Ecological drivers of inter- and intra-population variation of sexually-selected traits in brook trout (*Salvelinus fontinalis*)

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ABSTRACT

Although mating systems are generally considered fixed properties of species, ecological factors can play an important role in the degree to which sexual selection acts on mating-related traits in a population. Differences in the distribution of individuals can result in variation in sexually-selected traits. I explored the influence of ecological factors on population distribution and sexually-selected traits within and among four genetically isolated populations of brook trout in Cape Race, Newfoundland. Generally, females aggregate in areas with abiotic conditions that are optimal for offspring survival. Relationships between population density, sex ratio, and phenotypic variation suggest that social environment plays a large role in the distribution of brook trout within streams, as well as variation in the direction and magnitude of sexual size and colour dimorphism. Although these general trends were observed, variation among streams suggests that different factors are influencing the degree to which sexual selection operates on Cape Race.

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INTRODUCTION

Variation in Sexual Selection

Environmental factors may affect the opportunity for sexual selection to act on mating-related traits. Among populations, variation in environmental conditions can alter mating system structure by influencing variation in reproductive success (Andersson, 1994; Emlen & Oring, 1977; Koenig et. al., 2013). Although mating systems are generally considered static characteristics of species, ecological factors (both biotic and abiotic) can play an important role in the degree to which sexual selection can act within populations (Emlen & Oring, 1977). Inter-population differences in environmental conditions during mating can lead to different selective pressures, resulting in phenotypic variation among groups of the same species (Fujimoto et. al., 2014; Panhuis et. al., 2001; Reznick et. al., 1990; Romano et. al., 2017). These differences may be related to the spatiotemporal distribution and availability of resources or conditions favourable for mating and offspring survival, as well as mates themselves (Clutton-Brock & Vincent, 1991; Emlen, 1976).

In resource-based mating systems, whereby individuals seek out or guard resources necessary for offspring survival, when resources are clumped in space or time, mate distribution is often expected to follow. The operational sex ratio (OSR: the ratio of sexually active males to fertilizable females) has been used as a reliable measure for the intensity of sexual selection, and is directly affected by the spatiotemporal distribution of potential mates and resources critical for reproduction (Emlen, 1976; Emlen & Oring, 1977). Changes in social characteristics such as density or OSR can either facilitate or hinder the ability of one or a few individuals (usually males) to economically monopolize or defend potential mates or resources necessary for reproduction, leading to differences in competition intensity and the expression of sexually selected traits (Grant et al., 2000; Kvarnemo & Ahnesjo, 1996; Weir et. al., 2011). Generally, competition for mates becomes more intense as the OSR becomes increasingly skewed towards the non-limiting sex, although some research suggests that there is a limit to this as mates are no longer defendable if the sex ratio is extremely biased toward one sex (Weir et al., 2011).

In many animals, sexually-selected traits include size, weaponry, and nuptial colouration, which are used in both male-male competition and female mate choice (Andersson, 1994). These traits may be an honest signal of mate quality (Hamilton & Zuk, 1982), or signal the social status of an individual within the population (Parker, 1974). For example, red or orange pigment commonly seen in species of fishes and birds can indicate which males are dominant and guard territory or resources necessary for mating. This colouration is derived from carotenoids in the diet; animals cannot produce carotenoids naturally, and thus red or orange colouration can indicate which individuals can secure the best sources of food in a population (Hill, 1992). In populations where males compete directly for females, large body size offers an advantage during mate acquisition, and is used in direct intrasexual competition (Andersson, 1994).

Ecological factors may either enhance or curb sexual selection and lead to changes in sexually selected phenotypes within and among populations. (Myhre et. al., 2012; Reimchen, 1988; Seehausen, 1997). Seehausen et. al. (1997) demonstrated that increasing water turbidity in Lake Victoria interfered with mate choice because of constrained colour vision among populations of cichlids, relaxing sexual selection intensity for body colour. As various colour morphologies of cichlids within Lake

Victoria remain isolated via mate choice, this relaxation of sexual selection intensity brought on by a change in environmental condition led to a decline in cichlid diversity (Seehausen, 1997). This trend is also seen in Threespine Stickleback (*Gasterosteus aculeatus*), where male nuptial red colouration was duller in habitats where water clarity was reduced (Reimchen, 1988). Reimchen (1988) attributed these differences in nuptial colouration among populations to a diet limited in carotenoids as stained waters have low light penetration and low primary productivity, both of which are positively correlated with total carotenoid content in a given environment.

Breeding Habitat

Environments are typically heterogeneous and organisms detect patches of suitable habitat using physiological or social cues (Danchin et. al., 1998; Doligez et. al., 2003; Doligez et. al., 2002). The environmental conditions and resources required are likely to change for an individual as they grow and develop, so dispersal or migration to new environments at different life stages is expected to maximize fitness (Metcalfe et. al., 2008). During the reproductive season, individuals move to habitats with conditions favourable for mating, offspring survival or rearing. These migrations can be short-term and/or short-distance and within the individual’s current environment, or large scale between two distinct feeding and breeding locations (Bauer et al., 2011). Suitable environmental conditions for mating and offspring survival may include adequate resource availability, as well as physical traits such as temperature and photoperiod, and, for aquatic species, specific water chemistry characteristics. For instance, many bird species choose to nest in areas with high food abundance and vegetation cover, as it is beneficial for feeding offspring and minimizing predation (Doligez et al., 2002).

The spatial distribution of suitable mating habitats within an environment is also an important determinant of habitat choice and reproductive success. The ideal free distribution predicts that individuals can distribute themselves freely within patches of habitat and have perfect information about habitat quality (Fretwell & Lucas, 1969). This is often used as an explanation for spatial distribution patterns in proportion to food abundance, predator evasion, and mating habitat quality. However, in resource-based mating systems, competition for suitable breeding territory and can be intense, with the most fit individuals often actively excluding others from sites, resulting in a despotic distribution (Andrén, 1990; Fretwell, 1972). The abundance of resources within a habitat may also lead to a shift in mating system structure by altering intrasexual competition for resources required for offspring production. A decrease in nest-site abundance has been correlated with increased population density and intrasexual competition, and has been observed in populations of Sand Goby and Threespine Stickleback, suggesting abiotic conditions related to mating or nesting can alter sexual selection intensities among and within species (Forsgren et. al., 2006; Whoriskey & Fitzgerald, 1994).

Family Salmonidae & Brook Trout

The breeding patterns and mating systems of the family Salmonidae have been extensively studied (Baxter & McPhail, 2011; Blanchfield, 1998; Fleming, 1996, 1998; Fleming & Gross, 1994; Hutchings, 1994). The mating system of most salmonids is best described as a site-based competitive system, where males compete for access to females, and females compete for access to suitable spawning territories (Blanchfield, 1997; Blanchfield et. al., 2003; Fleming & Gross, 1994). However, because breeding systems are so closely linked to life-history and environmental conditions, variance across species and populations in sexually-selected phenotypes and behaviour is expected, and alternative reproductive tactics are seen in some cases (Gross, 1996; 1991; Tentelier et al., 2016; Thériault et. al., 2007).

Within salmonid mating systems, adult size is an important predictor of reproductive success (Gross, 1991; Quinn & Foote, 1994; Thériault et. al., 2007). Large body size in males facilitates intrasexual dominance and increases the opportunity to monopolize potential mates or ideal spawning territories, while large body size in females confers an advantage in fecundity, competition for nest sites, and nest site defense (Blanchfield et al., 2003; Fleming & Gross, 1994). Other sexually selected morphological traits that occur in males of many salmonid species include intense breeding colouration, large dorsal humps, and hooked snouts that are used during mate competition and are important determinants of reproductive success (Fleming & Gross, 1994).

During the reproductive season, females must gain access to optimal territory and construct nests where their eggs will eventually be fertilized and deposited to develop and hatch (Blanchfield, 1997). Males compete aggressively for access to females, and competition heavily relies on body size. The largest males occupy the closest positions to the females and guard spawning sites (Blanchfield et al., 2003; Fleming & Gross, 1994; Fleming, 1998; Tentelier et al., 2016). Dominant males tend to mate with many females due to their proximity to spawning sites, and smaller peripheral males occupy the edges of spawning sites and may attempt to sneak fertilizations (Fleming, 1996; Tentelier et al., 2016).

Brook trout (*Salvelinus fontinalis*) is a salmonid fish that is widely distributed in streams and lakes throughout North America. Native populations are found in the east;

however, naturalized and stocked populations have been established globally (MacCrimmon et. al., 2011). Similar to other salmonids, they exhibit a site-based competition mating system, and sexually selected traits such as large body size, dorsal humps (seen mostly in the largest individuals) and bright red abdominal colouration are important determinants of male reproductive success (Blanchfield et al., 2003). Extensive research has been conducted on the relationship between brook trout reproductive success and abiotic habitat characteristics (Blanchfield, 1997, 1998; Curry et. al., 1994; Curry et. al.,1995; Fraser, 1982). Offspring survival is dependent on the presence of upwelling groundwater, as it creates chemical and temperature gradients that allow eggs to overwinter successfully (Baxter & McPhail, 2011; Curry et al., 1995). Because offspring survival directly impacts the reproductive success of both males and females (Fleming, 1998), sites with significant groundwater upwelling can be seen as high quality, desirable habitats where individuals actively choose to spawn.

Study Location: Cape Race, Newfoundland

Cape Race is a coastal barren region in Southeastern Newfoundland, Canada. High winds, salt-spray, and nutrient-poor, acidic soil contribute to relatively sparse tree coverage. Instead, low-lying ericaceous shrubs, sedges, Sphagnum-bog pockets, and exposed bedrock dominate the landscape. Cape Race is characterized by a series of relatively short, low-order, parallel running streams that harbour populations of brook trout and other fish species (Atlantic salmon; *Salmo salar*, stickleback species; *Gasterosteus spp*., American eel; *Anguilla rostrata*). The streams at Cape Race are an example of a naturally fragmented habitat (Fraser et. al., 2014; Wood et. al., 2014), and have been studied extensively for upwards of thirty years from both genetic and life-history perspectives (Belmar-Lucero et al., 2012; Danzmann et. al., 1998; Hutchings, 1991, 1996).

Cape Race brook trout once existed as one large population 10,000-12,000 years ago, but diverged during the late-Wisconsonian glaciation, and have remained isolated ever since (Danzmann et. al., 1998). Immigration of trout between streams is highly unlikely, as there are no connections by lakes, and several streams terminate by waterfall directly into the Atlantic Ocean. Any individuals within these streams that leave cannot return, so populations are genetically distinct (Ferguson et. al., 1991). Due to their small individual body size, brook trout populations in Cape Race are free of anthropogenic exploitation and disruption. Small stream and population size facilitate comprehensive sampling, so reliable estimates of population size are available. The single-population origin of brook trout populations on Cape Race allows for the inference of selection pressures without the confound of different genetic and evolutionary factors, making Cape Race an ideal study site to investigate the evolution of sexually-selected morphologies, and variation in population distributions.

Previous research suggests that Cape Race brook trout populations exhibit interesting variation in life histories and growth rates among populations (Ferguson et. al., 1991; Hutchings, 1993). More recent studies have found noticeable differences in effective population sizes, distributions and morphologies and due to variation in environmental conditions within and among streams (Purchase & Hutchings, 2008; Wood et. al., 2014; Zastavniouk et. al., 2017). Purchase & Hutchings (2008) found that larger individuals inhabit higher-density locations within Freshwater River, a well-studied stream on Cape Race, suggesting that competition for high-quality spawning areas is present.

Research Objectives

This study aimed to further investigate inter- and intra-population variation in population density, morphology and colouration of spawning brook trout on Cape Race, Newfoundland. My goal was to link variation in sexually-selected traits and social structure of brook trout in natural populations to abiotic characteristics in their environments.

First, I explored the relationship between abiotic factors and variation in female density and body size. Quantifying density along an environmental scale is a common way of inferring habitat selection and estimating fish-habitat relationships within and among populations (Brown, 1984; Metcalfe et al., 2008; Purchase & Hutchings, 2008). Because more fish are expected in areas where fitness is maximized, I predict that female density during the spawning season will be highest in sites that have the abiotic characteristics necessary for successful offspring survival; differences in temperature, depth, and conductivity may be indicators of groundwater upwelling in specific areas. I predict that individuals within these densely populated areas will be larger than those in other areas within the stream because larger size confers a competitive advantage in contests over high-quality sites. In salmonid mating systems, females compete for access to spawning territories, and there is evidence that largest individuals occupy areas that have abiotic characteristics associated with offspring survival (Fleming, 1998; Purchase & Hutchings, 2008). I expect this relationship to be strongest in streams that are the most environmentally variable, and weakest in streams that have relatively similar abiotic characteristics throughout, as a homogenous stream will reduce competition for resources related to spawning.

Second, I asked whether variation in overall spawning density and OSR could predict variation in sexually-selected traits. Population characteristics such as density and OSR can influence the expression of sexually-selected traits by altering the intensity of mate competition, this can also lead to shifts in the direction and magnitude of sexual dimorphism of a population. Male brook trout are typically under stronger sexual selection pressures than females as a result of their mating system, so I expect density and OSR to have a more significant effect on male morphologies than female morphologies. Because density and OSR vary slightly among stream, I expect variation in both sexual size dimorphism and sexual colour dimorphism among stream. Within stream, I predict the largest and reddest males to occupy sites with high density and slightly male-biased OSR, as sexual selection pressures will be most intense in these areas.

Additionally, I wanted to explore any potential relationships between water turbidity and the expression of body colour in Cape Race streams. Using the concentration of chlorophyll-a as an indicator of phytoplankton content or water turbidity, and examined its effect on body redness of both sexes within each stream. Because turbid waters often reduce the expression of body redness in various species of fishes, I predict to see a decrease in the proportion of redness in individuals living in streams with highest chlorophyll-a concentrations.

METHODS

Stream & Site Selection

Four Cape Race streams were included in this study. From East to West, they were: Freshwater River, Ouananiche Beck River, Watern Cove River, and Cripple Cove River (Figure 1). These streams are relatively small in length (~2-3km) and depth (~20-30cm). Brook trout in these four systems spawn approximately one week apart (based on natural variation in water temperature change) and have been sampled regularly for almost thirty years (Bernos & Fraser, 2016; Hutchings, 1991, 1993; Hutchings & Gerber, 2002; Wood et. al., 2014). Colouration, body length, operational sex ratio, population density and effective population size vary markedly among the streams (Purchase & Hutchings, 2008; Wood et. al., 2014; Zastavniouk et. al., 2017). Spawning site locations are known based on previous studies (Purchase & Hutchings, 2008, D.J. Fraser & J.A. Wood, personal communication).

Ten sites per stream were sampled for this study. Using data from Purchase & Hutchings (2008), as well as unpublished data from D. J. Fraser, I was able to determine locations of known areas with high densities of spawning fish within the four streams. These spawning areas were treated as individual sampling sites within the streams. Other sites that did not have high densities of spawning fish (hereafter 'non-spawning sites') lengths were mapped upstream, downstream, and in between known spawning locations using consecutive 90m or 150m increments (Freshwater: 150m, Ouananiche Beck, Watern Cove, & Cripple Cove: 90m). The variation in site length is due to natural variation of stream length, accessibility, and location of previously known spawning locations. No ponds connected to the four streams were sampled, as per previous studies' methodologies (Purchase & Hutchings, 2008). The spawning period for brook trout depends on water temperature, with spawning usually beginning when temperatures drop to 10°C (Blanchfield, 1997), so temperatures at known spawning locations were monitored, and sampling began when water was 10°C or lower. Once a stream was ready to be sampled, an average of three sites per day were completed.

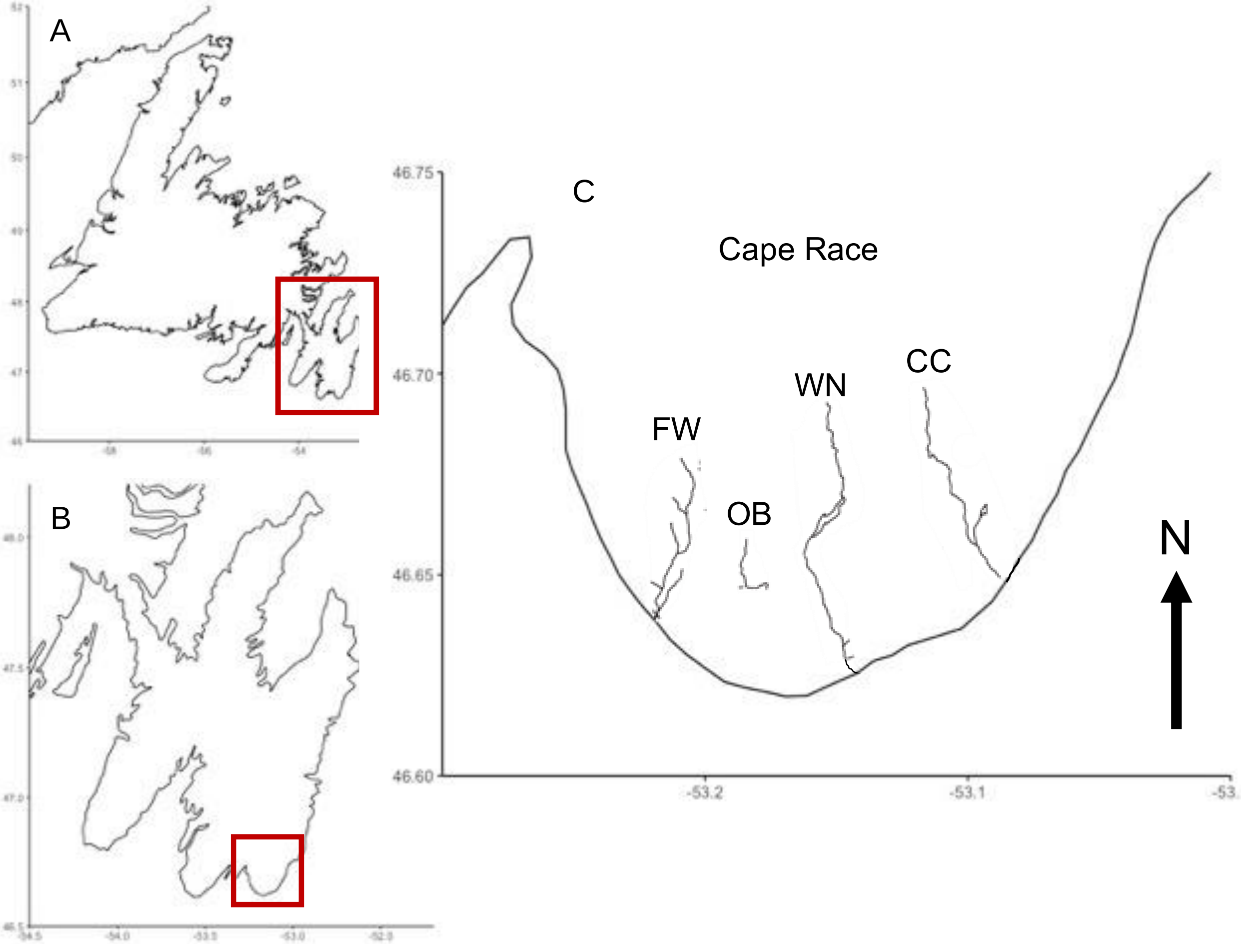

Figure 1. Map outlining the four streams sampled for this project. (A) Newfoundland Canada (B) Avalon Peninsula, Newfoundland (C) Cape Race, Newfoundland. From West to East, they are Freshwater River (FW), Ouananiche Beck River (OB), Watern Cove River (WN) and Cripple Cove River (CC). X-axis represents latitude, and y-axis represents longitude.

Fish Sampling

Each site was sampled using a Smith-Root Model LR-20 electrofisher using predetermined settings that maximized capture and minimized distress for the brook trout (325-345V and 50-55Hz). A single pass beginning at the most downstream point of each stream and site was performed to minimize the chance of repeatedly shocking the fish (Purchase & Hutchings, 2008). Individuals were captured using large dip nets, and placed into covered buckets with constant aeration and monitored for full recovery from the electrofishing procedure.

Once recovered, all fish were counted to provide density estimates. I sorted the fish based on sex and reproductive maturity. Reproductively mature males and females were sorted using external characteristics (e.g., sexual differences in the shape of the urogenital opening, soft abdomen (females), visible eggs beneath the body wall, and release of milt (males)). Immature individuals were placed in a different holding container. I calculated the operational sex ratio (OSR) for each site by dividing the number of sexually mature males to sexually mature females.

Standard length of mature males and females was measured for body size data and photographed for redness analyses. At sites where more than 40 sexually mature individuals were captured, a subset of 20 males and 20 females were sampled. The fish were anesthetized with clove oil (1mL oil in 1.5L of aerated stream water according to the SMU Animal Care Council Standard Operating Procedure: Use of Clove Oil for Fish Anesthesia and Euthanasia). I first measured each individual to the nearest 0.1cm on a measuring board, and then photographed them using an Olympus TOUGH TG5 camera mounted on a leveled stand to ensure that the camera is the same distance, zoom, and angle for every photograph. A colour reference (XRite© Photo ColourChecker Passport) and size reference was included in each photograph to allow for correction associated with ambient light differences among photos due to differences in time of day and weather.

Following measurement and photography, a small triangular fin clip was taken from the lower lobe of the caudal fin to mark the animal to ensure that the same fish is not measured and photographed on subsequent days, should an individual swim upstream to the next site. After fin clipping, the fish were placed in a covered aerated bucket to recover from anesthesia. They were checked periodically to ensure they had resumed normal breathing and swimming compared to their conspecifics who were not anesthetized. During this recovery period, abiotic measurements were taken to avoid disturbing the animals before capture or after release. After abiotic characteristics were recorded, all fish were checked to ensure they were fully recovered and not in distress, and then they were released 30-50m downstream. If after 10 minutes the fish were not recovered, they were euthanized following the SMU Animal Care Committee Standard Operating Procedure: Use of Clove Oil for Fish Anesthesia and Euthanasia. Throughout this field season, 2000 fish were caught, and only 10 had to be euthanized. All procedures were approved under the SMU Animal Care Protocol 19-11.

Abiotic Measurements

Abiotic measurements were taken from each site while anesthetized fish were recovering. Length, width, and depth were all measured using a 30m measuring tape. Depth was measured from the centre of each stream. Dissolved oxygen, temperature and conductivity were all measured using a YSI Pro2030 Dissolved Oxygen, Conductivity, Salinity Instrument**.** Velocity was measured by releasing a ball attached to a one metre string from an upstream position in the middle of the stream and recording the time required for the ball to travel one metre with the current. Substrate type and size was categorized at each interval using a Wentworth scale modified by Bain et. al., (1985) with the following categories: 0, vegetation; 1, sand, silt, or clay; 2, gravel (2–16 mm); 3, pebble (17– 64 mm); 4, cobble (65–256 mm); 5, boulder (>256 mm); and 6, bedrock (Bain et. al., 1985; Purchase & Hutchings, 2008).

In sites that were known spawning areas, abiotic characteristics were only measured once in the centre of the site, as known spawning areas were all between 5m-25m in length, and homogenous within sites. In sites that were not known spawning areas, lengths remained constant within streams (Freshwater: 150m; Ouananiche Beck, Watern, Cripple Cove: 90m), and all other abiotic characteristics were measured three times uniformly along the length of the site (Freshwater: 0m, 50m, 100m; Ouananiche Beck, Watern, Cripple Cove: 0m, 30m, 60m) and averaged to create one single site mean for each parameter, as abiotic characteristics throughout were not always constant. The characteristics for all four streams are summarized in Table 1.

Table 1. Means and ranges of abiotic characteristics of four Cape Race, Newfoundland streams during the sampling period. Substrate was categorized using a Wentworth scale modified by Bain et. al., (1985) with the following categories: 0, vegetation; 1, sand, silt, or clay; 2, gravel (2–16 mm); 3, pebble (17– 64 mm); 4, cobble (65–256 mm); 5, boulder (>256 mm); and 6, bedrock.

Stream & Sampled Length	DO_2 (%)	Width (m)	Conductivity (S/m)	Velocity (m/s)	Temperature (°C)	Depth (m)	Substrate
Freshwater (1218m)							
mean	101.23	2.93	56.26	0.262	11.62	0.187	4
range	95.2-106.23	2.24-4.53	49.46-59.66	0.101-0.388	10.03-14.43	0.146-0.26	3-5
Ouananiche Beck (820m)							
mean	91.03	4.49	41.29	0.169	9.97	0.243	5
range	69.93-103.7	1.42-7.52	37.73-44.03	0-0.243	8.5-11.6	0.124-0.573	4-5
Watern Cove (451m)							
mean	89.86	6.50	39.45	0.485	10.31	0.355	5
range	68.5-102.1	2.45-16.65	37.40-42.30	0-1.472	8.6-11.96	0.18-0.45	4-5
Cripple Cove (900m)							
mean	92.36	4.46	31.95	0.351	10.13	0.315	4
range	85.03-96.40	2.60-7.70	26.23-42.36	0.164-0.853	9.2-11.0	0.135-0.980	3-5

Chlorophyll *a* Analysis

A 500mL water sample was taken in the centre of the stream at each site, using the 0m increments for both spawning and non-spawning sites for analysis of chlorophyll *a* content at each site, to examine the relationship between water turbidity and red colouration. Water sample collection, filtration, and fluorometer analyses were performed according to the In Vitro Determination of Chlorophyll *a* and Pheophytin-a in Marine and Freshwater Algae by Fluorescence EPA Methods (Arar & Collins, 1997). All 500mL water samples were stored in opaque bottles until they could be filtered to minimize chlorophyll *a* pigment loss due to light. Filtering was performed within 24 hours of collecting the sample when possible, and completed in low-light. Unfortunately, a malfunction in the filtration apparatus and vacuum pump resulted in delayed filtration of 14 samples (4 from Watern Cove, 10 from Cripple Cove) by one week. Glass fibre filters were stored in a -20°C freezer until they could be extracted and analyzed.

All chlorophyll *a* extraction from the glass fibre filter samples was performed in subdued light. Using a mortar and pestle, I ground each sample individually in 4mL of aqueous 90% acetone to make a slurry. The slurry was poured into a 15mL Falcon tube wrapped in aluminium foil to omit light. An additional 6mL of acetone was used to rinse the mortar and pestle and subsequently added to the Falcon tube, which was vigorously shaken before placing the covered samples in a refrigerator (4°C) to steep for 23 hours. During the steeping period, the tubes were shaken once.

After the steeping period, samples were centrifuged for 15 minutes at 700 x g. The supernatant was pipetted into a separate labeled 15mL Falcon tube, to prevent re-mixing of the separated sample. I left the samples stand at room temperature for 30 minutes so they could return to ambient temperature before analyzing. Chlorophyll *a* content was measured using Turner Designs Trilogy Laboratory Fluorometer. The fluorometer was calibrated using a stored calibration appropriate for the Chlorophyll non-acidification mode used at room temperature, and was checked using a 5mL cuvette of 90% ethanol as a "blank" before use. Supernatant was transferred from the Falcon tube to a 5mL cuvette for analysis. Unfiltered chlorophyll *a* concentration (μg/L) was recorded and used in subsequent analyses.

Body Colour

Photographs of mature individuals taken at each sampling site were exported from RAW files as Exif-TIFF files in Olympus Viewer 3, a free photography editing software accessed from the Olympus website. I opened the images in ImageJ 1.52q, and set the global scale (mm) using the "Set Scale" function. Because all photos were taken from the same distance, angle, and zoom, I was able to use this scale for all analyzed photos. I measured redness using the RGB Measure plugin, which measures the mean, minimum, and maximum values of red, green, and blue present in a selected area of an image. It also records the mean, minimum and maximum brightness values (calculated by adding the corresponding red, green, and blue values together and then dividing by 3) present in a selected area of an image. To quantify body redness in each sampled mature individual, I first recorded the mean redness and mean brightness RGB values for a 5mm by 5mm square at the centre of the red colour checker in each image as a colour reference. Next, I landmarked an area on each individual by using the "Line" function. I drew two vertical, parallel lines using the anterior edges of the dorsal and anal fins as starting points to mark the anterior and posterior limits for RGB measurement. A horizontal line beginning at the lower jaw of each fish and ending at the caudal peduncle was used to mark the upper limit of measurement area, and finally a diagonal line beginning at the ventral side directly below the dorsal fin, lined up with the upper edge of the pelvic fin was used to mark the lower limit of measurement area (excluding pelvic fins; Figure 2). I used the RGB measure tool to obtain mean redness and mean brightness values in the area between all landmarks.

To correct for ambient light, I divided the mean redness value of each photograph of the colour checker by 255 (the "true red" value) to calibrate red values for each individual recorded. Each individual's mean redness value was then multiplied by the ratio value of its corresponding colour checker to correct for ambient lighting differences across all photos during the sampling period. To ensure that the red values were being quantified from a true red, rather than the brightness or "whiteness" of the individual, I multiplied each mean brightness value by 3 to get the total value for red, green, and blue. I then divided the mean redness value by the total colour value to get the proportion of red that was contributing to the total colour, which signifies the proportion of total colour (brightness) that is made up of true red colouration in the individual, rather than a high value coming from white areas of the individual.

To ensure that my landmarking and measurements were repeatable and reliable, this method was repeated again for forty individuals from Freshwater (twenty males, twenty females from sites FW1 and FW2). I plotted the repeated measurements against the first measurements for both the colour checker and proportion of redness values to check for correlations. The correlation between the first and repeated measurements of the colour checker values was 0.9955, and the proportion redness values had a correlation coefficient of 0.9596. These tight correlations between the first and second measurements suggest that my method for measuring red colouration is reliable.

Figure 2. Photograph of anesthetized male brook trout demonstrating digital landmarking used for body redness quantification. Area within black lines denotes area measured on each individual for mean redness and brightness values. Vertical, parallel lines were drawn using the anterior edges of the dorsal and anal fins as starting points to mark the anterior and posterior limits for RGB measurement. A horizontal line beginning at the lower jaw of each fish was used to mark the upper limit of measurement area, and finally a diagonal line beginning at the ventral side directly below the dorsal fin and lined up with the upper edge of the pelvic fin was used to mark the lower limit of measurement area.

Statistical Analyses

All analyses were carried out using R version 3.6.1 (R Core Team, 2019). Rather than combining the data of all sampled streams, I treated and analyzed each stream individually, as differences in both abiotic and biotic ecological factors were found among and within streams. Similarly, the separation of the streams leads to differences in the underlying genetic variation, which can affect the degree to which evolution can occur (either by drift or selection) within each stream. Variation in ecological factors may affect the intensity at which natural or sexual selection pressures act on populations, which can lead to differences in phenotypic traits (body size and colour) of individuals among streams. Generalized linear models (GLMs) were used to examine variation of abiotic characteristics, population density, OSR, body size and body colour among streams, whereas within stream analyses related to animal distribution were completed using linear regressions.

I used principal component analysis (PCA) to quantify abiotic variation within and among my sampled Cape Race streams, and to explore the relationships between abiotic and biotic variables within my data. Principal component analysis defines the dimensionality of a dataset (Benigni & Guiliani, 1994), and is often used as a tool in ecology to evaluate multivariate habitat relationships (Conner & Adkisson, 1977). By extracting the dominant patterns, PCA helps to simplify large datasets by creating new independent variables (principal components) (Wold, 1987). The first three principal components explained the majority of the variation within my data, and were used as dependent variables for all abiotic analyses. Abiotic variation was summarized by extracting principal components using the prcomp package in R.

The relationships between abiotic variation (PC1, PC2, and PC3), social or biotic environment (density, OSR, and their interaction), and sexually selected traits (body size and body colour) were determined by generalized linear models (GLMs). Data on an individual level were used for both body size and redness values, while spawning population densities and operational sex ratios were calculated for each of the ten sites within the four streams. To further explore these relationships and examine differences between males and females, body size and body colour were analysed separately by sex. Additionally, the relationships between raw chlorophyll-a and body redness within each stream were explored with GLMs.

I explored the variation of sexual size dimorphism (SSD) and sexual colour dimorphism (SCD) among streams, and their relationship with density and OSR with linear mixed effect models, with density and OSR as fixed effects and streams as a random effect, using the “lme4” package. Sexual dimorphism in traits associated with reproduction is often an important indicator of the direction and intensity of sexual selection pressures acting on a population (Fairbairn, 1997). I calculated SSD and SCD using the sexual dimorphism index (SDI) proposed by Lovich and Gibbons (1992), using the following equations:

SDI = (size of largest sex ÷ size of smallest sex) – 1, defined as positive when females are larger and negative when males are larger

This index of sexual dimorphism allows SSD and SCD values to be intuitive, as female biased dimorphism is indicated by a positive value, and male biased dimorphism a negative value (Lovich & Gibbons, 1992). In all analyses, model selection was determined using the Akaike Information Criterion (AICc) criteria, where models that differed from others by a delta value of 2 or greater were considered to best fit the data.

RESULTS

Do differences in abiotic factors explain the density and size distributions of females?

Principal Component Analysis for abiotic characteristics

The first three principal components accounted for 84.81% of the variation in the data and were used as dependent variables for subsequent analyses; these are summarized in Table 2. Briefly, the first principal component (PC1) summarized the relationship between dissolved oxygen and stream width; increasing values of PC1 indicate lower dissolved oxygen and wider stream sections. The second principal component (PC2), described the relatedness between water velocity and conductivity, whereby higher PC2 values indicate low conductivity and faster flowing water. The third principal component (PC3) summarized variation in stream temperature and depth and correlated positively with both of these variables.

Table 2. Percentage of total variance, abiotic contributions, and major trends of increasing PC values included for each principal component. Temperature, dissolved oxygen, conductivity, velocity, width, and depth were the abiotic parameters used in principal component analysis.

PC	% total variance explained	Contributions to the PCs						Major trends with increasing value of PC
		temp.	DO_2	cond,	velocity	width	depth	
PC1	43.91	20.15	27.05	14.19	5.41	20.13	13.07	↓DO_2, ↑width
PC2	25.42	0.02	2.53	28.33	51.65	3.20	14.27	↓conductivity, ↑velocity
PC3	15.47	40.24	0.22	10.25	0.17	17.02	31.72	↑temperature, ↑depth

Relationships between abiotic factors and female density and size

While streams varied in significantly in abiotic factors (Table S1, S2; Figure S1), I did not analyse the among-stream variation in female density, as female density can vary among streams for many reasons that are not related to the focus of my study. Thus, I examined within-stream variation in density to determine whether females were aggregating at sites with particular characteristics. The abiotic factors that best explained variation in female spawning density varied for each stream (Table 3). PC1 had a weak effect on variation in female density in Ouananiche Beck and Cripple Cove, whereby more females were found in wider areas of the stream with relatively low dissolved oxygen (Table 3). In Freshwater River, female density tended to increase with PC2, suggesting that females are aggregating in sites with high velocity and low conductivity (Table 3). Female density in both Watern Cove and Cripple Cove was highest in shallow sites with relatively low temperatures that are described by a negative relationship between density and PC3 (Table 3).

Table 3. Generalized linear models indicating the effects of abiotic factors (represented by principal components) on female densities in four Cape Race streams. Degrees of freedom (df), Akaike Information Criterion (AICc), the differences between the model with the lowest AICc and all other models (ΔAICC), and model weights (ωAICc) are included. The best models are highlighted in bold and differ from other models by a value of 2 or greater. R^2 values are included for the best models.

Model	df	AICc	ΔAICc	ωAICc	direction of relationship	R^2
Freshwater River						
PC2	**3**	**24.6**	**0.00**	**0.613**	**+**	**0.555**
PC1 + PC2	4	26.8	2.21	0.203		
intercept only	2	29.1	4.47	0.066		
PC2 + PC3	4	29.9	5.26	0.044		
PC1 + PC3	4	30.5	5.85	0.033		
PC1	3	30.9	6.29	0.026		
PC3	3	33.4	8.74	0.008		
PC1 + PC2 +PC3	5	33.5	8.91	0.007		
Ouananiche Beck						
intercept only	**2**	**26.6**	**0.00**	**0.500**		
PC1	**3**	**27.6**	**0.97**	**0.307**	**-**	**0.259**
PC2	3	29.9	3.34	0.094		
PC3	3	30.8	4.22	0061		
PC1 + PC2	4	33.3	6.71	0.017		
PC1 + PC3	4	33.5	6.91	0.016		
PC2 + PC3	4	35.9	9.34	0.005		
PC1 + PC2 +PC3	5	41.1	15.55	0.000		
Watern Cove						
intercept only	**2**	**30.3**	**0.00**	**0.464**		
PC3	**3**	**31.7**	**1.37**	**0.234**	**-**	**0.231**
PC2	3	33.0	2.76	0.117		
PC1 + PC2	4	33.5	3.24	0.092		
PC1	3	34.6	4.28	0.055		
PC2 + PC3	4	36.1	5.80	0.025		
PC1 + PC3	4	37.5	7.22	0.013		
PC1 + PC2 + PC3	5	41.7	11.38	0.002		
Cripple Cove						
PC3	**3**	**11.0**	**0.00**	**0.496**	**-**	**0.538**
PC1	**3**	**11.7**	**0.75**	**0.341**	**-**	**0.504**
intercept only	2	15.1	4.09	0.064		
PC1 + PC3	4	16.0	5.08	0.039		
PC2 + PC3	4	16.9	5.93	0.026		
PC1 + PC2	4	17.3	6.32	0.021		
PC2	3	18.3	7.37	0.012		
PC1 + PC2 + PC3	5	24.9	13.97	0.000		

Larger females were found in sites of Freshwater that are narrow, deep and have relatively high temperature, dissolved oxygen, and conductivity. A model including both PC1 and PC3 best explained the variation in female body size in Ouananiche Beck (Table 4), where larger females were found in wide, shallow sites of the stream with lower temperature and dissolved oxygen. Variation in Cripple Cove female body size was mostly affected by PC3, however weak effects of PC1 + PC3, PC2 + PC3, and PC1 were observed (Table 4). Thus, in this stream, large female body size was associated with increased temperature and depth. Surprisingly, although Watern Cove was the most variable stream in both social and abiotic factors, no models including abiotic factors best explained variation of female body size (Table 4).

Table 4. Generalized linear models indicating the effects of abiotic factors (represented by principal components) on body size of female brook trout in four Cape Race streams. Degrees of freedom (df), Akaike Information Criterion (AICc), the differences between the model with the lowest AICc and all other models (ΔAICC), and model weights (ωAICc) are included. Akaike Information Criterion (AICc) criteria was used for model selection. The best models are highlighted in bold, and R^2 values are included.

Model	df	AICc	ΔAICc	ωAICc	direction of relationship	R^2
Freshwater River						
PC1 + PC2 + PC3	**5**	**418.2**	**0.00**	**0.417**	+	**0.186**
PC1 + PC3	**4**	**419.2**	**0.99**	**0.254**		**0.163**
PC2 + PC3	**4**	**420.0**	**1.86**	**0.165**		**0.156**
PC2	3	420.7	2.50	0.119		
PC1 + PC2	4	422.7	4.52	0.043		
PC3	3	429.5	11.35	0.001		
PC1	3	433.8	15.58	0.000		
intercept only	2	434.1	15.92	0.000		
Ouananiche Beck						
PC1 + PC3	**4**	**464.2**	**0.00**	**0.683**	-	**0.199**
PC1 + PC2 + PC3	5	466.3	2.15	0.233		
PC3	3	469.3	5.14	0.052		
PC2 + PC3	4	470.4	6.17	0.031		
PC1	3	488.1	23.94	0.000		
PC1 + PC2	4	489.9	25.66	0.000		
intercept only	2	489.9	25.74	0.000		
PC2	3	491.9	27.75	0.000		
Watern Cove						
intercept only	**2**	**296.6**	**0.00**	**0.413**		
PC1	3	298.7	2.13	0.142		
PC2	3	298.7	2.13	0.142		
PC3	3	298.7	2.15	0.141		
PC1 + PC2	4	300.7	4.18	0.051		
PC1 + PC3	4	300.9	4.34	0.047		
PC2 + PC3	4	300.9	4.34	0.047		
PC1 + PC2 + PC3	5	303.0	6.40	0.017		
Cripple						
PC3	**3**	**481.6**	**0.00**	**0.241**	+	**0.023**
intercept only	**2**	**481.8**	**0.21**	**0.217**		
PC1 + PC3	**4**	**482.7**	**1.16**	**0.135**	+	**0.034**
PC2 + PC3	**4**	**482.9**	**1.30**	**0.126**	+	**0.032**
PC1	**3**	**483.2**	**1.62**	**0.108**	-	**0.007**
PC2	3	483.9	2.34	0.075		
PC1 + PC2 + PC3	5	484.7	3.15	0.050		
PC1 + PC2	4	484.8	3.24	0.048		

Do variation in density and operational sex ratio predict differences in sexually-selected traits?

AMONG STREAMS

Variation in OSR, density, size and colour

Density and operational sex ratio did not differ significantly among streams (Table 5; Figure 3A,B). Because differences in body size and colour may be attributable to a number of different factors, I attempted to control for among-stream variation by examining sexual dimorphism at each site; sites were used as replicates for the four streams to identify some possible differences in selective pressure owing to overall differences in stream characteristics during spawning. Sexual size dimorphism (SSD) varied among streams (Table 5; Figure 3C), whereby Freshwater river had female biased SSD but in Cripple Cove males were larger than females overall. In both Watern Cove and Ouananiche Beck, SSD values were close to zero, indicating that difference in body size between the sexes in these streams was minimal (Figure 3C). Sexual colour dimorphism (SCD) did not vary significantly among streams (Table 5), and had overall negative values, indicating that males in all streams were redder than females (Figure 3D).

Table 5. Generalized linear models indicating the effects of among stream differences on sexual size dimorphism (SSD) and sexual colour dimorphism (SCD) among and within Cape Race streams. Degrees of freedom (df), Akaike Information Criterion (AICc), the differences between the model with the lowest AICc and all other models (ΔAICC), and model weights (ωAICc) are included. Akaike Information Criterion (AICc) criteria was used for model selection. The best models are highlighted in bold.

Model	df	AICc	ΔAICc	ωAICc
Population density				
intercept only	**2**	**269.4**	**0.00**	**0.933**
stream	5	274.7	5.28	0.067
OSR				
stream	**5**	**-53.210**	**0.00**	**0.674**
intercept only	**2**	**-57.656**	**1.45**	**0.326**
sexual size dimorphism (SSD)				
stream	**5**	**-27.4**	**0.00**	**0.999**
intercept only	2	13.2	14.19	0.001
sexual colour dimorphism (SCD)				
intercept only	**2**	**-64.5**	**0.00**	**0.524**
stream	**5**	**-64.3**	**0.19**	**0.476**

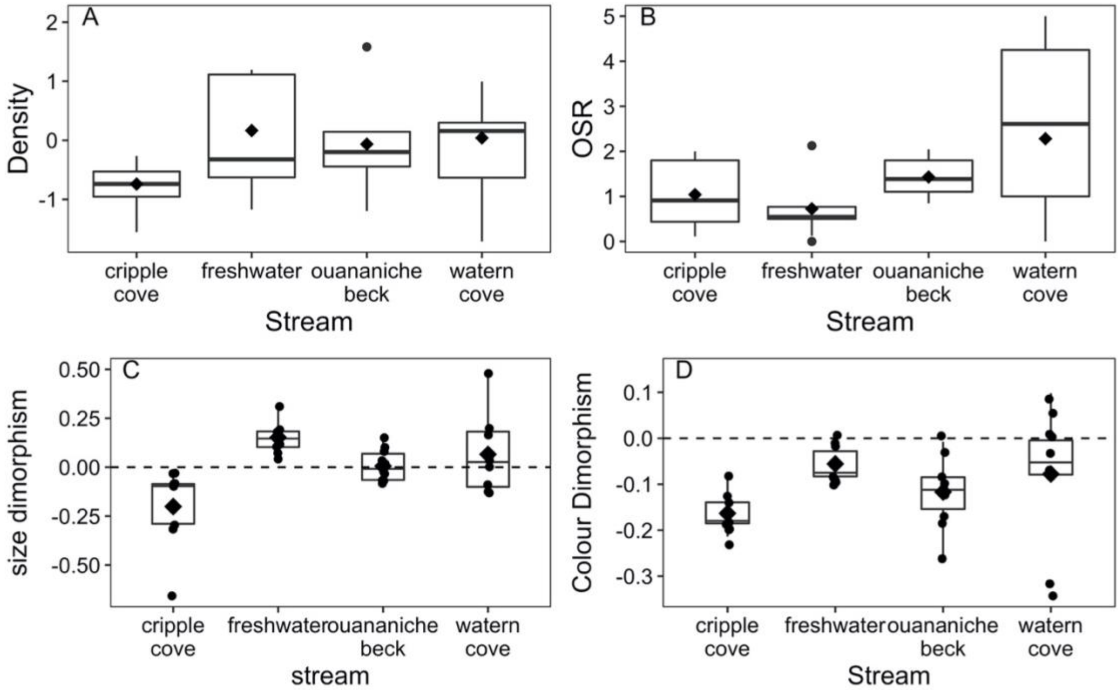

Figure 3. Variation in (A) spawning density, (B) operational sex ratio, (C) sexual size dimorphism, and (D) sexual colour across four studied Cape Race streams (from left to right: Cripple Cove, Freshwater, Ouananiche Beck, Watern Cove). Females are denoted by black fill and lines, males by grey fill and lines. Boxes represent 25th and 75th quartiles, whiskers represent 1.5 x the inter-quartile range, horizontal bars indicate the median, and means are denoted by diamonds.

Relationships between OSR, density and relative size and colour among streams

A combination of models indicated a weak effect on the variation of sexual size dimorphism (SSD) throughout Cape Race streams (Table 6). In all streams, SSD tends to become increasingly male-biased as OSR becomes increasingly male biased (Figure 4A), although this effect is weak. The model including only OSR had a slight effect on the variation of sexual colour dimorphism (SCD) among streams (Table 6; Figure 4B). SCD becomes increasingly male biased as OSR increases in Ouananiche Beck, Watern Cove, and Cripple Cove, with this relationship being strongest in Watern Cove. In Freshwater, SCD is negative, representing a male bias; however, as OSR becomes more heavily male biased the disparity in redness decreases (Figure 4B).

Table 6. Linear mixed effects models indicating the fixed effects of OSR and density on sexual size and colour dimorphism with stream as a random effect.Degrees of freedom (df), Akaike Information Criterion (AICc), the differences between the model with the lowest AICc and all other models (ΔAICC), and model weights (ωAICc) are included. Akaike Information Criterion (AICc) criteria were used for model selection. The best models are highlighted in bold.

Model	df	AICc	ΔAICc	ωAICc
sexual size dimorphism				
OSR	**4**	**-22.0**	**0.00**	**0.321**
density + OSR	**5**	**-21.2**	**0.78**	**0.218**
density	**4**	**-21.0**	**0.98**	**0.197**
intercept only	**3**	**-20.7**	**1.31**	**0.167**
density + density x OSR + OSR	6	-19.6	2.41	0.096
sexual colour dimorphism				
OSR	**4**	**-63.6**	**0.00**	**0.456**
intercept only	**3**	**-62.8**	**0.82**	**0.303**
density + OSR	5	-61.0	2.68	0.120
density	4	-60.4	3.22	0.091
density + density x OSR + OSR	6	-58.2	5.41	0.030

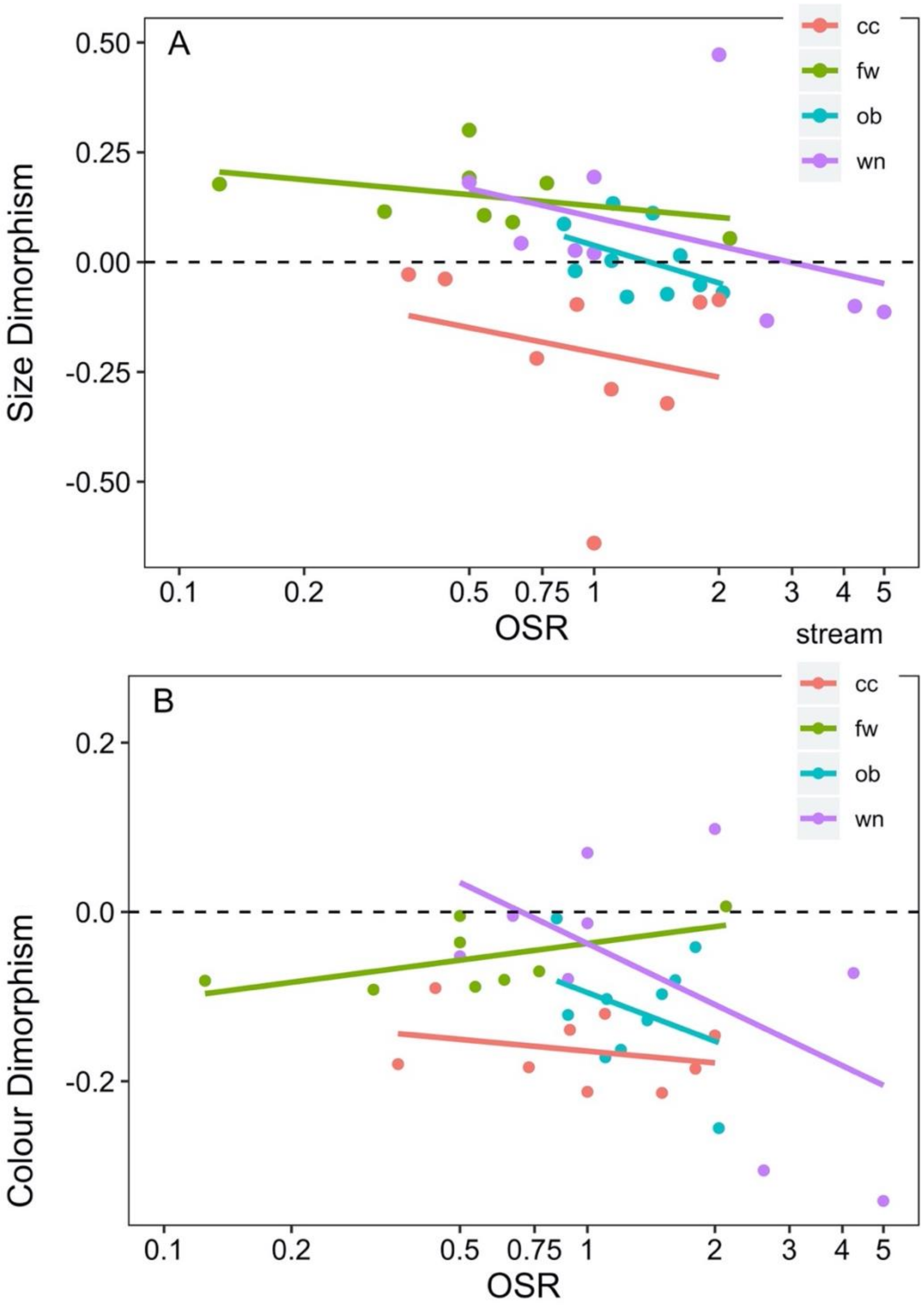

Figure 4. The relationship between (A) sexual size dimorphism, (B) sexual colour dimorphism, and operational sex ratio among four Cape Race streams. Points represent individual sample sites within streams, while lines indicate overall trends within streams.

WITHIN STREAMS

Relationships between OSR, density and sexually-selected traits in males

Body size

A model including both density and OSR was the best explanation for male body size in Freshwater (Table 7). As density increased in this stream, so did male body size (Figure 5A). The relationship between OSR and male body size was less distinct, however the largest average body sizes were found in sites of Freshwater with more male biased OSRs (>0.5) (Figure 5B). In Ouananiche Beck, density effectively explained the variation in male body size (Table 10). A trend of increasing male body size with density is seen in Figure 5C, while no obvious trends are visible in Figure 5D; a positive slope indicates that male body size may slightly increase with increasing OSR. Two models best explained variation in Watern Cove and Cripple Cove male body size; density and OSR, and OSR (Table 7). Male body size in these streams tended to first increase with increasing density and OSR, and then begin to decrease as density reached its greatest value, and OSR became heavily male biased (Figures 5E, 5F, 5G, 5H).

Redness

The model including OSR best explained male body colour in Freshwater (Table 7), where redness decreased as OSR became increasingly male biased (Figure 6B). There were no predictors of male body colour in Ouananiche Beck (Table 7); however, a weak positive relationship between density and colour suggests that males may be redder in sites with high density (Figure 6C). In Watern Cove, there was no effect of density or OSR on male body colour (Table 7; Figures 6E and 6F). Density had a slight effect on male body colour in Cripple Cove, where average redness decreased with increasing densities (Table 7; Figure 6G).

Table 7. Generalized linear models indicating the influence of OSR and density on the distribution of male body size and colour. Degrees of freedom (df), Akaike Information Criterion (AICc), the differences between the model with the lowest AICc and all other models (ΔAICC), and model weights (ωAICc) are included. Akaike Information Criterion (AICc) criteria was used for model selection. The best models are highlighted in bold, and R_2 values are included. Density x OSR models contain both the interaction and main effects.

			Body size			
River	Model	df	AICc	ΔAICc	ωAICc	R_2
Freshwater River	**density + OSR**	**4**	**271.7**	**0.00**	**0.450**	**0.122**
	density	3	272.3	0.54	0.344	
	density x OSR	5	273.9	2.16	0.153	
	intercept only	5	276.6	4.84	0.040	
	OSR	3	278.8	7.02	0.013	
Ouananiche Beck	**density**	**3**	**538.5**	**0.00**	**0.502**	**0.055**
	density x OSR	5	539.7	1.23	0.272	
	density + OSR	4	540.5	2.04	0.181	
	intercept only	2	544.3	5.82	0.0.27	
	OSR	3	545.2	6.73	0.017	
Watern Cove	**density + OSR**	**4**	**339.2**	**0.00**	**0.348**	**0.115**
	OSR	**3**	**339.6**	**0.42**	**0.282**	**0.045**
	density x OSR	5	340.5	1.33	0.178	
	intercept only	2	341.3	2.09	0.122	
	density	3	342.4	3.23	0.069	
Cripple Cove	**OSR**	**3**	**380.6**	**0.00**	**0.384**	**0.118**
	density + OSR	**4**	**381.2**	**0.62**	**0.282**	**0.135**
	density x OSR	5	381.6	1.05	0.228	
	density	3	383.3	2.72	0.098	
	intercept only	2	388.2	7.66	0.008	

Table 8 (Continued). Generalized linear models indicating the influence of OSR and density on the distribution of male body size and colour. Degrees of freedom (df), Akaike Information Criterion (AICc), the differences between the model with the lowest AICc and all other models (ΔAICC), and model weights (ωAICc) are included. Akaike Information Criterion (AICc) criteria was used for model selection. The best models are highlighted in bold, and R_2 values are included. Density x OSR models contain both the interaction and main effects.

			Redness			
River	Model	df	AICc	ΔAICc	ωAICc	R_2
Freshwater River	**OSR**	**3**	**-235.1**	**0.00**	**0.653**	**0.203**
	density + OSR	4	-233.1	2.04	0.236	
	density x OSR	5	-231.5	3.63	0.107	
	density	3	-224.7	10.39	0.004	
	intercept only	2	-221.3	13.88	0.001	
Ouananiche Beck	**intercept only**	**2**	**-358.0**	**0.00**	**0.402**	
	density	**3**	**-357.0**	**1.02**	**0.241**	**0.075**
	OSR	3	-356.1	1.89	0.157	
	density x OSR	5	-356.2	2.50	0.115	
	density + OSR	4	-354.6	3.12	0.084	
Watern Cove	**intercept only**	**2**	**-193.8**	**0.00**	**0.465**	
	OSR	3	-192.6	1.26	0.248	
	density	3	-191.7	2.12	0.161	
	density + OSR	4	-190.5	3.26	0.091	
	density x OSR	5	-188.6	5.22	0.034	
Cripple Cove	**intercept only**	**2**	**-276.8**	**0.00**	**0.367**	
	density	**3**	**-276.6**	**0.21**	**0.330**	**0.247**
	density + OSR	4	-274.8	2.04	0.133	
	OSR	3	-274.7	2.13	0.127	
	density x OSR	5	-272.6	4.28	0.043	

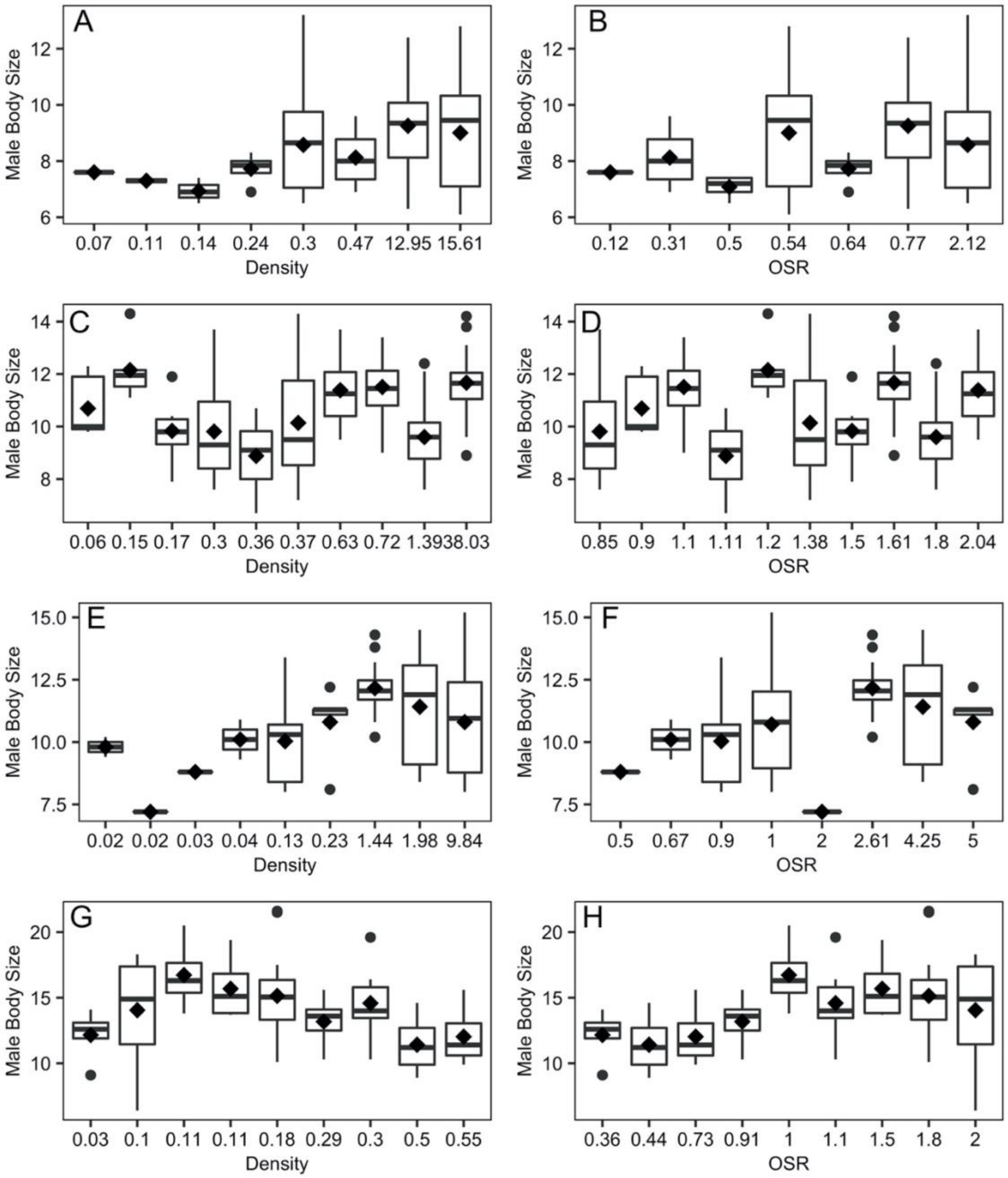

Figure 5. The relationship between male body size, total density, and OSR across four Cape Race streams. (A) Freshwater male body size and density (B) Freshwater male body size and OSR (C) Ouananiche Beck male body size and density (D) Ouananiche Beck male body size and OSR € Watern Cove male body size and density (F) Watern Cove male body size and OSR (G) Cripple Cove male body size and density (H) Cripple Cove male body size and OSR. Boxes represent 25th and 75th quartiles, whiskers represent 1.5 x the inter-quartile range, horizontal bars indicate the median, and means are denoted by diamonds.

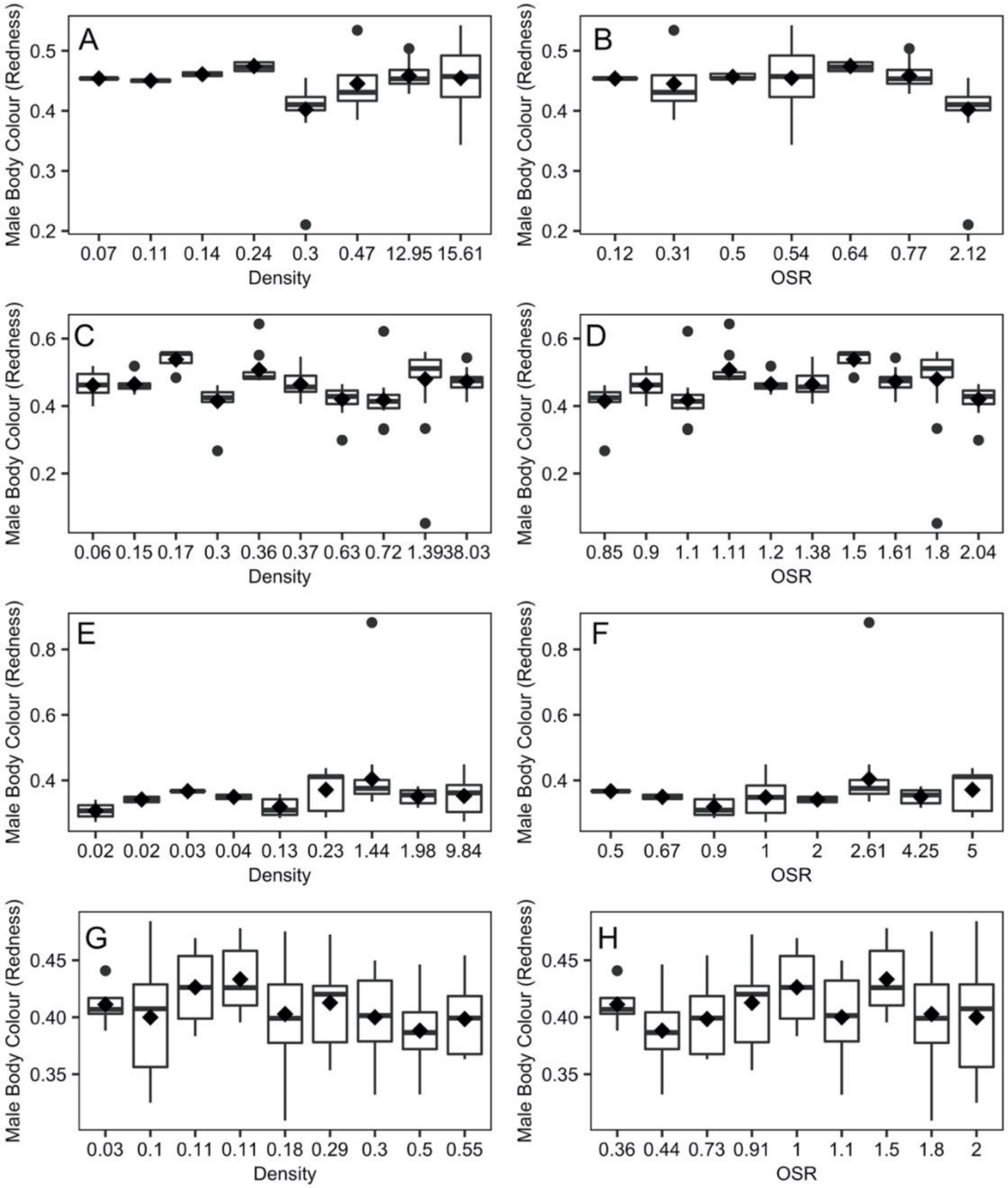

Figure 6. The relationship between male body colour, total density, and OSR across four Cape Race streams. (A) Freshwater male body colour and density (B) Freshwater male body colour and OSR (C) Ouananiche Beck male body colour and density (D) Ouananiche Beck male body colour and OSR € Watern Cove male body colour and density (F) Watern Cove male body colour and OSR (G) Cripple Cove male body colour and density (H) Cripple Cove male body colour and OSR. Boxes represent 25th and 75th quartiles, whiskers represent 1.5 x the inter-quartile range, horizontal bars indicate the median, and means are denoted by diamonds.

Relationships between OSR, density and traits in females

Body size

Three models effectively described variation in female body size in Freshwater: density, a combination of density and OSR, and a combination of density and OSR including their interaction (Table 8). Female body size in this stream tended to increase with increasing density, but decreased as density reached its peak value within Freshwater (Figure 7A). Female body size remains relatively constant over varying OSRs in Freshwater, however there is a slight increase as OSR becomes more heavily male-biased (Figure 7B). A model including density, OSR, and their interactive effects best explained female body size in Ouananiche Beck (Table 8). The patterns within Ouananiche Beck are not as strong as those within other streams, however positive relationships between female body size and both density and OSR suggest that female body size increases with these two parameters increase as well (Figures 7C and 7D). There were no predictors of variation in female body size in Watern Cove (Table 8; Figures 7E and 7F). Two models containing OSR best explained variation in female body size within Cripple Cove (Table 8), whereby female body size increases and then peaks at mid-range densities, and then decreases as density increases (Figure 7G). By contrast, body size increases with increasingly male-biased OSR (Figure 7H).

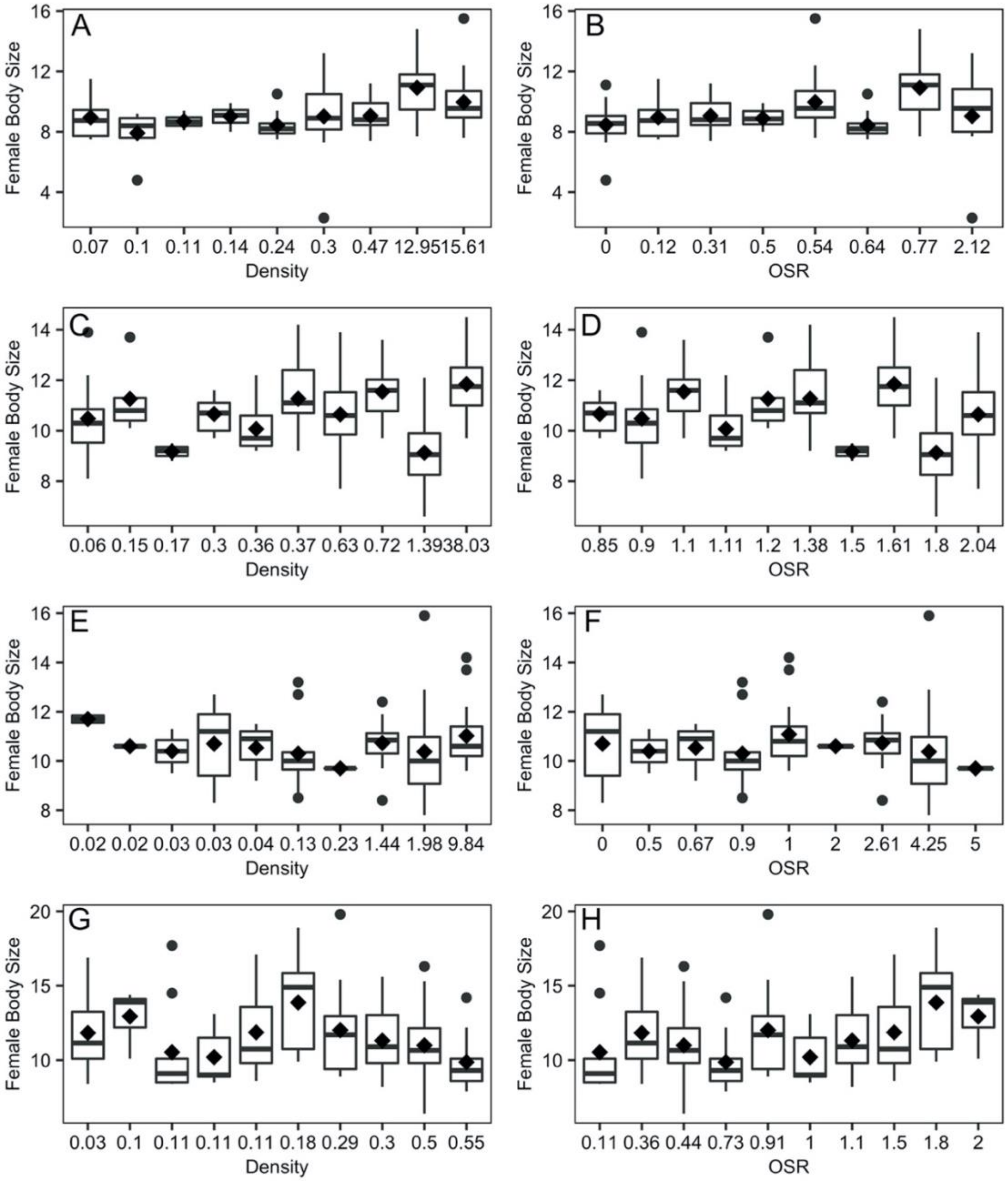

Figure 7. The relationship between female body size, total density, and OSR. (A) Freshwater female body size and density (B) Freshwater female body size and OSR (C) Ouananiche Beck female body size and density (D) Ouananiche Beck female body size and OSR € Watern Cove female body size and density (F) Watern Cove female body size and OSR (G) Cripple Cove female body size and density (H) Cripple Cove female body size and OSR. Boxes represent 25th and 75th quartiles, whiskers represent 1.5 x the inter-quartile range, horizontal bars indicate the median, and means are denoted by diamonds.

Redness

In Freshwater, three models explained variation in female body colour effectively; OSR, density and OSR, and density (Table 8). Redness increased with increasing OSR, but decreased when OSR became heavily male biased (Figures 8A and 8B). Density, OSR, and their interaction had an effect on Ouananiche Beck female body colour (Table 8). Although trends are less distinct in this stream, negative relationships between body colour, density and OSR suggest that females within this stream are most red in sites with low density and OSR (Figures 8C and 8D). In Watern Cove, models including density, and a combination of density and OSR best explained female body colour (Table 8), a positive relationship between redness and density, and a negative relationship with OSR suggest that females are redder in sites with high density and low OSR (Figures 8E and 8F). A weak effect of OSR could explain female body colour in Cripple Cove (Table 8), a negative correlation between the two suggests that females are less red in sites with highly male biased OSRs (Figure 8H).

Table 9. Generalized linear models indicating the influence of OSR and density on the distribution of female body size and colour. Degrees of freedom (df), Akaike Information Criterion (AICc), the differences between the model with the lowest AICc and all other models (ΔAICC), and model weights (ωAICc) are included. Akaike Information Criterion (AICc) criteria was used for model selection. The best models are highlighted in bold. Density x OSR models contain both the interaction and main effects.

			Body size			
River	Model	df	AICc	ΔAICc	ωAICc	R2
Freshwater River	**density**	**3**	**414.1**	**0.00**	**0.466**	**0.025**
	density x OSR	**5**	**414.7**	**0.66**	**0.336**	**0.025**
	density + OSR	**4**	**415.8**	**1.72**	**0.198**	**0.025**
	intercept only	2	434.1	20.00	0.000	
	OSR	3	434.8	20.72	0.000	
Ouananiche Beck	**density x OSR**	**5**	**468.3**	**0.00**	**0.984**	**0.074**
	density + OSR	4	476.6	8.30	0.015	
	density	3	482.7	14.40	0.001	
	OSR	3	489.2	20.84	0.000	
	intercept only	2	489.9	21.59	0.000	
Watern Cove	**intercept only**	**2**	**296.6**	**0.00**	**0.367**	
	density	**3**	**297.4**	**0.88**	**0.237**	**0.046**
	OSR	**3**	**297.6**	**1.07**	**0.215**	**0.013**
	density + OSR	**4**	**298.5**	**1.97**	**0.137**	**0.027**
	density x OSR	5	300.8	4.24	0.044	
Cripple Cove	**OSR**	**3**	**476.7**	**0.00**	**0.462**	**0.071**
	density + OSR	**4**	**477.3**	**0.60**	**0.343**	**0.085**
	density x OSR	5	479.5	2.80	0.114	
	density	3	481.5	4.74	0.043	
	intercept only	2	481.8	5.02	0.038	

Table 10 (Continued). Generalized linear models indicating the influence of OSR and density on the distribution of female body size and colour. Degrees of freedom (df), Akaike Information Criterion (AICc), the differences between the model with the lowest AICc and all other models (ΔAICC), and model weights (ωAICc) are included. Akaike Information Criterion (AICc) criteria was used for model selection. The best models are highlighted in bold. Density x OSR models contain both the interaction and main effects.

			Redness			
River	Model	df	AICc	ΔAICc	ωAICc	R2
Freshwater River	**OSR**	**3**	**-319.2**	**0.00**	**0.283**	**0.026**
	density + OSR	**4**	**-318.8**	**0.41**	**0.230**	**0.041**
	density	**3**	**-318.7**	**0.56**	**0.214**	
	intercept only	2	-318.5	0.74	0.196	
	density x OSR	5	-316.6	2.60	0.077	
Ouananiche Beck	**density x OSR**	**5**	**-426.8**	**0.00**	**1**	**0.214**
	density + OSR	4	-408.9	17.93	0	
	density	3	-406.9	19.91	0	
	intercept only	2	-400.4	26.39	0	
	OSR	3	-399.3	27.54	0	
Watern Cove	**density**	**3**	**-280.5**	**0.00**	**0.423**	**0.057**
	density + OSR	**4**	**-279.5**	**1.01**	**0.255**	**0.069**
	density x OSR	5	-278.5	1.97	0.158	
	intercept only	2	-277.7	2.82	0.103	
	OSR	3	-276.6	3.86	0.061	
Cripple Cove	**intercept only**	**2**	**-414.1**	**0.00**	**0.349**	
	OSR	**3**	**-414.0**	**0.10**	**0.332**	**0.021**
	density + OSR	**4**	**-412.2**	**1.90**	**0.135**	**0.024**
	density	**3**	**-412.1**	**1.96**	**0.131**	**0.002**
	density x OSR	5	-410.3	3.77	0.053	

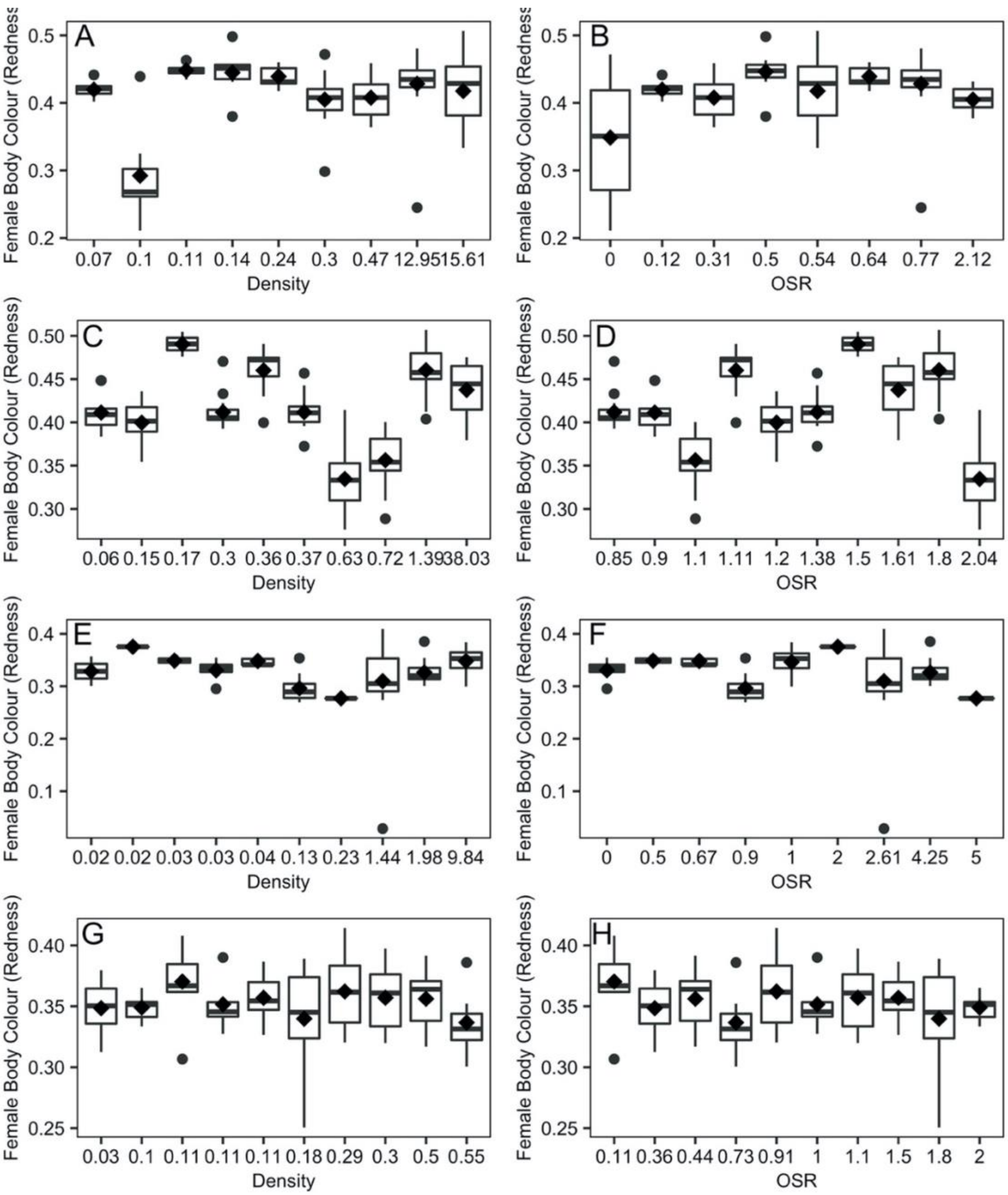

Figure 8. The relationship between female body colour, total density, and OSR across four Cape Race streams. (A-B) Freshwater female body colour and OSR, (C-D) Ouananiche Beck female body colour and density and OSR (E-F), Watern Cove female body colour and density and OSR, and (G-H) Cripple Cove female body colour and density and OSR. Boxes represent 25th and 75th quartiles, whiskers represent 1.5 x the inter-quartile range, horizontal bars indicate the median, and means are denoted by diamonds.

Chlorophyll-*a* Concentration

The model using chlorophyll-*a* as a predictor of redness had little to no effect on males or females when data was separated by sex in Freshwater, but a stronger effect was seen in both males and females of Watern Cove (Table 9). Redness increased with increasing chlorophyll-*a* concentrations in Freshwater males and females, and Watern males, but female redness in Watern Cove was negatively correlated with chlorophyll-a concentration (Table 9). Ouananiche Beck individuals' body colour was not explained by chlorophyll-*a* concentrations within the stream (Table 9). In Cripple Cove, effect of chlorophyll-*a* was stronger in males than it was females, with both sexes having slightly positive relationships with chlorophyll-*a* concentration, indicating that redness may increase with increasing chlorophyll-*a* concentrations (Table 9).

Table 11. Generalized linear models indicating the effects of chlorophyll-a (ug/L) on body colour for four sampled Cape Race streams. Degrees of freedom (df), Akaike Information Criterion (AICc), the differences between the model with the lowest AICc and all other models (ΔAICC), and model weights (ωAICc) are included. Akaike Information Criterion (AICc) criteria was used for model selection. The best models are highlighted in bold.

Model	df	AICc	ΔAICc	ωAICc
Freshwater male body colour				
intercept only	**2**	**-221.3**	**0.00**	**0.577**
chlorophyll-a (ug/L)	3	-220.6	0.62	0.423
Freshwater female body colour				
intercept only	**2**	**-318.5**	**0.00**	**0.712**
chlorophyll-a (ug/L)	3	-316.7	1.81	0.288
Ouananiche Beck male body colour				
intercept only	**2**	**-358.0**	**0.00**	**0.72**
chlorophyll-a (ug/L)	3	-356.1	1.89	0.28
Ouananiche Beck female body colour				
intercept only	**2**	**-400.4**	**0.00**	**1**
chlorophyll-a (ug/L)	3	-398.4	2.06	0
Watern Cove male body colour				
chlorophyll-a (ug/L)	**3**	**-201.9**	**0.00**	**0.982**
intercept only	2	-193.8	8.05	0.018
Watern Cove female body colour				
chlorophyll-a (ug/L)	**3**	**-279.0**	**0.00**	**0.657**
intercept only	2	-277.7	1.30	0.343
Cripple Cove male body colour				
chlorophyll-a (ug/L)	**3**	**-277.9**	**0.00**	**0.982**
intercept only	2	-193.8	8.05	0.018
Cripple Cove female body colour				
intercept only	**2**	**-414.1**	**0.00**	**0.737**
chlorophyll-a (ug/L)	3	-412.0	2.07	0.263

DISCUSSION

Do differences in abiotic factors explain the density and size distributions of females?

Relationships between abiotic factors and female densities were different for each stream, which may be a result of abiotic variation among streams. Trends in Cripple Cove and Freshwater suggest that females aggregate at sites with with low temperature and dissolved oxygen in Cripple Cove, and in Freshwater River they aggregate at sites with high conductivity These abiotic conditions may be indicative of groundwater seepage (Baxter & McPhail, 2011), a spawning habitat characteristic preferred by brook trout and beneficial for offspring survival (Curry et al., 1995; Curry et. al., 2008). Because groundwater temperature fluctuates less than stream water (Franken et. al., 2001), its seepage creates a temperature buffer within the nest site (Cardenas et. al., 2016), preventing eggs from freezing in the winter or potentially over-heating in the early-autumn (Baxter & McPhail, 2011). Although groundwater seepage is not a requirement for successful spawning and offspring survival, brook trout will readily seek out areas with groundwater seepage to spawn, even when substrate type and size is suboptimal for redd construction (Webster & Eiriksdottir, 1976).

Similar to female density, the relationships between female size and abiotic factors varied among streams as a result of abiotic differences within Cape Race. The largest females in Freshwater River were found in relatively slower, deeper areas with relatively higher temperature, conductivity, and dissolved oxygen compared to other sites. By contrast, in Ouananiche Beck the largest females were found in areas with relatively lower temperatures and dissolved oxygen values compared to other areas within this stream. No relationships between female body size and abiotic factors related to spawning were found in the other streams.

My results suggest that the largest females are aggregating in high densities in areas within Freshwater with abiotic factors optimal for spawning and offspring survival. This supports findings from Purchase and Hutchings' (2008) study of spatial variability within a spawning brook trout, and provides further evidence for despotic distribution within Freshwater river. When comparing high-density and low-density sites, the authors found that high-density sites had abiotic conditions indicative of groundwater seepage, and that the largest individuals were occupying these sites (Purchase & Hutchings, 2008). However, when examining the relationship between density and female body size, I found that the distribution of female body size in both Freshwater river and Cripple Cove first increases with increasing density, but decreases when densities are at their highest values within these streams. This decrease in body size suggests that competition for optimal spawning habitat in highest density areas may be too intense and not energetically favourable for large females, and a size advantage may only occur at moderately high spawning densities.

Because female salmonids typically select nesting habitat, I did not focus on relationships between abiotic characteristics and male density or size; however, these results are in Tables S3 and S4.

Do variation in density and operational sex ratio predict differences in sexually-selected traits?

AMONG STREAMS

Sexual dimorphism is a measure of differences in the expression of sexually-selected traits between males and females, and is common across many taxa (Andersson, 1994), and is influenced by both natural and sexual selection acting differently on both sexes (Hedrick & Temeles, 1989; Price, 1984; Young, 2005). For example, large female body size is favoured by natural selection, as fecundity is positively related to size, and large male body size is often sexually selected for via male-male competition or female mate choice. This metric is highly correlated with the intensity of mate competition within a species or population, and energetic allocations related to reproduction (Hedrick & Temeles, 1989; Lovich & Gibbons, 1992).

The direction and magnitude of dimorphism can be influenced by the mating system within a population (Weir et. al., 2016), and often results in intraspecific variation of sexual dimorphism among populations (Tamate & Maekawa, 2006; Young, 2005). Because both natural and sexual selection can influence SSD (Fairbairn, 1997; Ferguson & Fairbairn, 2000), and environmental factors can influence the intensities both selective pressures (Slatkin, 1984), habitat variability may constrain selective pressures that select for secondary sex traits such as large body size (Dobson & Wigginton, 1996). Variation in sexual dimorphism within and among populations may correlate with differences in the environment. For instance, conspicuous male secondary sex traits can sometimes raise the risk of predation (Andersson, 1994). In environments with high predation risk, males' colouration is duller than those inhabiting low-risk areas (Endler, 1980; Moodie, 1972).

Abiotic and biotic factors within in an environment can create a trade-off between survival and mate acquisition, constraining sexual selection and leading to intraspecific variation in sexual dimorphism among populations (Weese et. al., 2010).

I found that the direction of sexual size dimorphism (SSD) varied among streams, whereby Freshwater populations had female biased SSD, Cripple Cove populations had male biased SSD, and there was minimal size difference between the sexes in both Ouananiche Beck and Watern Cove. Generally, a female-biased or even SSD suggests that intrasexual competition for mates between males is not extreme, or that competition is more intense between females than males, whereas SSD is expected to become more male-biased with increasing intrasexual competition. A combination of density and operational sex ratio affected SSD among streams, suggesting that social environment is playing a role in the intensity of sexual size dimorphism in Cape Race brook trout populations. As expected, if OSR is an indication of the intensity of sexual selection, SSD becomes increasingly male biased with increasingly male biased OSRs.

Freshwater river has the most even OSR compared to the other streams, which may explain the female biased sexual size dimorphism, as a more even OSR reduces the intensity of intrasexual selection on males and large body size may not offer a significant competitive advantage in this environment. Contrarily, large body size in females may offer more of a competitive advantage in Freshwater than other streams. As previously mentioned, larger females in this river were found in areas with abiotic characteristics directly related to groundwater seepage, therefore, competition for optimal spawning territory by females may be more intense in Freshwater.

Males were redder than females in all streams. This result was expected, because body redness is a sexually selected trait among many salmonid species (Blanchfield, et. al., 2003; Fleming & Gross, 1994). As OSR becomes increasingly male biased, SCD becomes more extreme and male biased in Cripple Cove, Watern Cove, and Ouananiche Beck. Similar to large male body size, increased redness may be an advantage to males when intrasexual competition for mates is intense (Romano et al., 2017), as it would be in a spawning population with a male biased OSR. In Freshwater, SCD becomes less extreme and closer to zero as OSR becomes increasingly male biased. Other environmental factors may be affecting the intensity at which sexual selection can act on Freshwater populations, and limiting the expression of male red colouration in this stream (Reimchen, 1988; Seehausen, 1997).

Signals used to communicate reproductive information exist throughout the animal kingdom; however, their efficacy and expression is often directly dependent on the environment it is transmitted through (Morrongiello et. al., 2010). In aquatic habitats, turbid waters arise from substrate particles (silt or clay), or increased phytoplankton and algal growth, and can shift the colour and intensity of ambient light, altering the perception of visual signals used by several fish species (Dugas & Franssen, 2011). The effects of turbidity can therefore constrain visual signals specifically related to intra- or inter-sexual selection, leading to reduced species recognition in some cases (Castillo Cajas et. al., 2012; Seehausen, 1997). Reduced signal efficacy can also lead to reduced signal expression in populations living in turbid conditions (Wong et. al., 2007).

My results suggest that chlorophyll-*a* concentration, a measure of phytoplankton concentration or turbidity, has an effect on brook trout redness in both Watern Cove and

Cripple Cove, with redness increasing with increasing chlorophyll-a concentrations in males of both streams, and for Cripple Cove females. This trend was not initially predicted; however, some studies have found that red colouration becomes more intense with increasing turbidity. Wong et. al. (2007) found that threespine stickleback males in turbid water expressed redder colouration than their counterparts in clear water. Redness was also positively correlated with turbidity in a population of red shiners (Dugas & Franssen, 2011). These results were attributed to among-population variation in both carotenoid availability and intake, as well as differences in genetic or plastic responses to light environment during development. Interestingly, red shiners exhibit very similar mate competition to brook trout, in which males actively pursue females, whereas several studies that found a reduction of breeding colouration with increased water turbidity were completed using species of fishes where males guard fixed territories and resources for females (Castillo Cajas et al., 2012; Dugas & Franssen, 2011; Reimchen, 1988; Seehausen, 1997), so differences in mating system structure among species may also affect the expression and use of red breeding colouration.

WITHIN STREAMS

Large male body size is sexually selected for in salmonids as it offers an advantage during intrasexual competition for mates. Density and OSR affected the distribution of male body size differently among streams. Overall, OSR seems to have the greatest effect on male body size among streams, with males being largest in male-biased OSRs. In Cripple Cove and Watern Cove, an increase in male body size with increasingly male biased OSRs followed by a decrease in average size when OSR becomes heavily male-biased may be due to increased mate competition intensity (Kvarnemo & Ahnesjo, 1996; 2009). When the OSR is extremely skewed towards males and intrasexual competition for females is intense, it is no longer energetically favourable to compete directly, and a shift towards indirect intrasexual competition within these sites may occur, where body size is a less important determinant for reproductive success (Weir et al., 2011) and smaller males may gain some reproductive opportunities.

Noticeably, male redness tended to decrease as density increased in both Freshwater and Cripple Cove, and in all streams other than Watern Cove, female redness decreased with increasing density and OSR. When densities are high, sexually selected traits may not be as important for intrasexual or intersexual competition, and their advantage for mate acquisition may be reduced in these areas. Likewise, carotenoid availability may decrease in environments with high population densities, leading to duller colouration of individuals (Hill, 1992). Carotenoids cannot be produced by fishes, and are derived from the diet, and are therefore a limiting resource in aquatic habitats (Hill, 1992; Reimchen, 1988). Alternatively, other abiotic factors within the environment may be influencing the expression and distribution of redness in Cape Race brook trout such as chlorophyll concentration, water depth, temperature, and water chemistry (Tables S5, S6). Zastavniouk et. al. (2017) found several phenotype-environment relationships within Cape Race streams. These findings demonstrated an overall increase in female redness in warmer, deeper, and faster streams, and redness of both sexes increasing with increased water acidity (Zastavniouk et al., 2017), and suggest that natural selection may have an effect on the distribution and expression of redness within and among Cape Race brook trout populations.

GENERAL CONCLUSIONS

Differences in abiotic factors may partly explain the density and size distributions of female brook trout in Cape Race streams. Relationships between abiotic characteristics related to spawning territory suitability and female density were most notable in Freshwater and Cripple Cove rivers, where the highest densities of females were found in areas with abiotic characteristics related to groundwater seepage, and relatively cooler temperatures, which increase offspring survival. Females within these rivers may be actively choosing to aggregate in areas with favourable abiotic conditions to increase their reproductive success, leading to relatively higher densities than other areas during the reproductive season.

Because females must compete directly for optimal spawning territories, and large body size offers a competitive advantage, a relationship between abiotic conditions and large female body size may be expected. This trend held true in Freshwater and Ouananiche Beck rivers, where largest females were associated with abiotic factors directly related to suitable spawning habitats. Interestingly, Freshwater river had the most even operational sex ratio and a female-biased sexual size dimorphism, which may suggest that for females, large size is a particularly important trait to have within this river.

Variation in social environment (density and operational sex ratio) can predict differences in the distribution of sexually-selected traits of brook trout within Cape Race streams. Sexual size dimorphism varied among streams, and tended to become increasingly male skewed as OSR became increasingly male-biased, aside from Freshwater. This result can be explained by increased intra-sexual competition between males, where body size is important in securing dominance. The opposite trend in Freshwater can be related to the importance of female body size within this stream, as stated above. When examining male size alone, a decrease in size as OSR becomes extremely male-biased and may suggest that direct competition becomes too intense, and that individuals within sites exhibiting this trend may be opting for indirect means of competition. Likewise, female body size tends to decrease with increasing density, suggesting that competition for nest sites at high densities is too intense, and becomes energetically unfavourable. Overall sexual colour dimorphism (SCD) was male biased, meaning males were redder than females in all streams, which provides evidence that red body colouration is a sexually-selected trait among brook trout. OSR seemed to explain the distribution of SCD in all streams besides Freshwater, because SCD became more male skewed as OSR became increasingly male-biased. A decrease in male and female redness with increasing population densities may be explained by a limited availability of carotenoids within the environment, or the decreasing of importance of redness during mate competition at high densities.

General trends suggest that abiotic conditions directly related to spawning and offspring survival influence both female density and size distribution, whereas social conditions influence sexually-selected male phenotypes, and sexual dimorphism, and patterns regarding the influence of social conditions was complex. Overall, streams varied more markedly in the abiotic factors than biotic factors studied when comparing among streams. This abiotic variation can explain the divergence from general trends seen in female density and size, as well as sexually-selected male phenotypes. Because this variation is observed specifically within sexually-selected traits, I can conclude that ecological factors, both abiotic and social, are influencing the degree to which sexual selection can act on populations within Cape Race streams.

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SUPPLEMENTARY MATERIALS

Table S1. Table indicating sample site characteristics (length, width, depth), total number of individuals captured per site, and capture per unit effort (CPUE: individuals caught/hour) rates per site. All values reported for ten sites within four Cape Race streams (FW = Freshwater River, OB = Ouananiche Beck River, WN = Watern Cove River, CC = Cripple Cove River).

Stream	Sites									
FW	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
length (m)	150	10	150	8	150	150	150	150	150	150
width (m)	4.53	3.08	2.27	3.7	2.93	2.05	2.52	2.92	3.05	2.24
depth (m)	0.19	0.26	0.13	0.24	0.17	0.21	0.15	0.17	0.18	0.17
# captured	116	191	88	122	74	60	47	69	114	88
CPUE	58	191	44	122	37	60	47	55.2	91.2	58.6
OB	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
length (m)	90	90	90	90	90	90	10	90	90	90
width (m)	2.39	2.39	4.74	6.16	5.78	2.86	1.42	5.06	6.59	7.52
depth (m)	0.28	0.19	0.12	0.15	0.57	0.29	1.42	0.17	0.19	0.16
# captured	53	115	56	70	36	88	201	90	123	103
CPUE	42.4	92	44.8	56	36	70.4	201	72	98.4	68.7
WC	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
length (m)	15.5	90	90	90	90	6.8	16.2	13.1	27.5	51.6
width (m)	3.33	4.9	3.23	3.38	3.58	2.42	8.8	10.5	16.7	8.2
depth (m)	0.41	0.36	0.39	0.35	0.39	0.26	0.18	0.42	0.45	0.35
# captured	48	24	46	17	6	57	11	89	10	30
CPUE	48	32	61.3	34	12	76	22	71.2	20	40
CC	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
length (m)	90	90	90	90	90	90	90	90	90	90
width (m)	3.26	3.42	5.27	5.34	3.99	3.14	2.85	2.6	7.7	7.02
depth (m)	0.3	0.23	0.32	0.33	0.25	0.26	0.14	0.19	0.98	0.15
# captured	41	56	57	30	25	34	28	30	37	26
CPUE	54.6	74.6	76	60	50	45.3	56	60	49.3	34.7

Table S2. Generalized linear models indicating the effects stream on the variation in principal components, density, and operational sex ratio (OSR) for four sampled Cape Race streams. Degrees of freedom (df), Akaike Information Criterion (AICc), the differences between the model with the lowest AICc and all other models (ΔAIC), and model weights (ωAICc) are included. Akaike Information Criterion (AICc) criteria was used for model selection. The best models are highlighted in bold.

Model	df	AICc	ΔAICc	ωAICc
PC1				
stream	**5**	**140.5**	**0.00**	**0.999**
intercept only	2	154.9	14.37	0.001
PC2				
stream	**5**	**118.0**	**0.00**	**0.991**
intercept only	2	127.4	9.40	0.009
PC3				
stream	**5**	**109.8**	**0.00**	**0.629**
intercept only	**2**	**110.9**	**1.05**	**0.371**

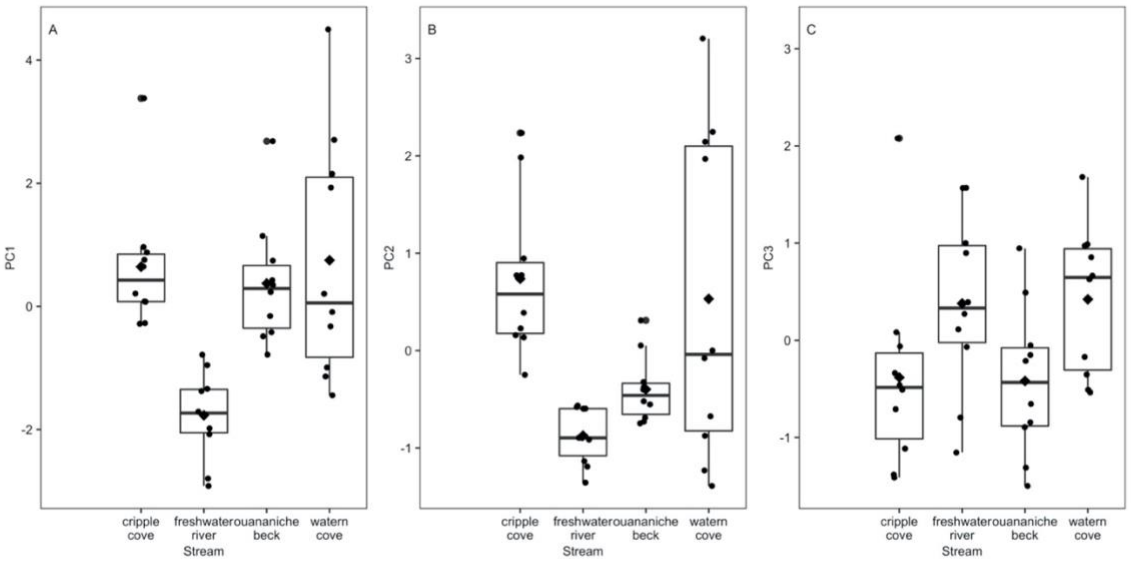

Figure S1. Variation in principal components describing abiotic conditions across four Cape Race Streams. The x-axis denotes each sampled stream (Left to Right: Cripple Cove, Freshwater, Ouananiche Beck, Watern Cove, while the y- axis represents (A) PC1 (B) PC2 (C) PC3. Boxes represent 25th and 75th quartiles, whiskers represent 1.5 x the inter-quartile range, horizontal bars indicate the median, and means are denoted by diamonds. Points represent ten sites within each stream.

Table S3. Generalized linear models indicating the effects of abiotic factors (represented by principal components) on male densities in four Cape Race streams. Degrees of freedom (df), Akaike Information Criterion (AICc), the differences between the model with the lowest AICc and all other models (ΔAICC), and model weights (wAICc) are included. Akaike Information Criterion (AICc) criteria was used for model selection. The best models are highlighted in bold.

Model	df	AICc	ΔAICc	ωAICc
Freshwater male density				
PC1 + PC3	**4**	**6.1**	**0.00**	**0.467**
PC2	**3**	**7.0**	**0.83**	**0.308**
PC1 + PC2 +PC3	5	9.5	3.32	0.089
PC1 + PC2	4	10.1	3.96	0.065
intercept only	2	11.2	5.01	0.038
PC2 + PC3	4	12.9	6.81	0.016
PC1	3	13.4	7.24	0.013
PC3	3	15.1	8.99	0.005
Ouananiche Beck male density				
intercept only	**2**	**28.2**	**0.00**	**0.503**
PC1	**3**	**29.3**	**1.10**	**0.290**
PC2	3	31.4	3.16	0.104
PC3	3	32.3	4.07	0.066
PC1 + PC2	4	34.9	6.72	0.018
PC1 + PC3	4	35.3	7.10	0.014
PC2 + PC3	4	37.4	9.14	0.005
PC1 + PC2 +PC3	5	43.9	15.69	0.000
Watern Cove male density				
PC3	**3**	**5.7**	**0.00**	**0.418**
intercept only	**2**	**6.1**	**0.40**	**0.343**
PC2	3	8.8	3.06	0.091
PC2 + PC3	4	9.8	4.10	0.054
PC1	3	10.3	4.60	0.042
PC1 + PC3	4	11.1	5.41	0.028
PC1 + PC2	4	11.4	5.74	0.024
PC1 + PC2 + PC3	5	17.6	11.91	0.001
Cripple Cove male density				
PC1	**3**	**12.7**	**0.00**	**0.695**
PC1 + PC2	4	15.4	2.67	0.183
PC3	3	18.0	5.21	0.051
PC1 + PC3	4	18.7	5.98	0.035
intercept only	2	19.2	6.50	0.027
PC2	3	23.0	10.29	0.004
PC1 + PC2 + PC3	5	23.8	11.06	0.003
PC2 + PC3	4	24.0	11.21	0.003

Table S4. Generalized linear models indicating the effects of abiotic factors (represented by principal components) on body size of male brook trout in four Cape Race streams. Degrees of freedom (df), Akaike Information Criterion (AICc), the differences between the model with the lowest AICc and all other models (ΔAICC), and model weights (wAICc) are included. Akaike Information Criterion (AICc) criteria was used for model selection. The best models are highlighted in bold.

Model	df	AICc	ΔAICc	ωAICc
Freshwater male body size				
PC1	**3**	**272.2**	**0.00**	**0.272**
PC2	**3**	**272.5**	**0.29**	**0.236**
PC1 + PC3	**4**	**273.1**	**0.82**	**0.180**
PC1 + PC2	**4**	**274.0**	**1.79**	**0.111**
PC2 + PC3	4	274.4	2.14	0.093
PC1 + PC2 + PC3	5	275.1	2.87	0.065
intercept only	2	276.6	4.32	0.031
PC3	3	278.6	6.37	0.011
Ouananiche male body size				
PC1 + PC3	**4**	**517.8**	**0.00**	**0.413**
PC2 + PC3	**4**	**519.2**	**1.40**	**0.205**
PC1 + PC2 + PC3	**5**	**519.4**	**1.53**	**0.192**
PC3	**3**	**519.4**	**1.57**	**0.189**
PC1	3	544.1	26.28	0.000
intercept only	2	544.3	26.48	0.000
PC1 + PC2	4	546.2	28.35	0.000
PC2	3	546.2	28.38	0.000
Watern Cove male body size				
PC2	**3**	**333.6**	**0.00**	**0.401**
PC1 + PC2	**4**	**335.3**	**1.69**	**0.172**
PC1	**3**	**335.6**	**1.97**	**0.150**
PC2 + PC3	4	335.8	2.21	0.133
PC1 + PC3	4	337.0	3.39	0.074
PC1 + PC2 + PC3	5	337.4	3.83	0.059
intercept only	2	341.3	7.68	0.009
PC3	3	343.4	9.84	0.003
Cripple Cove male body size				
intercept only	**2**	**388.2**	**0.00**	**0.280**
PC2	**3**	**388.5**	**0.24**	**0.248**
PC1 + PC2	**4**	**390.2**	**1.95**	**0.105**
PC3	3	390.3	2.07	0.099
PC1	3	390.4	2.13	0.096
PC2 + PC3	4	390.4	2.16	0.095
PC1 + PC3	4	392.0	3.76	0.043
PC1 + PC2 + PC3	5	392.5	4.23	0.034

Table S5. Generalized linear models indicating the effects of abiotic factors (represented by principal components) on body colour of male brook trout in four Cape Race streams. Degrees of freedom (df), Akaike Information Criterion (AICc), the differences between the model with the lowest AICc and all other models (ΔAICC), and model weights (wAICc) are included. Akaike Information Criterion (AICc) criteria was used for model selection. The best models are highlighted in bold.

Model	df	AICc	ΔAICc	ωAICc
Freshwater male body colour				
PC1	**3**	**-221.8**	**0.00**	**0.267**
intercept only	**2**	**-221.3**	**0.50**	**0.208**
PC2	**3**	**-220.8**	**0.99**	**0.162**
PC3	**3**	**-219.9**	**1.88**	**0.104**
PC1 + PC2	4	-219.5	2.24	0.087
PC1 + PC3	4	-219.5	2.25	0.087
PC2 + PC3	4	-218.7	3.05	0.058
PC1 + PC2 + PC3	5	-217.2	4.56	0.027
Ouananiche Beck male body colour				
PC1 + PC2 + PC3	**5**	**-382.5**	**0.00**	**0.713**
PC2 + PC3	4	-380.2	2.35	0.221
PC1 + PC2	4	-377.3	5.26	0.052
PC2	3	-374.7	7.81	0.014
PC3	3	-367.0	15.50	0.000
PC1 + PC3	4	-364.9	17.58	0.000
intercept only	2	-358.0	24.52	0.000
PC1	3	-355.9	26.61	0.000
Watern Cove male body colour				
PC1	**3**	**-194.4**	**0.00**	**0.285**
intercept only	**2**	**-193.8**	**0.57**	**0.214**
PC2	**3**	**-193.1**	**1.32**	**0.147**
PC1 + PC2	4	-192.2	2.21	0.094
PC1 + PC3	4	-192.2	2.21	0.094
PC3	3	-191.9	2.52	0.081
PC2 + PC3	4	-191.1	3.31	0.054
PC1 + PC2 + PC3	5	-189.9	4.48	0030
Cripple Cove male body colour				
intercept only	**2**	**-276.8**	**0.00**	**0.288**
PC2	**3**	**-276.6**	**0.24**	**0.256**
PC1	**3**	**-274.9**	**1.90**	**0.112**
PC3	3	-274.7	2.10	0.101
PC1 + PC2	4	-274.5	2.37	0.088
PC2 + PC3	4	-274.4	2.47	0.084
PC1 + PC3	4	-272.8	4.06	0.038
PC1 + PC2 + PC3	5	-272.5	4.29	0.034

Table S6. Generalized linear models indicating the effects of abiotic factors (represented by principal components) on body colour of female brook trout in four Cape Race streams. Degrees of freedom (df), Akaike Information Criterion (AICc), the differences between the model with the lowest AICc and all other models (ΔAICC), and model weights (wAICc) are included. Akaike Information Criterion (AICc) criteria was used for model selection. The best models are highlighted in bold.

Model	df	AICc	ΔAICc	ωAICc
Freshwater female body colour				
PC3	**3**	**-342.3**	**0.00**	**0.455**
PC1 + PC3	4	-340.9	1.37	0.230
PC2 + PC3	4	-340.3	2.00	0.168
PC1 + PC2 + PC3	5	-339.9	2.34	0.141
PC1 + PC2	4	-333.8	8.46	0.007
PC1	3	-326.6	15.63	0.000
PC2	3	-319.2	23.04	0.000
intercept only	2	-318.5	23.79	0.000
Ouananiche Beck female body colour				
PC1 + PC2 + PC3	**5**	**-504.9**	**0.00**	**0.999**
PC2 + PC3	4	-490.1	14.74	0.001
PC1 + PC2	4	-454.0	50.83	0.000
PC2	3	-446.4	58.47	0.000
PC3	3	-441.4	63.43	0.000
PC1 + PC3	4	-439.4	65.50	0.000
intercept only	2	-400.4	104.46	0.000
PC1	3	-398.5	106.36	0.000
Watern Cove female body colour				
PC2 + PC3	**4**	**-283.8**	**0.00**	**0.473**
PC1 + PC3	4	-281.7	2.17	0.160
PC1 + PC2 + PC3	5	-281.6	2.21	0.156
PC3	3	-280.3	3.50	0.082
PC1	3	-279.6	4.25	0.056
PC2	3	-278.4	5.38	0.032
intercept only	2	-277.7	6.16	0.022
PC1 + PC2	3	-277.4	6.42	0.019
Cripple Cove female body colour				
intercept only	**2**	**-414.1**	**0.00**	**0.351**
PC2	3	-412.7	1.40	0.175
PC3	3	-412.3	1.75	0.146
PC1	3	-412.1	1.97	0.131
PC1 + PC2	4	-410.5	3.52	0.060
PC2 + PC3	4	-410.5	3.54	0.060
PC1 + PC3	4	-410.2	3.85	0.051
PC1 + PC2 + PC3	5	-408.8	5.27	0.025