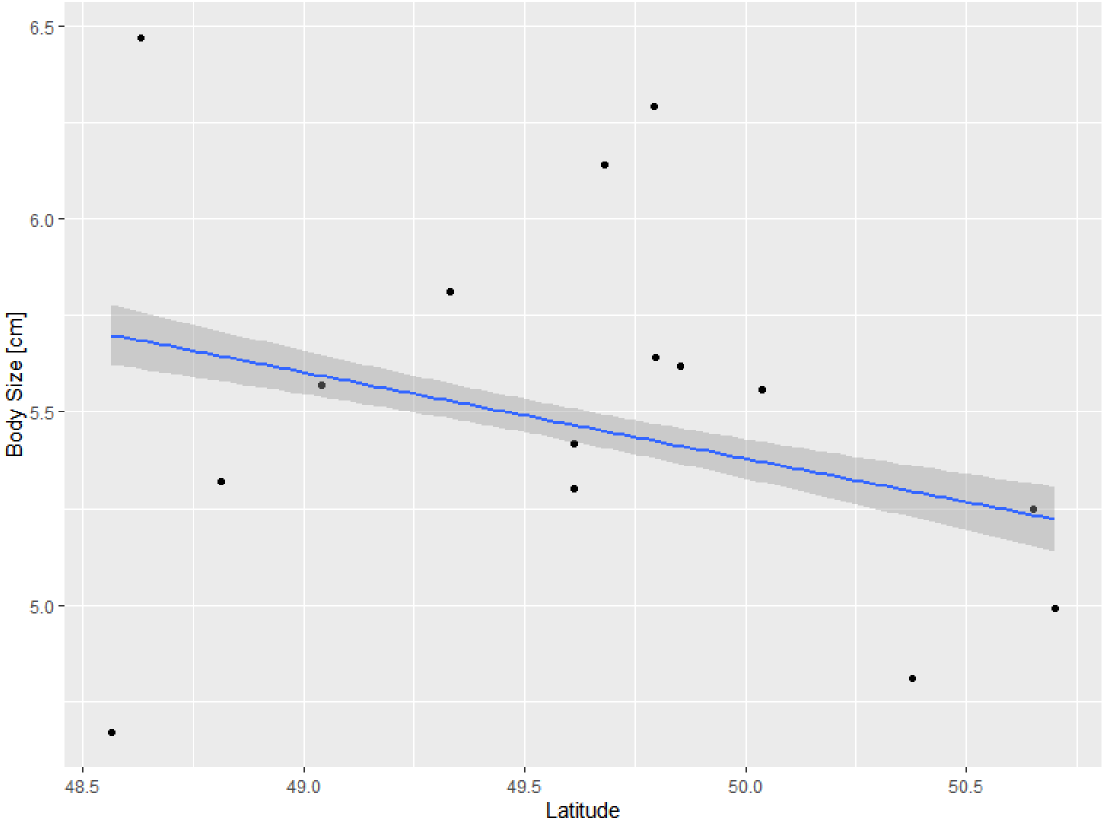

Figure A.4. The relationship between latitudinal coordinates of each of our sites and body size, represented by standard length (cm), to assess if our site followed Bergmann's rule. Correlation was not significant. Among my sites I found no support for Bergmann's rule, where body size, on average, increases with latitude. Removing the single outlier at the lowest latitude reduces the slope to zero. The relationship was assessed using the "two.b.pls" function in the *geomorph* package for R (Adams *et al.*, 2020).

Figure A.5. Map of the locations of my sites overlaid with the four oceanographic regions in the Southern Mainland coast of BC and around Vancouver Island, BC. The Strait of Georgia is in the yellow rectangle. The Juan de Fuca Strait is in the green rectangle and both Straits make up the Salish Sea. The western coast of Vancouver Island is in the red, and the northern coast is in the white rectangle. Map was constructed using Google Earth.

Appendix B

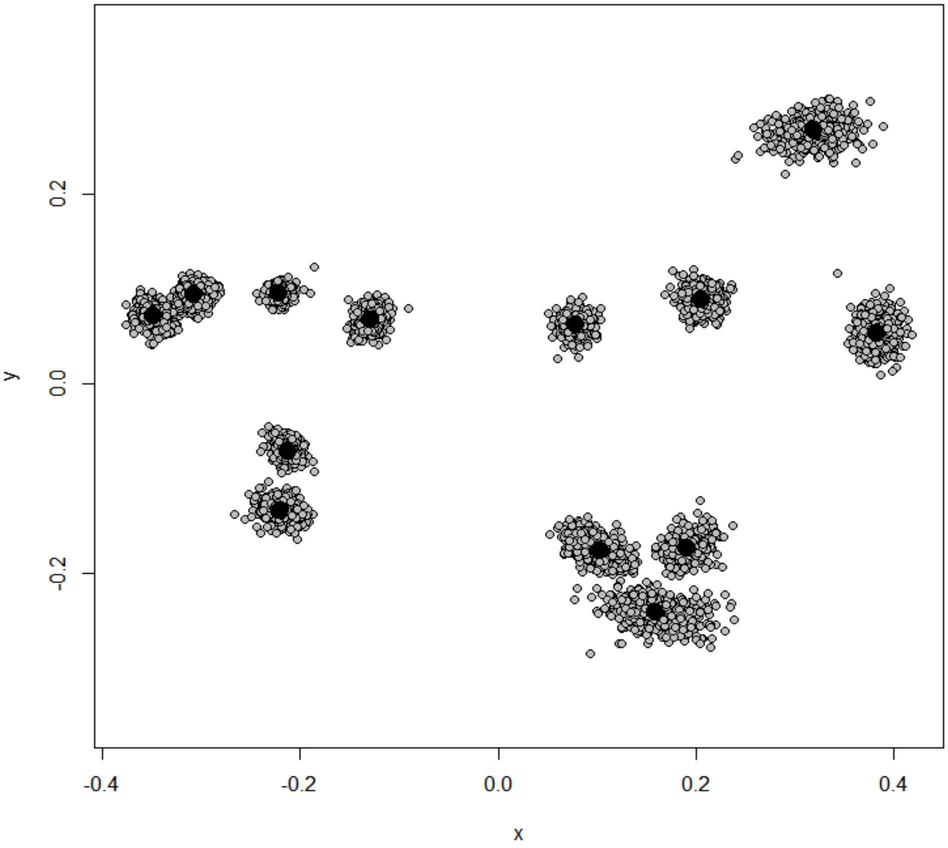

Figure B.1. All landmarks of individuals (*i.e.* Procrustes shape variables) superimposed on top of one another after Generalized Procrustes Analysis (GPA) of head shots. The snout of the individual is the left-most landmark, while the top of the head is the upper right-most landmark. For reference I have provided Figure 1, again, from the methods section below.

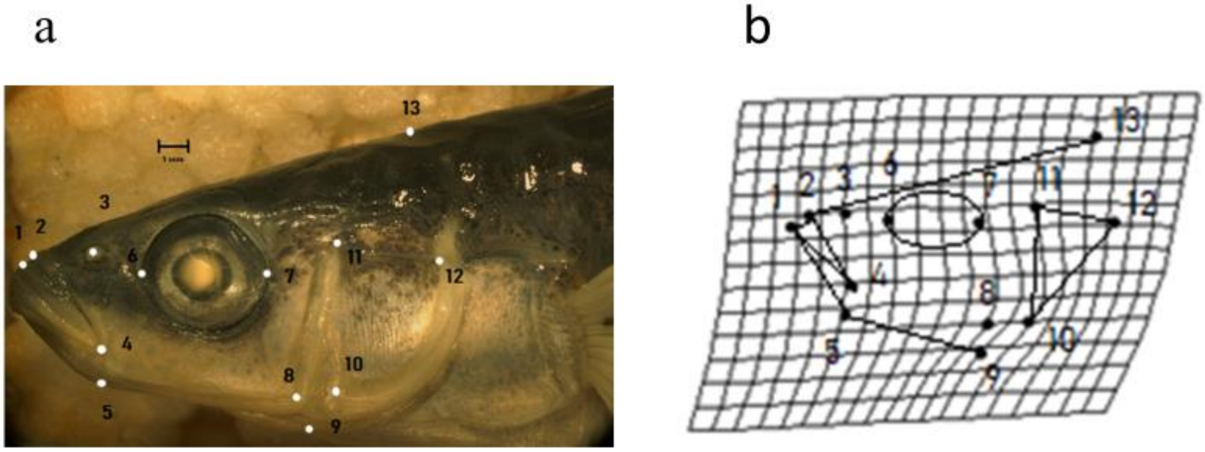

Figure 14. Analysis of stickleback head shape. Panel (a) shows the anatomical landmarks (1-13) used for geometric morphometric analysis. Panel (b) is a thin-plate spline deformation grid which represents variation in the shape of the specimen in panel (a).

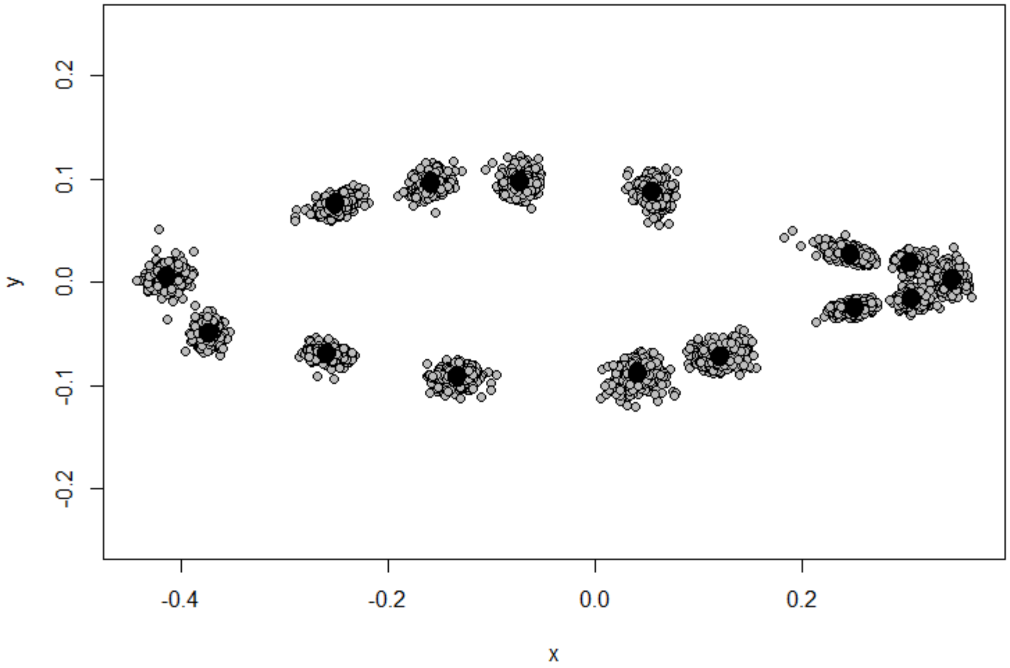

Figure B.2. All landmarks of individuals (*i.e.* Procrustes shape variables) superimposed on top of one another after Generalized Procrustes Analysis (GPA) of body shots. The snout of the individual is the left-most landmark, while the caudal fin is the right-most landmark.

Appendix C

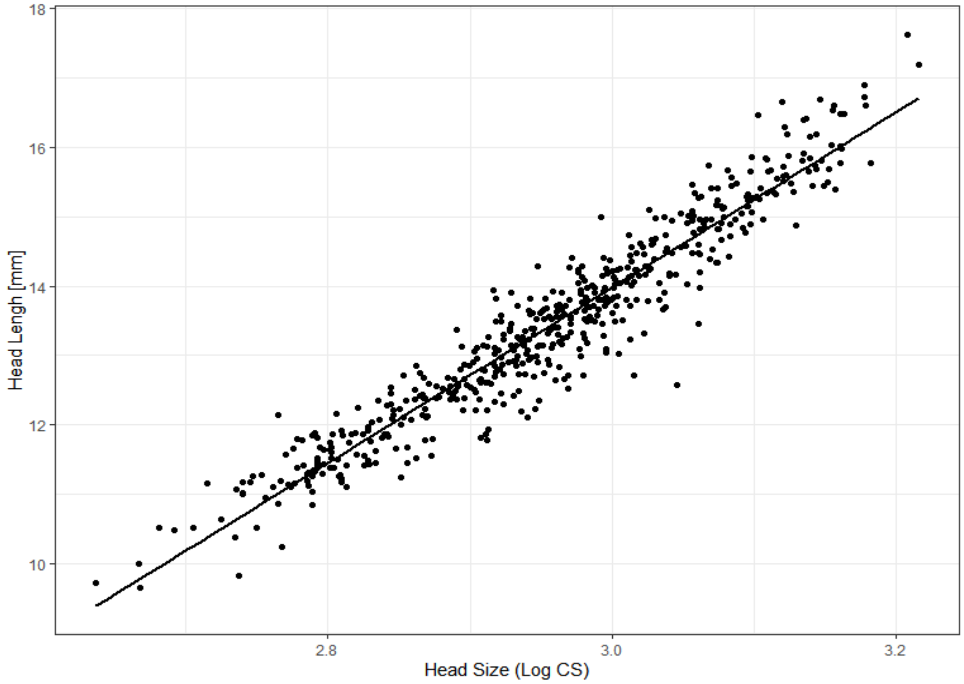

Figure C.1. The correlation (r-PLS = 0.958, *p* = 0.001) between head size (mm) and geometric head size (*i.e.* Log CS) which is a measure of head size used in shape analysis. CS was obtained following Generalized Procrustes Analysis (GPA), and each point represents one individual. Correlation was assessed using the “two.b.pls” function in the *geomorph* package for R (Adams *et al.*, 2020).

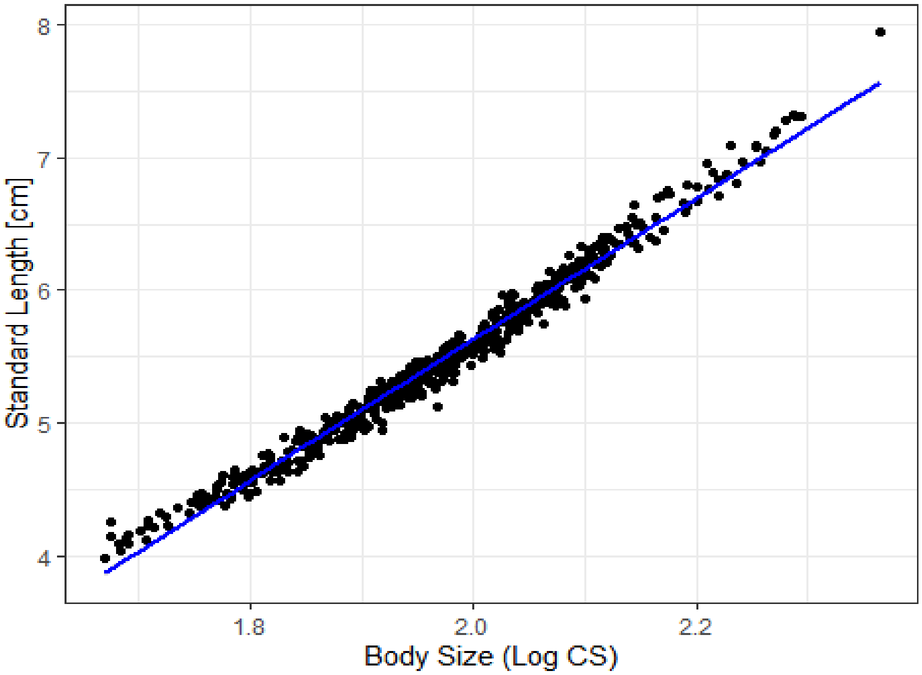

Figure C.2. The correlation (r-PLS = 0.991, $p = 0.001$) between body size (cm) and geometric body size (*i.e.* Log CS) which is a measure of body size used in shape analysis. CS was obtained following Generalized Procrustes Analysis (GPA), and each point represents one individual. Correlation was assessed using the "two.b.pls" function in the *geomorph* package for R (Adams *et al.*, 2020).

Appendix D

Table D.1. Model selection for linear mixed-effects models testing for region, habitat, and sex difference in head size. I built separate models using head size (mm) as the response variable, and sex, oceanographic region (Rgn), and estuarine habitat (Hbt) as fixed effects. Site was used as a random factor, with 15 levels. Each model has degrees of freedom (df), Log-Likelihood (logLik), AICc is the corrected Akaike information criterion values, while ΔAICc is the corrected Akaike information criterion delta values. Only the best models (ΔAICc <4) were considered and averaged. Weights (w) are the Akaike weights of each model and the intercept term (Int) represents the mean head size (mm) when all predictor variables are at the designated reference category (*i.e.* Sex (F), Region (SoG), Habitat (Lagoon)).

model	df	logLik	AICc	ΔAICc	W	Hbt	Region	Sex	Hbt*Sex	Rgn*Sex	Int
1	10	-790.87	1602.2	0.00	0.546		X	X		X	13.55
2	12	-789.92	1604.4	2.28	0.174	X	X	X		X	13.20
3	14	-788.09	1605.0	2.84	0.132	X	X	X	X	X	13.09
4	8	-795.59	1607.4	5.29	0.039	X		X	X		12.71
5	11	-792.53	1607.6	5.42	0.036	X	X	X	X		13.00
6	7	-796.97	1608.2	6.00	0.027		X	X			13.53
7	4	-800.27	1608.6	6.45	0.022			X			13.19
8	6	-798.63	1609.4	7.26	0.015	X		X			12.88
9	9	-795.94	1610.2	8.07	0.010	X	X	X			13.16
10	6	-812.37	1636.9	34.75	0.000		X				13.82
11	3	-815.54	1637.1	34.97	0.000						13.49
12	5	-813.70	1637.5	35.37	0.000	X					13.16
13	8	-811.20	1638.7	36.51	0.000	X	X				13.44

Table D.2. The top linear mixed-effects models following model selection with a response variable of head size (mm) for each model. The fixed effects included: region, habitat, and sex. Site was included as a random effect, with 15 levels. Only the models with ΔAICc < 4 were considered in my data interpretation. The variance explained by both fixed and random effects (R^2_c), and the variance explained by fixed effects (R^2_m). Akaike weights (w) are the weight of evidence in favour of each model. The intercept term (Int) represents the mean head size (mm) when all predictor variables are at the designated reference category (*i.e.* Sex (F), Region (SoG), Habitat (Lagoon)).

model	R^2m	R^2c	df	ΔAICc	w	Int
1	0.221	0.537	10	0.00	0.517	13.55
2	0.242	0.654	12	2.28	0.165	13.20
3	0.287	0.502	14	2.84	0.125	13.09

Table D.3. Model selection for LMM models testing for region, habitat, and sex difference in body size. I built separate models using standard length as the response variable, and sex, oceanographic region (Rgn), and habitat (Hbt) as fixed effects. Site was used as a random factor, with 15 levels. Each model has degrees of freedom (df), Log-Likelihood (logLik), AICc is the corrected Akaike information criterion values, while ΔAICc is the corrected Akaike information criterion delta values. Only the best models (ΔAICc <4) were considered and averaged. Weights (w) are the Akaike weights of each model, and the intercept term (Int) represents the mean head size (mm) when all predictor variables are at the designated reference category (*i.e.* Sex (F), Region (SoG), Habitat (Lagoon)).

model	df	logLik	AICc	ΔAICc	w	Sex	Region	Habitat	Sex*Rgn	Sex*Hbt	Int
1	14	-296.15	621.1	0	0.390	X	X	X	X	X	5.58
2	11	-299.61	621.7	0.62	0.287	X	X	X		X	5.54
3	12	-299.31	623.2	2.12	0.135	X	X	X	X		5.63
4	9	-303.16	624.7	3.56	0.066	X	X	X			5.60
5	10	-302.30	625.0	3.91	0.055	X	X		X		5.94
6	7	-306.04	626.3	5.18	0.029	X	X				5.90
7	8	-305.07	626.4	5.30	0.028	X		X		X	5.38
8	6	-308.57	629.3	8.19	0.007	X		X			5.44
9	4	-311.35	630.8	9.67	0.003	X					5.68
10	8	-330.65	677.6	56.46	0.000		X	X			4.38
11	6	-333.37	678.9	57.78	0.000		X				4.68
12	5	-336.31	682.7	61.63	0.000			X			5.29
13	3	-338.87	683.8	62.68	0.000						5.52

Table D.4. The top linear mixed-effects models following model selection with a response variable of body size (cm) for each model. The fixed effects included: region, habitat, and sex. Site was included as a random effect, with 15 levels. Only the models with ΔAICc < 4 were considered in my data interpretation. The variance explained by both fixed and random effects (R^2_c), and the variance explained by fixed effects (R^2_m). Akaike weights (w) are the weight of evidence in favour of each model. The intercept term (Int) represents the mean head length (mm) when all predictor variables are at the designated reference category (*i.e.* Sex (F), Region (SoG), Habitat (Lagoon)).

model	R^2m	R^2c	df	ΔAICc	w	Int
1	0.464	0.646	14	0.00	0.390	5.58
2	0.459	0.641	11	0.62	0.287	5.54
3	0.463	0.641	12	2.12	0.135	5.63
4	0.457	0.636	9	3.56	0.066	5.60
5	0.385	0.650	10	3.91	0.055	5.94

Appendix E

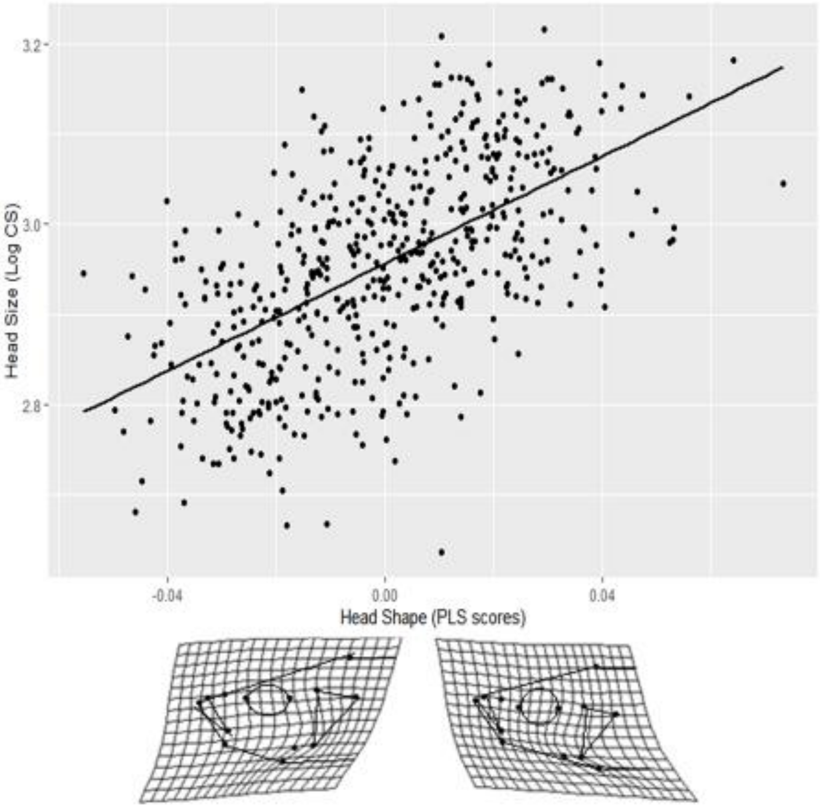

Figure E.1. The relationship (r-PLS = 0.587, $p = 0.001$) between head shape and geometric head size (*i.e*. Log CS). Each symbol represents a single specimen. Bottom panels show the shape deformation grids based on between mean shape differences between the minimum (left) and maximum (right) Partial Least Squares (PLS) scores.

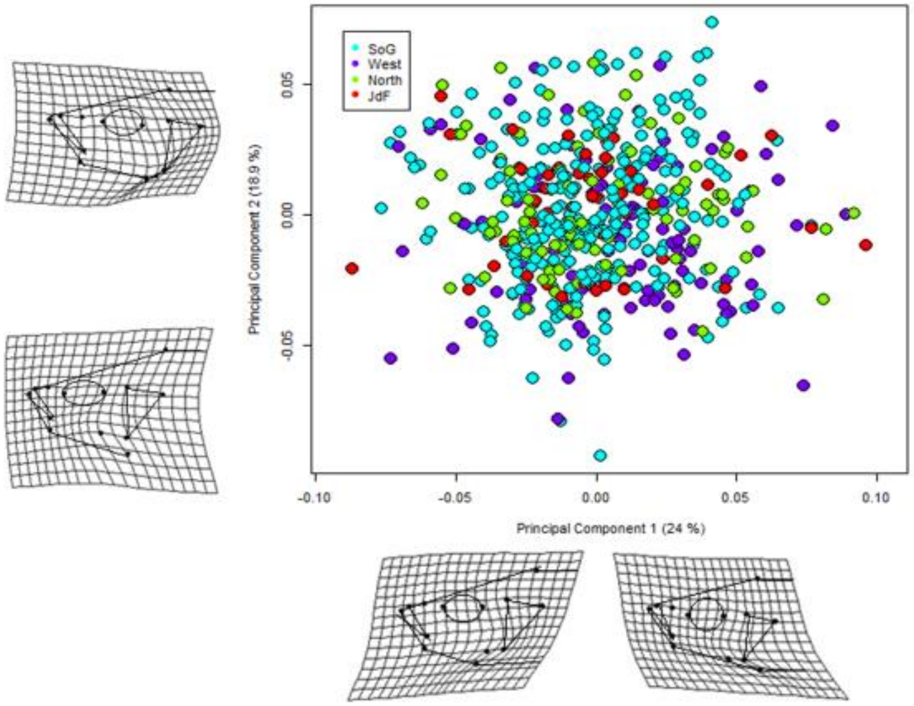

Figure E.2. Principal Component Analysis (PCA) of head shape. The head shape for each specimen was obtained from a set of thirteen anatomical landmarks (Fig. 1). The landmarks were converted into 26 shape variables by way of a Generalized Procrustes Analysis (GPA). These vectors of shape were used to carry out the PCA. Each data point represents data from the four oceanographic regions that surround Vancouver Island, BC: Strait of Georgia (light blue), west coast Vancouver Island (purple), north coast Vancouver Island (light green), and the Juan de Fuca Strait (red). Deformation grids were plotted with 1.5x magnification to facilitate visualization of head shape differences. The deformation grids represent the difference between the specimen on each end of each shape axis (Principal Component 1 and Principal Component 2). The effects of body size were removed from this analysis.

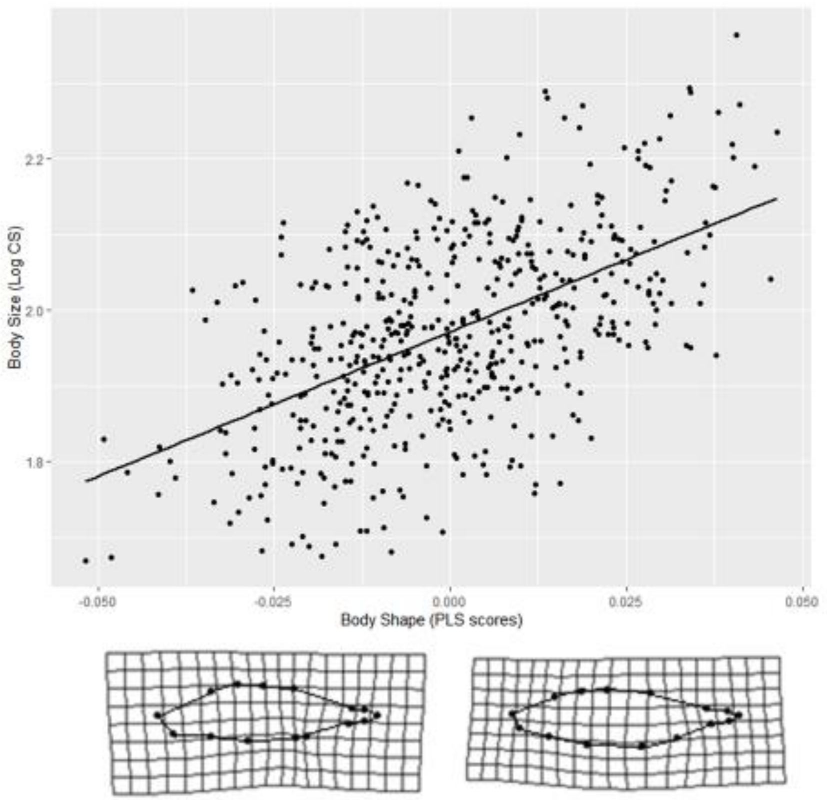

Figure E.3. The relationship (r-PLS = 0.543, *p* = 0.001) between body shape and geometric body size (*i.e*. Log CS). Each symbol represents a single specimen. Bottom panels show the shape deformation grids based on between mean shape differences between minimum (left) and maximum (right) Partial Least Squares (PLS) scores.

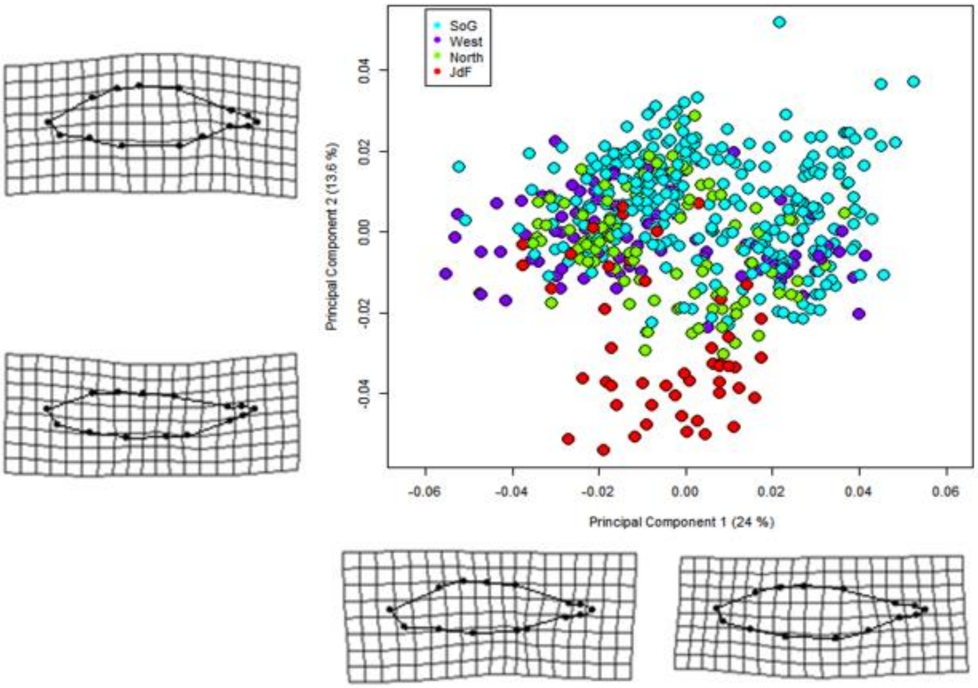

Figure E.4. Principal Component Analysis (PCA) of body shape. The body shape for each specimen was obtained from a set of fifteen anatomical landmarks (Fig. 2). The landmarks were converted into 30 shape variables by way of a Generalized Procrustes Analysis (GPA). These vectors of shape were used to carry out the PCA. Each data point represents data from the four oceanographic regions that surround Vancouver Island, BC: Strait of Georgia (light blue), west coast Vancouver Island (purple), north coast Vancouver Island (light green), and the Juan de Fuca Strait (red). The body shape variation is illustrated with the four deformation grids, which represent the differences between the specimen on each end of a shape Principal Component (PC) axis (PC1 and PC2)). The effects of body size were removed from this analysis.

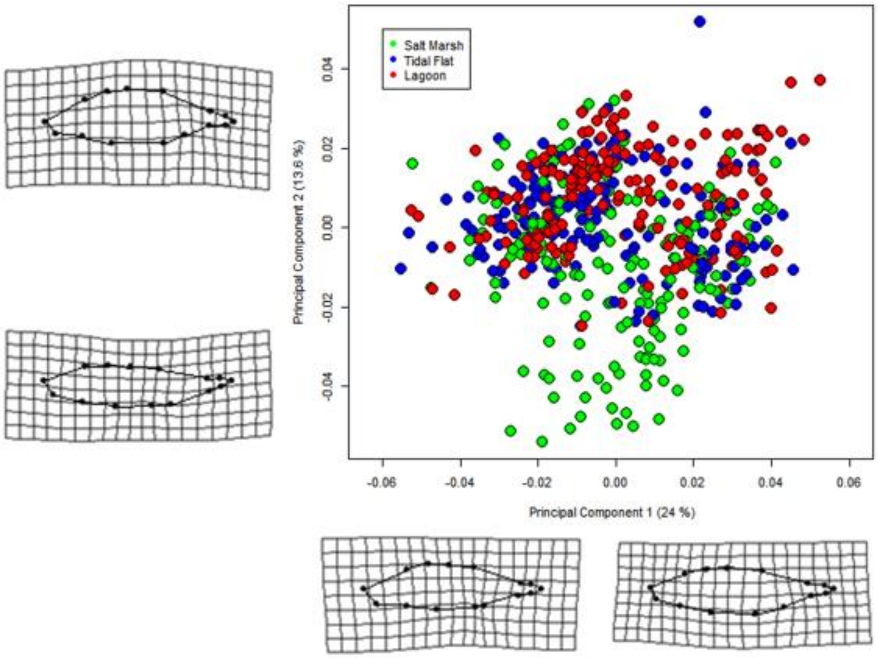

Figure E.5. Principal Component Analysis (PCA) of body shape. The body shape for each specimen was obtained from a set of fifteen anatomical landmarks (Fig. 2). The landmarks were converted into 30 shape variables by way of a Generalized Procrustes Analysis (GPA). These vectors of shape were used to carry out the PCA. Each point represents data from a specimen sampled from a habitat type, which include salt marshes (light green), tidal flats (dark blue), and lagoons (red). The body shape variation is illustrated with the four deformation grids, which represent the differences between the specimen on each end of a Principal Component (PC) axis (PC1 and PC2). The effects of body size were removed from this analysis.

Appendix F

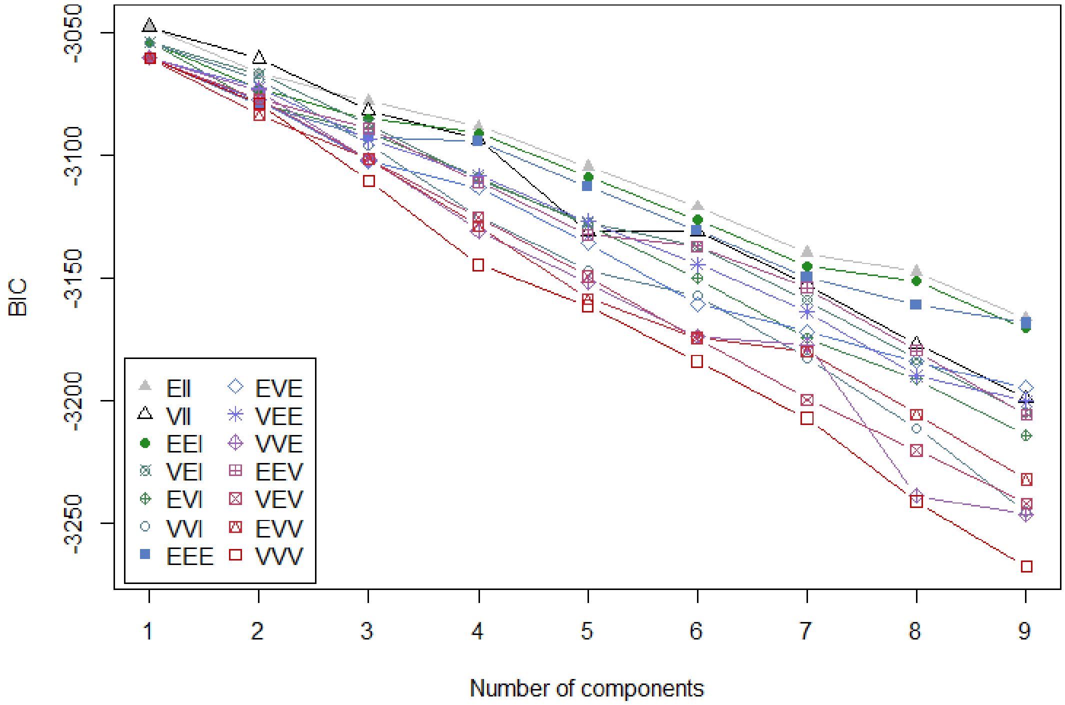

Figure F.1. Multivariate model-based clustering plot for the head dataset showing one component (cluster) for the fourteen possible model distribution structures tested for by *mclust*. The three letters of each model correspond to different characteristics of the model. In a multivariate setting, such as this, the volume is explained by the first letter, then shape, and orientation (Fraley *et al.*, 2012; Scrucca *et al.*, 2016). E stands for equal, V for varying, and I for identity. The head dataset was fit with an XXI model, indicating there was a single component identified in the dataset with a diagonal shape, multivariate volume, and normal orientation. The covariance parameterisation was selected using the Bayesian Information Criterion (BIC). The summary plot indicates that the top model (BIC = - 3047.692) had only one cluster for the head dataset, a strong indication that there was no clustering seen within the dataset.

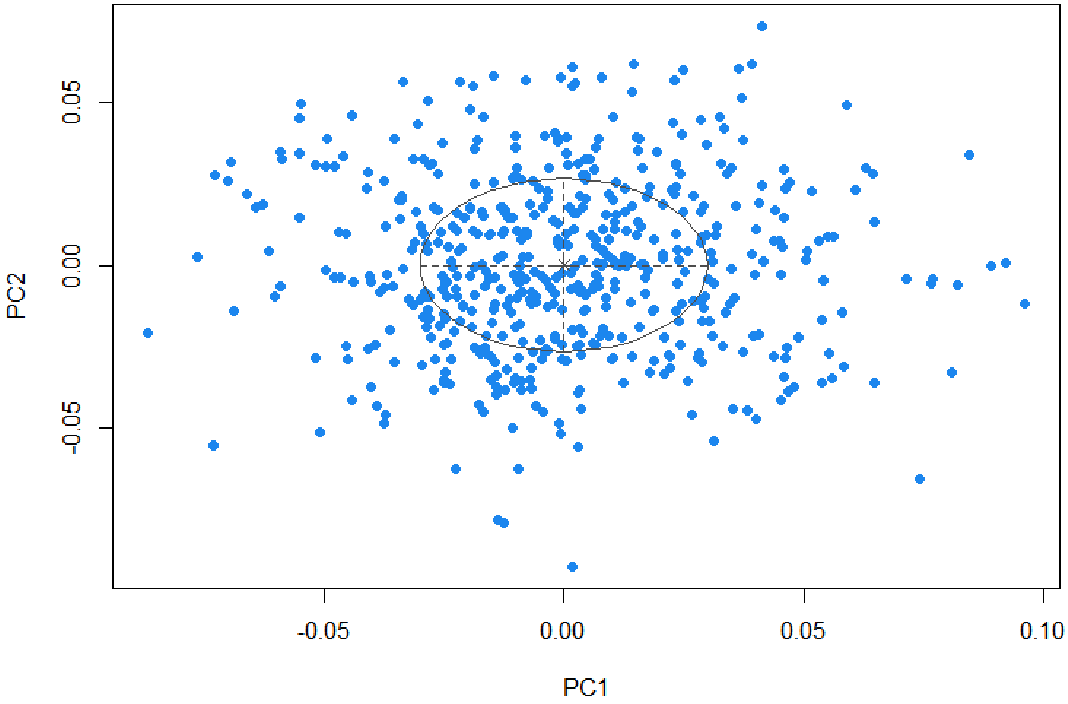

Figure F.2. Estimated classification plot for the head dataset fit with the XXI model from the *mclust* package in R (Scrucca *et al.*, 2016). No clustering was recognized following the Bayesian cluster analysis. The XXI model had the best fit (BIC = - 3047.692) of *mclust* models for datasets with a single component. Principal Component 1 (PC1) and Principal Component 2 (PC2) scores plotted on each axis were calculated with Principal Component Analysis (PCA) with *geomorph* package for R (Adams *et al.*, 2020).

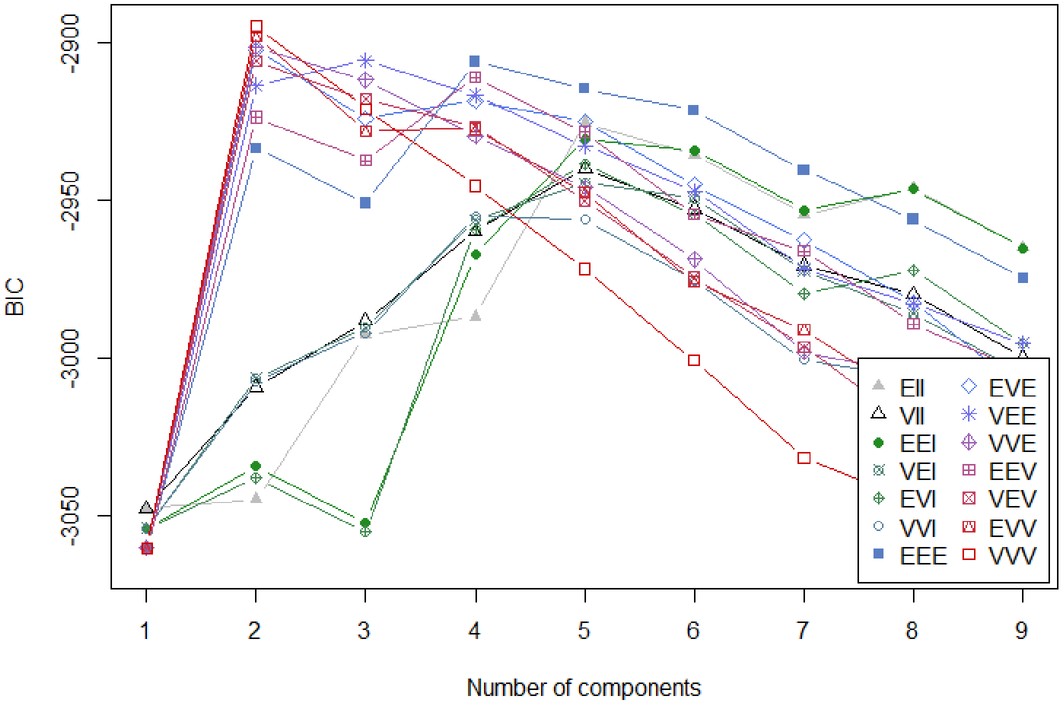

Figure F.3. Multivariate model-based clustering plot for the body dataset showing the best fit model, out of fourteen possible models, tested for by *mclust* (Adams et al. 2020). The three letters of each model correspond to different characteristics of the model. In a multivariate setting, such as this, the volume is explained by the first letter, then shape, and orientation (Adams *et al.*, 2020). E stands for equal, V for varying, and I for identity. The body dataset is best fit by the VVV model, indicating varying volume, varying shape, and varying orientation. The covariance parameterisation was selected using the Bayesian Information Criterion (BIC). The summary plot indicates that the top model (BIC = - 2894.888) had two clusters for the body dataset.

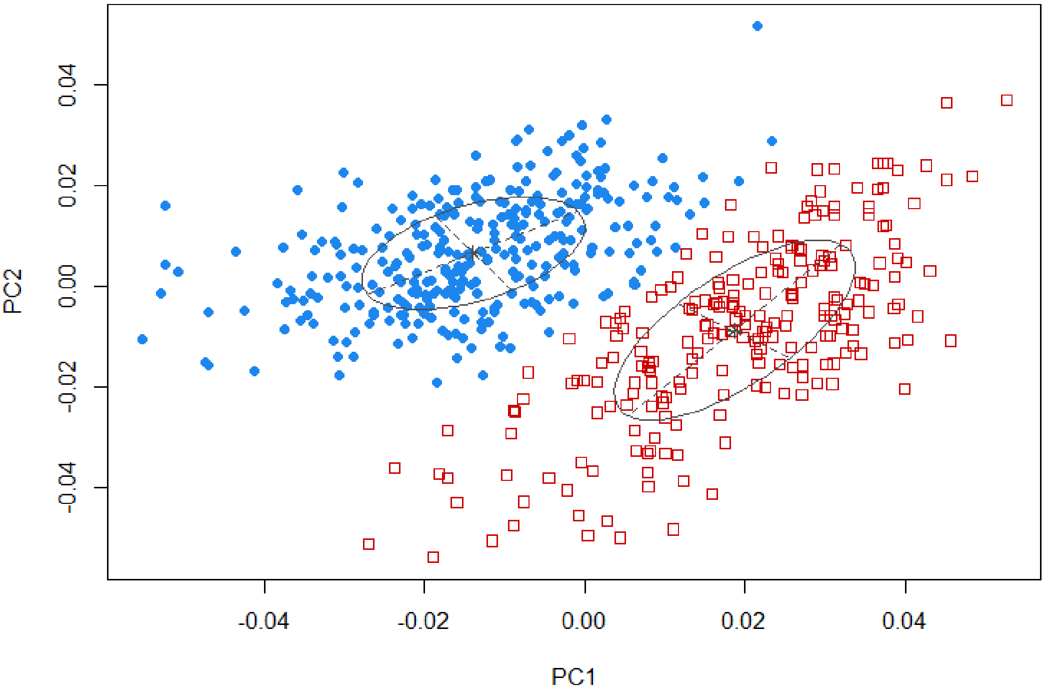

Figure F.4. Estimated classification plot of the body dataset fit with the VVV model from *Mclust* (*i.e.* varying volume, shape, and orientation model) (Fraley *et al.*, 2012; Scrucca *et al.*, 2016). The VVV model had the best fit (BIC = - 2894.888) of the fourteen fitted models. Principal Component 1 (PC1) and Principal Component 2 (PC2) scores plotted on each axis were calculated with Principal Component Analysis (PCA) with *geomorph* package for R (Adams *et al.*, 2020). The classification model provided an accurate recovery of the sex classes. The position of the blue and red points mirrors the cluster pattern of male and female data points seen in the PCA plot (Fig. 13).