# Disentangling the effects of biogeoclimatic factors and local stressors on breeding harlequin duck presence and abundance using field surveys, MaxEnt, and stable isotope turnover models

**by**

**Emily Missyabit McAuley**

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B.Sc.H., Carleton University, 2007

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## Declaration of Committee

| | |
|---|---|
| **Name:** | **Emily Missyabit McAuley** |
| **Degree:** | **Doctor of Philosophy** |
| **Thesis title:** | **Disentangling the effects of biogeoclimatic factors and local stressors on breeding harlequin duck presence and abundance using field surveys, MaxEnt, and stable isotope turnover models** |
| **Committee:** | **Chair:** Jane Fowler<br>Assistant Professor, Biological Sciences |
| | **Ronald C. Ydenberg**<br>Supervisor<br>Professor, Biological Sciences |
| | **Susan M. Bertram**<br>Supervisor<br>Professor, Biology<br>Carleton University |
| | **Jonathan Moore**<br>Committee Member<br>Professor, Biological Sciences |
| | **Wendy Palen**<br>Committee Member<br>Professor, Biological Sciences |
| | **Daniel Esler**<br>Examiner<br>Adjunct Professor, Biological Sciences |
| | **Grant Gilchrist**<br>External Examiner<br>Adjunct Professor, Biology<br>Carleton University |

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# Abstract

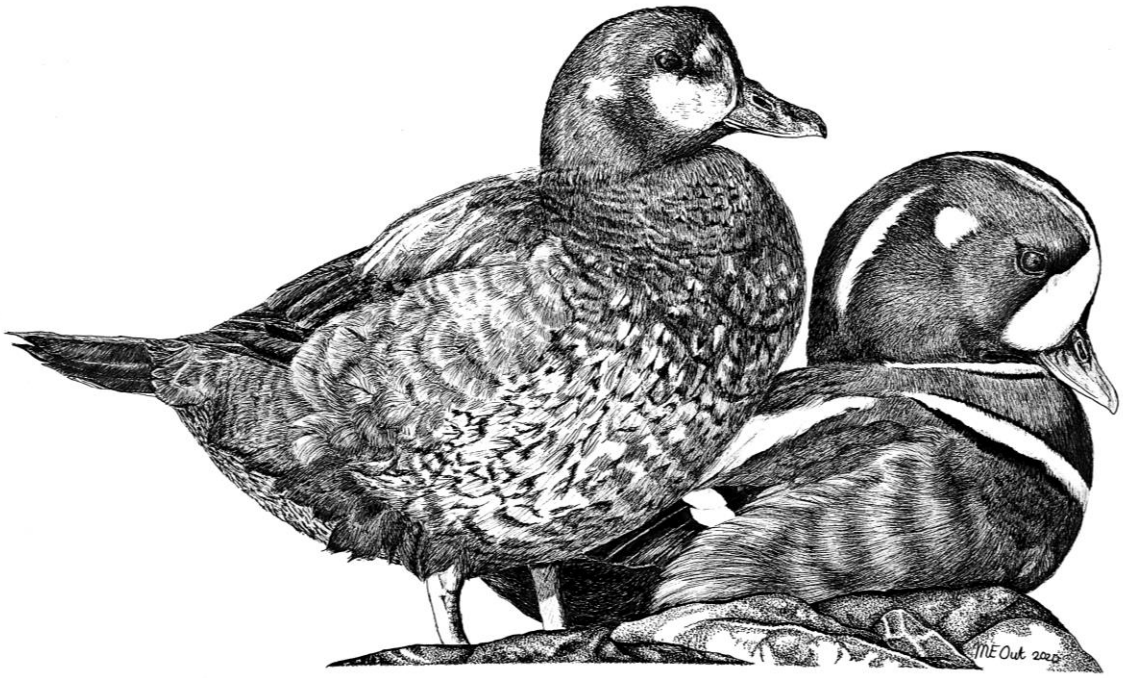
Harlequin ducks breed at low densities on montane streams across a large geographic region. Variation within and between watersheds can make it difficult to isolate the effects of local stressors within a broader suite of overlapping and interacting environmental factors influencing harlequin presence and abundance. For example, fish introductions may lower harlequin breeding habitat quality, either directly, through competition for food resources, or indirectly, by inducing antipredator behaviours in shared invertebrate prey or attracting predators, such as eagles. However, fish presence can be confounded with stream size, elevation, and reach gradient. To disentangle these effects, I used field surveys and complementary habitat suitability modeling in MaxEnt. I constructed candidate models using suites of biogeographic variables hypothesized to affect breeding harlequin duck presence and abundance. Proportion of grassland and shrubland was the best predictor of true absences in the field surveys, denoting unsuitable harlequin habitat. Annual temperature range contributed the most information to the final MaxEnt model, with low temperature range being most suitable (coast and mountains). Both analyses found habitat suitability increased with stream order, but order alone was insufficient in predicting harlequin distributions, likely because stream orders were fairly evenly distributed. Overall, climate and distance to coast appeared to constrain harlequin distributions at the broadest spatial scale while stream order seemed to be a subsequent environmental "filter." Lastly, I tested the feasibility of making inferences about breeding habitat using body tissue stable isotopes from recent arrivals to the wintering grounds. To account for the incorporation of wintering habitat isotopes, I created models of claw and blood cell isotopic turnover using flight feather regrowth as an index of time since arrival. Claw turnover mirrored blood cell turnover, but with a "lag" due to the time required for "current" claw tissue isotopes to grow from the base to the tip of the claw. In stream invertebrates collected during the field surveys, $\delta^{13}C$ decreased with distance from the coast while $\delta^{15}N$ increased with river order. As both factors were also associated with habitat suitability, isotope turnover back-calculations may offer a large scale method to examine harlequin duck breeding habitat usage.



**Keywords**: harlequin ducks, AIC, MaxEnt, stable isotopes, habitat suitability, isotope turnover model

# Dedication

For Jessie and Dean



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# Chapter 1. General Overview

## 1.1. Introduction

Harlequin ducks are small, Holarctic sea ducks that spend the majority of the year at sea but migrate inland to fast-flowing mountain streams to breed (Palmer, 1976). In North America, they have two distinct populations: one on the east and another on the west coast. Despite small population increases, the eastern North American population of harlequin ducks is considered a species of special concern, due to low numbers and their tendency to aggregate in open waters during winter, making them extremely susceptible to catastrophic events such as oil spills (COSEWIC, 2006). The western population is Yellow-listed in British Columbia (BC) and Alberta, Canada, where there is concern for its stability due to long-term declines in numbers (Smith et al. 2001). Harlequins are also considered a sensitive species in most of the north-western United States (Robertson & Goudie, 1999).

Harlequin ducks are sensitive in part due to their migratory behaviour, which makes them reliant on a variety of habitat types. For example, in western North America, harlequin ducks migrate hundreds of kilometers inland to breed in the coastal and interior mountains. Further, they respond to different habitat requirements throughout distinct pre-nesting, nesting, and brood-rearing periods (MacCallum et al., 2006, 2016; Robertson & Goudie, 1999). Pre-nesting tends to occur on some of the larger river reaches within a watershed (Bruner, 1997; MacCallum et al., 2016; Rodway, 1998) although there appears to be an upper limit (Rodway, 1998). Nesting occurs higher up in the same watershed, on smaller, headwater streams, where food may be less available, but nests are obscured by dense vegetative coverage (MacCallum et al., 2016). Brood-rearing occurs at an intermediate reach sizes and elevation within the same watershed. Selection of pre-nesting, nesting and brood-rearing sites therefore appear to each be driven by different sets of habitat requirements (MacCallum et al., 2016).

These changing habitat requirements expose migratory harlequin ducks to a variety of environmental conditions and stressors throughout the annual cycle. Habitat requirements can also vary over different spatial and temporal scales, the extent of which is strongly linked to the biology of the species (Mayor et al., 2009). Long-range

migrants, such as harlequin ducks, likely respond to a hierarchical set of habitat requirements, from the landscape scale, to the watershed, river reach, all the way down to the feeding or nesting site (Jones, 2001). Each of these scales and habitats are also being affected by humans, e.g. climate, terrain, vegetation, land use, and other human impacts, such as fish introductions. This can make it difficult to isolate the effects of local stressors within a broader suite of overlapping and interacting environmental drivers influencing species presence and abundance. For example, the introduction of fish to fishless waters across the harlequin duck breeding range has been suggested to lower habitat quality for harlequin ducks, either due to direct competition for food resources (freshwater invertebrates) (Goudie & Ankney, 1986; Robertson & Goudie, 1999) or indirectly, due to the antipredator behaviors or adaptations fish presence induces in these shared prey resources (LeBourdais et al., 2009). However, fish presence also tends to be confounded with stream size and gradient, since fish are more likely to be present in large, lower elevation reaches, making it difficult to disentangle the impacts of fish-induced food web effects from habitat differences that occur along the river continuum from headwaters to the ocean (Vannote et al., 1980), as well as differences among watersheds, for example, in terms of dominant land cover type.

The addition of fish has the potential to impact harlequin habitat quality directly, through competition that reduces the abundance or biomass of harlequin duck invertebrate prey (e.g. Forrester, 1994). Harlequins are income breeders: stream invertebrates are their main food source during the breeding season (Robert & Cloutier, 2001) and all of the nutrients used for egg production come from breeding ground nutrients, rather than fat storage of marine nutrients from the wintering season (Bond et al., 2007) and harlequin duck breeding densities have been shown to be related to invertebrate abundance and biomass (LeBourdais et al. 2009). Fish also have the potential to impact harlequin habitat quality indirectly, by inducing invertebrate antipredator behaviors or adaptations in these shared prey resources. Fish can cause invertebrates to switch from aperiodic to nocturnally-biased feeding and drift behaviors (Culp & Scrimgeour, 1993; Douglas et al., 1994; Flecker, 1992; Forrester, 1994; McIntosh & Townsend, 1996; Peckarsky et al., 1994) and becoming less active (e.g., hiding more, dropping activity such as drifting during the day and increasing it at night, choosing larger substrates to reside under, or digging deeper into the benthos). This can negatively impact harlequin abundance, given that they are visual, daytime predators

(LeBourdais et al., 2009; Robertson & Goudie, 1999). Fish presence and abundance can also result in invertebrates growing faster and emerging sooner at smaller body sizes (Peckarsky et al., 2001, 2002), homogenize invertebrate distribution throughout a reach, or dispersing from rivers with fish, all of which would reduce the availability of high value resource patches (Crowl et al., 1997).

The anthropogenic addition of fish to historically fishless waters therefore has the potential to be problematic for harlequin ducks, given the numerous high mountain lakes in the western harlequin duck breeding range that have been stocked with fish. For example, in western Canada, there are 1,464 lakes in the mountain National Parks, 95% of which were fishless prior to 1900 (Donald, 1987). Between 1900-1980, 305 (21%) of these lakes were stocked with some combination of cutthroat trout, *Salmo clarki*, rainbow trout, and brook trout, *Salvelinus fontinalis.* In the western U.S., more than 95% of the approximately 16,000 high mountain lakes (> 800 m elevation) would have been naturally fishless as they lie upstream of Pleistocene-age barriers to colonization (Bahls, 1992). This area has experienced extensive fish stocking. This is potentially concerning, as part of that area overlaps with the southern extent of the western harlequin duck breeding range, and harlequins still breed in this area. More than 60% of these lakes now contain trout; furthermore, 95% of deeper (>3 m) and larger (>2 ha) lakes contain trout (Bahls, 1992). Trout are still regularly stocked in 7000 mountain lakes (Knapp et al., 2001), and 70-80% of Sierra Nevada large lakes (>1 ha) have self-sustaining populations (Matthews & Knapp, 1999). This common practice of introducing fish to fishless waters across the harlequin duck breeding range is concerning if harlequin duck habitat quality is lowered by disrupting the availability of preferred insect food (LeBourdais et al., 2009).

Fish introductions can also indirectly impact harlequin breeding by attracting predators such as raptorial birds (Heath et al., 2006) or mustelids (Garwood et al., 2013) and allowing them to expand their ranges. Looking at a variety of habitat characteristics over different spatial scales may provide the best chance of isolating the effects of a single stressor on harlequin duck presence and abundance, while simultaneously enabling me to test and control for the effects of biogeoclimatic gradients and stressors.

## 1.2. Methods and Main Results

Given this problem of detecting an effect of fish while also needing to account for multiple interrelated environmental influences on harlequin duck distribution and abundance, in Chapter 2, I competed several categories of potential models (hypotheses) of harlequin duck presence and abundance based on streamside duck surveys, invertebrate sampling, and habitat measurements from several sites selected across southern BC and Alberta. Models were built using suites of related biogeographic variables representing several categories of factors hypothesized to affect breeding harlequin duck presence and abundance, including climate, terrain, vegetation and land use, invertebrate abundance and biomass, and human disturbance. This approach allowed me to examine the effects and relative importance of a range of biogeoclimatic variables that vary over different spatiotemporal scales on breeding harlequin duck density. It also allowed me to determine whether fish presence alters breeding harlequin duck densities relative to what would be predicted based on other biogeoclimatic variables that vary among and within watersheds. I also tested the same set of hypotheses on several measures of invertebrate abundance and biomass to understand how these biogeoclimatic factors may affect this important food resource within harlequin duck breeding habitat. I did this because: (1) invertebrates are the main food source for harlequin ducks during the breeding season; (2) if harlequins are mostly affected by invertebrates (and the effect of fish on invertebrates), then harlequins should be affected by the same environmental factors that affect invertebrates; and, (3) so that I could control for differences in these environmental factors when testing for the effects of site- and reach-level impacts, such as fish presence, and anthropogenic activities such as site disturbance, agricultural intensity, and urban areas, including roads and highways.

Chapter 2 revealed some support for each hypothesis of harlequin duck presence and abundance, but some were more explanatory than others. When examining the zero-inflation component of the harlequin models, one variable was consistently identified as the strongest predictor of true harlequin duck absences: the higher the proportion of grass and shrublands, the more likely that observed absences were true absences, and not simply failures to observe harlequin ducks during the streamside surveys. Thus, a high proportion of grass and shrublands in the area seemed to be an indicator of poor general habitat suitability. However, within suitable

watersheds, stream order was consistently the best predictor of harlequin abundance. Specifically, harlequin abundance was most strongly predicted by increasing stream order, or related variables. Stream width, which was positively correlated with stream order, and site slope, which was negatively correlated with order and width, were most often implicated in top models of harlequin abundance. This may have been due to invertebrate availability as several key taxa were also strongly related to stream size (order, width, site slope). Therefore, harlequin habitat suitability appeared to be first limited by land cover elements, then further refined by stream order. The addition of fish to the final harlequin duck presence and abundance models resulted in a non-significant positive effect of fish on harlequin duck abundance; however, the addition of fish did not provide more information than was already given by the more parsimonious models that included only an effect of stream order on abundance and percentage of grass, shrublands, and site slope on presence.

One concern that I had with Chapter 2 was that it necessarily focussed on only a handful of paired reaches. To improve site selection, I would recommend using mapping tools and habitat suitability algorithms, such as MaxEnt, to identify a subset of representative watersheds from across the western breeding range prior to surveying and sampling. I would then use provincial, territorial, and federal fish survey repositories to identify fishless reaches first, then match those to biogeophysically similar reaches within the same watershed. I would also increase the number of sites per reach to obtain a better overall measure of habitat suitability, rather than the characteristics of a single 100 m site. This would allow for a more biologically-relevant measurement (i.e. at the scale of harlequin duck reach or site selection) of habitat characteristics such as canopy, width, as well as invertebrate availability. I would also obtain water chemistry measurements for factors likely influencing invertebrates, such as pH and dissolved oxygen. I would survey for aerial predators while conducting the harlequin duck surveys to gain an indication of relative predation pressure within and across watersheds. I would consider adding a behavioural component to the duck surveys, such as observing the amount of time spent feeding, or the tendency to flush or continue feeding when disturbed, which could provide an integrated indication of both the amount of food available and the relative safety of the site. I would also consider using an invertebrate database such as the Canadian Aquatic Biomonitoring Network (CABIN) to obtain standardized, quality-controlled invertebrate data, either instead of or complementary to

the field samples. One limitation of the CABIN database is that it only provides estimates of the relative abundance of each taxon and not necessarily overall site abundance or biomass of invertebrates (i.e. food availability).

Since I was unable to sample the full range of potential sites, let alone the entire western harlequin duck breeding range, in Chapter 3, I tested the robustness of Chapter 2's results. I used MaxEnt to create habitat suitability models for breeding harlequin ducks using recent archives of publicly available sightings during the breeding season (May – August). I took this approach because looking at a broad range of environmental variable combinations may provide the best chance to disentangle the individual effects of impacts of interest (such as specific suspected human impacts), as well as identify the habitat characteristics most likely to exacerbate those impacts. My goal was to determine which environmental variables were most important to the western North American population of harlequin ducks using a larger observational dataset and continuous environmental layers to determine whether the same effects found in Chapter 2 persisted in a larger, presumably more geographically and environmentally representative dataset.

Similar to Chapter 2, I found support for all hypotheses (categories of biogeoclimatic variables) describing harlequin duck habitat suitability: climate, terrain, vegetation and land use, and human disturbance, but some variables were more explanatory than others. Annual temperature range contributed the most to the final model (which combined the top explanatory variables from each hypothesis, i.e., suite of environmental variables), suggesting that temperature range may determine habitat suitability at the largest spatial scale. This is consistent with other studies of species distributions (Baldwin, 2009). Ducks preferred areas with a low temperature range, which includes the coast and mountain areas. Outside of those areas, selection at finer spatial scales may not even occur. For example, a watershed that does not occur within the ideal temperature range should likely not be evaluated at the reach or site level; the entire region is simply not suitable.

Consistent with Chapter 2, stream order was important enough to be retained in the final harlequin duck models but was insufficient on its own to describe harlequin duck distribution. This may be due to the fact that streams of all orders were distributed fairly evenly across the geographic space. As such, the models in Chapter 3 suggest that

climate may constrain harlequin duck distributions at the broadest spatial scale, while stream order provides a subsequent environmental “filter” through which to refine habitat suitability. Interestingly, harlequins preferred stream orders 3-5 and 7, but seemed to disproportionately reduce their usage of sixth order streams, which were also found to be the most suitable for bald eagles. It appears harlequins may be avoiding these aerial predators, whose ranges may be expanded now due to the widespread introduction of fish. The distance to the coastline also reduced predicted habitat suitability, suggesting a migration distance limit. Future studies should discriminate between pre-nesting, nesting, and brood-rearing sightings and related biogeoclimatic variables and consider the amount of each habitat type available at the watershed level. This would better account for the range of habitat types required by harlequin ducks during the breeding season.

One of the other drawbacks of using streamside surveys to ascertain breeding ground information (as I did in Chapter 2) is that surveys could only be conducted on an average of 0.32 reaches per day (since the unit of replication is the reach, not the duck, on the breeding grounds) over a field season of 1-2 months. Thus, my technique of using streamside duck and habitat surveys necessarily limited the amount of information I could obtain across the breeding grounds. This low sample size problem was further exacerbated by the fact that I could only travel to a limited number of reaches, given the distance. As a result, I could only capture information on a small portion of the larger geographic range of the breeding grounds. I tackled this problem using a MaxEnt approach (Chapter 3), but I was also able to tackle it in a second way, using stable isotopes. Harlequin ducks have a behavioural characteristic associated with their migration that makes stable isotopes and appropriate tool for quantifying information about harlequin duck breeding grounds. Following breeding, harlequins aggregate in large numbers on their wintering grounds. As a result, they come from far and wide, mixing to a much greater extent than during the breeding season. Additionally, harlequin ducks are easily captured shortly after their arrival on the wintering grounds, as they complete a full flight feather moult. Because of this, I was able to capture 67 harlequin ducks in a very short (7 day) period. After capture, I collected body tissues (blood, claws, feathers, and fecal matter) for subsequent stable isotope analysis. Chapters 4 and 5 describe my methodological approach and how I was able to use these stable isotopes to back calculate potential breeding ground information. My back-calculations of

breeding ground isotopes enabled me to draw information from a larger geographic range than I could have using only my streamside surveys.

Stable isotopes are ideal for this sort of big picture overview of harlequins because harlequin diet changes between breeding grounds and wintering grounds. As harlequins migrate from inland freshwater environments to their marine wintering habitat, they experience a non-overlapping dietary shift in both $\delta^{15}N$ and $\delta^{13}C$ signatures (Bond et al., 2007). Marine environments tend to be more enriched in $\delta^{13}C$ than freshwater and terrestrial habitats. Further, both $\delta^{13}C$ and $\delta^{15}N$ tend to decrease with distance from marine inputs, with $\delta^{13}C$ being especially indicative of distance to coast (Chapter 5). Since harlequin ducks appear to select habitat based at least partially on distance to coast and stream order, and both appear to have effects on $\delta^{13}C$ and $\delta^{15}N$, I thought I may be able to make some inferences about harlequin duck breeding grounds using tissues collected on the wintering grounds.

Stable isotopes are naturally occurring, non-radioactive forms of elements that contain additional neutrons, making them heavier (Fry, 2006). They exist at predictable ratios relative to their more common, lighter forms, As a result, they can be used to trace the flow of nutrients through food webs and ecosystems. Stable isotopes provide a record of diet over different temporal scales, depending on the rate of tissue turnover (Tieszen et al., 1983). Tissues incorporate stable isotopes from the diet at rates proportionate to their respective rates of metabolism and growth (Boecklen et al., 2011). When individuals change their diet, for example, due to migration to a new habitat with different baseline isotope signatures, the isotope signature in the new diet becomes incorporated into their body tissues in a predictable way. In tissues that regenerate over time (e.g., blood), isotope signatures follow an exponential decay curve over time, wherein half of the old isotope signature is replaced by the new signature at a constant rate. Tissues with a lower rate of cellular replacement incorporate new isotope signatures at a lower rate following a dietary switch. Since biological processes modify these ratios in predictable ways, inferences can be made about dietary habits, habitat use, movement patterns, reproduction, spatial and temporal distribution, and trophic level, depending on the isotope and the growth and turnover rates of the tissues being analysed (Boecklen et al., 2011; Hobson, 1999). As a result, stable isotopes offer a glimpse into conditions in preceding seasons.

In contrast to continuously regenerating tissues, inert tissues, such as feathers, provide a stable (no turnover) record reflective of diet at the time of tissue formation. As a result, feather isotopes have been used to determine molting, breeding, and wintering locations of migratory birds at times outside of those periods. Claws also provide an intriguing glimpse into current and former conditions as they provide an inert record of diet at the time of growth, but they also grow continuously. Thus, claws contain within them a few months' worth of dietary records. Following a dietary shift, claws will consist of a gradient of old to new isotopic signatures, as new claw layers representing the new diet are slowly built up and migrate towards the distal tip of the claw (Hahn et al., 2014). As such, claw stable isotopes turn over more slowly than most tissues used for stable isotope analysis. Chapter 4 investigates the relative turnover rates for blood plasma, blood cells, and claws in a natural population of migratory sea ducks.

In Chapter 4, I modelled stable isotope turnover in harlequin duck claws, based on population-level isotopic "turnover" in claws and blood of recent arrivals on the wintering grounds. Many studies have been conducted with the intention of using claw stable isotope signatures in order to make inferences about pre-migration habitat, only to lament the rapid incorporation of isotopic signatures from the new, post-migratory diet (e.g., Bearhop et al., 2003; Hahn et al., 2014; Mazerolle & Hobson, 2005). A potential application of isotope turnover models is to use isotope turnover curves to obtain a back-calculated estimate of pre-migratory habitat isotope signatures. This approach could mitigate the problem of new dietary isotopes being incorporated into inert, but continuously growing tissues. I therefore conceptualized and explored a variety of turnover models in this chapter. The model that best fit the observed data was one in which claw isotope turnover mirrored, but lagged behind, blood cell isotopic turnover. The additional lag time was due to the time required for "current" claw tissue isotopes to migrate (via claw growth) from the base to the tip of the claw.

In Chapter 5, I used the isotopic turnover model that I developed in Chapter 4 and combined it with isotopes from the invertebrates that I had collected for Chapter 2 in order to explore the potential to draw inferences about breeding habitat associations based on isotopes sampled from harlequin ducks during the wintering season. Both $\delta^{13}C$ and $\delta^{15}N$ in invertebrates tended to decrease with distance from marine inputs, with $\delta^{13}C$ being especially indicative of distance to coast. $\delta^{15}N$ was most associated with river order and tended to increase with stream size, which has repeatedly been found to be a

variable of interest for harlequin duck breeding habitat site selection (e.g., Bond et al., 2007; Crowley, 1993; MacCallum et al., 2016). Since $\delta^{15}N$ increases with each trophic level, larger rivers with more trophic levels could have higher mean $\delta^{15}N$ signatures within their invertebrate communities. Since harlequin ducks appear to select habitat based at least partially on distance to coast and stream order, and both appear to have effects on $\delta^{13}C$ and $\delta^{15}N$, there appears to be some potential to make breeding ground inferences from wintering harlequin duck body tissues.

Future studies could include blood plasma isotopes as well, to account for different current dietary signatures in the wintering ducks, if any. Future studies could also consider using isotope maps as priors in habitat suitability algorithms such as MaxEnt and use back-calculated isotope values as "observations" to determine whether animals are disproportionately using habitat types associated with certain isotopic signatures.

## 1.3. Discussion

Harlequin duck distribution appeared to be associated at the broadest spatial scale with annual temperature range; harlequin habitat suitability was highest where annual temperature range was low. This type of habitat mainly occurred in coastal and montane regions and were associated with temperate needleleaf and mixed forests. Similarly, in the field surveys, a high proportion of grass and shrublands was the strongest predictor of harlequin absence. This habitat tended to occur at lower elevations and was associated with a continental climate, with relatively low amounts of precipitation and both higher mean annual temperature and temperature range. Within apparently preferred watersheds (coastal and montane), harlequin abundance increased with increasing stream size (order).

By definition, stream order is lowest in headwaters high up in the watershed and increases as one moves to larger, less steep reaches lower in the watershed. The increase in harlequin abundance observed with increasing stream order could be due larger streams representing more productive feeding habitat during the pre-nesting period. Harlequins are income breeders, acquiring all of the resources for egg production on the breeding grounds, rather than from winter fat stores (Bond et al., 2007); therefore, nutrient acquisition is of high importance during the pre-nesting period. Larger river

reaches were also found to contain higher numbers of scrapers and pupae than smaller reaches. These prey items may therefore be of value to pre-nesting harlequin ducks. Large pupae, such as *Glossosoma spp.*, in particular may represent an energy-rich source of nutrients that are often clustered in large groups, providing a high value, immobile source of energy. Additionally, small scraper abundance increased with river width, but only on streams containing harlequins. While this may suggest a preference for small scrapers, another interpretation could be that harlequin ducks release small scrapers from predation by large predators, such as stoneflies, which are known to be a preferred prey item (McCutchen, 2002). Additionally, harlequins may also use larger stream sections during the pre-nesting period as higher elevation reaches are still frozen (MacCallum et al., 2016).

The addition of fish to the final models of harlequin duck presence and abundance was negligible, providing no additional information to what was already given by the amount of coastal and montane habitat vs. continental habitat (presence) and stream order (abundance). If anything, there was a non-significant positive effect of fish on harlequin duck abundance. It is noteworthy that this result, though non-significant, is directly opposite to that predicted by one of the main hypotheses driving this study: that the addition of fish disrupts food webs in such a way that important invertebrate prey are less available to breeding harlequin ducks (LeBourdais et al., 2009). In fact, fish may provide some benefits to harlequin ducks, by providing them with additional food resources, such as eggs (e.g. Hunt, 1998; Smith et al. 1997), or even large nutrient subsidies, such as those provided by spawning anadromous species (Crowley, 1993).

While there was no direct indication of a negative association between fish and harlequins, nor between bald eagles and harlequins, the MaxEnt model indicated that harlequins seemed to use sixth order streams less than they did fourth or fifth order streams. Sixth order streams were determined to be the most suitable for bald eagles, potentially signalling a weak tendency for harlequins to avoid these streams more than expected. While fish may provide nutrient subsidies to harlequin ducks in some cases, fish introductions may increase predation pressure from raptors and terrestrial predators, such as mustelids. Eagle numbers have been on the increase since their habitat was federally protected and DDT (dichloro-diphenyl-trichloroethane) banned in 1970s. While eagle numbers returning to what they once numbered should not necessarily be bad for harlequins per se, fish have now been introduced across the landscape and at higher

elevations than prior to the bald eagle crash. This may result in increased eagle numbers as well as range expansion, such as to watersheds that were previously fishless, or had very little fish. If this is the case, harlequins may be affected by multiple stressors, particularly in high elevations where fish are more likely to be introduced, potentially disrupting food webs or attracting additional predators. The highest elevation reaches are also already smaller streams, which are less preferred by harlequins (Chapters 2 and 3) with more variable flows which can lead to lower invertebrate stocks (Bond et al., 2007). As such, fish introductions may add to other sources of lowered habitat suitability for harlequins breeding on these reaches, and should be considered within the local and historic context, rather than attempting to identify a single effect of "fish" range-wide. Additionally, previous studies have also found harlequin duck presence and abundance to be determined more strongly by habitat features associated with predation than by food availability (e.g. Heath et al., 2006; MacCallum et al., 2016). Open canopy and with dense streamside vegetation may facilitate vigilance for, but also nearby cover from, aerial predators during the pre-nesting period (Heath & Montevecchi, 2008; MacCallum et al., 2016).

Future studies of harlequin duck breeding season habitat should begin by first determining broad scale habitat suitability as determined by climatic restrictions. Watersheds should then be screened by the overall availability of suitable pre-nesting, nesting, and brood-rearing habitat, using both vegetation (e.g. forested vs. grass-shrubland areas) and terrain variables. Within suitable watersheds, habitat should then be evaluated along linear river lengths, looking at the overall availability of suitable feeding sites (pre-nesting), covered nesting sites (nesting) and brood-rearing habitat as determined by canopy and streamside vegetation (MacCallum et al., 2016). These habitat associations may also differ among watersheds that experience different levels of predation pressure. For example, landscape features that facilitate predator avoidance or vigilance may be of higher importance where predation pressure is high, whereas features that influence prey availability may take precedence where predation pressure is lower.

Migratory birds may have a metapopulation structure (Esler, 2000). Heath et al., (2006) found that, in the eastern North American population, harlequin duck habitat and prey availability did not differ between source and sink populations of harlequin ducks. Rather, sink populations occurred in areas with higher densities of nesting ledges for

raptorial birds. As such, birds of prey may limit harlequin ducks from using otherwise suitable breeding habitat. Source populations were also larger and less variable among years (Heath et al. 2006). If breeding density is correlated with local population stability, my findings may suggest that larger order rivers tend to carry more stable, possibly source populations of harlequin ducks. This may be because larger rivers tend to have less variable flow and correspondingly higher invertebrate biomass than smaller rivers (Bond et al. 2007). Heath found that habitat requirements were prioritized differently for sources population (where food availability was most important) and sink populations (where availability of raptorial nesting ledges, and associated birds of prey, were most associated with harlequin duck density).

Populations that are in less favourable habitats act as sinks as they lose individuals due to mortality or emigration. However, these sink populations can be replenished by source populations. Since habitat requirements for harlequin ducks have been found to differ between source and sink populations (Heath 2006), it may not make sense to expect the effect of fish to be consistent across the entire landscape. It may be more important to examine the effect of fish in each context (source vs. sink being only one possible categorization or context), how prevalent each context is currently, and how that prevalence has changed as a result of past fish introductions. In other words, prior to widespread human-facilitated fish introductions, what proportion of watersheds would have historically been sources and what proportion of watersheds would have historically been sinks?

It would also be important to consider which watersheds already had fish and which ones did not prior to fish introduction. What was the effect of introducing fish into those different types of watersheds? Adding extra fish to a system that already had fish would be much different, all else being equal, than the effect of adding fish to a place that had no fish in it whatsoever. Further, the effect would also be modified by whether the fish species being added were native, introduced, or invasive, and the history of introduction and ongoing stocking. After all, the effect of adding one additional species to a waterway with 15 other species already present would likely be different than the effect of adding three species to a waterway with only two benthic feeding species. Future research should also consider the impact of benthic versus column feeding fish. Many sports fish, which humans love to introduce and continuously stock in waterways for recreational fishing, are aggressive, visual hunters, that feed actively in the water

column, moving quickly back and forth through fast flowing water and catching drifting and floating invertebrates. While this makes fishing more fun, it may also increase the impact of fish, both direct and indirect, on harlequin food sources. Benthic feeding fish, on the other hand, are relatively systematic hunters, feeding on slower moving crawlers and clingers who cling to the benthos surface. As a result, benthic feeding fish may have less of a competitive impact on harlequin food supply. Therefore, the effects of fish on harlequin ducks should depend on a number of factors that my models did not consider. Regardless, the fact that there are so many potential ways that fish might affect food webs through various prey- and predator-mediated relationships means that their presence and stocking efforts must be taken into account for any management-related decision-making or in consideration of other impacts.

Given all this, it may be useful to investigate whether the widespread introduction of fish was done in such a way that (potential) sink populations have now increased and source populations decreased, leading to a decrease in overall population numbers. To address this, future studies should first determine which biogeoclimatic variables in the western population distinguish source from sink by determining which variables are most important in a source and whether they differ from the most important variables in a sink. Using these environmental predictors combined with local estimates of population size and stability, it should be possible to determine the probability that a sub-population is a sink or a source. Using local knowledge, historical surveys, and past biogeoclimatic data, it may then be possible to investigate the extent to which the proportion of sinks to sources has changed. Finally, it may even be possible to supplement this estimate with stable isotope signatures from breeding ground tissues or use isotope turnover models to account for incorporation of new dietary isotopes and back-calculate to pre-migratory isotopic signatures. This could be done with both current studies capturing live animals, to make inferences about current conditions, combined with analysis of museum specimens, to make inferences about past conditions. Management-related decision-making on the Yellow-listed western population in BC and Alberta could then focus on protecting the subset of watersheds that are already source populations, and improving the quality of current sink watersheds, with the goal of turning some into sources.

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# Chapter 2. Effects of biogeoclimatic factors and local stressors on freshwater invertebrates and harlequin ducks during the breeding season

## 2.1. Introduction

Migratory species respond to a range of habitat requirements throughout the year (Newton, 2004). This can make it difficult to isolate the effects of local stressors within a broader suite of overlapping and interacting environmental drivers influencing species presence and abundance. Habitat requirements also vary over different spatial and temporal scales, the extent of which is strongly linked to the biology of the species (Mayor et al., 2009). Long-range migrants, such as harlequin ducks, likely respond to a hierarchical set of habitat requirements, from the landscape scale, to the watershed, river reach, all the way down to the feeding site (Jones, 2001).

Habitat requirements can also change throughout a single season. For example, during the breeding season, harlequin ducks inhabit and respond to different habitat requirements through distinct pre-nesting, nesting, and brood-rearing periods (MacCallum et al., 2006, 2016; Robertson & Goudie, 1999). Depending on the period, harlequins use reaches from shallow, headwater streams, to intermediate sized rivers. Of all the stream reaches used throughout the breeding season, pre-nesting occurs on the largest river reaches (Bruner, 1997; MacCallum et al., 2016; Rodway, 1998b). Nesting occurs higher up in the same watershed, on smaller, headwater streams, where food may be less available, but nests are obscured by high vegetative coverage (MacCallum et al., 2016). Finally, brood-rearing occurs at an intermediate reach size and elevation within the same watershed. Selection of pre-nesting, nesting and brood-rearing sites appear to each be driven by different sets of habitat requirements (MacCallum et al., 2016).

These overlapping and interacting habitat requirements can make it difficult to separate the effects of local stressors from the environmental gradients with which they might be confounded. For example, the introduction of fish to fishless waters across the harlequin duck breeding range has been suggested to lower habitat quality for harlequin ducks, either due to direct competition for food resources (freshwater invertebrates) (R. I. Goudie & Ankney, 1986; Robertson & Goudie, 1999) or indirectly, due to the

antipredator behaviors or adaptations fish presence induces in these shared prey resources (LeBourdais et al., 2009).

However, fishless reaches that can be used as reference sites in impact studies are relatively rare, especially for mid-sized rivers preferred by breeding harlequin ducks (Bruner, 1997; E. F. Cassirer et al., 1996; Machmer, 2001; Morneau et al., 2008; R. L. Wallen & Groves, 1988; Richard L Wallen, 1987). Following the last glaciation, fish would have recolonized river systems from the bottom up. The higher up in the watershed they reached, the more likely they would be to encounter an increasing density of barriers such as waterfalls, prohibitively steep gradients, and underground or intermittent flows. Additionally, humans are more likely to introduce fish to the most accessible rivers, which tend to be lower in elevation with low terrain ruggedness. As such, fish presence tends to be confounded with other environmental variables known to be important to breeding harlequin ducks, such as stream width and gradient and elevational position within the watershed.

Furthermore, the effects of stressors could change across the species' distribution, dependent on both broad-scale and local environmental contexts. The relative importance of different biogeoclimatic drivers in determining species usage of a habitat patch can be context-dependent. For example, while MacCallum et al. (2016) found that invertebrate prey biomass had some effect on harlequin duck pre-nesting distributions within watersheds, features that facilitated vigilance for predators, such as a clear line of sight up and downstream and little to no overhead canopy, were more important. Similarly, Heath et al. (2006) found no difference in habitat or invertebrate characteristics between harlequin duck source and sink populations, but found that the availability of nesting ledges and associated densities of raptorial birds was the main factor determining whether a local population was low and unstable, or high, stable and a source of emigrants.

Harlequins are income breeders: stream invertebrates are their main food source during the breeding season (Robert & Cloutier, 2001) and all of the nutrients used for egg production come from breeding ground nutrients, rather than fat storage of marine nutrients from the wintering season (J. C. Bond et al., 2007). Availability of this important food resource is also tied to harlequin breeding propensity (Bengtson & Ulfstrand 1971). In years of low food availability, many females defer breeding (Bengtson, 1972).

Variation in aquatic insect abundance among years is highly correlated with variation in productivity (number of young) (Bengtson, 1972; Gardarsson & Einarsson, 1994, 2004). The addition of fish can cause invertebrates to switch from aperiodic to nocturnally-biased feeding and drift behaviors (Culp & Scrimgeour, 1993; Douglas et al., 1994; Flecker, 1992; Forrester, 1994; McIntosh & Townsend, 1996; Peckarsky et al., 1994) and has caused mayflies to develop faster and emerge sooner and at smaller sizes (Peckarsky et al., 2001, 2002). Fish presence also tends to homogenize invertebrate distribution throughout a reach, reducing the availability of high value resource patches (Crowl et al., 1997). All of these changes can decrease prey availability or biomass for harlequin ducks, which are visual, daytime predators (LeBourdais et al., 2009; Robertson & Goudie, 1999).

Food web disruptions, such as those caused by the introduction of fish to previously fishless mountain streams can affect harlequin ducks by disrupting the availability of preferred insect food (LeBourdais et al., 2009). Alternatively (or additionally), fish introductions can also indirectly impact harlequin breeding by attracting predators, such as raptorial birds (Heath et al., 2006) or mustelids (Garwood et al., 2013). Looking at a variety of habitat characteristics over different spatial scales may provide the best chance of isolating the effects of a single stressor on harlequin duck presence and abundance, while simultaneously enabling me to test and control for the effects of biogeoclimatic gradients and stressors.

I collected field data on habitat characteristics, invertebrate abundance, biomass and density at multiple watersheds across the breeding range of harlequin ducks in western Canada. Within these watersheds, I then compared harlequin duck presence and abundance on streams with and without fish. This large-scale approach allowed me to (1) examine the effects and relative importance of a range of biogeoclimatic variables that vary over different spatiotemporal scales on breeding harlequin duck presence and abundance, and (2) determine whether fish presence alters harlequin duck presence and abundance relative to what would be predicted based on other biogeoclimatic variables that vary among and within watersheds. I also tested the same set of hypotheses on several measures of invertebrates. I predicted that, if harlequin duck presence and abundance are mostly driven by different components of invertebrate availability, then harlequins should be affected by a similar set environmental variables as those that affect invertebrates. I also tested for this explicitly by testing the effects of

select invertebrate variables on harlequin duck presence and abundance. Conversely, I predicted that, if harlequins experience habitat restrictions through mechanisms unrelated to invertebrates, the sets of variables affecting harlequins and invertebrates should be different, or not completely overlap.

Identifying the habitat and invertebrate variables that most affect harlequin duck presence and abundance then allowed me to control for differences in those variables when testing for site- and reach-level impacts such as fish presence, and anthropogenic activities such as site disturbance, agricultural intensity and urban buildup. For example, large, low-elevation streams are more likely to contain fish and be impacted by anthropogenic activities; therefore, I needed to control for any potential biogeoclimatic biases inherent to impacted vs. non-impacted sites, as well as account for effects from multiple impacts.

### 2.1.1. Hypotheses

Harlequin duck presence and abundance may be directly and indirectly affected by a variety of abiotic and biotic factors (Table 2.1). Previous studies have found benthic macroinvertebrate abundance or biomass to have positive effects on harlequin duck presence (MacCallum, 2016; Rodway, 1998b) and abundance (Bond et al., 2007; LeBourdais et al., 2009). However, habitat features facilitating predator vigilance may be of higher importance where predation pressure is relatively high (e.g. Heath et al., 2006; Heath & Montevecchi, 2008; MacCallum, 2016). Additionally, among and within watershed differences in other biogeoclimatic factors, such as climate, topography, vegetation, and land use, must all be considered or, at least, controlled for, when attempting to isolate the effects of specific potential stressors, such as food web effects of fish or impacts of land use changes such as agriculture or urbanization. As such, I considered several non-mutually exclusive hypotheses pertaining to the presence and abundance of harlequin ducks in BC and Alberta, the potentials mechanisms for which are explored in more detail below.

**Table 2.1 List of hypotheses considered in this Chapter, along with proposed mechanisms of how they might affect invertebrate or harlequin duck abundance.**

| Hypothesis | Effect | Mechanism | Predictions | Studies |
|---|---|---|---|---|
| Climate | Indirect | Mediated through changes to invertebrate prey | Harlequin abundance will be affected by the same climate variables as their invertebrate prey | (Soulliere & Thomas, 2009) |
| | Direct | Risk of nest flooding | Harlequins will avoid areas with high precipitation during the breeding season | (MacCallum et al., 2016) |
| Terrain and physical stream attributes | Direct | Harlequins will prefer clear, fast-flowing reaches that facilitate visibility of benthic invertebrate prey | Harlequins will prefer mid-sized rivers with 1-7% gradient reaches and fast-flowing water | (E. F. Cassirer et al., 1996; R. Ian Goudie & Gilliland, 2008) |
| | Indirect | Stream flow will affect invertebrate biomass or abundance through its effects on food and oxygen delivery | Both invertebrate and harlequin abundance will be affected by river size and gradient | (McCabe, 2010) |
| Land cover | Predation | Canopy cover reduces visibility of predators | Harlequins will avoid sites with high canopy cover | (Heath et al., 2006; Heath & Montevecchi, 2008; MacCallum et al., 2016) |
| | Nesting habitat | Forested areas are required for nesting | Harlequins will be positively associated with forest cover | (MacCallum et al., 2016) |
| Human disturbance | Site disturbance | Human activity disrupts harlequin duck breeding | Harlequins will avoid areas with high human activity, such as cities, roads, bridges, and trails | (Kuchel, 1977; Soulliere & Thomas, 2009; Richard L Wallen, 1987) |
| | Land use – agriculture | Agriculture may (i) increase invertebrate availability through light and nutrient inputs; or (ii) disrupt breeding through loss of riparian vegetation and nesting habitat | In areas with high agricultural intensity: (i) both invertebrate and harlequin abundance will be higher than expected; or (ii) harlequin abundance will be lower than expected. | (Soulliere & Thomas, 2009) |

| Hypothesis | Effect | Mechanism | Predictions | Studies |
|---|---|---|---|---|
| Fish | Direct | Fish (salmonids and catostomids) eggs provide an additional energy resource | Harlequins will be associated with streams where fish lay eggs, especially spring spawning species | (W. A. Hunt, 1998; Smith, 1997) |
| | Indirect – invertebrates | Fish will reduce (i) invertebrate densities directly, through predation; or (ii) reduce invertebrate availability by inducing anti-predator behaviours that reduce feeding activity during the day | (i) Both invertebrate and harlequin abundance will be lower where fish are present; or (ii) Harlequin abundance will be lower than predicted by environmental factors where fish are present | (LeBourdais et al., 2009) |
| | Indirect – predators | Fish attract common predators, such as bald eagles and mustelids | Where fish are present, invertebrate prey may be less important and canopy cover (which can reduce detection of avian predators) may be more important in predicting harlequin duck usage or abundance than stream sections where fish are not present | (Heath et al., 2006; MacCallum, 2016; Chapter 3) |

Invertebrate growth and metabolism require a strict range of temperatures. As such, climate and temperature are quite likely to contribute to invertebrate abundance, biomass and density. Likely, mean temperature will need to be within a certain range and not be subject to high daily fluctuations. Dissolved oxygen levels also decrease with increasing water temperature. Pollution-sensitive species, such as those consumed by harlequin ducks during the breeding season (e.g. ephemeroptera, plecoptera, trichoptera), are highly sensitive to low oxygen levels. Therefore, water temperatures must be not too low (for growth and metabolism), but also not too high (in order to provide adequate oxygen). Additionally, high temperatures during winter season means invertebrates that overwinter as larvae could deplete energy stores due to overly high metabolism. Precipitation, another large component of climate, affects flow, which must be within a certain range as well. Too much precipitation, especially during spring runoff, or occurring with high variability, can cause scouring floods, damaging both invertebrates and the substrates that they inhabit. Not enough precipitation can cause low flows which may reduce standing stocks of many invertebrate species which require a certain amount of food and oxygen to be carried to them by the current, or even cause death through desiccation in the case of drought.

Climate, which is partly related to geographic position (e.g. latitude) and topography (e.g. elevation) can be confounded with physical stream structure and longitudinal patterns within watersheds, which can also affect species distributions. For example, stream gradient (steepness) and, thus, water velocity, tends to decrease with elevation. Water velocity affects the amount of oxygen and food carried to invertebrates, as well as the rate of particulate deposition. In slow flows, fine particulates can accumulate between larger stream substrates, such as cobbles. The observed preference of harlequin ducks for fast-flowing runs, riffles and rapids may be due to the high visibility of the benthos in which they search for invertebrate prey (R. Ian Goudie & Gilliland, 2008). Discharge, on the other hand, is determine both by water velocity and stream size; while stream order is determined by topography (Strahler, 1952), width and discharge also depend on precipitation, and tend to increase from top to bottom within watersheds as more tributaries converge and flow into the mainstem (McCabe, 2010). Velocity is also affected by the stream channel, as determined by substrate and riparian vegetation. High peak flows, due to large precipitation or snowmelt events, combined with thin width restricted by bedrock channels, for example, can result in scouring floods

that displace large amounts of benthic materials, cleaning substrates of algae and invertebrates and reducing standing invertebrate stocks by half or more. Flooding can also directly impact harlequin duck reproduction by washing out nests, which tend to be built on the ground, on in-stream islands (Bruner, 1997; Robertson & Goudie, 1999). Thus, it can be difficult to determine whether the apparent preference of harlequin ducks for larger stream sizes within a watershed (e.g. MacCallum et al., 2016) is due to more open forest canopy allowing for easier aerial predator vigilance or other benefits, such as decreased flow variability leading to more stable standing invertebrate stocks (e.g. LeBourdais et al., 2009).

Climate and terrain effects can be further complicated by their associated effects on vegetation. Small headwater forest streams have closed canopies and are dominated by allochtonous input (leaves and other organic matter produced outside the stream). As such, headwater streams tend to be dominated by associated shredders (Cummins et al., 1989), which break down this coarse, organic leaf litter during feeding, and gatherer-collectors that feed on the resulting fine particulate matter (Vannote et al., 1980). Further down in the watershed, river width increases and the canopy opens up, resulting in increased light input and algal growth. In these mid-sized reaches, the proportion of algae-eating scrapers increases. Further down still, rivers deepen and light cannot penetrate to the bottom. Autochtonous input (algal growth) decreases and collectors (filtering and collecting organisms) dominate. The proportion of predators remains about the same from headwaters to lower reaches since these organisms only require other organisms on which to feed, and are not affected by the type of input at the base of the food web (Vannote et al., 1980). Thus, harlequin ducks may be adapted to feed on invertebrate communities that dominate in the intermediate-sized rivers selected during pre-nesting.

In addition to affecting organic matter input and shading, the surrounding vegetation (or lack thereof) can also affect both invertebrates and harlequins. Forests reduce runoff and moderate peak flows by absorbing precipitation, and decrease water temperature through shading. Colder temperatures result in higher dissolved oxygen, important for invertebrates, especially for the sensitive species consumed by harlequin ducks, such as Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies) (Bengtson, 1972; F. Cassirer & Groves, 1994; Rodway, 1998a; Richard L Wallen, 1987). Deciduous forests provide high, seasonal inputs of allochtonous organic

matter relative to evergreen species, which do not shed their leaves annually; however, deciduous forests require warm and wet growing seasons for leaf replacement. Therefore, the surrounding vegetation and forest type will have an effect on the amount of allochtonous inputs available for invertebrate growth and metabolism, but will, itself, depend on climate. Additionally, while harlequins require forest and understory vegetation during incubation and brood-rearing (MacCallum et al., 2016; Robertson & Goudie, 1999), canopy cover can actually hinder detection of avian predators, such as eagles, by harlequins (Heath et al., 2006; MacCallum et al., 2016). Therefore, harlequins may avoid feeding along reaches with thick or closed canopy, especially during the brood-rearing period.

Deforestation as a result of urban development, agricultural activities, resource extraction and forestry harvesting activities results in increased runoff, erosion and sediment load, while also decreasing allochtonous inputs as a result of canopy removal. While canopy removal can result in increased light for algal growth, sediment can clog invertebrate gills and feeding organs of filtering organisms and reduces the amount of light penetrating to the stream bottom. Suspended sediments also directly reduce prey visibility for harlequin ducks (E. F. Cassirer et al., 1996). Canopy removal also decreases shading, resulting in increased water temperatures, decreasing the amount of dissolved oxygen available to invertebrates. Thus, deforestation can have both direct and indirect effects on harlequin ducks. Agricultural activity further contributes organic nutrients from fertilizers. Both these and other sources of pollution, such as industry or sewage disposal, can increase both primary and secondary production (standing invertebrate stocks), while too much can cause excessive algal growth and even eutrophication (Chislock et al., 2013).

Broad climatic trends tend to drive species distributions at the largest scales (Pearson et al., 2004) while regional variables, such as vegetation, human activity and stream density within a watershed, may determine which areas are most suitable within the broader climate-determined range. Within suitable watersheds, further restrictions can occur at the river reach scale, such as availability of loafing sites and instream islands (E. F. Cassirer et al., 1996), and visibility up and downstream, and above, to scan for predators (Heath et al., 2006; MacCallum et al., 2016). Finally, within reaches, variables that influence feeding behavior and prey availability, such as turbidity, velocity,

depth, substrate size, may best predict the areas in which harlequins spend the most time feeding (E. F. Cassirer et al., 1996; Machmer, 2001; Richard L Wallen, 1987).

In this chapter, I tested several non-mutually exclusive hypotheses to explain variation in harlequin duck presence and abundance among streams in several watersheds across the breeding range (Table 2.1). I competed hypotheses related to the effects of suites of biogeoclimatic factors (climate, terrain, vegetation, human disturbance, fish presence) on harlequin duck abundance. Some variables might affect harlequins indirectly by inducing changes in freshwater invertebrates (the main food source for harlequin ducks during the breeding season), or may directly impact the ducks themselves. In order to determine whether variables were acting on ducks directly, or indirectly via invertebrates, I tested the same set of hypotheses on both invertebrates and ducks in order to determine the extent to which each suite of biogeoclimatic variables (i.e. hypothesis) affected harlequin duck presence and abundance directly and the extent to which their effects were mediated through influences on invertebrates.

## 2.2. Methods

### 2.2.1. Site selection

Watersheds were selected from across southern British Columbia (BC) and Alberta (AB), Canada (Figure 2.1). Most of the initial selection of specific river sections resulted from correspondence with local provincial biologists about the probability of fish and harlequin duck presence on streams in the area. Finding fishless river sections proved to be the limiting factor for field site selection as streams that were large enough for harlequins to breed on tended to contain naturally-occurring or stocked fish. As such, fishless river sections were identified first, then paired or grouped with nearby fish-bearing river sections. I tried to match similar habitat characteristics among paired or grouped streams, and also selected areas with either historic harlequin sightings or containing reasonably suitable harlequin duck breeding habitat based on physical characteristics. Harlequins tend to use intermediate-sized rivers during the pre-nesting period and feed primarily in riffles, runs and rapids (R. Ian Goudie & Gilliland, 2008). They also prefer reaches with medium to large sized substrates that offer plenty of surface areas to which larval insects can attach, as well as sufficient flow to provide

oxygen to filter-feeding insects (Goudie & Gilliland, 2008). I tried to consistently sample hand-sized cobbles within riffle, run or rapids habitat, and not in pools. Neighboring fish-bearing streams were always easier to find as fish-bearing streams were often larger and constituted more suitable breeding habitat than fishless streams. This reinforced the importance of controlling for habitat and invertebrate prey differences between fishless and fish-bearing rivers.

As could be expected from the heterogeneous nature of stream habitats, invertebrates were patchily distributed along river sections (pers. obs.). Harlequins were also patchily distributed and were repeatedly observed using the same feeding sites (pers. obs.), as has been reported in the literature (E. F. Cassirer et al., 1996; B. Hunt & Ydenberg, 2000; Kneteman & Hubbs, 2000; Robertson & Goudie, 1999). To account for the patchy nature of stream habitats, stream "reaches" were defined *post hoc,* anchored around each invertebrate sampling site; some reaches contained more than one invertebrate sampling site (Figure 2.1). Reaches were delineated using an equal upstream and downstream distance from existing invertebrate sampling sites. I wanted to choose a standard distance for all reaches that would select a relatively homogeneous stretch of river habitat, within which the average invertebrate availability could be assumed to be relatively constant. I further felt that the distance should also be reflective of a single unit of selection for harlequin duck feeding site selection.

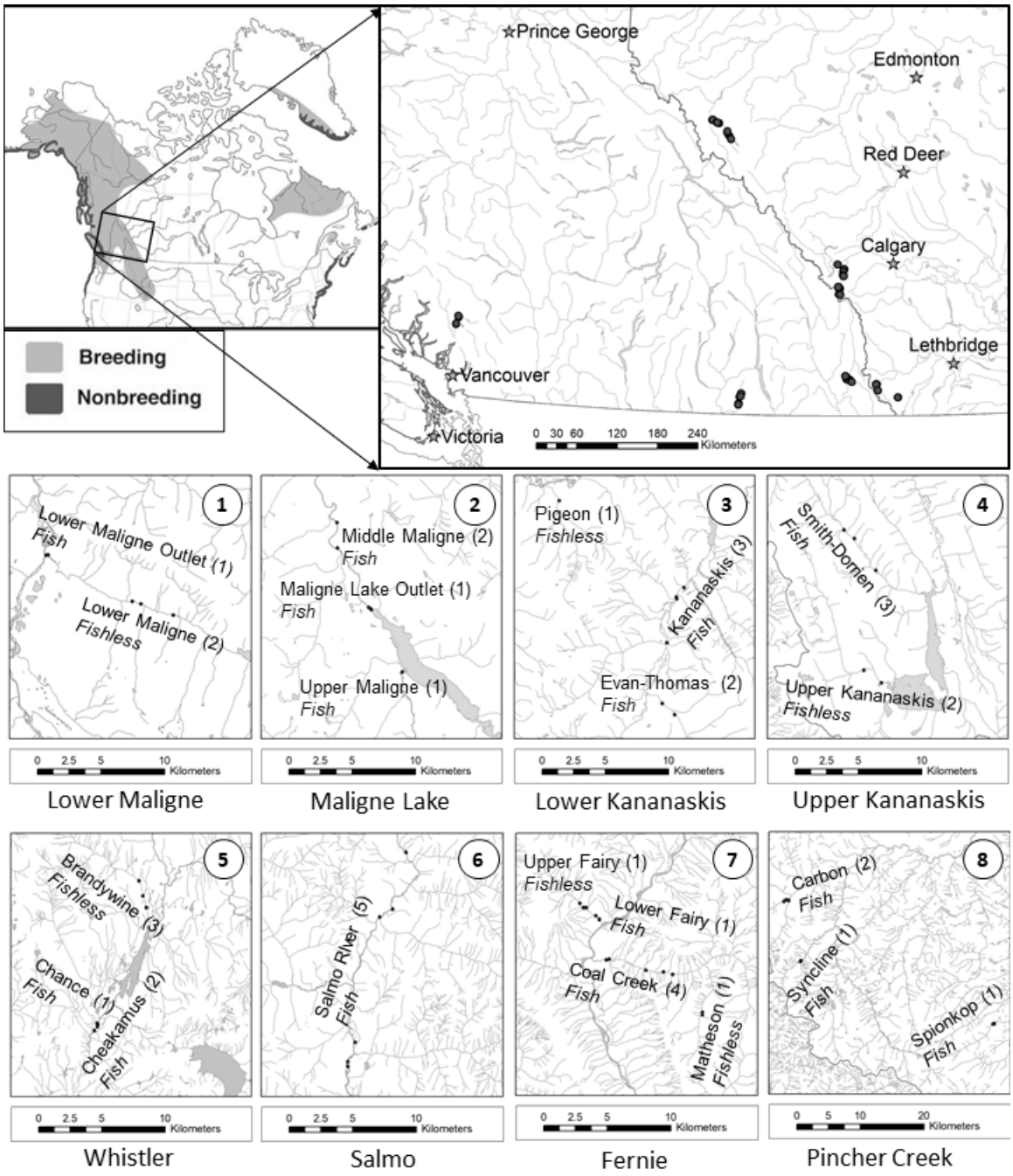


**Figure 2.1 Map of watersheds (circles 1-8) and river sections where invertebrate and harlequin duck surveys were conducted.**

Note: River sections (unique names in lower 8 panels; n = 22) were defined as continuous waterways uninterrupted by a fish barrier or change in fish status (fish vs. fishless). Numbers in brackets indicate the number of unique ~1.1 km reaches (n = 43) surveyed for harlequins along that river section. Individual dots indicate field sites where I collected invertebrate samples and habitat measurements (n = 67).

I selected an upstream/downstream distance of 550 m, for a total reach length of 1.1 km. This was partly based on a small number (n = 13) of recaptures from a previous

study of harlequin ducks in coastal BC, which found that harlequin ducks were recaptured or re-sighted within 115-937 m (mean ± SD = 548 ± 297 m) of initial capture/sighting (Ydenberg, Esler, Bond, LeBourdais, unpub data). These findings agreed with a separate study in Glacier National Park (Montana, USA) which found that harlequin duck home ranges varied from 1-2 km of linear river length, equivalent to an upstream/downstream distance of 500-1,000 m (Kuchel, 1977).

Finally, I compared how the designation of harlequin duck reach presence changed with reach length (Figure 2.2). Increasing the reach length past 1.1 km produced diminishing returns when identifying harlequin duck occupancy. In other words, most harlequins were within 550 m upstream or downstream of invertebrate sampling sites (or further than 3,000 m); increasing the total reach length past 1.1 km did not change the status of harlequin occupancy for almost all reaches. Thus, I felt that 1.1 km was a reasonable estimate of feeding site selection and/or home range size for harlequin ducks. Finally, it should be noted that 1.1 km was a maximum reach length. If a reach was interrupted by a downstream tributary, the tributary was considered to be the end point for that reach, since tributaries contribute large amounts of nutrients, sediments and invertebrates, thereby invalidating the definition of a homogeneous river reach.

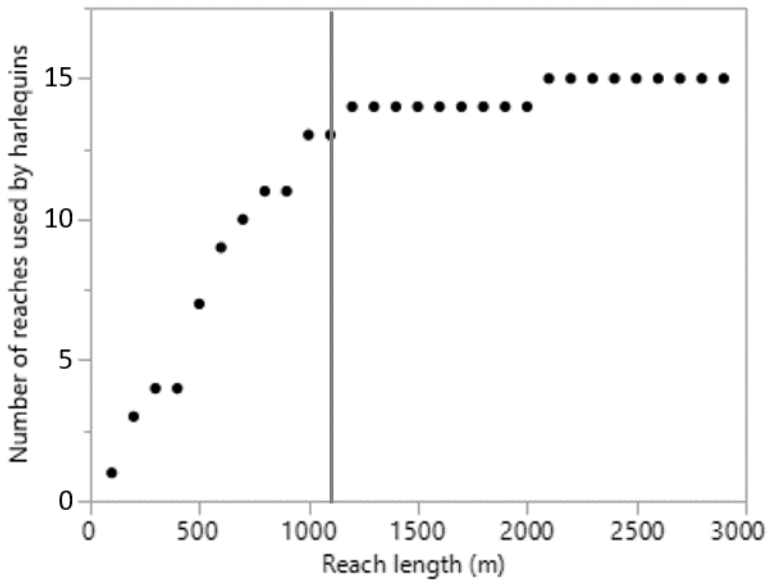


**Figure 2.2 The number of reaches in which harlequin ducks were observed relative to the definition of reach length (total number of reaches: n = 43).**

Note: Using 1.1 km as the standard reach length identified 13 reaches as being used by harlequins and 30 unused.

## 2.2.2. Harlequin duck surveys

Each river reach was surveyed for harlequin ducks and sampled for invertebrates. Streamside surveys were conducted on foot following the harlequin duck survey protocol described in the Inventory Methods for Riverine Birds provincial field manual (British Columbia et al., 1998). Date, time, elevation and coordinates of all harlequin duck sightings were recorded using Garmin GPS units. In addition to harlequin presence, I also estimated relative harlequin abundance along reaches. Lone individuals were assumed to represent a pair, consistent with previous waterfowl studies (Dzubin, 1969; Heath et al., 2006). Since reach length could vary depending on the presence of downstream tributaries, reach length was used as an offset in generalized linear models of harlequin abundance. Due to the large number of “unused” reaches, I used a zero-inflated Poisson model, with reach length as an offset, to model harlequin presence and abundance simultaneously, while also accounting for excess zeros. I suspected that some unused reaches may have been surveyed in within watersheds that were unlikely to contain harlequin ducks in the first place, which would inflate the number of “unused” reaches in the dataset.

### 2.2.3. Habitat measurements

Some habitat measurements were made in the field, while others were estimated using remote sensing data or hydrological analysis in ArcMap 10.5. Others, such as river width, were estimated in the field but were improved, especially for the widest rivers, using satellite imagery in Google Earth. Site slope was estimated using topographic analysis in Google Earth, by calculating the change in elevation over a 100 m river length at each invertebrate sampling site.

Land cover and land use was determined from the North American Land Change Monitoring System (NALCMS, 2010) which provides a harmonized view of the physical cover of Earth's surface across the continent based on 2010 Landsat satellite imagery (Latifovic et al., 2017). The NALCMS provides 19 classes of land cover (Table 2.2) using the Land Cover Classification System standard developed by the Food and Agriculture Organization of United Nations. I used ArcMap 10.5 to calculate the proportion of each land cover class within a 5 km-radius circle around each invertebrate site. Similar categories were combined when their overall coverage was low, i.e. for moss, grass and shrub lands (hereafter, grass-shrublands). Just as habitat characteristics are nested within the hierarchical structure of watersheds, my habitat measurements capture spatial variation over different scales (Table 2.3). To account for this nested hierarchy, both invertebrate and harlequin models contained random effects of river reach, nested within river section, nested within watershed (“Sub-Drainage” *sensu* Water Survey of Canada, 2016).

**Table 2.2 The 19 classes of land cover from the 2010 North American Land Change Monitoring System (Latifovic et al., 2017).**

| | |
|---|---|
| **1** | **Temperate or sub-polar needleleaf forest** |
| **2** | **Sub-polar taiga needleleaf forest** |
| 3 | Tropical or sub-tropical broadleaf evergreen forest |
| 4 | Tropical or sub-tropical broadleaf deciduous forest |
| **5** | **Temperate or sub-polar broadleaf deciduous forest** |
| **6** | **Mixed forest** |
| 7 | Tropical or sub-tropical shrubland |
| **8** | **Temperate or sub-polar shrubland** |
| 9 | Tropical or sub-tropical grassland |
| **10** | **Temperate or sub-polar grassland** |
| **11** | **Sub-polar or polar shrubland-lichen-moss** |
| **12** | **Sub-polar or polar grassland-lichen-moss** |
| **13** | **Sub-polar or polar barren-lichen-moss** |
| **14** | **Wetland** |
| **15** | **Cropland** |
| **16** | **Barren lands** |
| **17** | **Urban** |
| **18** | **Water** |
| **19** | **Snow and Ice** |

* Of the 19 classes, only 15 are found in Canada, as indicated in bold.

**Table 2.3 Habitat measurements, their respective units, approximate spatial and temporal scales, and method of measurement.**

| Spatial scale | Variable | Units | Method |
|---|---|---|---|
| SubDrainage (50-100 km) | 2013 Flood | Affected/unaffected (geographic), before/after (temporal) | ArcMap 10.5 |
| River Section (5-50 km) | Fish presence | Present/absent | Local biologists, known fish barriers |
| | Land cover and land use | Proportion of land cover types (NALCMS 2010) within a 5 km-radius | ArcMap 10.5 |
| | Climate | Mean, minimum, maximum, and monthly temperature and precipitation variables by survey year (2011, 2012, 2015)<br><br>Temperature and precipitation trends such as mean diurnal range, annual range and seasonality (coefficient of variation) | (McKenney et al., 2011) |
| Reach (~1.1 km) | Harlequin duck presence, abundance | Present/absent, Pairs/km | Field surveys (British Columbia et al., 1998) |
| | Latitude, longitude | Decimal degrees | Field (Garmin GPS) |
| | Elevation | Meters | Field (Garmin GPS) |
| | Order | Denotes the "hierarchy" of streams from headwaters (first order) downstream; stream order increases by 1 each time two stream sections with the same order meet (Strahler, 1952) | ArcMap 10.5 |
| | Magnitude | Similar to order but denotes, additively, the number of upstream tributaries contributing to each stream section (Shreve, 1967) | ArcMap 10.5 |

| Spatial scale | Variable | Units | Method |
|---|---|---|---|
| Site (100 m) | Site Slope | Percent gradient | Google Earth |
| | Width | Mean width from 3 measurements along 100 m of stream length | Field + Google Earth |
| | Canopy Coverage | Percent vegetation overhang | Field |
| | Human Disturbance (trails, roads, bridges) | 4 = crossing river<br>3 = adjacent to river (<20 m)<br>2 = within 50 m radius of site<br>1 = within 100 m radius of site<br>0 = none (>100 m) | Field + Google Earth |
| | Date | Year<br>Day of year<br>Degree days, percentage of growing season | Calculated using R v. 3.5.1 |
| Rock (<1m) | Time of day | Hours since sunrise | Field |
| | Air temperature | Degrees Celsius | |
| | Rock volume | Nearest 25 ml | |
| | Rock surface area | 13.875 x log(volume in ml)$^{3.60}$ = surface area in cm$^2$ | (McCutchen, 2002) |
| | Invertebrate measurements | Presence, abundance, density, functional feeding group, life stage (larva, pupa, adult), case-building | Field collection + laboratory identification (Clifford, 1991; Merritt et al., 2008) |

### 2.2.4. Aquatic invertebrate collection and identification

The dominant substrate in most of the reaches surveyed were large, hand-sized cobbles (pers. obs.). Harlequin ducks wade upstream to feed, picking out insects from between and on top of these cobbles, or dive and dig around in these rocks, often flipping them over with their bills in order to find prey (Robertson & Goudie, 1999). Given this foraging behavior, I chose a sampling approach that would best approximate the availability of only those prey items that would be accessible to harlequins, *sensu* LeBourdais et al. (2009). Specifically, I planted a 900 µm aquatic D-net on the stream bottom, downstream from a hand-sized cobble. I then picked up the cobble allowing any invertebrates jumping off to be washed into the net. I then picked up the net and carefully scrubbed the rock for remaining invertebrates. I washed the net into an 8.5 L bucket then poured the water through a 500 µm sieve. I removed debris and picked out invertebrates, euthanizing and preserving them in 70% ethanol. These preserved samples were stored in plastic vials. I sampled 5-10 rocks at each site, moving in an upstream direction to avoid disturbing sites and invertebrates prior to sampling. I estimated the volume of each rock to the nearest 25 ml by measuring the water displaced in a 3 L plastic, volumetric bucket. I then calculated the surface area of each rock as per McCutchen (2002):

$$Surface\ area\ = 13.875\ \times \log\left(volume^{3.603}\right)$$

In 2011 and 2012, invertebrates from each individual rock were stored in individual vials in order to investigate the effect of rock size on invertebrate functional feeding group and size classes. In 2015, 80% of samples were collectively stored with invertebrates from five rocks together in a single large vial, as this greatly expedited processing time in the field; however, for the remaining 20%, invertebrates from each individual rock were stored in a single vial so that the effect of rock size could still be examined for a subset from each site.

Preserved invertebrates were identified to functional feeding group (usually Family) using a dissecting microscope following Merritt, Cummins, & Berg (2008). For samples collected in Alberta, identification was also conducting using Merritt et al. (2008) for the first few samples from each site, or the first time a new taxon was encountered; however, identification of further samples was then expedited following Clifford (1991),

which is based on Merritt et al. (2008) but only contains taxa present in Alberta. Following identification, samples were then dried for 24 hours at 30°C and weighed to the nearest 0.01 mg. Each taxon was weighed individually from each sample or sub-sample, allowing me to investigate differences in mean individual mass by taxon and functional feeding group. A subset of invertebrate samples was used for stable isotope analysis (Chapters 4 and 5).

### 2.2.5. Statistical analyses

The invertebrate data were much more numerous than the harlequin duck data, since at least five rocks were sampled at one or more sites each along each reach. Harlequin ducks, in contrast, only had a single presence and abundance value per reach. As such, invertebrate models were built using a nested random effect structure of site, nested within reach, nested within river section (fish-bearing or fishless), nested within sub-drainage (Water Survey of Canada, 2016). Harlequin duck models, on the other hand, were created using mean habitat values for each reach.

There were a large number of invertebrate variables, including counts and masses, and separated by size classes and functional feeding groups. In order to gain a better understanding of the interrelatedness and collinearity of the various methods of tabulating and categorizing the invertebrate data, I conducted a principal component analysis on all the invertebrate count and mass variables relating to life stage (larvae, pupae), body size (small < 1 cm in length < large) and functional feeding group (predator, scraper, filterer-collector, gatherer-collector, shredder). Principal components with eigenvalues greater than 1 were then rotated using Varimax rotation in factor analysis, to ease interpretation. Rather than using the resulting factors (rotated principal components) in the habitat analyses, I simply used this process to gain a better understanding of the underlying trends and relationships among the numerous invertebrate variables.

I used the factor analysis to identify invertebrate variables that were representative of different aspects of invertebrate abundance, biomass, and density. After I selected the dependent invertebrate variables, the first step in model selection was to identify the appropriate control variables to account for differences in sampling date and season (Table 2.4). Candidate models were evaluated using Akaike's

information criterion, adjusted for small sample sizes (AICc) (Hurvich & Tsai, 1989). Control variables from the most parsimonious, strongest supported model for each invertebrate variable were used as the null model in subsequent rounds of model selection for habitat-related hypotheses (Table 2.4). Due to the smaller sample size for harlequin duck surveys, the null harlequin duck models contained an intercept only and did not control for sampling date.

**Table 2.4 Control variables considered for use in each null invertebrate model.**

| Potential Control Variable | Description | Range | Source |
|---|---|---|---|
| Degree Days | Degree days above zero since the start of the growing season (at least five days in a row >5°C after March 1) (natural log scale) | 0-620 degree-days | (McKenney et al., 2011) |
| Day of Growing Season | Number of days since the start of the growing season | 6-91 days | |
| Percent Growing Season | Number of days since start of growing season divided by total length of growing season | 4.35-33.8% | |
| Mean rock surface area | Surface area of rocks sampled for freshwater invertebrates | 23.0-102.5 $cm^2$ | Field obs. |
| Time of day | Hours past sunrise | 6.0-15.5 h | |

Note: For seasonal variables, such as degree days and percent growing season, quadratic terms were also included during model selection.

I modelled the selected invertebrate variables using generalized linear mixed models. Count data were modelled using a poisson distribution, unless overdispersion was detected, in which case count data were modeled using a negative binomial distribution. Residuals were checked to confirm the best fitting distribution. Continuous data, such as biomass and mean body size, were difficult to model due to a heavily right-skewed distribution of very small values. I took the natural log of biomass and mean body size to reduce the leverage of a few large values. Many count were difficult to model as well, due to zero-inflation. Rather than using more complicated zero-inflation models, I instead chose to model presence or absence using a binomial distribution for these less frequently observed invertebrate types: large larvae and pupae (length > 1 cm), presence and abundance of case-building individuals, and presence and mass of pupae.

Only 13 of the 43 reaches contained harlequin duck sighting(s). As such, the harlequin duck data were appeared heavily zero-inflated. I created null models of harlequin counts using only an offset term of logged survey distance and different distributions. I compared poisson, negative binomial, zero-inflated poisson and zero-inflated negative binomial distributions using Bayesian information criterion (BIC), which gives a stronger penalization to model complexity than AICc (Schwarz, 1978). The zero-inflated poisson model was the strongest supported and was used in all subsequent models of harlequin abundance.

Due to the high number of environmental variables, hypotheses were tested using a staged approach (e.g. English et al., 2017; Maccallum et al., 2016; Murray et al., 2015). Each hypothesis was tested by competing a suite of biogeoclimatic variables related to that hypothesis within an information theoretic framework, using AICc (Hurvich & Tsai, 1989). Prior to model selection, all independent variables were rescaled by subtracting the mean and dividing by two standard deviations (Gelman, 2008). Due to the high level of intercorrelation among many environmental variables, variable combinations were only included in the same model when they had a Pearson correlation coefficient of less than 0.6 ($\alpha = 0.05$). Variables from the most parsimonious, strongest supported model(s) were then competed using a final global model for each dependent measure of invertebrate presence, abundance and density, and for harlequin duck presence and abundance. Models within $\Delta AICc = 2$ of the best supported model (lowest AICc value) were considered to have similar support. Smaller models that were nested inside larger models with similar support ($\Delta AICc < 2$) were considered most parsimonious; the additional term in the larger model was considered uninformative, as its inclusion makes no difference to AICc (Arnold, 2010; Burnham & Anderson, 2002).

It was difficult to keep track of all the correlations among different environmental variables in order to clearly understand what each model was describing, biologically. It was also difficult to separate the effects of climate, from its dependencies on terrain and topography, which themselves determine hydrology, from the association of all these variables with both land cover (vegetation) and land use; often human activities have an environmental component as well. In order to more holistically describe the correlated and collinear suites of environmental variables, I ran a principal component analysis on the top supported variables from all of the harlequin duck models. I also added a few of the variables that were important to the invertebrate variables identified in the final

harlequin duck models (pupae presence and small scraper abundance). The resulting factors revealed some underlying habitat types and climatic regions driving variation within groups of variables. This allowed me to make more sense of how the strongest effects of individual variables might actually be driving harlequin duck distributions within the context of broader ecosystem trends, which is the level that ducks might be most affected at and to which they may pay the most attention.

Statistical analyses were performed using JMP (version 13.1.0, SAS Institute Inc.) and R (version 3.5.1, the R Foundation for Statistical Computing).

## 2.3. Results

### 2.3.1. Invertebrate Abundance, Biomass and Density

Principal component analysis of 38 measures of invertebrate abundance, biomass and density (biomass per surface area of substrate) yielded 10 eigenvectors with eigenvalues greater than 1. These components were rotated using Varimax rotation to obtain ten orthogonal factors that, together, explained 91.4% of the total variance in the included invertebrate measurements (Table 5). Total invertebrate counts were mainly driven by numbers of small larvae, which consisted mainly of small cased individuals, such as chironomids, and small scrapers, such as mayflies and some caddisflies (INV1). Total biomass per sample experienced very similar loadings to density (biomass by surface area); as such, it seemed that most of the variation in density was driven by differences in total biomass, rather than differences in rock size. Biomass and density appeared to be mostly driven by numbers of large larvae, especially large scrapers (INV2) and large predators (INV4). The remaining factors represented individual size classes within life stages and functional feeding groups, but none contributed much to overall count or biomass. Therefore, I chose a few individual invertebrate variables (dependent variables listed in Table 6) that loaded strongly onto the factors most associated with overall invertebrate abundance or biomass (INV1, INV2 and INV4). I the tested the same set of duck hypotheses on these selected variables to identify and determine the extent to which invertebrate variables respond to the same biogeoclimatic drivers as harlequins.

**Table 2.5 Factor analysis of the main invertebrate measurements considered for inclusion in harlequin duck breeding habitat models.**

| | Factors | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **INV1** | **INV2** | **INV3** | **INV4** | **INV5** | **INV6** | **INV7** | **INV8** | **INV9** | **INV10** |
| Larvae Small (n) | **0.874** | 0.165 | 0.052 | 0.061 | 0.374 | -0.040 | 0.106 | 0.161 | 0.062 | 0.096 |
| Larvae Large (n) | 0.222 | **0.868** | -0.026 | 0.253 | 0.013 | 0.039 | 0.233 | -0.060 | 0.131 | 0.035 |
| Larva Total (n) | **0.867** | 0.210 | 0.049 | 0.073 | 0.367 | -0.037 | 0.117 | 0.154 | 0.068 | 0.096 |
| Larvae Mass | 0.277 | **0.557** | 0.034 | **0.737** | 0.139 | 0.034 | 0.041 | 0.067 | 0.066 | 0.004 |
| Pupae Small (n) | 0.091 | 0.162 | -0.049 | 0.009 | 0.016 | **0.960** | 0.066 | -0.015 | 0.008 | 0.006 |
| Pupae Large (n) | -0.009 | 0.067 | **0.963** | 0.004 | 0.023 | 0.027 | 0.008 | 0.022 | -0.042 | -0.016 |
| Pupae Total (n) | 0.089 | 0.165 | 0.066 | 0.009 | 0.020 | **0.955** | 0.062 | -0.013 | 0.004 | 0.006 |
| Pupae Mass | 0.290 | 0.027 | **0.579** | 0.081 | -0.109 | **0.672** | -0.020 | -0.034 | -0.003 | -0.033 |
| Cased Small (n) | **0.911** | 0.032 | 0.135 | 0.100 | -0.177 | 0.100 | 0.019 | -0.117 | 0.112 | -0.040 |
| Cased Large (n) | 0.173 | 0.114 | **0.816** | 0.008 | 0.024 | -0.036 | 0.022 | -0.014 | **0.479** | 0.037 |
| Cased Total (n) | **0.908** | 0.034 | 0.155 | 0.100 | -0.175 | 0.098 | 0.019 | -0.116 | 0.124 | -0.039 |
| Cased Mass | **0.603** | 0.092 | **0.496** | 0.122 | -0.139 | 0.371 | -0.008 | -0.068 | 0.345 | -0.024 |
| PR Small (n) | 0.091 | 0.045 | 0.031 | -0.032 | 0.065 | 0.114 | **0.903** | 0.161 | 0.031 | 0.087 |
| PR Large (n) | 0.124 | 0.237 | -0.058 | **0.560** | -0.023 | -0.088 | **0.600** | -0.131 | -0.050 | -0.072 |
| PR Total (n) | 0.123 | 0.139 | -0.003 | 0.212 | 0.041 | 0.041 | **0.950** | 0.062 | 0.005 | 0.034 |
| PR Mass | -0.104 | -0.016 | -0.043 | **0.927** | -0.042 | -0.048 | 0.172 | -0.049 | -0.041 | -0.025 |
| SC Small (n) | **0.941** | 0.231 | 0.061 | 0.088 | -0.027 | 0.082 | 0.030 | 0.061 | 0.068 | 0.025 |
| SC Large (n) | 0.133 | **0.953** | -0.016 | 0.001 | -0.006 | 0.133 | -0.017 | 0.013 | -0.021 | 0.050 |
| SC Total (n) | **0.931** | 0.274 | 0.060 | 0.085 | -0.026 | 0.087 | 0.028 | 0.060 | 0.066 | 0.027 |
| SC Mass | **0.467** | **0.774** | 0.082 | 0.123 | 0.003 | 0.285 | -0.072 | 0.062 | 0.008 | 0.005 |
| FC Small (n) | 0.027 | 0.025 | -0.026 | -0.026 | 0.075 | 0.014 | 0.044 | 0.071 | -0.005 | **0.989** |
| FC Large (n) | 0.311 | 0.097 | 0.056 | -0.003 | -0.003 | -0.094 | 0.036 | -0.049 | **0.881** | 0.087 |
| FC Total (n) | 0.143 | 0.060 | -0.002 | -0.016 | 0.066 | -0.023 | 0.051 | 0.047 | 0.334 | **0.919** |
| FC Mass | 0.079 | 0.069 | 0.013 | 0.056 | 0.014 | 0.092 | -0.016 | 0.005 | **0.876** | 0.160 |
| GC Small (n) | 0.116 | -0.043 | -0.018 | 0.002 | **0.955** | 0.028 | 0.103 | 0.014 | 0.001 | 0.098 |
| GC Large (n) | 0.019 | -0.073 | -0.006 | 0.270 | 0.214 | -0.058 | -0.058 | -0.052 | 0.029 | 0.035 |
| GC Total (n) | 0.116 | -0.044 | -0.018 | 0.006 | **0.955** | 0.027 | 0.102 | 0.013 | 0.002 | 0.098 |
| GC Mass | 0.056 | 0.155 | -0.003 | 0.123 | **0.795** | -0.044 | -0.088 | 0.108 | -0.014 | -0.074 |
| SH Small (n) | 0.104 | -0.010 | -0.012 | -0.031 | 0.058 | -0.026 | 0.082 | **0.976** | -0.016 | 0.058 |
| SH Large (n) | -0.001 | 0.090 | **0.961** | 0.003 | 0.033 | 0.017 | 0.012 | 0.024 | -0.048 | -0.017 |
| SH Total (n) | 0.102 | 0.005 | 0.140 | -0.009 | 0.061 | -0.017 | 0.079 | **0.970** | -0.026 | 0.054 |
| SH Mass | 0.349 | -0.056 | **0.768** | 0.082 | -0.078 | 0.085 | -0.022 | 0.155 | -0.047 | -0.009 |
| Total Small (n) | **0.870** | 0.179 | 0.047 | 0.063 | 0.368 | 0.081 | 0.112 | 0.162 | 0.060 | 0.098 |
| Total Large (n) | 0.226 | **0.849** | 0.219 | 0.236 | 0.032 | 0.035 | 0.228 | -0.044 | 0.145 | 0.031 |
| Total (n) | **0.861** | 0.222 | 0.059 | 0.074 | 0.361 | 0.081 | 0.122 | 0.155 | 0.067 | 0.097 |
| Total Mass | 0.349 | **0.534** | 0.161 | **0.687** | 0.107 | 0.152 | 0.038 | 0.051 | 0.109 | -0.007 |
| Density (mg/m$^2$) | 0.287 | **0.514** | 0.117 | **0.756** | 0.058 | 0.119 | 0.096 | 0.093 | 0.096 | -0.021 |
| Eigenvalue | 12.5 | 4.2 | 4.0 | 2.8 | 2.4 | 2.3 | 2.1 | 1.9 | 1.4 | 1.2 |
| Cum. variance explained (%) | 32.9 | 43.9 | 54.5 | 62.0 | 68.2 | 74.1 | 79.6 | 84.5 | 88.2 | 91.4 |

Small individuals are less than 1 cm in length; large individuals are 1 cm or more in length.
Functional feeding groups include: PR (predator), SC (scraper), FC (filterer-collector), GC (gatherer-collector) and SH (shredder).

Loadings > |0.40| are emboldened to aid with interpretation.
Cumulative percent refers to the percentage of total variance explained by that factor, in addition to each preceding factor.

All of the invertebrate variables examined were affected by climate, especially temperature (Table 2.6). In general, multiple measures of invertebrate abundance increased with increasing annual or quarterly temperatures, monthly temperatures, or mean temperature of the growing season (Table 2.6). Invertebrate abundance and biomass were most strongly associated with temperature related variables and, to a lesser extent, latitude and elevation; however, latitude and elevation were not found in any of the final invertebrate models.

**Table 2.6 Linear and generalized linear mixed models of the effects of biogeoclimatic variables, including fish and harlequin presence, on invertebrate presence, abundance and biomass per surface area of rock (mg/m$^2$).**

| Final Invertebrate Models | K | logLik | AICc | ΔAICc | wt |
|---|---|---|---|---|---|
| Total invertebrate abundance (models = 309) | | | | | |
| MeanTempGrowingSeason + AprPrecipitation* + MayPrecipitation | 12 | -745.7 | 1517.3 | 0 | 0.359 |
| MeanTempGrowingSeason + AprPrecipitation* + OctPrecipitation* | 12 | -746.5 | 1519.1 | 1.73 | 0.151 |
| (Null) logDegreeDays + RockSurfaceArea | 9 | -756.7 | 1532.5 | 15.12 | 0 |
| Log biomass (mg/m$^2$) (models = 58) | | | | | |
| FebMaxTemp | 9 | -234.6 | 488.3 | 0 | 0.355 |
| JunMaxTemp | 9 | -235.3 | 489.8 | 1.51 | 0.167 |
| FebMinTemp | 9 | -235.4 | 489.9 | 1.58 | 0.162 |
| MarMaxTemp | 9 | -235.4 | 490.0 | 1.68 | 0.153 |
| (Null) logDegreeDays | 8 | -239.4 | 495.8 | 7.43 | 0.009 |
| Abundance of large larvae/pupae (> 1 cm) (models = 36) | | | | | |
| JunMaxTemp | 9 | -332.9 | 685.0 | 0 | 0.298 |
| OctMaxTemp | 9 | -333.5 | 686.2 | 1.18 | 0.165 |
| JulMaxTemp | 9 | -333.6 | 686.3 | 1.29 | 0.156 |
| MarMaxTemp | 9 | -333.6 | 686.4 | 1.33 | 0.153 |
| (Null) logDegreeDays + RockSurfaceArea | 8 | -339.2 | 695.3 | 10.21 | 0.002 |
| Large larvae/pupae present/absent (models = 142) | | | | | |
| PercentDeciduous + Fish* + PercentDeciduous:Fish | 11 | -63.4 | 150.5 | 0 | 0.288 |
| SepPrecipitation + MeanTempGrowingSeason + Harlequin + SepPrecipitation:Harlequin | 12 | -62.9 | 151.8 | 1.30 | 0.150 |

| Final Invertebrate Models | K | logLik | AICc | ΔAICc | wt |
|---|---|---|---|---|---|
| PercentGrassShrubLand* + PercentTaigaNeedleleaf + Fish* + PercentTaigaNeedleleaf:Fish | 12 | -63.1 | 152.1 | 1.68 | 0.124 |
| (Null) logDegreeDays + logDegreeDays$^2$ | 8 | -72.2 | 161.3 | 10.80 | 0.001 |
| Abundance of cased individuals (models = 106) | | | | | |
| MeanTempGrowingSeason | 9 | -296 | 610.4 | 0 | 0.212 |
| AprMaxTemp | 9 | -296 | 610.4 | 0.02 | 0.210 |
| (Null) logDegreeDays | 8 | -303 | 622.2 | 11.82 | 0.001 |
| Cased individuals present/absent (models = 31) | | | | | |
| AprMaxTemp | 8 | -86.3 | 189.5 | 0 | 0.321 |
| MeanTempGrowingSeason | 8 | -86.7 | 190.3 | 0.78 | 0.218 |
| MayMaxTemp | 8 | -86.7 | 190.4 | 0.82 | 0.213 |
| (Null) logDegreeDays | 7 | -91.6 | 197.9 | 8.33 | 0.005 |
| Proportion of Predators (by abundance) (models = 16) | | | | | |
| MeanDailyTempRange | 10 | -265.5 | 552.3 | 0 | 0.187 |
| Isothermality | 10 | -266.0 | 553.5 | 1.17 | 0.104 |
| (Null) logLarvaAbundance + DayOfGrowingSeason + DayOfGrowingSeason2 | 9 | -267.7 | 554.5 | 2.16 | 0.064 |
| Proportion of Scrapers (by abundance) (models = 303) | | | | | |
| PrecipitationSeasonality + Width + Harlequin + Width:Harlequin | 12 | -540.1 | 1106.3 | 0 | 1 |
| (Null) logLarvaAbundance + logDegreeDays | 8 | -556.1 | 1129.0 | 22.75 | 0 |
| Log Mean Invertebrate Body Mass (mg) (models = 326) | | | | | |
| PrecipitationWarmQuarter | 8 | -183.7 | 384.3 | 0 | 0.102 |
| PrecipitationDryQuarter + PrecipitationWetPeriod | 9 | -182.9 | 385.0 | 0.73 | 0.071 |
| PrecipitationDryQuarter + GrowingSeasonPrecipitation | 9 | -183.0 | 385.1 | 0.83 | 0.067 |
| AnnualPrecipitation | 8 | -184.7 | 386.3 | 1.97 | 0.038 |
| (Null) | 7 | -185.8 | 386.4 | 2.09 | 0.036 |
| Pupae Abundance (models = 90) | | | | | |
| PrecipitationWetPeriod + logCanopyOverhang | 11 | -155.7 | 335.0 | 0.45 | 0.109 |
| DecMaxTemp | 10 | -158.1 | 337.5 | 2.95 | 0.031 |
| SepMaxTemp | 10 | -158.1 | 337.7 | 3.10 | 0.029 |
| NovMaxTemp | 10 | -158.5 | 338.5 | 3.93 | 0.019 |
| (Null) PercentGrowingSeason + PercentGrowingSeason2 + RockSurfaceArea | 9 | -165.3 | 349.7 | 15.16 | 0 |
| Pupae Presence/Absence (models = 733) | | | | | |
| MarMaxTemp + SiteSlope* + Order* | 10 | -45.0 | 111.5 | 1.26 | 0.140 |

| Final Invertebrate Models | K | logLik | AICc | ΔAICc | wt |
|---|---|---|---|---|---|
| (Null) logDegreeDays | 7 | -55.8 | 126.4 | 16.16 | 0 |

Only models with strong support (ΔAICc < 2) are shown, plus the null model for comparison.
Independent variables that were found to be important for both invertebrates and harlequins are denoted with an asterisk (*).
Control variables for each response variable are only listed next to the null model for simplicity, but were included in all models for that set.
Smaller models nested within larger models with similar support (ΔAICc < 2) were considered to be most parsimonious; thus, models containing additional, non-explanatory variables are not shown.

A few invertebrate variables were also affected by precipitation; total abundance, abundance of large individuals, proportion of scrapers, mean body mass and abundance of pupae were all affected by precipitation variables (Table 2.6). High precipitation in April and May was associated with lower overall numbers of invertebrates, but a higher probability of presence of large individuals. Precipitation seasonality tended to decrease invertebrate numbers, especially scrapers (Table 2.6). Seasonality increased with precipitation extremes, i.e., low precipitation during the dry season and/or high precipitation during the wet season. Precipitation during the dry period appeared to drive the negative association of seasonality with invertebrate numbers; low precipitation in the dry period was negatively associated with invertebrate numbers. Conversely, high precipitation during the wet period, and throughout the year, was positively associated with mean body mass and number of pupae. Furthermore, mean body mass was likely directly affected by number of pupae, due to the large energetic stores required by pupae for metamorphosis.

### 2.3.2. Harlequin Duck Presence and Abundance

Harlequin presence and abundance were positively associated with April, May and October precipitation and mean temperature during the wet quarter (Table 2.7). Probability of harlequin ducks using a reach decreased with increasing grass and shrub land, while abundance was highest in areas with high proportions of temperate needleleaf forest (Figure 2.2). Harlequin presence and abundance were consistently positively associated with habitat characteristics related to stream size, such as higher order, magnitude and width, and lower gradient (Figure 2.2). The only invertebrate variable affected by stream size was the proportion of scrapers (Figure 2.3).

**Table 2.7 Zero-inflated models of harlequin duck abundance along stream reaches.**

| Model | K | logLik | AICc | ΔAICc | wt |
|---|---|---|---|---|---|
| Climate (models = 67) | | | | | |
| Abundance ~ OctPrecipitation* + Flood + OctPrecipitation:Flood \| Zeros ~ Intercept only | 5 | -51.1 | 113.9 | 0 | 0.437 |
| Abundance ~ MeanTempWetQuarter + Flood + MeanTempWetQuarter:Flood \| Zeros ~ Intercept only | 5 | -51.2 | 114.1 | 0.24 | 0.388 |
| Abundance ~ Intercept only \| Zeros ~ AprPrecipitation* + MarPrecipitation | 5 | -52.1 | 115.8 | 1.9 | 0.169 |
| (Null) | 2 | -59.0 | 122.3 | 8.39 | 0.007 |
| Terrain and physical attributes (models = 556) | | | | | |
| Abundance ~ Order* \| Zeros ~ SiteSlope* | 4 | -50.8 | 110.7 | 0.97 | 0.207 |
| Abundance ~ Order* + SiteSlope* \| Zeros ~ DistanceToCoast | 5 | -50.0 | 111.7 | 2.00 | 0.124 |
| (Null) | 2 | -59.0 | 122.3 | 12.56 | 0.001 |
| Land Cover (models = 697) | | | | | |
| Abundance ~ PercentTemperateNeedleleafForest* \| Zeros ~ PercentGrassShrubLand* | 5 | -49.8 | 111.1 | 0 | 0.67 |
| (Null) | 2 | -59.0 | 122.3 | 11.12 | 0.003 |
| Human Disturbance (models = 42) | | | | | |
| Abundance ~ Intercept only \| Zeros ~ PercentAgriculture | 3 | -56.7 | 120 | 0 | 0.354 |
| (Null) | 2 | -59.0 | 122.3 | 2.27 | 0.114 |
| Invertebrate prey (models = 299) | | | | | |
| Abundance ~ SmallShredderCount + sqrt(PredatorMass) + sqrt(SmallScraperCount) \| Zeros ~ Intercept only | 5 | -48.0 | 107.7 | 0 | 0.379 |
| Abundance ~ SmallShredderCount + sqrt(SmallScraperCount) + sqrt(TotalLarvaMass) \| Zeros ~ Intercept only | 5 | -48.3 | 108.3 | 0.65 | 0.274 |
| Abundance ~ SmallShredderCount + sqrt(SmallScraperCount) + LargePredatorCount \| Zeros ~ Intercept only | 5 | -48.7 | 109 | 1.3 | 0.198 |
| Abundance ~ SmallShredderCount + sqrt(PredatorMass) + PupaePresent \| Zeros ~ Intercept only | 5 | -49.0 | 109.5 | 1.88 | 0.148 |
| (Null) | 2 | -59.0 | 122.3 | 14.61 | 0 |
| Final combined models (models = 836) | | | | | |
| *Abundance ~ Order* \| Zeros ~ PercentGrassShrubLand* + SiteSlope** | 5 | -44.6 | 100.9 | 0 | 0.366 |
| *Abundance ~ Order* + Fish* \| Zeros ~ PercentGrassShrubLand** | 5 | -45.2 | 101.9 | 1.03 | 0.219 |

| Model | K | logLik | AICc | ΔAICc | wt |
|---|---|---|---|---|---|
| *Abundance ~ PupaePresent + Fish* \| Zeros ~ PercentGrassShrubLand** | 5 | -45.2 | 101.9 | 1.04 | 0.218 |
| *Abundance ~ sqrt(SmallScraperCount) + Fish* \| Zeros ~ PercentGrassShrubLand** | 5 | -45.3 | 102.1 | 1.24 | 0.197 |
| (Null) | 2 | -59.0 | 122.3 | 21.37 | 0 |

Only models with strong support (ΔAICc < 2) are shown, plus the null model for comparison.
Independent variables that were found to be important for both invertebrates and harlequins are denoted with an asterisk (*).
Smaller models nested within larger models with similar support (ΔAICc < 2) were considered to be most parsimonious; thus, models containing additional, non-explanatory variables are not shown.

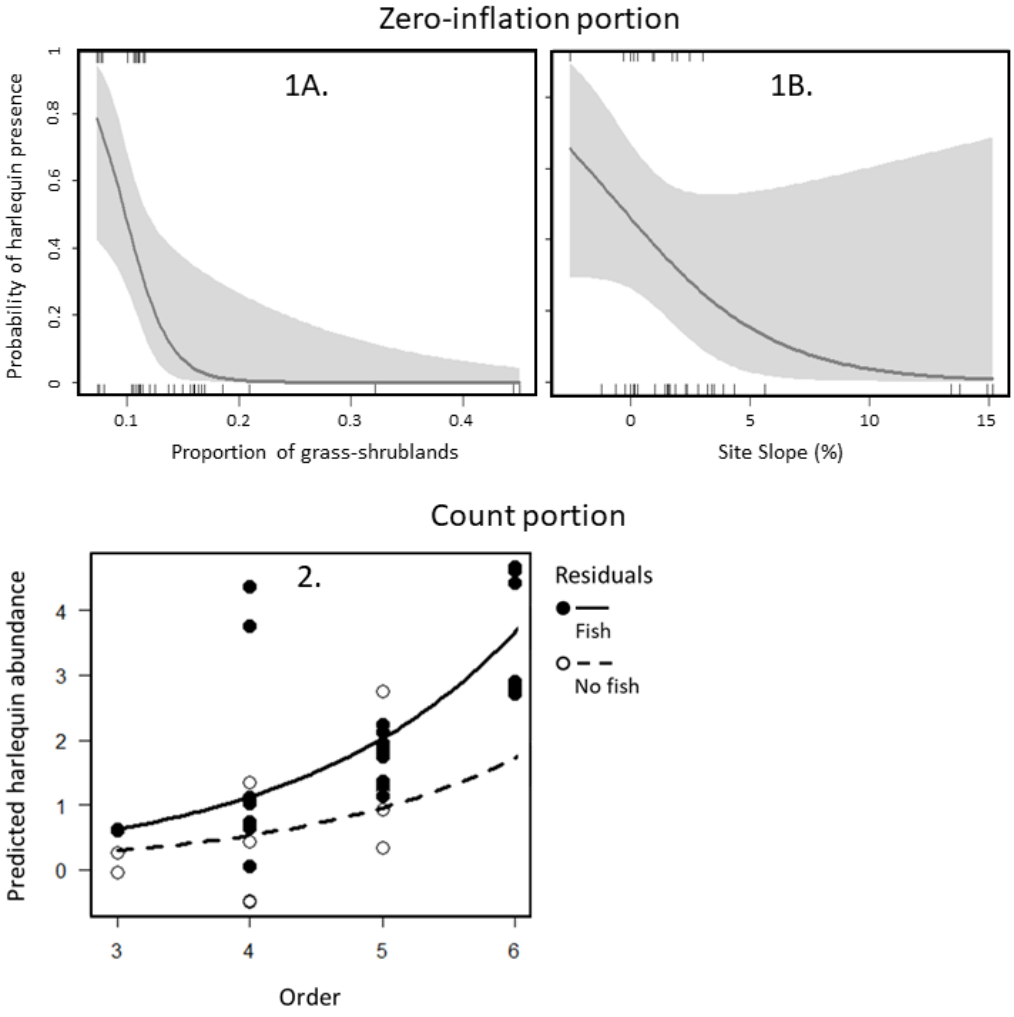


**Figure 2.3 Conditional probabilities and predicted abundance from the final zero-inflated harlequin duck model(s).**

Note: Proportion of grass-shrublands was retained as the best predictor of true harlequin absences in all final harlequin models (Panel 1A). In one of the final harlequin models, site slope also contributed additional information about harlequin absence (Panel 1B): the higher the proportion of grass-shrublands and the steeper the site slope, the more likely that a count of zero

was likely to be true. Hash marks denote observed presences and absences and shading indicates the 95% confidence interval on predicted probabilities. In the count portion of the model, stream order was consistently one of the strongest predictors of duck abundance (Panel 2). Circles indicate the residuals of observed relative to predicted values in reaches containing fish (black circles, solid line) and no fish (open circles, dashed line).

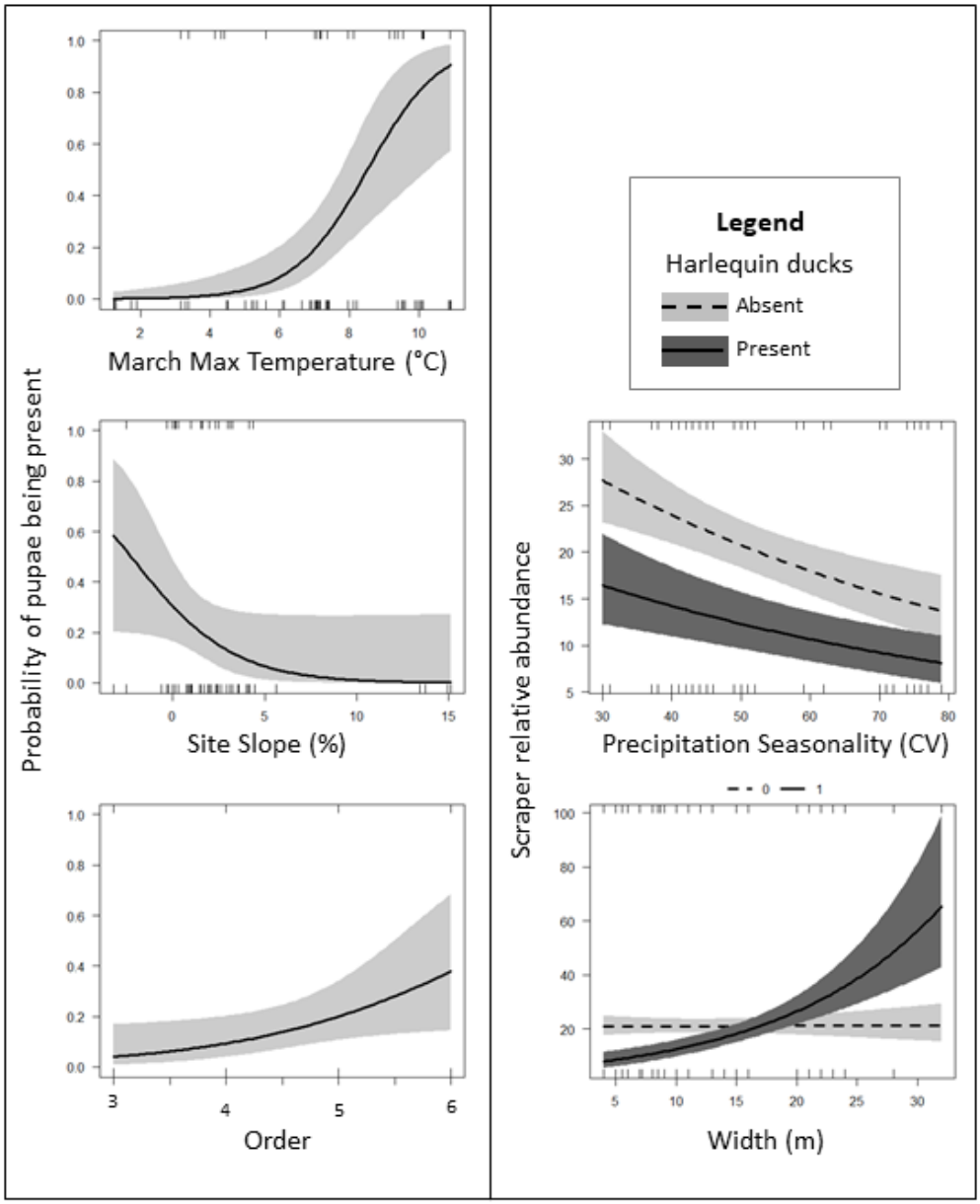


**Figure 3. The marginal effects of stream size (order and width) on invertebrates found to be associated with harlequin duck abundance.**

Note: The predicted probability of pupae presence increased with increasing stream order and March maximum temperature, and decreased with increasing site slope. Hash marks indicate raw observations. Precipitation seasonality decreased scraper abundance. On reaches occupied by harlequins, scraper abundance increased with width. On unoccupied reaches, scraper

abundance was unaffected by width. Hash marks denote positive (top) and negative (bottom) residuals of predicted values relative to observed values.

It was difficult to interpret the individual variable effects on harlequin duck abundance, in a large part because my model selection design allowed for a great deal of flexibility in variable selection. There were so many different climate variables, in particular, that, rather than arbitrarily removing highly correlated variables *a priori*, I instead still allowed some correlated variables to be competed against each other in separate models, but never allowing them to appear in the same model. There were not a lot of duck data points over which to assess variable importance and effects (n = 43). This meant that the final ducks models could only contain, at maximum, four environmental variables, using a "10 observations per independent variable" rule of thumb (Peduzzi et al., 1996). As such, arbitrarily reducing the number of variables prior to building the models ran the risk of arbitrarily discarding the candidate variables that better fit the observed data, instead keeping related variables that are still indicate a relationship, but less strongly, with duck abundance. This could potentially attribute less importance to a hypothesis than is, in reality, more important to harlequins than predicted by the model's support.

While this allowed me to explore a greater number of variables, and give all variables a chance to be evaluated in at least one model, this meant that, following model selection, I was left to interpret the effects of, temporally, relatively fine scale climatic variables. I was also aware that there were many obvious correlations within the climate, terrain, vegetation, human impact and fish variables and likely there were less obvious but still collinear effects within the data set as well.

In order to be able to more holistically describe the correlated and collinear suites of environmental variables, I ran a principal component analysis on the top supported variables from all of the harlequin duck models. In addition to the reasons above, this analysis was also done partly to determine whether there were latent, underlying environmental factors within the suite of biogeoclimatic variables that described ecosystem-level features that perhaps drive harlequin duck habitat usage at a scale of more importance to the wide-ranging, long-lived species. Thus, this factor analysis aimed to describe underlying trends in the biogeoclimatic data that would best explain the effects of individual variables on duck abundance. In addition to the best supported variables from the harlequin duck models, I also added several environmental variables

that were important to pupae and scrapers, which were the two invertebrate variables identified as important in the final harlequin duck models.

Principal component analysis of the best supported variables explaining harlequin duck presence and abundance under each hypothesis (Table 2.7), plus selected variables explaining pupae presence and scraper abundance, yielded 7 eigenvectors with eigenvalues greater than 1. Together, these principal components explained 85.2% of the total observed variance in the variables. Varimax rotation reduced the 27 selected variables down to seven factors (ENV1 to ENV7) that revealed some habitat types and other underlying patterns in the variables (Table 2.8). ENV1 described habitats with high precipitation throughout much of the year, but with a distinct dry period, and was positively associated with low elevation, coastal mixed and temperate needleleaf forests. High values of ENV2 described taiga needleleaf forest habitat that occurs at high elevations and latitudes, while low values described mixed forest and deciduous forest habitats, which tend to co-occur in the south with urban build-up. Positive values of ENV3 described an association between wetlands, grass-shrublands and croplands, while negative values described high order streams in temperate needleleaf forest.

Stream size variables mostly loaded onto a single factor together; stream order and width were positively associated with ENV4, along with April precipitation, while site slope was negatively associated with ENV4.

Most of the invertebrate variables in the factor analysis were not highly correlated with the other environmental variables (ENV5-7). ENV5 described the positive association between small scrapers and pupae. The sixth factor (ENV6) mainly described the abundance and biomass of large predatory larvae, while the seventh (ENV7) described small shredder abundance.

**Table 2.8 Factor analysis of the best supported variables explaining harlequin duck presence and abundance under all proposed hypotheses.**

| | Factors | | | | | | |
|---|---|---|---|---|---|---|---|
| **Variable** | **ENV1** | **ENV2** | **ENV3** | **ENV4** | **ENV5** | **ENV6** | **ENV7** |
| April Precipitation | **0.528** | -0.113 | -0.146 | **0.682** | -0.272 | 0.023 | -0.093 |
| March Precipitation | **0.905** | -0.239 | 0.053 | -0.127 | -0.078 | 0.161 | -0.162 |
| Mean Temp Wet Quarter | -0.372 | **0.820** | -0.284 | -0.019 | -0.059 | -0.079 | 0.044 |
| October Precipitation | **0.932** | -0.115 | -0.095 | -0.177 | -0.047 | 0.102 | -0.143 |
| Precipitation Seasonality (CV) | **0.683** | 0.294 | -0.088 | 0.375 | 0.050 | -0.052 | 0.397 |
| Precipitation (wet period) | **0.978** | 0.071 | -0.077 | 0.074 | -0.005 | 0.089 | -0.046 |
| Precipitation (dry period) | **-0.743** | 0.253 | 0.315 | -0.313 | 0.185 | 0.128 | -0.194 |
| 01_TemperateNeedleaf | **0.472** | -0.045 | **-0.774** | 0.133 | 0.076 | -0.139 | 0.197 |
| 02_TaigaNeedleleaf | **-0.401** | **0.830** | -0.001 | 0.101 | 0.101 | -0.104 | -0.151 |
| 05_Deciduous | -0.131 | **-0.889** | 0.204 | 0.264 | -0.015 | -0.088 | -0.038 |
| 06_MixedForest | **0.751** | **-0.602** | -0.131 | 0.020 | -0.039 | 0.046 | -0.117 |
| 08-13_GrassShrub | -0.218 | -0.223 | **0.664** | -0.155 | 0.032 | 0.137 | 0.206 |
| 14_Wetlands | -0.104 | -0.028 | **0.944** | -0.008 | -0.001 | -0.170 | -0.009 |
| 15_Croplands | -0.024 | -0.267 | **0.816** | -0.101 | 0.105 | -0.223 | 0.049 |
| 17_Urban | -0.050 | **-0.700** | -0.013 | -0.325 | 0.179 | -0.242 | 0.268 |
| Latitude | -0.117 | **0.859** | -0.175 | 0.158 | -0.124 | -0.064 | 0.288 |
| Elevation | **-0.742** | **0.551** | 0.193 | -0.216 | 0.040 | 0.085 | -0.069 |
| Distance to Coast | **-0.893** | 0.049 | 0.257 | -0.116 | 0.260 | -0.067 | 0.048 |
| Order | -0.046 | 0.080 | **-0.426** | **0.683** | 0.242 | -0.267 | -0.007 |
| Site Slope | 0.029 | 0.044 | -0.063 | **-0.579** | -0.049 | -0.265 | -0.388 |
| Width | 0.065 | 0.099 | -0.097 | **0.884** | 0.040 | -0.104 | -0.004 |
| Sqrt(Small Scraper Count) | -0.105 | -0.199 | 0.128 | -0.140 | **0.772** | 0.110 | 0.273 |
| Small Shredder Count | -0.119 | 0.041 | 0.062 | 0.062 | 0.319 | -0.121 | **0.838** |
| Large Predator Count | 0.119 | -0.051 | -0.081 | -0.123 | 0.174 | **0.863** | 0.015 |
| Sqrt(Predator Biomass) | 0.057 | 0.071 | -0.052 | 0.033 | -0.097 | **0.877** | -0.092 |
| Pupae Present/Absent | -0.114 | -0.038 | -0.038 | 0.059 | **0.822** | -0.118 | 0.114 |
| Sqrt(Pupae Biomass) | -0.077 | 0.061 | -0.013 | 0.113 | **0.885** | 0.100 | -0.024 |
| Eigenvalue | 8.06 | 5.02 | 3.06 | 2.28 | 1.85 | 1.66 | 1.06 |
| Cumulative Percent of Variance Explained (%) | 29.9 | 48.5 | 59.8 | 68.3 | 75.1 | 81.2 | 85.2 |

Loadings > |0.40| have been emboldened for clarity.
Cumulative percent refers to the percentage of total variance explained by that factor, in addition to each preceding factor.

Grass-shrublands was consistently identified as the strongest discriminator of true from false zeros (Table 2.7). The higher the proportion of grass-shrublands and, in one model, the steeper the site slope, the more likely that a count of zero was likely to be true (Figure 2.2). In the count portion of the model, stream order was consistently one

of the strongest predictors of duck abundance (Figure 2.2). The presence of fish tended to increase predicted harlequin counts relative to their prediction based on other environmental variables, such as stream order (Figure 2.2).

Only two of the invertebrate dependent variables examined, presence of pupae and proportion of scrapers, were also affected by stream size variables (Figure 2.3). Interestingly, these same invertebrate variables were identified as important in the final harlequin duck models as well (but only if fish presence was also included) (Table 2.7). The predicted probability of pupae presence increased with increasing stream order and March maximum temperature, and decreased with increasing site slope (Figure 2.3). Precipitation seasonality decreased scraper abundance (Figure 2.3). Scraper abundance increased with river width when harlequins were present (Figure 2.3). On reaches unoccupied by harlequin ducks, scraper abundance was unaffected by width.

## 2.4. Discussion

I found some support for each hypothesis of harlequin duck presence and abundance, but some were more explanatory than others (Table 2.7). When examining the zero-inflation component of the harlequin models, one variable was consistently identified as the strongest predictor of true harlequin duck absences: the higher the proportion of grass and shrublands, the more likely that observed absences were true absences, and not simply failures to observe harlequin ducks during the streamside surveys. Thus, percentage of grass and shrublands in the area seemed to be an indicator of general habitat suitability (harlequins present or not). However, within suitable watersheds, stream order was consistently the best predictor of harlequin abundance (Table 2.7; Figure 2.2).

Harlequin preference for increasing stream size was consistent with previous studies that found harlequins to prefer intermediate sized streams, third to fifth order (Bruner, 1997) and above (Morneau et al., 2008). While it was not evident within the range of stream sizes I selected, there is evidence for upper limits to the size of streams that harlequins will use for pre-breeding, nesting, and brood-rearing. Rodway (1998b) found that the streams used by harlequins throughout the breeding season had a mean width of 22 ± 21 (SD) m, whereas larger streams, with a mean width of 48 ± 40 (SD) m were not used. Here, I found harlequins used streams with a mean width of 16.65 ± 6.3

(SD) m while unused streams had a width of 12.45 ± 7.6 (SD) m. My field sites had relatively narrow widths due to the emphasis on finding fishless streams, which tend to be higher up in watersheds and smaller than streams containing fish.

There are a few potential explanations for the observation that harlequins seem to prefer increasing stream sizes (up to a point). The first possibility is that preferred stream sizes are associated with certain invertebrate prey communities. The only invertebrate variables directly affected by various measures of stream size were scrapers (stream width) and presence of pupae (stream order and site slope). The proportion of scrapers increased with stream width, consistent with the river continuum concept; higher order rivers are wide enough to preclude complete canopy closure, opening the stream up to light while also reducing leaf inputs, increasing algal growth and associated scraping organisms (Vannote et al., 1980). Interestingly, scrapers only increased with river width in reaches used by harlequins. Thus, harlequins may prefer stream sections with large numbers of scrapers. Alternatively, harlequins may release scrapers from predation pressure by consuming large predatory invertebrates, such as stoneflies (e.g. McCutchen, 2002).

There was otherwise not strong support for harlequin duck presence or abundance to be linked directly to various components of invertebrate abundance. Almost all final invertebrate models contained climate variables, especially those related to temperature. In contrast, none of the final harlequin duck models contained any climate variables, and even the final harlequin duck climate-only models only contained precipitation-related variables, as opposed to the temperature-related variables associated with most invertebrate variables. Thus, there did not seem to be a lot of support for the hypothesis that harlequin ducks select certain habitats due to the invertebrate communities associated with those habitats.

It made sense that invertebrate variables were mostly affected by temperature variables. Air temperature and stream water temperature are tightly linked. Water temperature affects invertebrate growth rates (and metabolic requirements), as well as dissolved oxygen levels in the river. Warm water holds less dissolved oxygen, and this has both a latitudinal component, and altitudinal; as elevation rises, air pressure and oxygen solubility drop, but dissolved oxygen is somewhat balanced out by the lower temperatures at high elevations. Latitude, elevation and temperature variables were all

found to affect measures of invertebrate abundance and biomass, but only temperature (and precipitation) variables were retained in the final invertebrate models.

Two invertebrate variables were explicitly identified in the final harlequin duck models: presence of pupae and number of small scrapers. Both variables were positively related to duck abundance, suggesting that pupae and small scrapers may be preferred prey items for harlequin ducks. However, scrapers were often found in higher proportions on streams without ducks, except for the widest streams (Figure 2.3). Either some wide streams are able to host increased scrapers for some reason, or harlequins could be feeding on a third trophic level between ducks and scrapers: predators. The abundance and total biomass of predatory invertebrates, mostly driven by abundance of large individuals (length > 1 cm), was negatively related to both harlequin duck abundance and stream order. Harlequin ducks may therefore be preferentially feeding on large, high value predatory invertebrates, releasing smaller scrapers from predation pressure. These large predators may be preferred by harlequin due to their mobile hunting strategy and large size making them highly visible, as well as energetically rewarding. Suppression of predatory invertebrates, either directly, by reducing their densities, or indirectly, by reducing their activity, can have large positive effects on scraper abundance, even cascading down to the level of algae. This could explain the positive relationship between harlequin and small scraper abundance.

Alternatively, the presence of fish in all of the sixth order streams could have cascading effects on food web interactions. Not only do fish release scrapers from predation pressure from predatory invertebrates, their presence can also cause mayflies to develop faster and emerge sooner and at smaller sizes, in order to escape predation pressure in the larval aquatic habitat (Peckarsky et al., 2001, 2002). Thus, the increased presence of scrapers on the widest streams could result from a combination of compounded effects from fish presence. This appeared to be favorable for harlequins; harlequin abundance increased with small scraper abundance, especially on the widest streams, which also happened to contain fish.

The probability of pupae being present also increased with increasing stream order and decreasing slope (Figure 2.3), which are both related to stream size. Slope tends to decrease with increasing order; at decreasing elevations within a watershed, streams grow larger and less steep. Thus, perhaps harlequins prefer higher order

streams because they prefer consuming scrapers and pupae. Indeed, case-building caddisfly *Glossosoma* spp. larvae (which are scrapers) and pupae were particularly numerous in some fish-bearing river sections. *Glossosoma* pupae are immobile and carry large energy reserves to meet the demands of metamorphosis, making them a potential high value prey item for both ducks and fish. *Glossosoma* greatly reduce the costs of fish predation by building stone cases around themselves, increasing gape-limitation of fish predators. These cases are portable at the larval stage, making *Glossosoma* a very successful scraper species where fish are present (Kuhara et al., 1999). Other scraping taxa, such as *Baetis* spp., which is normally an exploitative competitor with *Glossosoma*, will abandon patches to *Glossosoma* when fish are present, due to the extreme advantage of the case-building taxa in greatly reducing predation pressure (Kuhara et al., 1999). Glossosoma also dilute the individual risk of predation by building "colonies" of stone-cased pupae together in order to spread predation risk across the group. However, *Glossosoma*, especially pupae, have been found to be a preferred prey item for harlequin ducks (Richard L Wallen, 1987), which could explain the positive effect of fish on duck abundance.

Harlequin duck apparent preference for larger stream size could also be due to the open view of the sky, which should increase a harlequin's ability to scan for aerial predators (Heath et al., 2006; MacCallum et al., 2016). However, if ability to scan the open sky is one of the key basis for habitat selection, I would have expected canopy coverage or width to be a stronger predictor of harlequin abundance than stream order; however, neither were identified in the land cover or terrain models, respectively, of harlequin abundance. Additionally, one might expect avian predation pressure, e.g. from eagles, to be higher where fish are present, since fish are a common prey item for avian predators, causing harlequins to avoid streams with fish; however, I found the opposite. If anything, harlequin abundance increased where fish were present, even when I controlled for stream size.

This is an important finding, as it fails to support one of the key hypotheses that drove my work: that the introduction of fish to fishless waters across the harlequin duck breeding range lowers habitat quality for harlequin ducks (either through direct competition for food resources or through antipredator behaviors or adaptations that fish presence indirectly induces in shared prey resources) (LeBourdais et al., 2009). Not only are fish apparently not negatively impacting harlequins, potentially fish eggs, or

beneficial changes induced in the invertebrate community are positively affecting harlequins where fish are present. For example, Crowley (1993) found that harlequins used the largest salmon spawning streams for nesting, likely benefitting from the rich nutrient subsidy. Salmon spawning also provides nutrient subsidies in stream, to invertebrates, where the marine nutrients they transport to freshwater ecosystems can be traced through stable isotopes into the stream invertebrate community and, subsequently into the interconnected terrestrial ecosystem within which the stream system is embedded, both through carcass decomposition, but also terrestrial insect subsidies as freshwater larvae pupate and emerge as terrestrial adults to breed.

It should be noted that the analysis presented here did not attempt to distinguish between freshwater and anadromous fish, benthic or column feeding species, or introduced and naturally-occurring populations. Future studies will need to consider these differing effects as well. This might allow for finer scale distinctions to be made across field sites with different populations of fish, reducing the need to find completely fishless river sections as reference sites, instead allowing harlequin habitat preferences to be determined over a fish gradient. In my study, fishless streams tended to be small and fish bearing streams were the largest, which directly confounded tests of fish effects with the strongest predictor of harlequin abundance: large stream order.

Grassland shrubland classes were combined because of their low individual values; combining them still only resulted in a mean percentage of 13.74% (± 8.28 SD) of lands within a 5 km radius. However, all four reaches in one watershed, Pincher Creek (Figure 2.1), had the highest percentages of grass and shrublands (range: 32.2-45.1%) and also had zero harlequin observations. As such, the percentage of grass and shrublands in a watershed may determine its overall suitability for harlequin duck usage at the highest hierarchical scale: the higher the proportion of grass and shrublands in the area, the more unsuitable the overall habitat may be for harlequins. Grass and shrublands provide little of the forest habitat required for nesting (E. F. Cassirer et al., 1993); thus, it is possible that the Pincher Creek watershed does not provide sufficient nesting habitat for harlequin ducks. Indeed, factor analysis revealed that grass and shrublands were negatively associated with percentage of temperate needleleaf forest (ENV2; Table 2.8), which was, itself, positively associated with harlequin abundance in the land cover models.

Grass and shrublands were also positively associated with both wetlands and agriculture (ENV2; Table 2.8), both of which were found to have negative associations with harlequin duck presence and abundance. Grasslands and wetlands are both natural habitats that tend to be disproportionately disrupted by agricultural activities, due to their association with large, flat expanses of rich soils with few trees to remove (Coombs & Thie, 1979). Thus, the grasslands-wetlands-agricultural matrix found in the Pincher Creek watershed, which borders on the transition to the Canadian prairies, may simply be overall unsuitable habitat for harlequin ducks. However, public sightings have been recorded in the area in the past, as well as since the surveys were conducted (i.e. post-May 2015). These sightings suggest that, while the area can be used by harlequin ducks, it likely serves as a low abundance, low stability population that can approach local extinction in some years (Heath et al., 2006; Heath & Montevecchi, 2008), especially when compounded with additional stressors, such as the 2013 flood.

There was a negative association between the 2013 flood and harlequin duck abundance in the climate-only models (Table 2.7). Harlequin productivity has been linked to low winter precipitation (i.e. snowpack) and early spring runoff; less snow contributing to spring runoff and earlier runoff means that peak flows are over far before harlequin nest initiation, lowering nestling mortality due to flooding (Kuchel, 1977). However, the watersheds most affected by the 2013 flood (Lower Kananaskis, Fernie, Pincher Creek) also happened to contain higher percentages of grass and shrublands than other sites. Therefore, it was difficult to distinguish between the effects of grass and shrublands over flood, but grass and shrublands was retained in the final harlequin models, rather than flood. Alternatively, grasslands could be a result of frequent flooding, which can lower standing invertebrate stocks (Poff et al., 1997; Power et al., 2008) and flood harlequin nests (J. Bond et al., 2007). However, land cover was consistently supported as a more explanatory predictor of harlequin duck presence than climate.

Indeed, no explicit climate effects were retained in the final harlequin models, including the 2013 flood. However, all of the harlequin duck models retained grass and shrublands in the zero-inflated portion and small scraper count and presence of pupae in the count portion, all of which are likely to be influenced by climate. In fact, the proportion of grass and shrublands may provide an integrated index of ecosystem suitability for harlequin ducks, which performs better than climate effects that vary more in space and time. Highly temporally and spatially variable environmental drivers are

more likely to produce direct effects in organisms that respond to environmental cues on a similar scale, such as invertebrates. Harlequin ducks live much longer and slower lives, over much greater geographic distances than do freshwater invertebrates. It makes sense that harlequin ducks would be better predicted by a holistic, integrated measure of biogeoclimatic variation.

Factor analysis also revealed a negative association between the percentage of grass and shrublands and river order (ENV2; Table 2.8), the latter of which was identified as the strongest predictor of harlequin abundance. Since river order is determined solely by topography (see Methods), the relatively small river orders of streams observed in areas with lots of grass and shrublands was probably due to their relatively flat topography. Rather than constraining stream flow to a steep and narrow path, as seen in mountain areas, the flat topography of grass and shrublands spreads water flow across a wide and shallow area with slow flows. Harlequin ducks prefer clear, fast-flowing streams during the breeding season (Kuchel, 1977; Robertson & Goudie, 1999); therefore, the strong association between percentage of grass and shrublands and harlequin duck absence could be due to a lack of suitably sized, fast-flowing streams; however, grass and shrublands, rather than stream size itself, was consistently the best predictor of harlequin duck absence (Table 2.7).

### 2.4.1. Future studies

Future studies of harlequin duck breeding season habitat should begin by first determining broad scale habitat suitability as determined by climatic restrictions. Watersheds should then be screened by the overall availability of suitable pre-nesting, nesting, and brood-rearing habitat, using both vegetation (e.g. forested vs. grass-shrubland areas) and terrain variables. Within suitable watersheds, habitat should then be evaluated along linear river lengths, looking at the overall availability of suitable feeding sites (pre-nesting), covered nesting sites (nesting) and brood-rearing habitat as determined by canopy and streamside vegetation (MacCallum et al., 2016). These habitat associations may also differ among watersheds that experience different levels of predation pressure. For example, landscape features that facilitate predator avoidance or vigilance may be of higher importance where predation pressure is high, whereas features that influence prey availability may take precedence where predation pressure is lower. Future studies could investigate whether the relative importance of different

categories of environmental predictors depends on the presence or abundance of predators or predator-related features.

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# Chapter 3. MaxEnt habitat suitability models for breeding harlequin ducks in British Columbia and Alberta

## 3.1. Introduction

Species distributions are driven by biotic and abiotic factors that vary over different spatial and temporal scales (Mayor et al., 2009). The effects and relative importance of these environmental drivers is tightly linked to the biology of each species. Long-range migratory species, such as harlequin ducks, experience a particularly large variety of habitat restrictions over different spatial scales (Newton, 2004). For such highly mobile species, habitat selection likely involves a hierarchical process, from broad-scale habitat suitability to selection depending on the availability of local habitat structures within larger areas, such as watersheds, all the way down to the nest-site (Heath et al., 2006). In order to isolate the effects of local variance in biological and physical factors on species presence and abundance, studies should account for broader geographic habitat differences, such as those that vary most at the watershed or regional level.

Additionally, many factors affecting species distributions, such as climate and terrain, are intricately interlinked, changing together over geographic space, as well as modifying the effects of one another on habitat suitability for different species (Hijmans et al., 2005). As such, it can be difficult to disentangle correlated and collinear effects of various environmental factors from underlying differences driving climatic, topographic and hydrological patterns. Failure to consider these broad scale effects or account for their differences among regions, such as watersheds, can make it difficult to isolate the effects of site-level characteristics within the context of broader geographic drivers of distribution, such as climate. Characterizing the effects of human-induced stressors can be especially problematic, as these effects are often widespread but diffuse (occurring at low densities), and are often correlated with confounding factors that can also affect distribution.

Looking at a broad range of environmental variable combinations may provide the best chance to disentangle the individual effects of impacts of interest (such as specific suspected human impacts), as well as identifying those habitat characteristics

most likely to exacerbate those impacts. However, this strategy presents the logistical problem of surveying presence and abundance of the species of interest and characterizing environmental variables at a large enough spatial extent, over the same temporal window, to allow such inferences. Additionally, perhaps the most difficult challenge is separating true absences from failures to observe.

Presence-only modeling attempts to address these issues by using a machine learning algorithm to build probability distributions using large repositories of “sighting” data, along with remote sensing or broad-scale climatic modeling data to estimate habitat suitability. The algorithm builds model rules by contrasting known presence points against “background sites” (i.e. sites chosen at random from where presence is unknown) over a variety of environmental variables. One can choose from a variety of algorithms (modeling approaches, e.g. BioClim, DOMAIN, GAP, GARP, GAM, GLM, MaxEnt, random forest, ANN, SRE, MARS) or use them simultaneously and compare the results (ensemble modeling). I chose to use Maximum Entropy Species Distribution Modelling (MaxEnt), which has been used in over 1000 published studies of species distribution and has been shown repeatedly to perform well (Baldwin, 2009; Costa et al., 2010; Elith et al., 2011; Merow et al., 2013) in addition to being open access and user-friendly. MaxEnt uses presence only data and an assortment of environmental variables to predict the probability distributions of habitat suitability given the environmental variables (Phillips et al., 2006). It can be used over any spatial extent, even worldwide, as long as each environmental variable has the exact same spatial coverage.

To my knowledge, to date, virtually all of the studies of harlequin duck breeding habitat preferences have been local, e.g., (Bengtson, 1972; Bengtson & Ulfstrand, 1971; Bond et al., 2007, 2007; Bruner, 1997; Crowley, 1993; Heath, 2001, 2001; LeBourdais et al., 2009; MacCallum, 2001; Machmer, 2001; Rodway, 1998). In fact, there appears to be only one regional study of harlequin duck habitat preferences: Heath et al., (2006) found that harlequin duck habitat and prey availability did not differ among source and sink populations of harlequin ducks. Rather, sink populations occurred in areas with higher densities of nesting ledges for raptorial birds. As such, birds of prey may limit harlequin ducks from otherwise suitable habitat. However, Heath et al.’s (2006) regional study was conducted on the eastern North American population, which is isolated from the western population that is the focus of this dissertation. Additionally, the glacially

carved canyons through high subarctic plateau inhabited by the birds in the Heath et al., (2006) study is quite dissimilar from the montane habitat that I studied.

Given the lack of regional habitat suitability studies for the western population of harlequin ducks, I created a regional model of harlequin duck habitat suitability for British Columbia (BC) and Alberta (AB) in western Canada. Since the western North American population of harlequin ducks are isolated from the eastern population, my goal was to determine which environmental variables were most important to the western North American population of harlequin ducks. I selected BC and AB so that my results would be directly applicable to birds breeding in the provinces in which all of my field work data were collected. Therefore, the broad-scale analysis conducted here would pertain to the same population (or metapopulation), and thus, be comparable, to the analysis of harlequin duck habitat suitability examined at the reach-level in the field (Chapter 2). However, this regional analysis allowed me to investigate harlequin duck distribution at over an unprecedented combination of biogeoclimatic variables, increasing the opportunities to disentangle their individual and interrelated effects.

The environmental predictors I included came from four foundational categories of species distribution (van Gils et al., 2014): climate, terrain, vegetation, and human impacts. Model building and variable selection were conducted in a series of steps. The first step was to model climate, since it typically constrains distribution at the broadest level (Baldwin, 2009). Most of these effects are likely to be related to food (freshwater macroinvertebrates), such as timing of ice-out and peak runoff, length of growing season, effects of temperature and day length on growth rate, and the frequency, severity, timing and length of droughts and scouring floods (see Chapter 2 for a summary of these effects).

The second step was to model harlequin duck habitat suitability based on terrain related variables only. Terrain variables typically denote positional data, such as altitude and distance inland, which can also have strong effects on climate, influencing species distributions. For example, elevation can be used as a proxy for ambient temperature and precipitation. Elevation, slope and stream order can also give an idea of position within a watershed, from headwater streams to medium-sized, high productivity rivers, to deeper and slower large rivers. The river, and its related riparian habitat, changes along this longitudinal gradient in predictable ways (Vannote et al., 1980). Small headwater

streams are colder and faster, have closed canopies, and obtain the majority of their organic matter from allochtonous sources (i.e. terrestrial plants). Compared to headwaters, medium sized rivers have shallower slopes, are wider, more open to light, produce more algae and contain associated scraping invertebrates. The largest rivers are slow, which is not conducive to the harlequin's first escape strategy – floating quickly downstream. They are also deep, reducing visibility of prey items.

The third step was to combine climate and terrain data into a single model, given both climate and terrain data may give interrelated indications of food (freshwater invertebrate) availability for harlequin ducks.

The fourth step was to add human impact factors to the final combined model. Vegetation cover type can be considered an integrated index of ecosystems (van Gils et al, 2012), and can also provide indications of land-use, both direct, e.g. cropland and urban buildup, and indirect, e.g. a lack of forest cover in areas of logging and resource extraction. Thus, I added a categorical layer of land cover type to the combined climate and terrain model from step 3. Urban areas can affect ducks indirectly, by harming invertebrate communities through increased peak flow, and water temperature due to water impervious surfaces, such as concrete, contribute point source pollution and can also directly influence ducks through increased disturbance and habitat destruction. Agricultural activities remove riparian forest, increasing erosion and decreasing canopy cover and terrestrial organic inputs, as well as nesting habitat.

Additionally, harlequin duck distribution can also be influenced by predators and competitors (Heath et al., 2006; LeBourdais et al., 2009; MacCallum et al., 2016). For example, widespread fish introductions to previously fishless waters, and increased fish abundance where fish stocking activities occur, can disrupt local food webs. Fish have the potential to reduce the quality of otherwise suitable breeding habitat for harlequin ducks by (1) competing, directly or indirectly, for aquatic invertebrate prey (LeBourdais et al., 2009), or (2) by attracting higher densities of shared predators, such as eagles. However, it is difficult to confirm the presence (or absence) and abundance of fish in a single river reach, much less all rivers across BC and AB (as my regional analysis required). Given this difficulty, I chose to use sightings of bald eagles, *Haliaeetus leucocephalus*, as an indicator of potential predators and competitors. Bald eagles are a potential indicator of fish, given fish are a key resource for bald eagles (Buehler, 2000),

and access to foraging areas is an important determinant of bald eagle breeding habitat (Jackman et al., 2007; Thompson & McGarigal, 2002). In some areas, loss of fish stocks has displaced populations of avian and mammalian predators which fed upon them (Spencer et al., 1991). Bald eagles are an even more appropriate surrogate, as eagles are a known predator of harlequin ducks (reviewed in Heath, 2001). Given the potential negative impact of fish and/or eagle presence on Harlequin duck habitat suitability, the fifth step was to add bald eagle presence. My overall goal with this five step modelling approach was to identify the most important drivers of harlequin duck regional distribution from among climate, terrain, vegetation and human impact factors.

## 3.2. Methods

### 3.2.1. Presence Data

Harlequin duck presence data were downloaded from NatureCounts.ca (all available records from April-August from 1970-2019 in any inventory: BC Breeding Bird Atlas, Project Nestwatch, Alberta Bird Records, Great Backyard Bird Count) and from GBIF.org (Global Biodiversity Information Facility: a publicly available electronic repository of biodiversity inventories), with the exception of eBird data. Though available through GBIF.org, eBird data were not included in the training portion of the modeling procedure because there is no protocol or requirement for eBird sightings to be validated or confirmed. In contrast, iNaturalist, another inventory open to the public and available for download through GBIF.org, contains “research grade” presence data; these sightings require a photographic specimen which must then be validated by another iNaturalist registered user. eBird data were, however, used as “test” data to evaluate the model fit; once MaxEnt had built the model using the training data from NatureCounts and the more rigorously validated sources on GBIF.org, the eBird data were used as “test” data using MaxEnt’s built-in test setting.

### 3.2.2. Sampling bias

MaxEnt assumes that all locations in geographic space are equally likely to be sampled (Elith et al., 2011; Merow et al., 2013; Phillips et al., 2006). As such, it assumes that all locations in environmental space are sampled proportionately to their frequency (e.g. if 80% of the area of interest is mountainous terrain, then mountainous terrain is

assumed to receive 80% of the sampling effort). However, sampling bias often occurs, especially in databases containing public sightings, with higher sampling "effort" in areas that are highly accessible to humans, e.g., along roads, coastlines, and near urban centres, where terrain is smooth and flat or gently inclined. Observations are probably also biased towards areas with internet access (i.e. in the south).

To control for sampling bias, I used MaxEnt to create a model for a behaviorally similar taxon using environmental factors that were likely to affect sampling effort, rather than habitat requirements (Merow et al., 2013). American dippers, *Cinclus mexicanus*, are behaviorally similar to harlequin ducks during the breeding season, with dippers having the investigatory advantages of being easier to spot and more abundant than harlequins. As such, dippers tend to be an indicator that an area may be suitable for harlequins (i.e. you will see dippers before you see harlequins; pers. obs.). Dippers and harlequins are so behaviorally similar that the B.C. Ministry of Environment, Lands and Parks lists them in the same, "Inventory Methods for Riverine Birds," field guide, along with belted kingfishers (British Columbia et al., 1998). However, dippers and harlequins additionally share another habitat-related characteristic in consuming, nearly exclusively, freshwater macroinvertebrates during the breeding season (Robertson & Goudie, 1999; Willson & Kingery, 2011). Thus, anywhere that dippers have been reported can reasonably be assumed to represent a potential harlequin duck sighting as well, thereby serving as a proxy of sampling effort. The resulting layer of sampling effort was then used in MaxEnt's built-in "bias layer" setting for the harlequin duck models. Dipper sighting data were retrieved from the same repositories (i.e. NatureCounts and GBIF.org minus eBird records) and date ranges (April-August 1970-2019) as harlequin duck data.

### 3.2.3. Environmental data

For the climate variables, I selected from the 19 bioclimatic variables contained in the WorldClim version 2 dataset. The bioclimatic variables in the WorldClim 2 dataset are derived from monthly temperature and rainfall values in order to generate more biologically meaningful variables. The bioclimatic variables represent annual trends (e.g., mean annual temperature, annual precipitation) seasonality (e.g., annual range in temperature and precipitation) and extreme or limiting environmental factors (e.g., temperature of the coldest and warmest month, and precipitation of the wet and dry quarters). WorldClim also contains monthly averages, minimums and maximums for

temperature and precipitation. All of these variables represent average values over the period 1970-2000 (Fick & Hijmans, 2017). I included six monthly variables, identified as being potentially important to harlequin ducks in Chapter 2, in addition to the 19 bioclimatic variables.

I derived several terrain-related attributes from the 30 arc-second (approx. 1-km) resolution version of Canada3D, a digital elevation model (DEM) produced by the Canadian Forestry Service, Ontario region. Canada3D was derived from the cells of the Canadian Digital Elevation Data (CDED) at the 1:250 000 scale. I used ArcMap 10.5's Surface toolset (Spatial Analyst extension) to obtain layers of aspect (compass direction of the face of the surface, i.e. whether a hillside faces north or south) and slope (degree of incline) from the Canada3D DEM.

I used ArcMap 10.5's Hydrology toolset (Spatial Analyst extension) to create a raster layer defining waterways and their respective stream orders from the Canada3D DEM. Stream order provides an estimate of position within a watershed, from headwater streams (first order) to mainstem rivers (e.g. approx. sixth or seventh order). Headwater streams are given a value of 1, and stream order increases when streams of the same order intersect: two first order streams meet to create a second order stream; two second order streams meet to create a third order stream, and so on (Strahler, 1952).

To estimate stream order, anomalies and outliers in the DEM were first filled to create a depression-less DEM, which was then used to calculate flow accumulation and direction. Since the resolution of the DEM was relatively coarse (1-km resolution vs. the 30-meter resolution typically used for hydrological analysis), cells denoting waterways were defined as those with a flow accumulation of greater than 1 cell (vs. 500 or more in typical hydrological analysis). After calculating the Stream Order of cells with accumulation greater than 1 cell, cells were effectively "buffered" by running the Focal Statistics tool to determine the maximum Stream Order within a circular, 1-cell radius "Neighborhood" (approx. 1 km) around each cell. Cells with a flow accumulation of 1 or less were assigned a Stream Order of 0 (i.e. terrestrial), using ArcMap's raster calculator (Figure 3.1).


**Figure 3.1 Stream order as calculated at the 1-km resolution using ArcMap 10.5's Hydrology toolset.**

Note: Map represents a small subsection of full extent used for MaxEnt model building.

To categorize land cover, I used the 30-m resolution North American Land Change Monitoring System (NALCMS) 19 class layer for 2010. I resampled this layer at 30 arc-seconds (approx. 1 km) resolution, to match the resolution of the previously included climatic and terrain layers. Individual land cover categories were considered to be positive predictors when the predicted suitability was greater than 0.5 for that category, e.g. (van Gils et al., 2014).

Bald eagle sighting data were downloaded from the same date ranges and repositories as harlequin duck data. To reduce the sampling bias in these data, I used the Sampling Design Tool for ArcGIS 10 to reduce sightings proportionate to their proximity to roads, urban centres, and human population density. I did this by creating a MaxEnt model for the bald eagle sightings using (1) a layer of distance to roads and cities and (2) a layer of human population density. This resulting model essentially gives an estimate of sampling bias or detection probability due to those variables. I then created 20 categories of "habitat suitability" from the continuous layer by multiplying the MaxEnt model output (continuous value from 0.0-1.0) by 20 and rounding up. I then instructed the Sampling Design Tool to subsample the sightings so that the sightings were thinned until each category contained approximately the same amount of sightings

(500). Ten categories (with the lowest probabilities of detection) contained no sightings or fewer than 500 (100-200 instead).

The sightings were then considered to have been somewhat thinned to remove sampling bias, as expected from distance to roads and cities as well as human population density. The remaining samples were then used to create a raster layer of 0s (no sighting) and 1s (sighting reported within a 2-cell radius) at the same resolution as the other environmental layers (30 arc-seconds). The resulting raster layer of 0s and 1s was then smoothed by using the Focal Statistics tool to calculate the mean cell value within a 2-cell radius. Thus, cell values were 1 in cells containing one or more sightings, then faded to zero within approximately a 3-cell radius, unless another cell containing one or more sightings was encountered. This layer was expected to provide some local indication of eagle presence, likely affected at some positive rate by fish presence.

However, it would also be reasonable to expect eagle sightings to be positively correlated with harlequin duck sightings due to the tendency for birders tending to report bird “lists” rather than single species occurrences. Similarly, most bird inventory protocols, such as the Breeding Bird Atlas, require point count or sighting data for all species encountered, not just focal species. Such surveys are usually submitted with a single set of geographic coordinates representing the entire survey, even for a 10x10 km survey area. As such, it is quite likely for harlequin duck and bald eagle observations to coincide in geographic space, especially since both species are highly associated with water. As described above, I tried to account for this by (1) thinning the bald eagle sightings and (2) creating a small 3-km radius circle with a gradient of “detection” probability centered around each retained sighting, as described above.

In addition to the steps above that were taken to reduce the odds of encountering a 1-to-1 correspondence between harlequin duck and bald eagle sightings, I also created a MaxEnt model of bald eagle habitat suitability to add to the model of harlequin duck presence. This reasoning behind this was to enable the model to distinguish between (1) suitable eagle habitat (from the MaxEnt layer) containing eagles (from the thinned bald eagle sighting layer) and (2) apparently suitable eagle habitat (MaxEnt layer) without eagles (sighting layer) in order to potentially identify areas with and without fish, respectively.

Similar to the harlequin duck models, I used a similar species, the common raven, *Corvus corax*, to create a sampling bias layer based on distance to roads and cities and human population density. Rather than reducing the models stepwise, I instead used the full suite of climatic and terrain variables considered for inclusion in the harlequin duck models, with the exception of distance to coast. Since distance to coast was explicitly included in the harlequin duck models to test the hypothesis that their breeding range is constrained by migration costs (whether time, distance, or energy), I did not want to risk confounding the eagle layer with this second hypothesis. Rather than containing any local information about actual eagle presence, density or behavior, the resulting layer gives a broad-scale estimation of potential habitat usage by eagles, to examine whether the underlying ecology of bald eagle distribution constrains harlequin duck distribution at a much larger, near range-wide scale.

**Table 3.1 Candidate variables included in variable selection following elimination of highly correlated variables (Pearson correlation coefficient > 0.70).**

| Category | Variable | Definition | Source |
|---|---|---|---|
| Climate | Annual Mean Temperature | Mean of mean monthly temps | WorldClim 2 (Fick & Hijmans, 2017) 1970-2000 |
| | Isothermality | (Mean of monthly (max temp - min temp)) / Temperature Annual Range * 100 | |
| | Temperature Annual Range | Max Temperature of Warmest Month - Min Temperature of Coldest Month | |
| | Mean Temperature of Warmest Quarter | Degrees Celsius | |
| | Precipitation Seasonality | Coefficient of Variation | |
| | April Precipitation | Mm | |
| | June Precipitation | Mm | |
| Terrain | Elevation | Metres above sea level | Canada 3D Digital Elevation Model (2001) |
| | Aspect | Compass direction of hillside | |
| | Slope | Degree of incline | |
| | Stream Order | Strahler Order (see text for definition and methodology) | Calculated using ArcMap 10.5 |
| | Distance to Coast | Euclidean distance to nearest ocean | |

| | | | |
|---|---|---|---|
| Human Impact | Land Cover | Categorical (Needleleaf, Deciduous, Mixed Forest, Grassland, Shrubland, Wetlands, Croplands, Barren, Urban, Water, Snow) | North American Land Change Monitoring System (2010) |
| | Bald eagle habitat suitability | MaxEnt model | April-August (1970-2019) sightings from NatureCounts.ca and GBIF.org |
| | Bald eagle sighting | Within 3 km radius | |

### 3.2.4. Model fit

I ran MaxEnt version 3.4.1 (Phillips et al., [Internet]) with settings as follows: random test percentage = 0; regularization multiplier = 1; maximum number of background points = 10,000. For ease of interpretation, I selected only linear, quadratic and product features. I used 10-fold cross-validation to evaluate model generality (the ability of the model to identify attributes of the species distribution). The threshold-independent area under the curve (AUC) of the receiver operating characteristic (ROC) plot (Fielding & Bell, 1997) was used to evaluate model fit. AUC can range from 0.5 (no better than random) to 1. Models with an AUC > 0.7 are considered to have good discriminative power – the ability to discriminate presence from reference points (Hosmer & Lemeshow, 1989), which, in the case of presence-only data, refers to background points (Merow et al., 2013).

### 3.2.5. Variable selection

I used a hierarchical stepwise approach to variable selection (Baldwin, 2009; Pearson et al., 2004; Van Gils et al., 2012), using four categories of environmental predictors: climate, terrain, vegetation, and human impact (van Gils et al., 2014).

Climatic variables were examined first, as they typically constrain distribution at the broadest geographic scale (i.e. the highest hierarchical level) (Pearson et al., 2004). It should be noted that WorldClim variables are correlated and collinear for multiple reasons: they change together due to underlying associations with elevation, latitude, and longitude. Additionally, the layers themselves are mainly interpolations between scattered weather stations. The algorithms that interpolate these meteorological point data are often based on underlying differences in topology and geographic position, and thus, the resulting interpolated layers often share similar geographic gradients. Despite

this, WorldClim variables are the most commonly used climate variables in MaxEnt terrestrial modelling (Booth et al., 2014).

I used ArcMap 10.5's Band Collection Statistics tool (Spatial Analyst extension) to calculate the correlation matrix for WorldClim 2's bioclimatic variables, as well as mean temperature and precipitation for a few key months identified as potentially affecting harlequin duck presence and abundance in the reach-level analyses (Chapter 2). Climatic variables that had Pearson correlation coefficients >0.7 were eliminated by retaining the variable with the strongest biological explanation or the variable which would retain the broadest variety of climatic predictors. For example, if one variable needed to be eliminated from amongst annual precipitation, mean annual temperature, and precipitation during the dry season, I retained one precipitation and one temperature variable, rather than retaining two measures of precipitation and none of temperature. I chose a relatively high cut-off value for the correlation coefficient due to the already inherently high correlations among variables due to the way in which climate point data are interpolated across geographic surfaces to create gapless raster layers.

After removing highly correlated variables, the full climate model retained seven of the 19 bioclimatic variables from the WorldClim 2 dataset, plus April and June precipitation (Fick & Hijmans, 2017) (Table 3.1). MaxEnt uses an iterative process to maximize model "gain". Gain is a penalized likelihood function, closely related to a likelihood (deviance) statistic. Exponentiating the gain gives the likelihood ratio of an average presence to an average background point; thus, model gain provides a measure of the model's ability to distinguish between presence and background (random) locations (Elith et al., 2011; Merow et al., 2013). "Redundant" predictors (those with the lowest calculated percentage contribution to model gain as calculated by MaxEnt) were eliminated stepwise until the most parsimonious model with an AUC greater than 0.8 was obtained, as in (e.g. Baldwin, 2009; Van Gils et al., 2012; van Gils et al., 2014). The same process was repeated using only variables relating to terrain (elevation, slope, aspect, ruggedness, distance to coast) and stream physical structure (stream order). The remaining variables from these two analyses were then combined into a single model, and reduced stepwise as before. To test for the effect of human disturbance, I lastly added the land cover layer (which contained both Urban and Crop categories) and eagle sighting layers (to test for a mechanism explaining the relatively low abundance of harlequins on river reaches containing fish).

## 3.3. Results

The relatively coarse resolution (1 km) caused the stream order analysis to miss most first order streams, identifying only second order streams and higher. Thus, each stream order estimate was 1 less than would typically be calculated. For example, the largest river in Figure 1, the Fraser River, is labelled as an eighth order river on the map, where it is typically considered to be a ninth order river. Regardless, this method yielded quite reliable estimates of river order, given the resolution (1 km) was more than 30 times coarser than what is typically used (30 m) for this type of hydrological analysis.

The sampling bias layer (Figure 3.2), produced by using MaxEnt to model American dipper observations against human population density and access to urban centres, had an AUC of 0.767 (AUC > 0.70 is considered to have good discriminative power; Merow et al., 2013). Probability of detection was mostly influenced by distance to roads and cities (relative contribution of variable to MaxEnt model: 82.6%) and decreased quickly as distance increased. Human population density only increased the regularized gain by 17.4%, with detection probability increasing sharply with human population density.

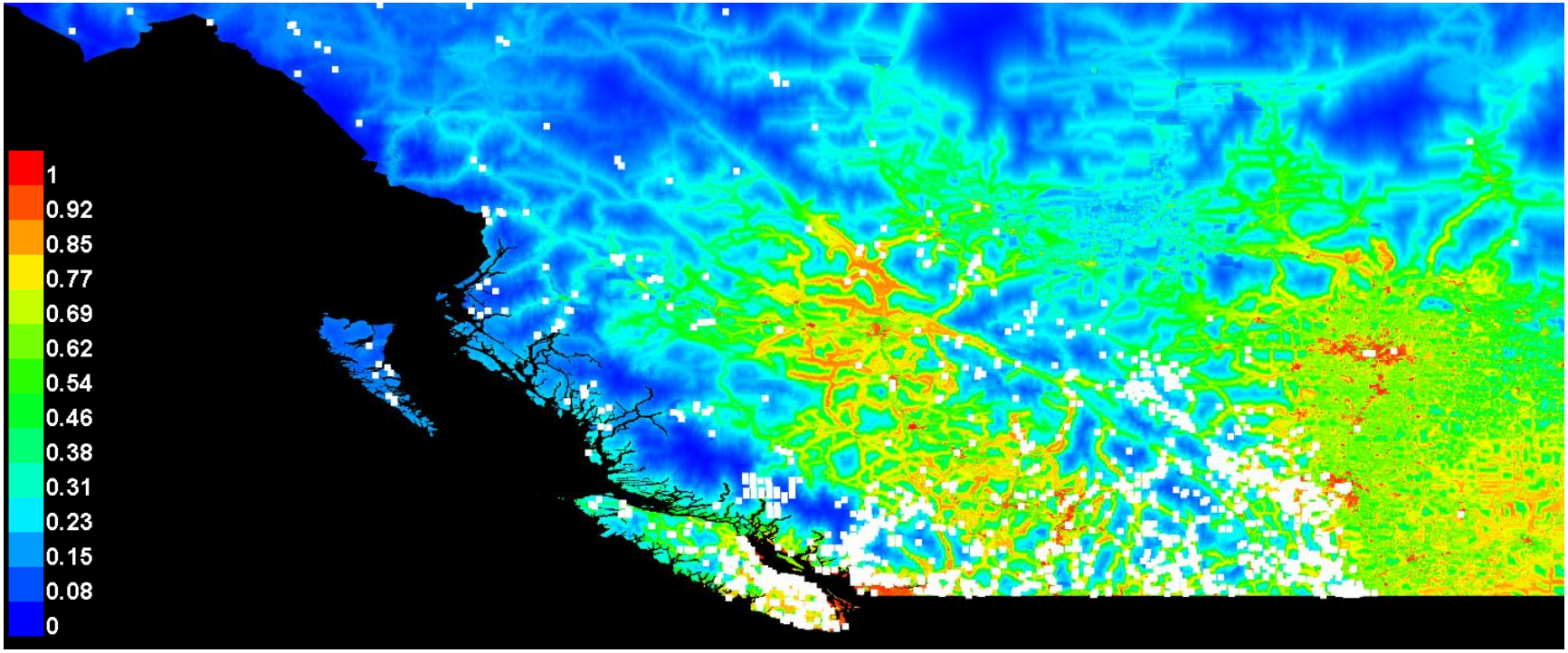



**Figure 3.2 Predicted "sampling bias" layer for harlequin ducks using American dippers, *Cinclus m.*, as a behaviourally-similar species. Habitat suitability is shown in BC and AB given (1) distance to roads and cities and (2) human population density.**

Note: Dark blue has the lowest probability of detection (0-0.08) while red has the highest (0.92-1.0). Detection probabilities increased along roadways and towards cities, especially those with high population densities. White dots indicate the *Cinclus m.* observations used to train the model (n = 1,913). This map shows the full geographic extent of all analyses presented in this Chapter.

The climate-only harlequin duck model (Table 3.2; Figure 3.3) was comprised of only four variables following stepwise reduction. All but one (precipitation seasonality) were related to temperature. Annual temperature range contributed the most gain to the model (49.6%). Precipitation seasonality was highly correlated with distance to coast (Pearson coefficient = 0.675) and was eliminated during the stepwise procedure when combining climate and terrain variables. Presumably, the distance to coast variable was better at distinguishing presence from background locations, which can also be seen in its large contribution to model gain (64.2%) in the terrain-only model (Figure 3.4). Slope and aspect contributed very little to model gain (2.3% and 1.3%, respectively). Presumably, the effects of these variables were better captured by other environmental factors, such as stream order (rather than slope). The final combined climate and terrain model (Figure 3.5) contained only three variables; annual temperature range, distance to coast, and stream order (Table 3.2).

**Table 3.2 Most parsimonious species distribution models for harlequin ducks in British Columbia and Alberta with an AUC > 0.80 for: (1) climate only, (2) terrain only, and (3) climate and terrain combined.**

| Model | Variable | Environmental suitability | Percent variable contribution to model (%) | Model AUC |
|---|---|---|---|---|
| **Climate** | Annual Temperature Range | Increases where temperature range is low (coast and mountains) | 49.6 | 0.817 |
| | Mean Annual Temperature | Suitability increases where mean temperature is high (large rivers and coast) or, to a lesser extent, low (mountains) | 29.1 | |
| | Precipitation Seasonality | Low to intermediate seasonality most suitable; high seasonality decreases suitability | 12.8 | |
| | Isothermality | Increases suitability | 8.5 | |
| **Terrain** | Distance to Coast | Decreases with distance from coast | 64.2 | 0.824 |
| | Stream Order | Orders 4, 5, 7 most suitable | 18.7 | |
| | Elevation | Increases at low (coastal) and high (montane) elevations | 13.5 | |
| | Slope | Suitability highest at intermediate values, lowest at low and steep slopes | 2.3 | |
| | Aspect | Little effect – slight increase at intermediate values | 1.3 | |

| Model | Variable | Environmental suitability | Percent variable contribution to model (%) | Model AUC |
|---|---|---|---|---|
| **Climate + Terrain** | Annual Temperature Range | Increases where temperature range is low (coast and mountains) | 45.6 | 0.816 |
| | Distance to Coast | Decreases with distance from coast | 31.0 | |
| | Stream Order | Orders 4, 5, 7 most suitable | 22.5 | |

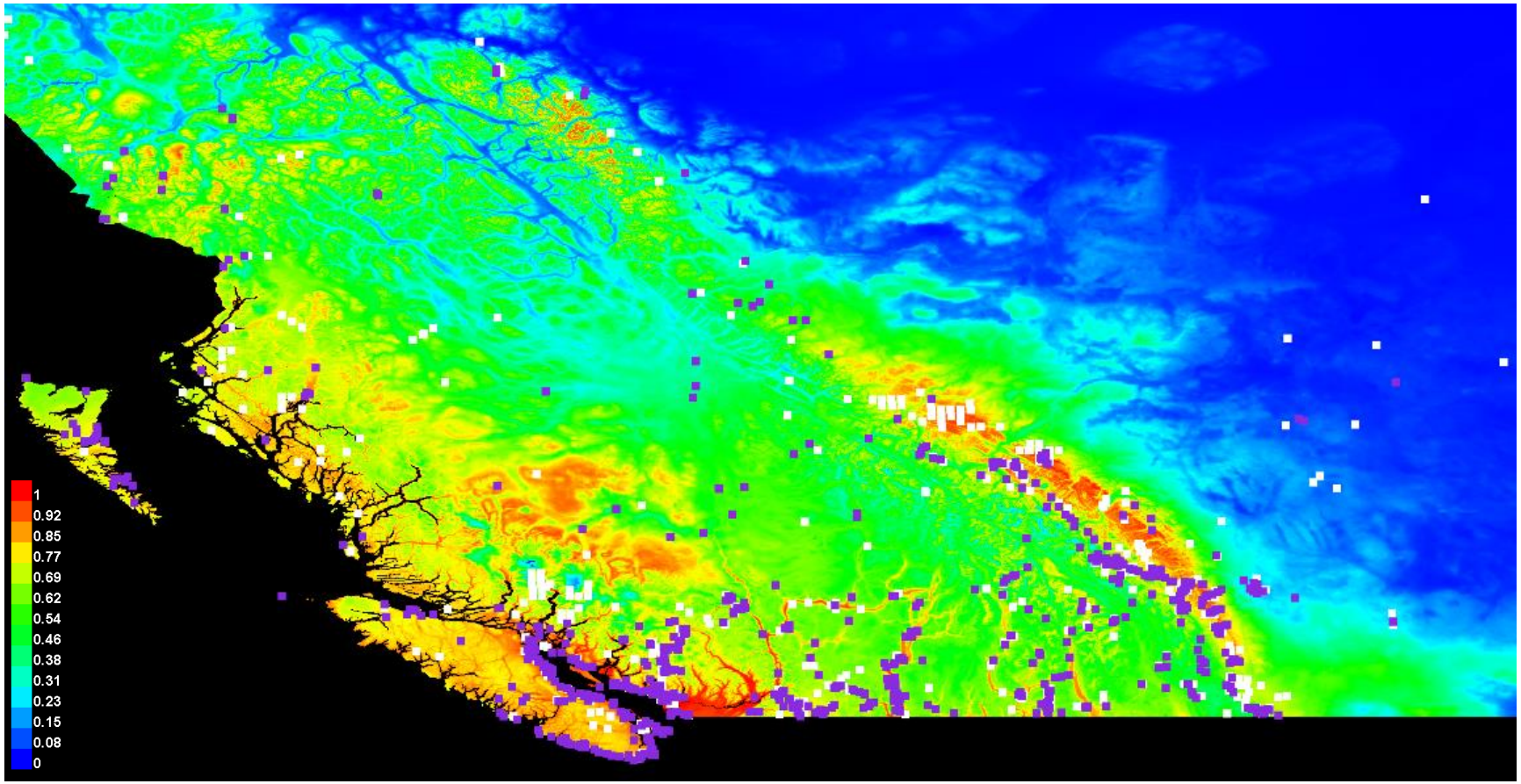



**Figure 3.3 Climate-only model of harlequin duck, *Histrionicus histrionicus*, presence data (white dots) using: Annual Temperature Range, Mean Annual Temperature, Precipitation Seasonality, and Isothermality.**

Note: Dark blue has the lowest probability of detection (0-0.08) while red has the highest (0.92-1.0). Purple dots are test data from e-Bird sightings. For visibility, this map displays a slightly reduced longitudinal extent than that used in all analyses presented in this Chapter (all of BC and AB).

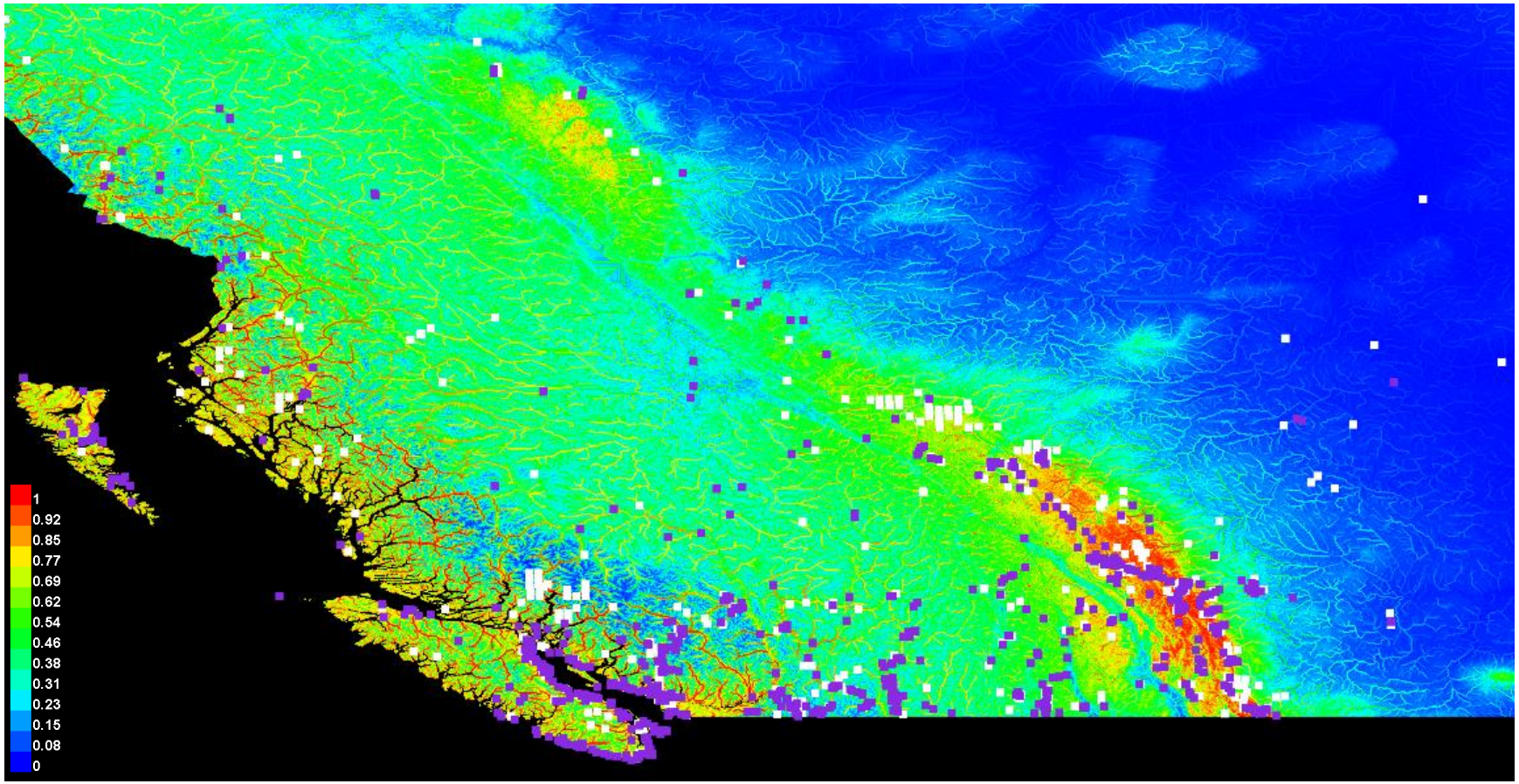



**Figure 3.4 Terrain-only model of harlequin duck, *Histrionicus histrionicus*, presence data (white dots) using: Distance to Coast, Stream Order, Elevation, Slope, and Aspect.**

Note: Dark blue has the lowest probability of detection (0-0.08) while red has the highest (0.92-1.0). Purple dots are test data from e-Bird sightings. For visibility, this map displays a slightly reduced longitudinal extent than that used in all analyses presented in this Chapter (all of BC and AB).

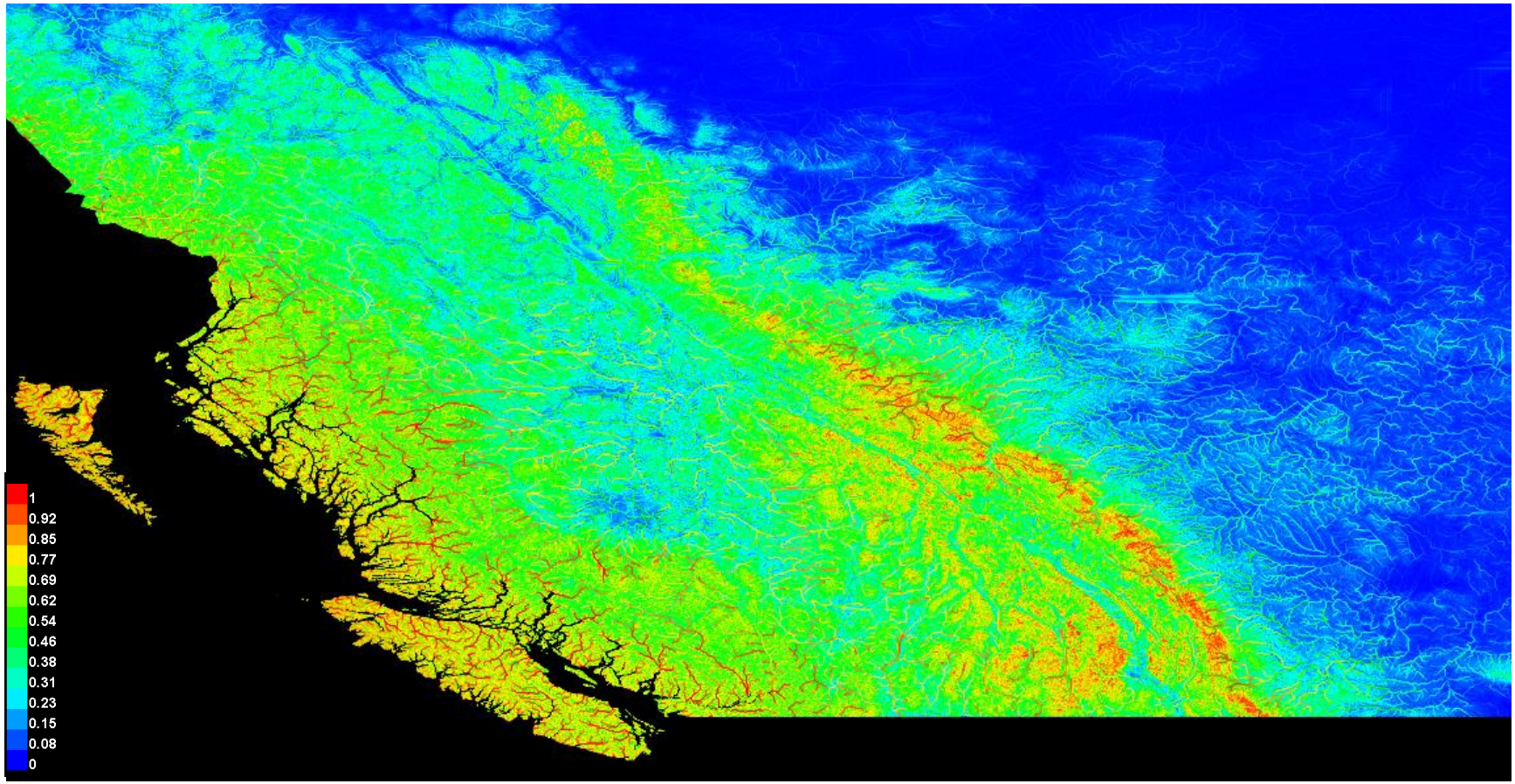



**Figure 3.5 Climate and terrain model of harlequin duck, *Histrionicus histrionicus*, habitat suitability using: Annual Temperature Range, Distance to Coast, and Stream Order.**

Note: Dark blue has the lowest probability of detection (0-0.08) while red has the highest (0.92-1.0). Map shows mean values from ten-fold cross-validation. For visibility, this map displays a slightly reduced longitudinal extent than that used in all analyses presented in this Chapter (all of BC and AB).

Land cover was not more important than the three climate and terrain variables already included in the model, but it did provide some contribution to model gain (8.9-15.3%, depending on the model; Table 3.3). Land cover classes that increased habitat suitability included: barren land, urban buildup, water, and snow. Classes that decreased suitability included croplands and deciduous forest (

Figure 3.6).

Annual temperature range contributed the most to the final model (without eagles added), followed by distance to coast, stream order, and land cover. Habitat suitability tended to decrease with annual temperature range and distance to ocean (Figure 3.8). Adding the bald eagle sightings layer increased model gain and was the most important variable in any model in which it was included (e.g. Table 3.3). The result of including this variable was the creation of high “points” of habitat suitability across the landscape, wherever bald eagle sightings were recorded (Figure 3.7); thus, this factor may provide more of an indication of bird report “hotspots” rather than being representative of a biotic interaction. For this reason, model interpretation was performed both with and without the bald eagle sightings layer (Table 3.3).

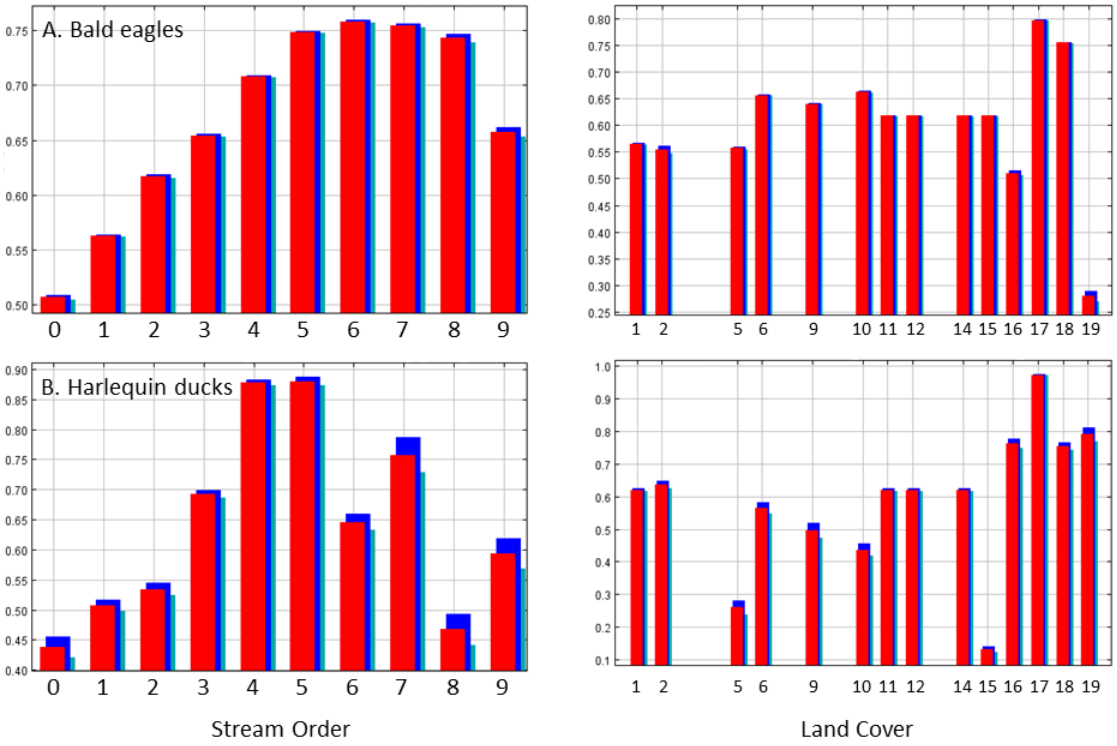
The bald eagle habitat suitability models created with MaxEnt tended to have much lower AUC values (0.61-0.70, depending on the variables included) than the harlequin duck models. Climate variables contributed the most to model AUC, with terrain variables providing little additional information (Table 3.4). Regardless of the method of evaluating model fit, Mean Annual Temperature was consistently identified as the largest contributor to model gain (Table 3.4). In fact, a model built using only mean annual temperature was almost as good at discriminating between presence and background locations as the full model (Table 3.4). Stream order was the second-most important variable, with eagle habitat suitability highest in orders 5-8 (

Figure 3.6).

**Table 3.3 Species distribution models for harlequin ducks in British Columbia and Alberta using climate, terrain, vegetation, and human impact variables. The only difference between models A and B is the inclusion/omission of the Bald Eagle Observations layer.**

| Model | Variable | Environmental suitability | Percent variable contribution to model (%) | Model AUC |
|---|---|---|---|---|
| A. Climate + Terrain + Vegetation + Human impacts | Bald eagle observations (thinned to reduce sampling bias) | Increases with bald eagle sightings | 35.5 | 0.849 ± 0.018 |
| | Annual Temperature Range | Increases where temperature range is low (coast and mountains) | 25.2 | |
| | Distance to Coast | Decreases with distance from coast | 18.0 | |
| | Stream Order | Orders 4, 5, 7 most suitable | 10.2 | |
| | Land cover | Decreases with crop cover and deciduous forest. Increases with barren land, urban buildup, water, and snow. | 8.9 | |
| | Bald eagle MaxEnt habitat suitability layer | Increases in areas with low suitability for bald eagles (high elevation) and areas with high suitability for bald eagles (coasts) | 2.2 | |
| B. Climate + Terrain + Vegetation + Human impacts | Annual Temperature Range | Increases where temperature range is low (coast and mountains) | 36.8 | 0.831 ± 0.023 |
| | Distance to Coast | Decreases with distance from coast | 27.5 | |
| | Stream Order | Orders 4, 5, 7 most suitable | 18.6 | |
| | Land cover | Decreases with crop cover and deciduous forest. Increases with barren land, urban buildup, water, and snow. | 15.3 | |
| | Bald eagle MaxEnt habitat suitability layer | Increases in areas with low suitability for bald eagles (high elevation) and areas with high suitability for bald eagles (coasts) | 1.8 | |

Model fit was evaluated using 10-fold cross-validation; as such, model AUC values are means ± standard deviation for the ten runs.




Land cover classes are defined as follows (NALCMS 2010):

| | |
|---|---|
| 1 | Temperate or sub-polar needleleaf forest |
| 2 | Sub-polar taiga needleleaf forest |
| 5 | Temperate or sub-polar broadleaf deciduous forest |
| 6 | Mixed forest |
| 8 | Temperate or sub-polar shrubland |
| 10 | Temperate or sub-polar grassland |
| 11 | Sub-polar or polar shrubland-lichen-moss |
| 12 | Sub-polar or polar grassland-lichen-moss |
| 13 | Sub-polar or polar barren-lichen-moss |
| 14 | Wetland |
| 15 | Cropland |
| 16 | Barren lands |
| 17 | Urban |
| 18 | Water |
| 19 | Snow and ice |

**Figure 3.6 Responses of (A) bald eagles and (B) harlequin ducks to stream order and land cover, using a MaxEnt model built using only that variable.**

Note: Red bars show the mean response of the 10 replicate Maxent runs while blue bars (two shades) show the mean ± one standard deviation.

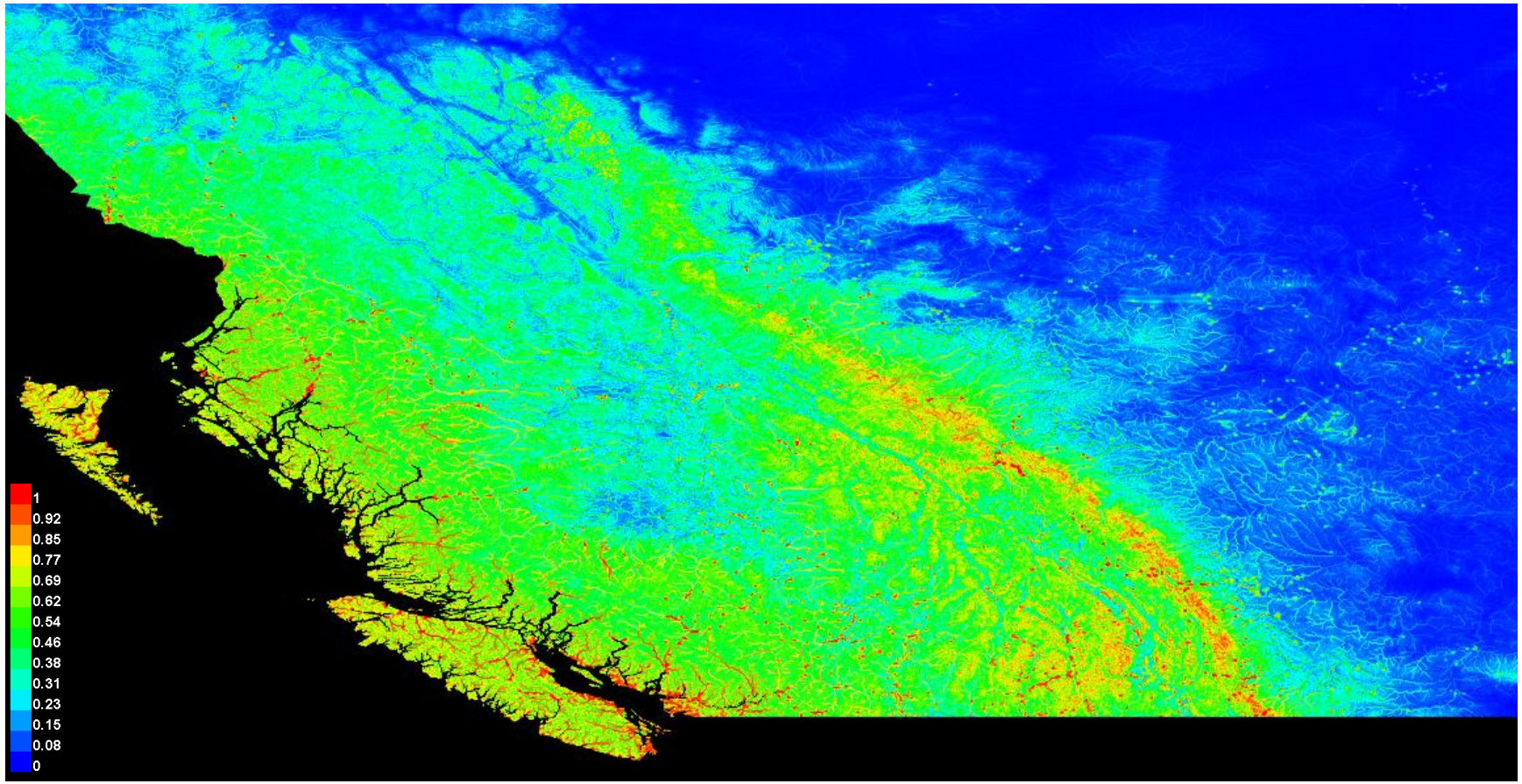



**Figure 3.7 Habitat suitability model of harlequin ducks, *Histrionicus histrionicus*, using: annual temperature range, distance to coast, stream order, bald eagle sightings and bald eagle habitat suitability.**

Note: Dark blue has the lowest probability of detection (0-0.08) while red has the highest (0.92-1.0). Map shows mean values from ten-fold cross-validation. For visibility, this map displays a slightly reduced longitudinal extent than that used in all analyses presented in this Chapter (all of BC and AB).

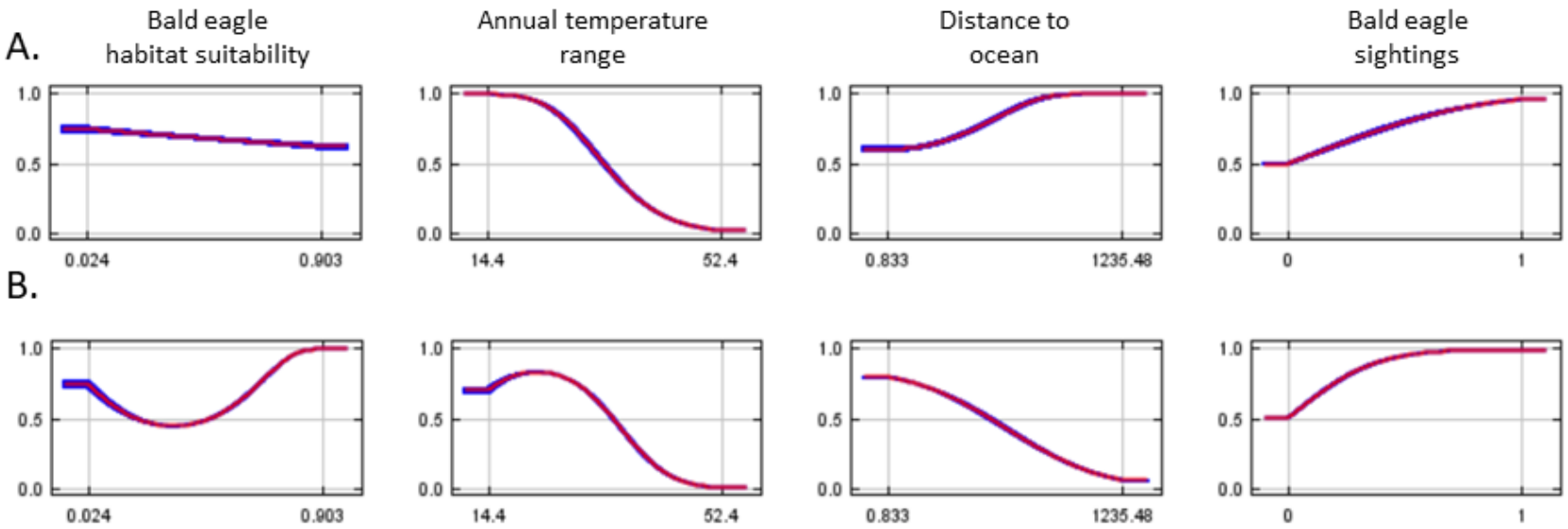



**Figure 3.8 Response curves showing the effect of individual environmental variables on predicted harlequin duck habitat suitability.**

Note: These curves show how each continuous environmental variable affects the Maxent prediction (categorical response shown elsewhere). The curves in (A) show how the predicted probability of harlequin duck presence changes as each environmental variable is varied, keeping all other environmental variables at their average sample value (marginal response). In contrast, each curve in (B) represents a different model, namely, a MaxEnt model created using only the corresponding variable. Each curve reflects the dependence of predicted suitability on both the selected variable and on dependencies induced by correlations between the selected variable and other variables. They may be easier to interpret if there are strong correlations between variables. Curves in both (A) and (B) show the mean response of the 10 replicate MaxEnt runs (red) and the mean ± one standard deviation (blue).

**Table 3.4 Full and single-variable species distribution models for bald eagle sightings during the breeding season (April-August) in BC and AB.**

| Model | Variable | Percent variable contribution to model (%) | Model AUC |
|---|---|---|---|
| Full | Mean Annual Temperature | 65.1 | 0.690 |
| | Stream Order | 13.8 | |
| | Elevation | 5.7 | |
| | Annual Temperature Range | 4.6 | |
| | Land cover | 4.1 | |
| | Mean Temperature of Warmest Quarter | 4.0 | |
| | Slope | 1.8 | |
| | Precipitation Seasonality | 0.7 | |
| | June Precipitation | 0.1 | |
| | April Precipitation | 0.1 | |
| | Aspect | 0 | |
| | Isothermality | 0 | |
| Single variable | Mean Annual Temperature | 100.0 | 0.680 |

Adding the bald eagle habitat suitability layer (Figure 3.9) to the harlequin duck model did not contribute much additional gain (1.8-2.2%). However, jackknife tests of variable importance (which are different from the heuristic estimate of importance described so far) showed that bald eagle habitat suitability used alone to model harlequin duck distribution, performed almost as well as distance to ocean combined with land cover. However, removing the bald eagle habitat suitability layer did not reduce the gain of the model, suggesting that the bald eagle layer does not contain much additional information relative to what is already provided by the other variables (Elith et al., 2011).

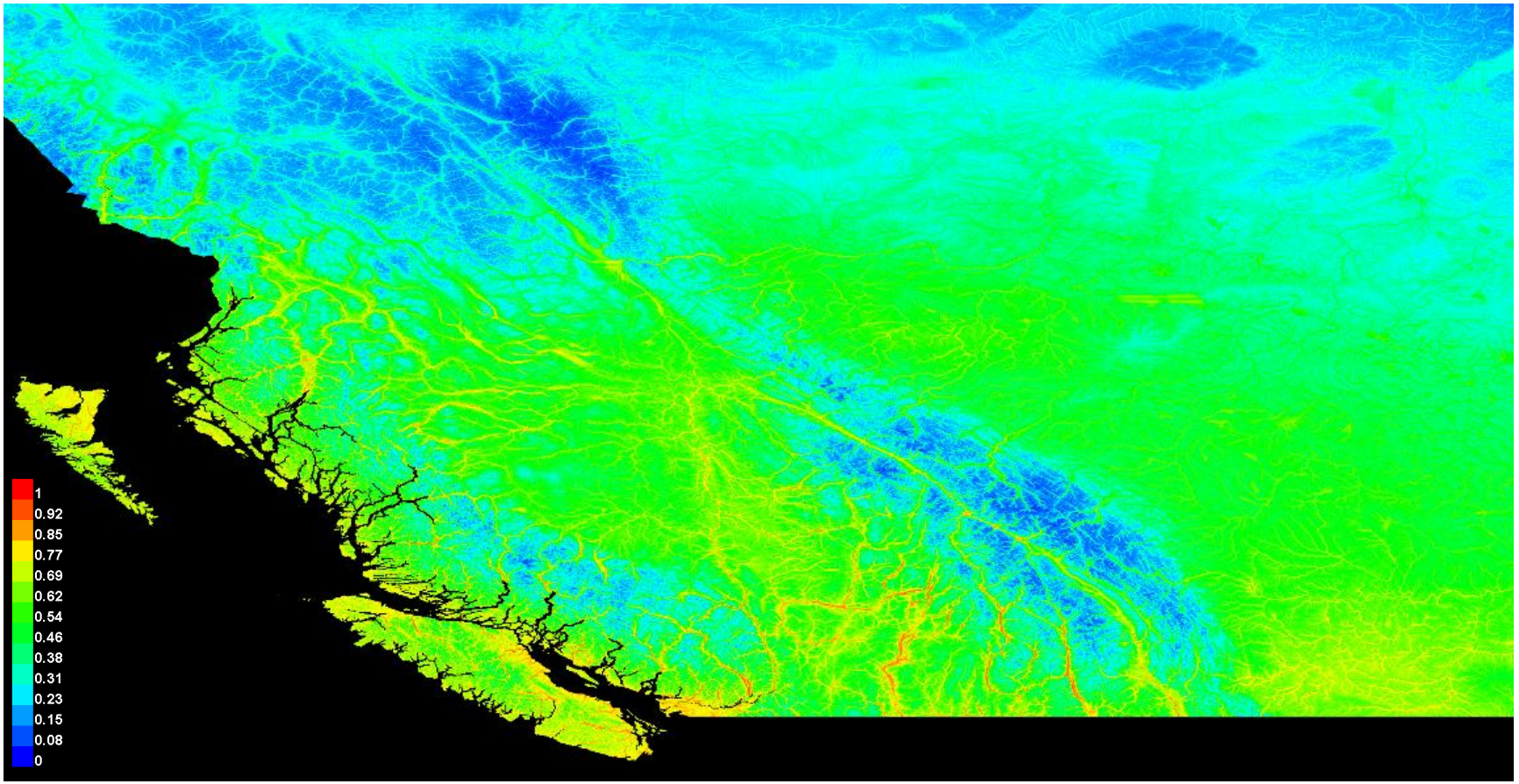



**Figure 3.9 Habitat suitability model of bald eagles, *Haliaeetus leucocephalus*, using: annual Temperature Range, Mean Annual Temperature, Precipitation Seasonality, Isothermality, Distance to Coast, Stream Order, Elevation, Slope, and Aspect.**

Note: Dark blue has the lowest probability of detection (0-0.08) while red has the highest (0.92-1.0). Map shows mean values from ten-fold cross-validation. For visibility, this map displays a slightly reduced longitudinal extent than that used in all analyses presented in this Chapter (all of BC and AB).

For both harlequin duck and bald eagle layers, standard deviations of AUC were small (0.008-0.023), indicating consistent performance across replicates. e-Bird test data consistently identified the same top contributors as training data, though the relative of lesser contributing variables often changed.

## 3.4. Discussion

Overall, the MaxEnt harlequin duck models performed well at discriminating between presence and background locations (AUC > 0.80). The suitability maps (Figures 3.3-3.5) showed similar geographic ranges as a map of probability of observation produced by the BC Breeding Bird Atlas (Figure 3.10). Climate tended to predict harlequin duck habitat suitability well on its own (Table 3.2), likely because it incorporates a number of biogeoclimatic features. For example, temperature provides an integrated measure of elevation, longitude and distance inland (Fick & Hijmans, 2017; Hijmans et al., 2005).

The terrain variables alone were less effective at predicting harlequin duck habitat suitability than models with other variables included (i.e. climate, vegetation, human impacts). Terrain variables appeared to only have an indirect effect on harlequin duck distributions, acting mainly through their influence on more biologically relevant variables, such as temperature and precipitation (Elith & Leathwick, 2009). The only exception was distance to coast, which greatly increased AUC for any model to which it was added (Table 3.2). Only three variables were required in the final climate-terrain model to adequately (AUC > 0.80) distinguish harlequin duck presence from background locations: annual mean temperature, distance to coast, and stream order (Table 3.2).

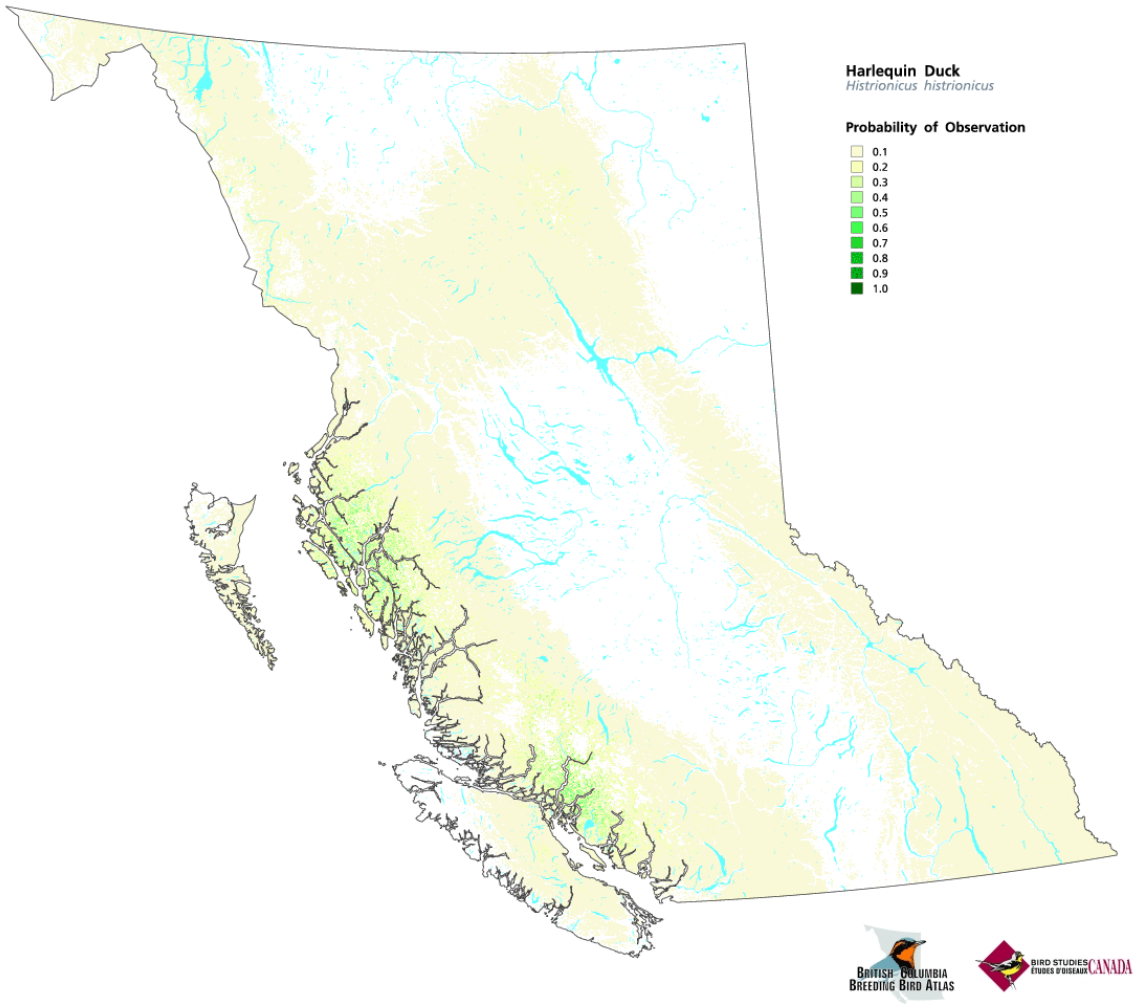



**Figure 3.10 Probability of observing harlequin ducks during the 2008-2012 BC Breeding Bird Atlas**
Image credit: Burger, A.E., 2015.

Annual temperature range was repeatedly identified as an important predictor of harlequin duck habitat suitability, with predicted suitability declining with increasing temperature range (Table 3.3; Figure 3.8). From visual inspection of maps, temperature range appeared to be lowest along the coast, and in montane areas, both of which are known (and can be seen in Figure 3.3-Figure 3.4) to be well-used by harlequins. The climate-only model predicted high habitat suitability where the temperature range was low (coast and mountains) while the terrain-only model predicted the highest suitability at low (coastal) and high (montane) elevations (Table 3.2). In the final combined climate and terrain model, elevation was eliminated during the stepwise procedure, with annual temperature range being retained as the variable contributing most to model gain (Table

3.2). Thus, it appeared that only one of those two variables were needed, with temperature range having the largest effect on model gain.

From correlation analysis, temperature range appears to be driven by both minimum temperature (Pearson coefficient = -0.94) and, to a lesser but still large extent, by maximum temperature (Pearson coefficient = 0.71). Habitat suitability was highest where temperature range was low; thus, temperature extremes seem to reduce habitat suitability. This could be due to accumulation of precipitation (snowpack) during the cold season, leading to high peak flows during spring freshet. High peak flows can be damaging to both invertebrates (through substrate habitat displacement, mechanical damage, and increased sediment load which can clog gill and feeding structures) and nesting habitat for harlequins, such as in-stream islands. A large flooding event in southern BC and AB in 2013 caused local harlequin duck population loss across the affected area (pers. obs.; S. Humphries, pers. comm.). Spring freshet can be a particularly sensitive time for such disturbances, as harlequins will often be engaged in pre-nesting and nesting activities during this time (Robertson & Goudie, 1999).

Stream order was important enough (10.2-18.6%; Table 3.3) to be retained in the final harlequin duck models, but was insufficient on its own to describe harlequin duck distribution. This may be due to the fact that streams of all orders were distributed fairly evenly across the geographic space (e.g. Figure 3.1). One might expect stream order to be negatively associated with elevation, as was found in Chapter 2. However, at the broad geographic extent examined here, the Pearson correlation coefficient between stream order and elevation was only -0.3. As such, it appears that climate is the first driver of distribution, with stream order then being added as an additional “filter” on top of the restrictions defined by climate (note the difference between Figure 3.3 and Figure 3.5).

While stream order is an index of watershed size, it is determined solely through topography and not precipitation or substrate type (Strahler, 1952). As such, it does not offer insights into potentially important factors such as flow regime, nor direct measurement of river depth or width, which can be further affected by bedrock restriction and the degree of overland flow of water that is moderated by surrounding vegetation. The increase in harlequin abundance observed with increasing stream order could be due larger streams representing more productive feeding habitat during the pre-nesting

period. Harlequins are income breeders, acquiring all of the resources for egg production on the breeding grounds, rather than from winter fat stores (Bond et al., 2007); therefore, nutrient acquisition is of high importance during the pre-nesting period. Larger river reaches were also found to contain higher numbers of scrapers and pupae than smaller reaches. These prey items may therefore be of value to pre-nesting harlequin ducks. Large pupae, such as *Glossosoma spp.*, in particular may represent an energy-rich source of nutrients that are often clustered in large groups, providing a high value, immobile source of energy. Additionally, small scraper abundance increased with river width, but only on streams containing harlequins. While this may suggest a preference for small scrapers, another interpretation could be that harlequin ducks release small scrapers from predation by large predators, such as stoneflies, which are known to be a preferred prey item (McCutchen, 2002). Additionally, harlequins may also use larger stream sections during the pre-nesting period as higher elevation reaches are still frozen (MacCallum et al., 2016). In areas with high predation pressure, habitat features associated with predator avoidance may be of higher importance (Heath et al., 2006; MacCallum et al., 2016). In such watersheds, larger order rivers with dense streamside vegetation may offer cover while still allowing an open view to scan for aerial predators.

Land cover had a smaller effect on harlequin duck distribution than either climate or terrain, but still contributed 8.9-15.3% to model gain, depending on the model (Table 3.3). Croplands had the largest negative impact on harlequin duck habitat suitability out of all the categories (

Figure 3.6). This can occur for a number of reasons. In some areas, agricultural activities removes forest and riparian habitat, which is needed for nesting habitat, while in other places it replaces or disturbs wetlands and grasslands. Grasslands have strong root systems that add to bank stability. Erosion eventually leads to wider, shallower flows and increased sediment load. These can alter invertebrate communities as well as directly affect harlequin duck habitat. I suspected that agriculture might be associated with unsuitable, flat terrain in the prairies (southeastern portion of Figure 3.3, Figure 3.4, Figure 3.5, Figure 3.7), but the negative effect of cropland persisted even when other variables were accounted for (i.e. in the marginal response curves).

**Broadleaf deciduous forest was also associated with a negative impact on harlequin duck habitat suitability (Table 3.3;**

Figure 3.6). This could be due to the climatic area in which this forest type grows (low elevation, higher temperature, longer growing season), but could also be attributed to the amount of canopy cover this type of forest provides. While some forested area and canopy cover is required for nesting as well as river bank stability, MacCallum (2016) found that harlequins avoid areas with high canopy cover as it may obscure avian predators, such as eagles, increasing the energy and time required for vigilance, as well as the risk of adult and juvenile predation. This fits well with Heath's (2006) finding that harlequin sink populations in Labrador are determined by the local availability of raptorial nesting ledges, and associated birds of prey. I also found a small, negative effect of canopy cover on harlequin duck presence and abundance at the reach level (see Chapter 2).

**Furthermore, all the land cover categories associated with positive effects on harlequin distribution are canopy-less classes: barren lands, urban buildup, water features, and snow and ice (**

Figure 3.6). While this is likely not the main driver of these positive associations, it is an interesting finding that does not contradict the negative effect of canopy cover found elsewhere (Chapter 2; MacCallum et al. 2016). Alternatively, the positive effect of urban buildup could be a result of not fully controlling for sampling bias. At the reach-level (Chapter 2), I also found a positive effect of man-made features such as roads, trails, and bridges on both invertebrate abundance and biomass, as well as pupal invertebrate abundance. Areas with these man-made features often have reduced canopy cover, receive more light and could be more productive as a result.

Bald eagle sightings were always positively associated with harlequin duck sightings (Table 3.3; Figure 3.8). This could be due to incomplete removal of sampling bias in both the eagle and duck observations, that tends to cluster sightings near roads and cities. Indeed, urban buildup was also a positive predictor of harlequin presence (

Figure 3.6). Additionally, harlequins and eagles may share some habitat preferences, such as water features and undisturbed habitat. Furthermore, eagles may also be attracted to areas with large numbers of harlequins, as a prey item. However, eagle diet tends to be fish-dominated; thus, fish as prey is a more likely driver of eagle

distribution than harlequin ducks, a much less common prey item. It is still important to note, however, that eagles are not required to kill harlequins to have demographic consequences. Induced antipredator behaviors, such as increased vigilance, can lower carrying capacity of a system where the same amount of finite resources will take longer to harvest due to time lost scanning for predators (Lima & Dill, 1990). These effects can be exacerbated in areas with low visibility, such as smaller order streams or sites with increased canopy cover (MacCallum et al., 2016).

Likely, harlequin duck habitat selection is hierarchical, from landscape-level (climate), to watershed (stream order), finally selecting feeding and nesting sites based on their local characteristics (see Chapter 2), such as availability of boulders for resting between feeding bouts, reduced canopy cover to facilitate predator vigilance (MacCallum et al., 2016), and the availability of nesting habitat (in-stream islands, understory vegetation). It is possible that the 1 km resolution that I utilized here was too coarse to identify areas affected by fish (eagles). Using a finer resolution (Pearson et al., 2004) of eagle presence, at the watershed scale, may be a better test of the effects of fish and eagles.

Alternatively (or additionally), it is also possible that, at least in some areas, fish provide a benefit to harlequin ducks. For example, while spawning salmon disturb sediments (harming invertebrates and increasing erosion), they also provide one of the largest natural resource subsides to both freshwater and terrestrial ecosystems (Janetski et al., 2009). Spring spawners, such as rainbow trout, which have been introduced extensively throughout the Rocky Mountains (and the world), provide concentrated resource subsidies to harlequin ducks, and other species, in the form of eggs. Indeed, harlequin ducks have been repeatedly observed feeding on rainbow trout eggs and fry.

The addition of the eagle habitat suitability layer (as opposed to actual eagle sightings) to the harlequin duck models did not change the positive association between eagle sightings and harlequin duck habitat suitability. However, harlequin habitat was found to be more suitable both in areas that had: (1) high suitability for eagles and (2) low suitability for eagles. Thus, it appears that harlequins and eagles overlap in some areas (e.g. along the coast) but that harlequins are also present in large numbers in other areas that are not suitable for eagles (e.g. at high elevations). In fact, stream order 6, which was the most suitable order for eagles, experience a strong dip in suitability for

harlequin ducks, given that order 4, 5, and 7 were found to be the most suitable for harlequin ducks. It could be that harlequins avoid sixth order rivers (keeping in mind that these are usually defined as seventh order; see Methods) due to the high probability of encountering bald eagles on these streams. Interestingly, the lower order rivers (here, defined as fourth and fifth order) are also most likely to have remained uncolonized by fish, due to an increasingly larger overall number of barriers to pass on the way up (e.g. canyons, waterfalls, underground segments), as well as an increasing tendency to become smaller, less productive and steeper with increasing elevation.

Another possibility is that the bald eagle habitat suitability layer was poorly defined. Indeed, AUC values for bald eagle models were consistently lower than the harlequin duck models (Table 3.2 and Table 3.4). Perhaps the harlequin's strict tenacity to rivers during the breeding season (J.P. Heath et al., 2006; pers.obs.) and their relatively small breeding ranges (see Chapter 2) contributed to more accurate observation coordinates and a correspondingly high AUC. Bald eagles, on the other hand, have slightly larger breeding ranges, and would likely be migrating large distances at this time of year as well. Additionally, bald eagles are much more well-known to the public, and soar at much higher altitudes than harlequins, making them much easier to observe and report. However, for the same reasons, bald eagle sightings may also be more likely to be misidentified, or reported with lower geographical precision (or both). The resolution of my models (1-km) does, however, allow for a fairly large margin of error in coordinates. Another possibility is that bald eagles, an apex predator whose populations and distributions have made large, recent recoveries worldwide, is quite ubiquitous and not easily modeled.

Habitat preference studies for highly vagile organisms such as harlequin ducks should examine habitat effects at all spatial scales that would be relevant to that specific organism. However, many harlequins, particularly females, exhibit philopatry to their natal watersheds. As such, for females in particular, habitat "selection" likely does not occur directly at the regional level, but as a consequence of demographic processes determining source and sink populations. Environmental conditions in some areas appears to lead to higher productivity and stability (leading to emigration), and lower productivity and stability, leading to immigration or, at other times, local extinctions, in others (Heath et al., 2006). The results of these MaxEnt models suggest that habitat suitability is defined at the highest level by broad climatic factors. Within the watershed,

harlequins exhibit high site fidelity (i.e. favourite feeding sites) as well as clubbing behaviour (unusually high group numbers at particularly productive feeding sites). Pairs also return to the same reaches year after year (pers. obs.) and also display some degree of territoriality; behaviorally, they must be selecting for some attributes at the reach and even site levels.

One factor that was not considered here is the distinction between pre-breeding, nesting, and brood-rearing habitats. MacCallum (2016) found that harlequin ducks use larger reaches during the pre-breeding stage, likely where food availability is higher, or where rivers have thawed following winter. Some pairs will nest in the same areas used for pre-breeding, while most move upstream to higher, smaller reaches for nesting and brood-rearing (MacCallum et al., 2016). Brood-rearing also occurs on these smaller reaches, until hens slowly move brood back down to the main stem, late in the season, just before migrating back to the coast. Presumably, the larger reaches are more energetically profitable (prey-wise) but potentially more dangerous for juveniles, which MacCallum (2016) suggests require low banks with vegetation cover to provide easy access to cover when predators are seen. Thus, the July-August harlequin observations (nesting and brood-rearing stage) should potentially be excluded from future models of harlequin duck pre-breeding habitat. Alternatively, any future pre-breeding habitat modeling should consider including the amount of nearby and suitable nesting and brood-rearing habitat as a potential constraint on the selection of pre-breeding habitat. However, I included the July-August observations here since all breeding bird atlas data, which represented more rigorous and controlled sampling design, was often expressed as aggregate data from throughout the breeding season, defined as April-August.

### 3.4.1. Conclusions

The models presented here suggest that climate may constrain harlequin duck distributions at the broadest spatial scale, which is consistent with other studies of species distributions (Baldwin, 2009). Stream order provides another environmental “filter” through which to refine habitat suitability. Harlequin ducks seem to prefer rivers that are large enough to have openings in the overhanging vegetation. This may be to facilitate vigilance for predators, as well as allow for adequate light penetration for algal growth and associated invertebrate prey (see Chapter 2). Some human-induced factors appear to reduce harlequin habitat quality, such as agricultural activities. Fish

introductions may also have a detrimental effect, as fish presence was found to lower harlequin duck breeding presence and abundance (Chapter 2; LeBourdais et al. 2009) below what would be expected given other environmental variables. Additionally, fish introductions may attract common predators, such as eagles, which harlequins seem to avoid in some areas (by breeding at higher elevations than eagles) and congregate with in others (such as along the coast). Other human-induced factors may increase habitat suitability, such as urban buildup, which may further reduce canopy coverage.

### 3.4.2. Future studies

Future studies should consider parsing observations and environmental data temporally and investigate whether environmental variables can explain not only harlequin presence per se, but also changes in presence and distribution over time. Future studies could also examine harlequin presence and distribution over the entire breeding range in order to consider the full range of biogeoclimatic combinations encountered by breeding harlequin ducks in western North America. Since harlequins are Holarctic, it may even be informative to create a northern hemisphere-wide model, to best identify the most important factors driving harlequin duck distributions worldwide.

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# Chapter 4. Using isotope turnover models to back-calculate breeding ground isotopes in harlequin duck claw tips and blood cells

## 4.1. Introduction

Events throughout the entirety of an animal's annual life cycle can affect its condition, success, and, ultimately, its fitness. A review by Ryan Norris & Marra (2007) revealed non-negligible carry-over effects between seasons in migratory species. Oppel & Powell (2008) further argued that in order to understand factors influencing populations of migratory species on the breeding grounds, it is crucial to determine the influence of preceding events outside the breeding season. However, capturing carry-over effects between seasons can be especially difficult in migratory species, which often travel large distances, disperse over large ranges in at least one season of the annual cycle, and can inhabit dissimilar habitats during the breeding and non-breeding seasons. The effects of these movements make it difficult to make general observations about habitat selection and usage, habitat connectivity and population dynamics at a population- or landscape-level.

Stable isotopes offer a means of uncovering a glimpse of conditions in preceding seasons. For example, isotope studies in migratory birds have revealed that the earliest arrivals to breeding grounds appeared to feed in the highest quality wintering grounds in the preceding season (Stuart Bearhop et al., 2004; Gill et al., 2001; Gunnarsson et al., 2005), are in better condition at both stopover sites (Stuart Bearhop et al., 2004) and upon arrival to the breeding grounds (Marra et al., 1998), and also use higher quality habitat during the breeding season (Gunnarsson et al., 2005). Carryover effects, such as timing of arrival, can have strong implications for breeding success and fitness, as early breeding ground arrival is usually associated with higher breeding success (Aebischer et al., 1996; Currie et al., 2000; McKellar et al., 2013; Norris et al., 2004; Rockwell et al., 2012; Saino et al., 2004; Sergio et al., 2007) which can ultimately lead to consequences on population dynamics (Mazerolle & Hobson, 2005; Newton, 2006).

### 4.1.1. Stable isotopes

Stable isotopes are naturally occurring, non-radioactive forms of elements that contain additional neutrons, making them heavier (Fry, 2006). They exist at predictable ratios relative to their more common, lighter forms and can be used to trace the flow of nutrients through food webs and ecosystems. Stable isotopes provide a record of diet over different temporal scales, depending on the rate of tissue turnover (Tieszen et al., 1983). For example, blood, muscle, and liver samples are commonly used body tissues that incorporate stable isotopes from the diet at rates proportionate to their respective rates of metabolism and growth (Boecklen et al., 2011). Since biological processes modify these ratios in predictable ways, inferences can be made about dietary habits, habitat use, movement patterns, reproduction, spatial and temporal distribution, and trophic level, depending on the isotope and the growth and turnover rates of the tissues being analysed (Boecklen et al., 2011; Hobson, 1999).

Commonly used tissues for stable isotope research include blood, muscle, liver, bone, hair, feathers, and claws. When individuals change their diet, for example, due to migration to a new habitat with different baseline isotope signatures, the isotope signature in the new diet becomes incorporated into their body tissues in a predictable way. In tissues that regenerate over time, such as blood components (plasma and blood cells), liver, or brain, isotope signatures follow an exponential decay curve over time, wherein half of the old isotope signature is replaced by the new signature at a constant rate (turnover rate constant, *k*). Tissues with a higher rate of cellular replacement, such as the liver, incorporate new isotope signatures at a higher rate following a dietary switch than slower turnover tissues, such as blood cells. Tissue turnover through time is calculated as:

$$Y_{(t)} = Y_{new} + (Y_{old} - Y_{new})e^{-kt} \qquad (1)$$

Where $Y_{(t)}$ is the current ratio of an isotope (such as $\delta^{15}N$ or $\delta^{13}C$) in a tissue of an individual at the time of capture and *t* is the time since dietary switch (e.g. arrival). The turnover rate can be converted to a half-life (λ) by the equation:

$$\lambda = \frac{\ln(2)}{k} \qquad (2)$$

This half-life (λ) is equal to the amount of time at which half of the tissue cells would have reached the end of their natural lives and been replaced. Thus, tissue turnover rates depend on the rate of tissue replacement (due to metabolism and growth).

### 4.1.2. Isotope turnover models

Turnover rates also vary between species and by isotope (Boecklen et al., 2011; Hobson, 1999). For example, $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ may all turn over at different rates within a single tissue and species. There are also tissue-, species-, and isotope-specific differences in discrimination factors: the amounts by which the tissue consistently differs from the isotopic signature of the diet (Tieszen et al., 1983). For example, the turnover rates for $\delta^{13}C$ and $\delta^{15}N$ within a single tissue can differ, due to differences in the elemental composition of the molecules being produced during growth and metabolism, as well as the fact that the body will utilise different dietary components to produce different tissues types (referred to as isotopic routing) (Boecklen et al., 2011). As a result, stable isotope analysis typically requires conducting rigorously controlled laboratory dietary studies in order to determine the turnover rates for various tissues. Once these values have been ascertained, species-, tissue- and isotope-specific turnover curves can be used to estimate previous unknowns, such as the number of days since arrival following migration.

For example, Phillips & Eldridge (2006) built a single-tissue isotope turnover model by using established turnover rates for plasma and blood cells to calculate the number of days since arrival (i.e. since dietary switch). To build their single-tissue isotope turnover model they also needed to know, *a priori*, the isotopic signatures of both the pre- and post-migration dietary isotopic signatures. As such, they were able to use the established turnover curves for $\delta^{13}C$ in blood plasma and blood cells to estimate the number of days since arrival for each individual based on the $\delta^{13}C$ signatures of their simultaneously sampled blood plasma and blood cells. Phillips and Eldridge's (2006) single-tissue isotope turnover model also allowed them to make other inferences about, for example, the condition and dietary habits of individuals that were early to arrive on the post-migratory grounds relative to later arrivals.

The drawback of using Phillips and Eldridge's (2006) single-tissue isotope turnover model methodology is that it does not allow for a wide range of either pre- or

post-migratory isotope signatures because their model's methodology assumes that the old isotope is the same for every individual. Further, their model's methodology requires knowledge of the isotopic signature of the old diet, which is often impractical, particularly for species that migrate long distances. To solve some of these problems, Klaassen et al. (2010) built a dual-tissue isotope turnover model which is more practical in that it only requires knowledge of the isotopic signature of the new diet, a value which is much more easily estimated in post-migratory individuals.

A potential application of these isotope turnover models is to use the isotope turnover curves to back-calculate to the pre-migratory isotope signatures. For example, this could allow one to determine whether certain individuals are migrating from wet or dry habitats, as can be inferred from $\delta^{13}C$ levels (Huiskes et al., 2006), or coastal or interior environments using $\delta^{2}H$. When dual tissue isotope turnover is used, the turnover rates in the different tissue must be different in order to allow for comparisons between tissues. Further, the turnover rates cannot be so fast that the tissue has already equilibrated, or is close to equilibration, with the new diet. The closer the tissue is to equilibration, the smaller the isotopic difference between turnover curves from different pre-migratory habitats; thus, the more difficult it becomes to distinguish or accurately back-calculate between the isotopic signatures of pre-migratory habitats.

Use of either the single- or dual-tissue model requires knowledge of the isotope-specific turnover rate for each tissue. This, in turn, entails running rigorously controlled diet-switching studies in a laboratory setting. These experiments are unlikely to be feasible, particularly for species at risk. Using inert tissues in place of tissues with higher turnover rates can solve some of these problems.

### 4.1.3. Isotopes in inert tissues

In contrast to continuously regenerating tissues, such as blood and liver, inert tissues, such as feathers and hair, provide a stable (no turnover) record reflective of diet at the time of tissue formation. For example, feather isotopes have been used to determine molting, breeding, and wintering locations of migratory birds at times outside of those periods (Cherel et al., 2000; Hobson et al., 2001; Lott et al., 2003; Mazerolle & Hobson, 2005; Oppel & Powell, 2008), as well as feeding habits, locations and dietary shifts (Hedd & Montevecchi, 2006; Hobson & Clark, 1992; Mizutani et al., 1990;

Thompson & Furness, 1995). However, making inferences about behaviour from feather isotope records requires detailed knowledge of feather molt timing and extent so that the captured isotope signatures can be attributed to the correct time period.

When molt timing is unknown, or does not line up with the time period of interest, claws provide another possibility. Claws provide an inert record of diet at the time of growth, but grow continuously; thus, representing a few months' worth of dietary records. Following a dietary shift, claws will consist of a gradient of old to new isotopic signatures, as new claw layers representing the new diet are slowly built up and migrate towards the distal tip of the claw (Hahn et al., 2014). As such, while claws turn over more slowly than most tissues used for stable isotope analysis, some authors have noted the difficulty in ensuring that claws were sampled early enough to be composed of materials solely representative of the previous diet, especially if the species experiences a relatively long outward migration or visits a wide variety of stopover sites (S. Bearhop et al., 2003, p. 2003). This problem can be addressed if isotopic turnover in claws can be accurately modeled. Here, I take advantage of an interesting molt habit in harlequin ducks which allowed me to investigate the relative turnover rates for blood plasma, blood cells, and claws in a natural population of migratory sea ducks without the need for rigorously controlled laboratory determination of turnover rates and tissue-specific discrimination factors.

### 4.1.4. Study species

Harlequins disperse over a relatively large geographic region during the breeding season, and nest at relatively low densities within watersheds. Males do not provide co-parental care and instead depart the breeding grounds during egg incubation. A couple of months later, within a short window in the fall, females and young of the year join the males on the wintering grounds; they are thought to migrate directly from the breeding grounds (Gregory James Robertson & Goudie, 1999). As harlequins migrate from inland freshwater environments to their marine wintering habitat, they experience a non-overlapping dietary shift in both $\delta^{13}C$ and $\delta^{15}N$ signatures (Bond et al., 2007). Marine environments tend to be less depleted in $\delta^{13}C$ than freshwater and terrestrial habitats. Marine animals tend to be 7‰ more enriched in $\delta^{13}C$ than freshwater and terrestrial animals because the source of carbon in marine food webs, bicarbonate, is approximately 7‰ more enriched in $\delta^{13}C$ than carbon dioxide, which is the source of

fixed carbon in freshwater and terrestrial systems (Craig 1953; Chisholm, Nelson & Schwartz 1982; Fry, Scanlan & Parker 1983). As soon as harlequin adult females arrive on the marine wintering grounds, undergoing a dietary shift, they also experience a complete flight feather molt, rendering them flightless for a period of 20-40 days (Palmer, 1976). Their feathers steadily grow over the next month (G. J. Robertson et al., 1997).

### 4.1.5. Goals of study

I used a series of nonlinear regressions to model single-isotope, single-tissue turnover curves for both $\delta^{13}C$ and $\delta^{15}N$ in blood cells, using ninth primary feather growth as an index of time. I then compared these turnover curves to the results from single-isotope, dual-tissue nonlinear regressions, which incorporated claw signatures in addition to blood cells. My dual-tissue models depend on the nature of isotope turnover in claws, which has not been well-elucidated. I therefore used a series of turnover functions that included (1) an exponential decay function, (2) a linear function, or (3) an exponential function. Additionally, any of these three functions may or may not be preceded by a "time lag", wherein the claw material grown after dietary switch has not yet migrated to the sampled distal claw tip (Hahn et al., 2014). In order to visualize these predictions, I created simple models described by the equations and turnover rate estimates (based on both literature and visual inspection of the isotope signatures observed in harlequin duck tissues relative to feather growth in this study) to produce predicted claw isotope turnover curves. I expand upon these turnover functions, their rationales, and they predictions they make, in the Methods.

These dual-tissue models are my own and no one else (to my knowledge) has utilized this approach. Given this, the primary goal of this chapter was to test whether my modelling approach was appropriate. The secondary goal of this chapter was to (a) estimate the turnover rates for $\delta^{13}C$ and $\delta^{15}N$ in both tissues (blood cells and claws), (b) elucidate the pattern of turnover for both $\delta^{13}C$ and $\delta^{15}N$ in claws and the amount of time for new material to migrate to the tip (lag), (c) estimate the new isotopic equilibrium for both isotopes in all tissue types and (d) use all the preceding information to perform a back-calculation to estimate the pre-migratory isotopic signatures for each individual (i.e. the signature of the old diet). My back-calculation to the pre-migratory isotopes is also novel. Inferences made here through stable isotopes in tissues grown during the

breeding season could yield insights into harlequin duck habitat usage during the breeding season, in a large number of individuals from across the entire breeding range.

## 4.2. Methods

### 4.2.1. Capture and sampling

Harlequin ducks were captured at five sites on Hornby Island, BC, (Figure 4.1) in the Strait of Georgia from September 7-13, 2012. Ducks undergoing wing molt were captured by corralling them with kayaks and inflatable zodiacs into drive traps (Gregory J. Robertson et al., 1998), while flighted individuals were caught by either standard or floating mist-nets (Kaiser et al., 1995). Capture and sampling were carried out under permit from Environment Canada (scientific permit # BC-12-0061 and banding permit # 10759 A), Simon Fraser University's (SFU) Biosafety permit # 187-2012B and our field methods were approved by SFU's Animal Care Committee (Protocol # 1010B-11).

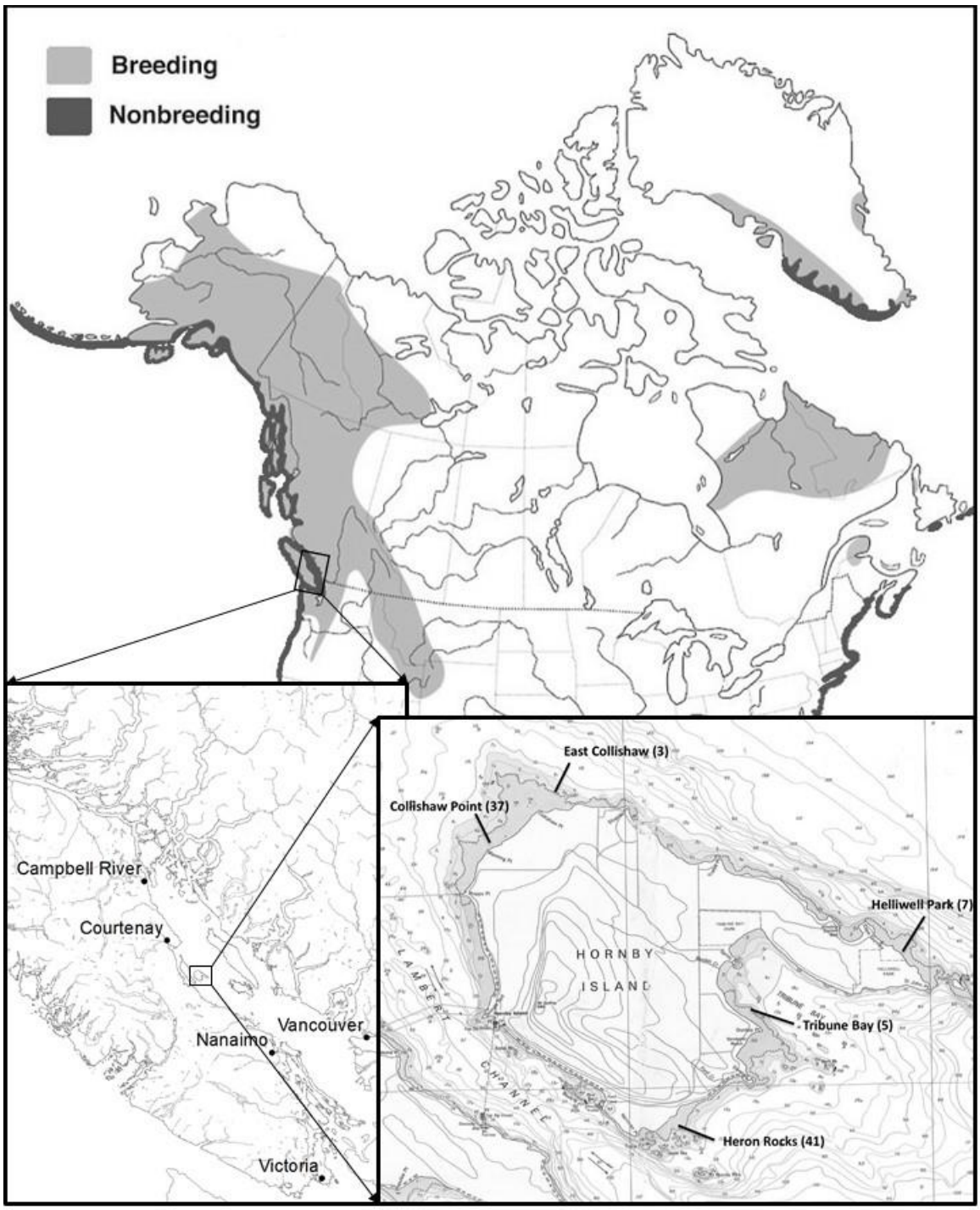


**Figure 4.1 Capture locations and capture numbers for five field sites on Hornby Island, British Columbia, Canada relative to North American range for harlequin ducks.**

Images: North American range (top) panel is adapted from (Gregory James Robertson & Goudie, 1999). Hornby Island (bottom-right) panel provided by K. Wright.

### 4.2.2. Sample collection

All individuals captured were sexed and aged by cloacal examination using the depth of the Bursa of Fabricius (Mather & Esler, 1999) and plumage characteristics. Standard banding measurements were taken using calipers (to the nearest 0.1 mm): tarsus, culmen, wing chord (notched, natural).

We collected each bird's blood by piercing the brachial vein with a sterile 24-gauge needle. We collected the blood with a heparinized Natelson tube and then transferred it to a heparinized 2.0 ml plastic vial and stored it in a cooler on ice. We collected 0.5-1.5 ml of blood from each bird; the target volume was 1.0-1.5 ml, but birds often gave less than 1.0 ml, which was still usable for blood cell stable isotope analysis. We clipped the apical 1-2 mm of the longest (middle) claw from either the left or right foot using fingernail clippers. These claw clippings were stored in labelled, 2.0 ml plastic vials. Fecal samples were collected opportunistically and stored in 2.0 ml plastic vials.

Within 6 h of field collection, blood samples were centrifuged to separate plasma and cellular components. Plasma was drawn off using a micropipette from the precipitate cellular blood fraction and stored in separate heparinized 1.5 ml plastic tubes. All tubes, including fecal samples, were then frozen at -20°C within 12 h of collection.

### 4.2.3. Stable isotope analysis

Plasma, blood cell, and fecal samples were dried at 60°C for 48 h, then left to equilibrate with the ambient humidity for at least 24 h. Dried samples were then homogenized using a mortar and pestle. Homogenized fecal samples were rinsed with a 2:1 chloroform:methanol solution to remove lipids (Bligh & Dyer, 1959), dried for at least 24 hours under a fume hood, then further treated with a few drops of 0.1N HCl solution without rinsing to remove carbonates and left to dry for a further 24 hours under a fume hood. Claw samples were rinsed with a 2:1 chloroform:methanol solution to remove surface contaminants, dried in a fume hood for at least 24 hours, then prepared whole for stable isotope analysis.

Between 0.3-0.4 mg of homogenized plasma, 0.8-1.8 mg of homogenized blood cells, 1.1-2.2 mg of homogenized fecal samples, and 0.2-2.2 mg of whole claw tip samples, respectively, were weighed into tin cups for stable carbon and nitrogen isotope

analysis. Samples were sent for dual $\delta^{13}C$ and $\delta^{15}N$ isotope analysis at either (1) the Stable Isotope Facility at the University of California (Davis, CA, USA), using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK); or, (2) the Environmental Isotope Laboratory at the University of Waterloo (Waterloo, ON, Canada) using a 4010 Elemental Analyzer (Costech Instruments, Italy) coupled to a Delta Plus XL (Thermo-Finnigan, Germany) continuous flow isotope ratio mass spectrometer (CFIRMS).

### 4.2.4. Predictions

If feather growth occurs at a relatively constant rate, it should provide an estimate of time since arrival at the wintering ground. For example, harlequin ducks molting in the Strait of Georgia too took 30 (males) to 31 (females) days to regrow their flight feathers, representing growth rates of approximately 4.33 (males; ninth primary 130 mm) and 3.87 (females; ninth primary 120 mm) mm/day (G. J. Robertson et al., 1997). However, their estimates were made by recording the amount of time that it took for wings to be fully grown, then dividing the fully grown wing length by the number of days; they did not measure feather growth repeatedly on the same individuals to determine whether the growth rate was constant over time. However, this still provides a very useful estimate of time represented by mm of feather growth (i.e. 1 day/3.87 mm or 0.258 day/mm).

Using feather growth (approx. 3.87 mm/day) as an index of time, I predicted that blood and claw isotopes would turn over with time (i.e. feather length) from low (freshwater signature) to high (marine) (Figure 4.2). There is little information on the rate of claw growth in wild sea ducks; however, the claw growth rate for captive waterfowl (mallard *Anas platyrhynchos*, pintail *Anas acuta*, and lesser scaup *Aythya affinis*) range from 0.06 to 0.13 mm $d^{-1}$. As such, Oppel and Powell (2008) expected king eider claws, which are 9 to 10 mm in length, to incorporate isotopic information and reach the distal claw tip (~1 mm) within 70 to 170 days following a dietary shift. This period is much longer than the time for blood cells to reach equilibrium with a new diet, which is estimated to be a few days to weeks (Klaassen et al., 2010; Lourenço et al., 2015; Oppel & Powell, 2010). Therefore, I predicted that claw isotope incorporation would be slower than that of blood; as such, claw isotope signatures for both $\delta^{13}C$ and $\delta^{15}N$ should be lower in claws than in blood within individuals, as well as in general, across the sample population.

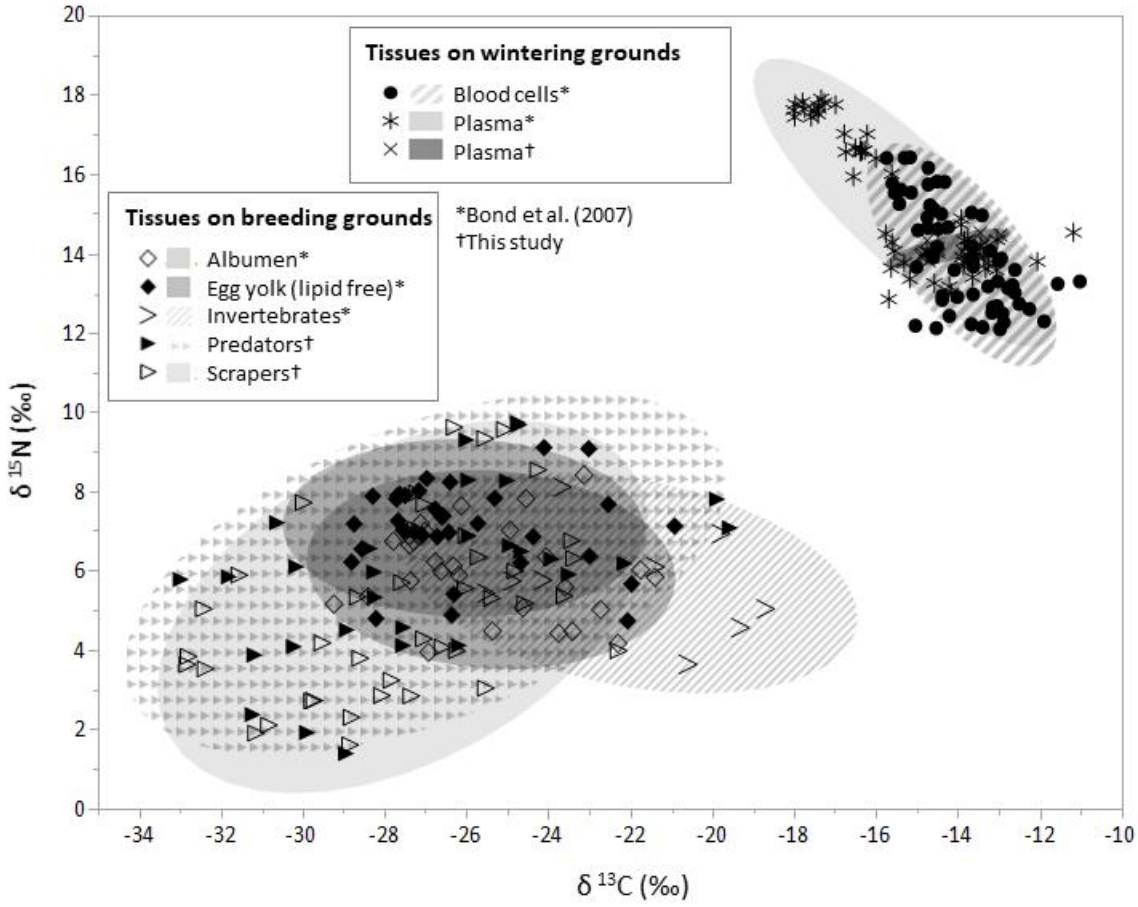


**Figure 4.2 Biplot of $\delta^{13}C$ and $\delta^{15}N$ signatures in various harlequin duck body tissues and prey items throughout the annual cycle. Samples from the breeding season (freshwater) are distinctly depleted in both isotopes (lower left quadrant) while wintering ground (marine) isotopes are distinctly enriched (top right quadrant).**

Note: Invertebrate (food) $\delta^{15}N$ values have been increased by 3.5‰ in order to make them directly comparable to harlequin ducks, assuming ducks are one trophic level higher than their invertebrate prey.

Claws, like other keratin-based tissues, have also been found to experience higher tissue-diet discrimination than blood (Boecklen et al., 2011; Lourenço et al., 2015). As such, claws should equilibrate to a higher value for both $\delta^{13}C$ and $\delta^{15}N$ than blood cells. Further, individuals whose tissues are either still at equilibrium with the old (new arrivals) or have equilibrated to the new diet (longest time since arrival as measured by feather length) should have claw signatures higher than blood cell signatures for both $\delta^{13}C$ and $\delta^{15}N$.

### 4.2.5. Single-tissue models

Bearhop et al. (2002) note that a number of isotope studies have been conducted under the assumption that $\delta^{13}C$ and $\delta^{15}N$ turn over at approximately the same rate within a single tissue. While only a few studies have simultaneously looked at $\delta^{13}C$ and $\delta^{15}N$ turnover rates within the same tissue, their findings have been inconsistent. Depending on the species, tissue, and diet, $\delta^{15}N$ has been found to turn over at the same rate (Stuart Bearhop et al., 2002), faster (Hilderbrand et al., 1996), and slower (Carleton & Rio, 2005) than $\delta^{13}C$ within the same tissue. To test this, I used nonlinear regressions to model single isotope, single tissue turnover curves for both $\delta^{13}C$ and $\delta^{15}N$ in blood cells, using ninth primary feather growth as an index of time. These single-isotope, single-tissue models were used to obtain initial estimates of the turnover rates for $\delta^{13}C$ and $\delta^{15}N$ in blood cells, which were then compared to the results from single-isotope, dual-tissue nonlinear regressions, which also incorporated claw signatures.

### 4.2.6. Dual-tissue models

The dual-tissue models depend on the nature of isotope turnover in claws, which has not been well-elucidated. The first possibility, as modeled in several avian claw isotope turnover studies (Hopkins III et al., 2013; Lourenço et al., 2015), is that claw isotope turnover follow the same exponential decay, but slower, as the cellular fraction of blood. The mechanism for this could be that the proteins used for claw growth are incorporated from blood products, which experience exponential decay; thus, claw turnover may mimic, but lag behind, cellular blood turnover (e.g. Figure 4.3 – Panel A).

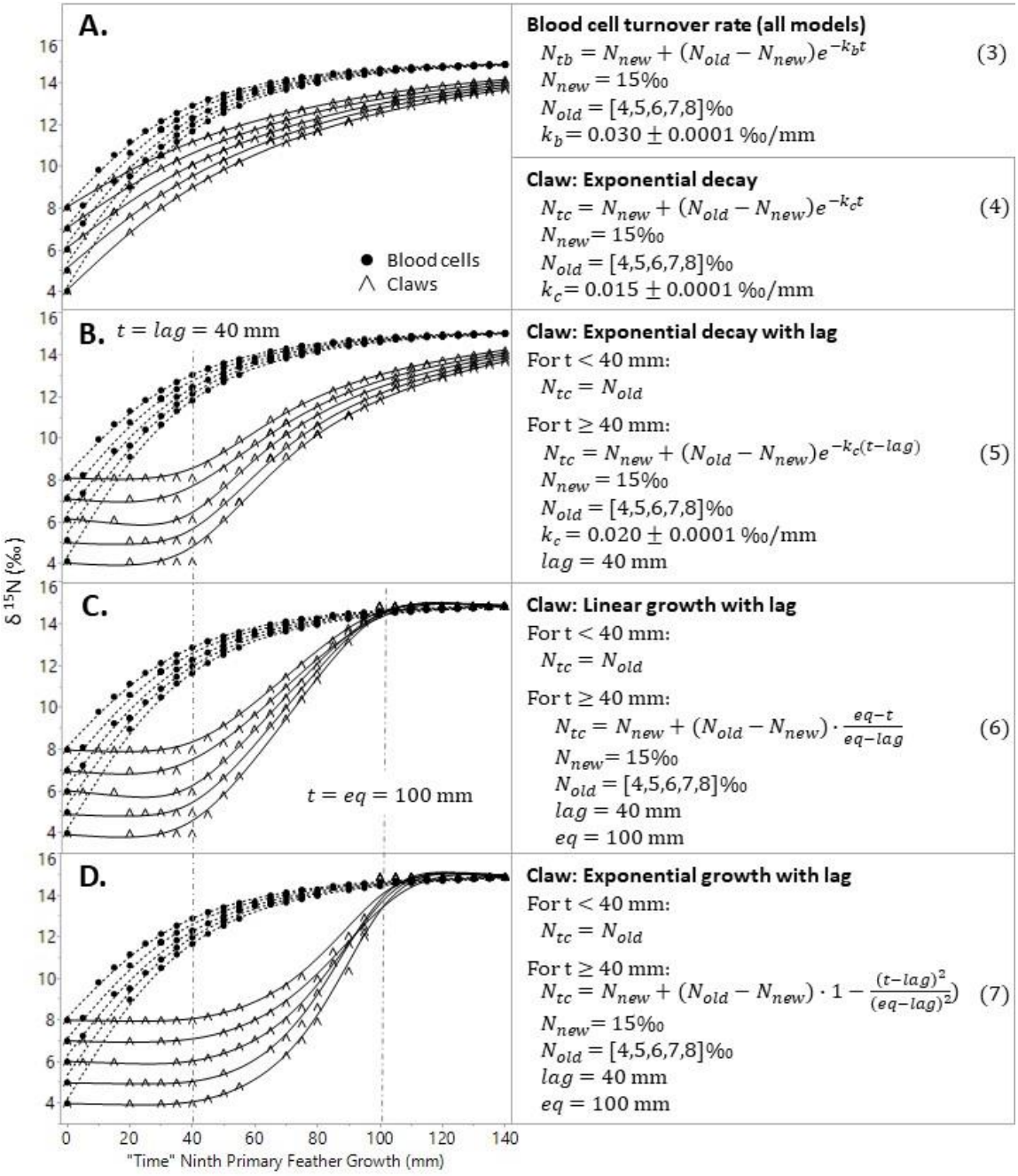


**Figure 4.3 Hypothetical turnover curves for $\delta^{15}N$ in blood cells (closed circles, dashed lines) and claws (upright arrows, solid lines), with four possible turnover curves for claws: exponential decay, exponential decay with lag, linear turnover with lag, and exponential growth with lag.**

Notes: (A) claw isotope turnover follows an exponential decay curve, similar to that of blood cells, but slower; (B) claws experience a lag (no change) in turnover, followed by the same exponential decay as A; (C) claws experience a lag before incorporating the new isotope signature in a linear way, with all individuals reaching equilibrium around the same time; and, (D) claws experience a lag before turning over with exponential growth of the new isotope within the distal claw tip, all reaching equilibrium around the same time. See text for further explanation of modeling approaches and variables used.

However, this slow exponential decay function may be inaccurate because claws should not experience cellular replacement with a half-life in the way that blood components or liver cells do. Instead, avian claws experience continuous growth (Lucas and Stettenheim 1972, Ethier et al. 2010); thus, keratin growth should be traceable as a gradient from the oldest to the most recently formed keratin in claws (Hahn et al., 2014). As such, claw "turnover" may follow a linear gradient directly related to the rate of claw growth (Figure 4.4 – Panel B). Since turnover is related to claw growth only, all claws should reach equilibrium with the new diet at approximately the same time. The slope of the linear turnover "curve" will depend on the magnitude of the difference between the old and new diets (Figure 4.4Figure 4.3 – Panel C).

However, while mammalian claws grow mostly longitudinally, avian claws tend to grow both from the centre of the claw (laterally) and, to a lesser extent, longitudinally; thus, avian claws are thought to experience conical growth (Hahn et al., 2014) (Figure 4.4 – Panel C). Additionally, harlequin ducks use their claws extensively, both to dig in the substrate for prey, as well as for moving along river and ocean beds. Thus, their claw tips should be more worn down than a proportionately sized passerine claw. As such, I created a simple, exponential model incorporation of a new dietary isotope through growth into the worn, distal claw tip, based on the volume of a truncated cone (Figure 4.3 – Panel D).

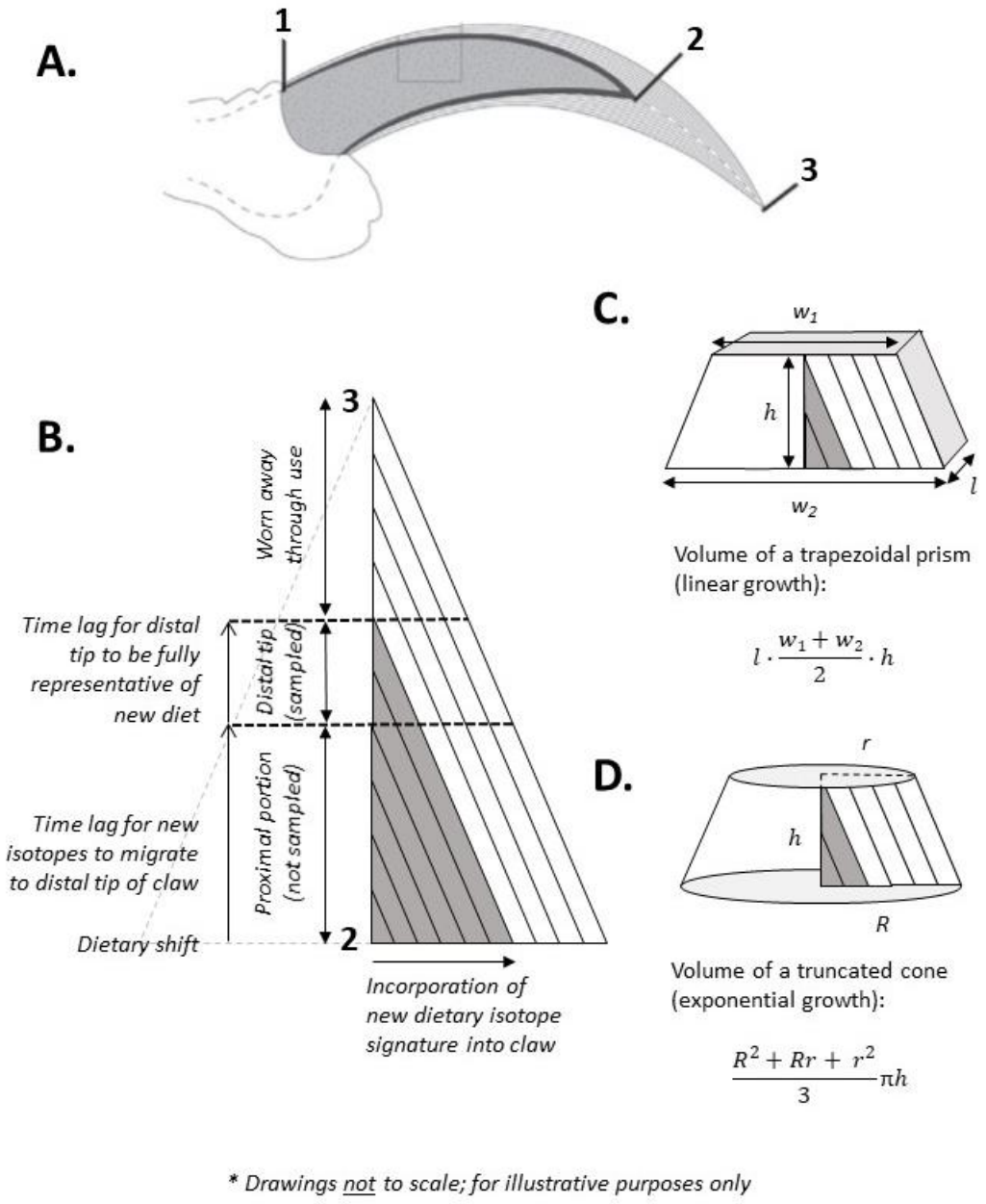


**Figure 4.4 Hypothesized claw growth in harlequin ducks.**
Image: Panel A is adapted from Fig. 1 in Hahn et al. (2014); it depicts a schematic longitudinal section of a passerine back toe, including the conical bone (distance 1-2) and keratinous claw tip (distance 2-3).
Notes: Panel B illustrates the layered growth of claw tissue following a change in dietary isotopes at point (2). Panel C shows incorporation of the new dietary isotope into the distal claw tip following mainly longitudinal claw growth, as is typical for mammals; this relationship is modelled using a linear equation. Panel D shows incorporation of new dietary isotopes into the distal claw tip through conical growth (both longitudinal and lateral growth); this relationship is modelled using an exponential equation.

In addition to the nature and, thus, shape of the turnover curve itself, there may also be a time-lag between the existing distal portion (already formed upon arrival to a new habitat, i.e. at the time of dietary switch) and the amount of time that it takes for the first bit of newly grown claw (keratin) to arrive within the sampled portion of the claw (Hopkins III et al., 2013). Hahn et al. (2014) correctly point out that this lag is almost never accounted for in studies of claw isotope turnover.

In both the linear (Figure 4.3 – Panel C) and exponential models (Figure 4.3 – Panel D), since isotopic "turnover" is hypothesized to occur purely due to physical growth, claw tips from different individuals should experience approximately the same temporal "lag" ($time = lag$) between dietary switch and the amount of linear growth required for the new claw material to migrate to the proximal (sampled) claw tip. Similarly, all claws should also reach equilibrium with the new diet at approximately the same time ($time = eq$); the amount of time for the entire proximal claw tip to be composed of new tissue.

As such, I predicted that claw isotope turnover in harlequin ducks, as plotted against primary feather growth, will either follow an (1) exponential decay, (2) linear, or (3) exponential growth turnover function; and additionally, that any of these three functions may or may not be preceded by a "time lag", wherein the claw material grown on the new diet has not yet migrated to the sampled distal claw tip. In order to visualize these predictions, I created simple models described by the equations and initial turnover rate estimates (based on both literature and visual inspection of the isotope signatures observed in harlequin duck tissues relative to feather growth in this study) to produce the hypothetical curves depicted in Figure 4.3.

Statistical analyses were performed using JMP (version 13.1.0, SAS Institute Inc.) and R (version 3.5.1, the R Foundation for Statistical Computing).

As can be seen in equations 4-7 (Figure 4.3), the isotopic signature in the claw (in these examples, $N_{tc}$, but the same could be said for $C_{tc}$) at time, $t$, is always dependent on the magnitude of the difference between the old and new diets ($N_{old} - N_{new}$). Assuming that this difference is the same for blood cells as it is for claws (as was assumed in Klaassen's 2010 model), equation (3) can be rearranged as:

$$(N_{old} - N_{new}) = \frac{N_{tb} - N_{new}}{e^{-k_b t}} \qquad (8)$$

The right-hand side of equation 8 can now be substituted into equations 4-6 in place of the term, $(N_{old} - N_{new})$. These equations can now be rearranged to solve for $N_{tb}$ without a need to know $N_{old}$ for each individual (Table 4.1). Additionally, in lag models, the actual measured value of $N_{tc}$ can be substituted for $N_{old}$ when time < lag (i.e. the signature in the claw will be at equilibrium with the old diet when the time since dietary shift is less than the time needed for new claw tissues to migrate to the distal tip of the claw). These equations were then used as the underlying models for nonlinear regressions, solving for $N_{tb}$ as measured in the ducks in this study, where $N_{tc}$ is as measured, $t$ is equal to wing length minus 53 mm, and $N_{new}$, $k_b$ and $k_c$ are estimated as population-level model parameters.

**Table 4.1** **Predicted $\delta^{15}N$ signature in blood cells, $N_{tb}$, at time, $t$, under various claw turnover scenarios. Each model was used in a nonlinear regression to (1) estimate population-level model parameters for $N_{new}$, $k_b$, and $k_c$, and (2) to determine which claw turnover model best explained the observed $N_{tb}$ and $N_{tc}$, given the time, $t$, since dietary shift, as estimated by ninth primary feather regrowth.**

| Claw turnover model | $N_{tb}$ | Equation |
|---|---|---|
| Exponential decay | $N_{tb} = (N_{tc} - N_{new})e^{-(k_c - k_b)t}$ | (4a) |
| Exponential decay with lag | When $t < lag$: $N_{tb} = N_{new} + (N_{tc} - N_{new})e^{-k_b t}$ | (5a) |
| | When $t \geq lag$: $N_{tb} = N_{new} + (N_{tc} - N_{new})\frac{e^{-k_b t}}{e^{-k_c(t-lag)}}$ | (5b) |
| Linear growth with lag | When $t < lag$: $N_{tb} = N_{new} + (N_{tc} - N_{new})e^{-k_b t}$ | (6a) |
| | When $lag \leq t < eq$: $N_{tb} = N_{new} + (N_{tc} - N_{new})\frac{eq - lag}{eq - t}e^{-k_b t}$ | (6b) |
| | When $t \geq eq$: $N_{tb} = N_{tb}$ | (6c) |
| Exponential growth with lag | When $t < lag$: $N_{tb} = N_{new} + (N_{tc} - N_{new})e^{-k_b t}$ | (7a) |
| | When $lag \leq t < eq$: $N_{tb} = N_{new} + \frac{(N_{new} - N_{tc})(lag - eq)e^{-k_b t}}{eq - lag}$ | (7b) |
| | When $t \geq eq$: $N_{tb} = N_{tb}$ | (7c) |

* Once claws have reached $t = eq$, they are now equilibrated with the new diet and have no additional information to provide regarding turnover rates or $N_{old}$, hence equations 6c and 7c.

** These same conceptual models were used to estimate the same parameters for $\delta^{13}C$, using $\delta^{13}C$ signatures in those same tissues and individuals.

## 4.3. Results

Ninth primary feather regrowth, calculated as $growth = wing\ chord\ length - 53\ mm$, appeared to act as a reliable index of time; blood cell and claw isotope values generally increased with feather length, and variation decreased, indicating turnover to the higher marine signatures, which were also evident in the already equilibrated plasma signatures (Figure 4.3). One interesting exception was noted wherein an individual (bird band no. 1015-06357) exhibited unexpectedly low isotope signatures in both blood cells and claws relative to its very long wing measurements (wing chord = 192 mm, the second longest wing measurement; equivalent to 139 mm of feather regrowth). Upon reviewing photographs of this individual, along with its tail growth status (old tail present, i.e. not yet molted), it was determined that this individual had incorrectly had its old (previous year) flight feathers measured, which it was still in the process of molting. As such, rather than being at the high end of time since arrival, this was one of the newest arrivals in our sample. I therefore adjusted this individual's wing length to 68 mm (i.e. 15 mm of growth; denoted by arrows in Figure 4.3) in order to place it with the other three individuals who were noted as still being in the process of molting their flight feathers.

Visual inspection of blood cell and claw isotope signatures relative to feather growth suggested that the discrimination factor for claws is higher than that of blood cells (Figure 4.5). Four individuals also appeared to have reached equilibrium with the new diet in both blood cells and claws, as both $\delta^{13}C$ and $\delta^{15}N$ signatures in claws had risen above that of blood cells; these individuals also exhibited the longest wing length of all the individuals sampled (Figure 4.5). While claw signatures were consistently lower than blood cells prior to approximately 100 mm of feather growth, after that point, claw signatures quickly rose above that of blood cells. The fact that claw signatures eventually overtook blood signatures suggests that, at equilibrium with the old diet, claws would have also expressed higher isotope signatures than blood cells. Based on the differences observed in $\delta^{13}C$ and $\delta^{15}N$ signature in claws and blood cells of these four presumably equilibrated individuals, the difference in discrimination factors between claws and blood cells were estimated to be $\delta^{15}N_{claws\text{-}blood}$ = 0.748 ± 0.148‰ (mean ± SD) and $\delta^{13}C_{claws\text{-}blood}$ = 0.743 ± 0.101‰ (Table 4.2). For ease of calculation in subsequent turnover modeling, $\delta^{13}C$ and $\delta^{15}N$ signatures in claws were adjusted to be directly comparable to those of blood by subtracting 0. 748‰ and 0.743‰, respectively.

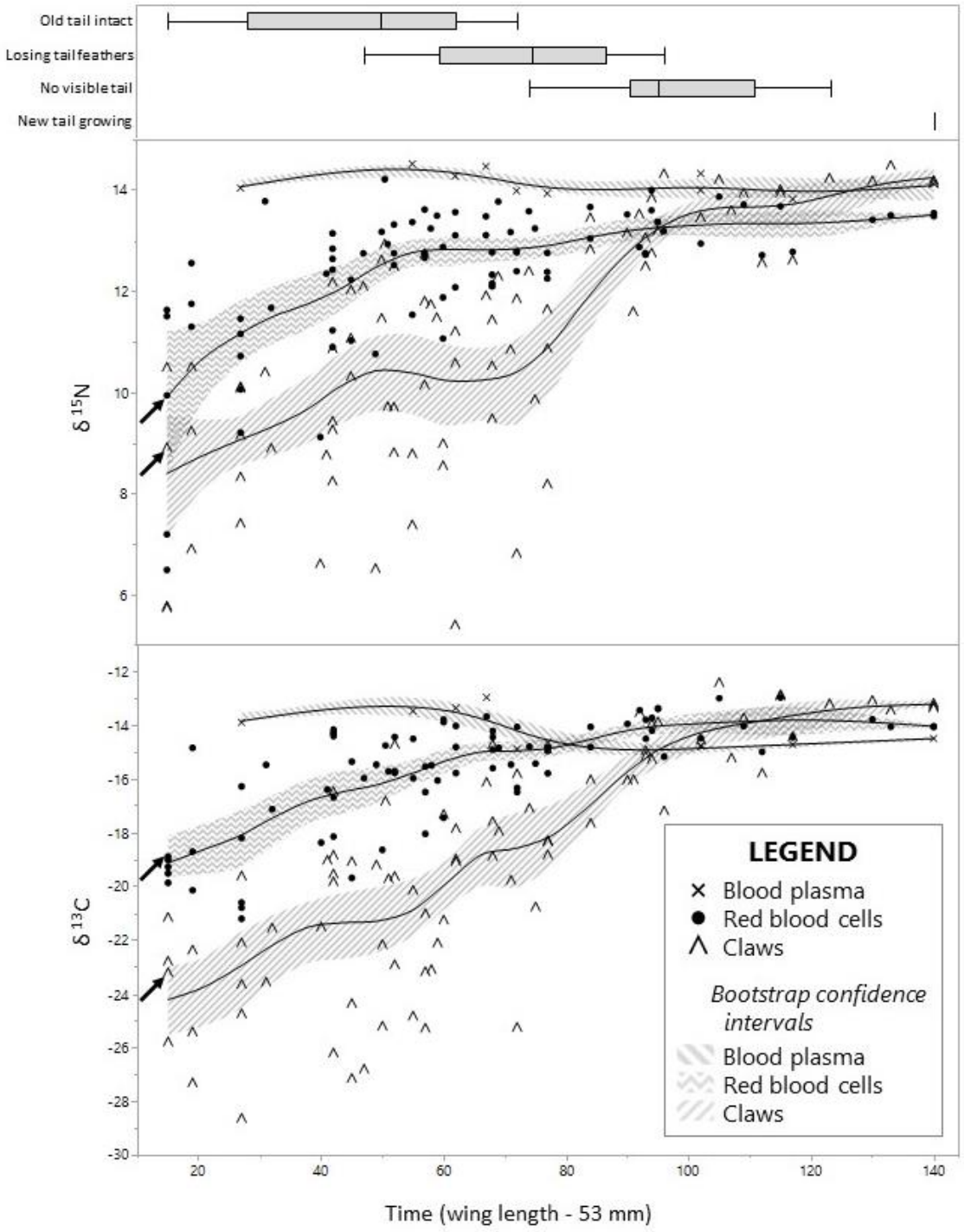


**Figure 4.5 Plot of stable isotope signatures in blood components and claws of harlequin ducks relative to wing and tail molt. Regrowth of the longest (ninth) primary feather should be linearly related to time since dietary switch, i.e. time since arrival on the wintering grounds.**

Note: Arrows denote a single individual (bird band no. 1015-06357) whose flight feathers were just starting to molt and was initially measured as $growth = 192 - 53 = 139\ mm$, but, based on tail molt, was adjusted to just 15 mm of growth. Whiskers on box plots of tail status indicate minimum and maximum measured values.

**Table 4.2** **$\delta^{13}C$ and $\delta^{15}N$ signatures in blood cells and claws in four individuals that appear to have reached equilibrium with the new (marine) diet in both tissues. One individual (band no. 1015-14928) had two claw samples. The mean discrimination factor between claws and blood was used in further analyses to "adjust" the measured isotope value for claws to be comparable to that of blood cells.**

| | $\delta^{15}N$ (‰) | | | $\delta^{13}C$ (‰) | | |
|---|---|---|---|---|---|---|
| **Band Number** | **Blood cells** | **Claws** | **$\delta^{15}N_{claws\text{-}blood}$** | **Blood cells** | **Claws** | **$\delta^{13}C_{claws\text{-}blood}$** |
| 1015-06346 | 13.418 | 14.197 | 0.779 | -13.773 | -13.053 | 0.720 |
| 1015-06348 | 13.506 | 14.500 | 0.994 | -14.048 | -13.420 | 0.628 |
| 1015-14927 | 13.549 | 14.204 | 0.655 | -14.059 | -13.159 | 0.900 |
| 1015-14928 | 13.488 | 14.140 | 0.652 | -14.028 | -13.328 | 0.700 |
| 1015-14928 | 13.488 | 14.148 | 0.659 | -14.028 | -13.262 | 0.766 |
| **Mean** | | | 0.748 | | | 0.743 |
| **Standard deviation** | | | 0.148 | | | 0.101 |

Although claw isotope signatures were higher than blood cell signatures in long-winged individuals (i.e. those who had been on the wintering grounds for a relatively long amount of time), the same was not true for those at the "early" end of wing molt. Individuals at the "early" end of wing molt already exhibited blood cell signatures higher than those of their claws (Figure 4.5), indicating that they had already been on the wintering grounds long enough for their blood cell signatures to rise above that of claws despite increased diet-tissue discrimination for both $\delta^{13}C$ and $\delta^{15}N$ in claws relative to blood cells.

## 4.3.1. Single-tissue turnover models

I used single-isotope, single tissue nonlinear regressions to estimate the turnover rates for $\delta^{13}C$ and $\delta^{15}N$ in blood cells. Approximate starting estimates of $lag$ (40 mm) and $eq$ (100 mm) were obtained from visual examination of claw turnover versus feather growth (Figure 4.5). Using no constraints (except for reasonable starting values), both models converged easily and to reasonable estimates (Table 4.3).

**Table 4.3. Initial values and estimates from converged nonlinear regression models of $\delta^{13}C$ and $\delta^{15}N$ turnover in harlequin duck blood cells (single tissue model).**

| Variable name | Description | Initial values (‰) | Model estimates (‰) | Approx. Standard Error |
|---|---|---|---|---|
| *All variables are estimated as the mean for the entire sample population | | | | |
| $N_{blood(old)}$ | Blood at equilibrium with old diet | 9.0 | 7.332 | 1.173 |
| $N_{blood(new)}$ | Blood at equilibrium with new diet | 15 | 13.423 | 0.272 |
| $k_{bloodN}$ | Turnover rate (vs. feather growth) | 0.03/mm | 0.0366/mm | 0.009 |
| $k_{bloodN}$ | Turnover rate (vs. time = 3.87 mm/day) | | x 3.87 mm/day = 0.142/day | 0.0348 |
| $C_{blood(old)}$ | Blood at equilibrium with old diet | -22 | -25.009 | 1.095 |
| $C_{blood(new)}$ | Blood at equilibrium with new diet | -12 | -13.225 | 0.686 |
| $k_{bloodC}$ | Turnover rate (vs. feather growth) | 0.03/mm | 0.0225/mm | 0.006 |
| $k_{bloodC}$ | Turnover rate (vs. time = 3.87 mm/day) | | x 3.87 mm/day = 0.0871/day | 0.0232 |

The single-tissue turnover models did not converge for exponential decay in claws; therefore, either the nonlinear exponential decay turnover model needs, for some reason, to be constrained more strictly than the blood models did (i.e. by constraining the $N_{new}$ and $C_{new}$ values to a certain number) or an exponential decay model is not suitable for claw isotope turnover (Figure 4.6).

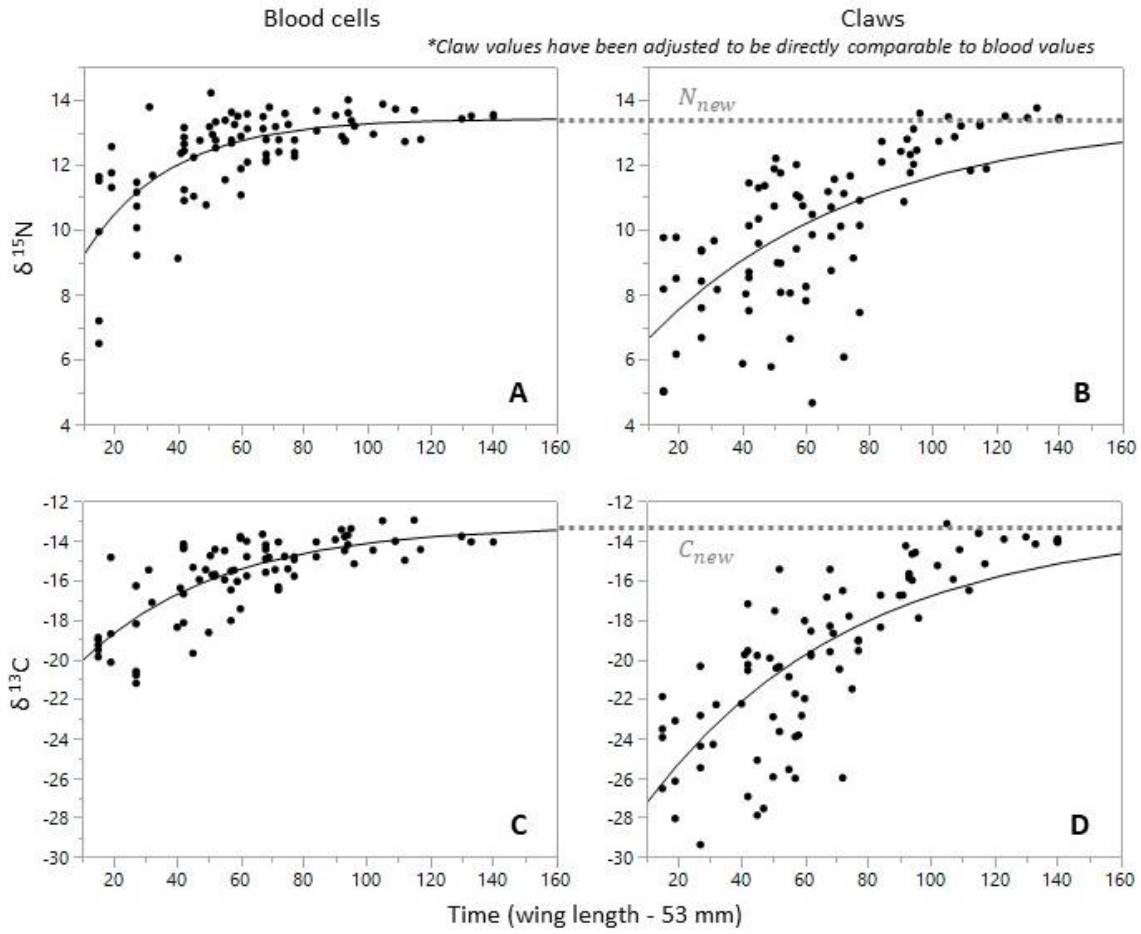


**Figure 4.6 Nonlinear regression curves for $\delta^{13}C$ and $\delta^{15}N$ turnover (exponential decay) in blood cell and claws.**

Notes: Here, $\delta^{13}C$ and $\delta^{15}N$ signatures in claws have been adjusted by subtracting $\delta^{15}N_{claw\text{-}blood}$ = 0.748‰ and $\delta^{13}C_{claw\text{-}blood}$ = 0.743‰, respectively. Blood cell turnover curves (A and C) converged without any constraints. Claw turnover curves (B and D) only converged when $N_{new}$ (B) or $C_{new}$ (D) were constrained (dashed lines); here, $N_{new}$ and $C_{new}$ were constrained to 13.423‰ and -13.225‰, as estimated from the blood cell turnover models (A and C, respectively).

## 4.3.2. Dual-tissue turnover models

I used nonlinear regressions of dual-tissue turnover models for exponential decay with lag, and linear growth with lag to determine which model best suited the data (models in Table 4.1). The exponential decay model (with lag for claws) converged (Table 4.4).

**Table 4.4 Initial values and estimates from converged nonlinear regression models of $\delta^{13}C$ and $\delta^{15}N$ turnover in harlequin duck blood cells (dual tissue model). Time was estimated based on ninth primary flight feather regrowth.**

| Variable | Description | Initial values | Model estimates | Approx. Standard Error |
|---|---|---|---|---|
| Lag | Time (in feather growth) required for new claw material to migrate to tip | 20 mm | 61.279 mm | 8.854 |
| $N_{new}$ | Blood (and adjusted claw) at equilibrium with new diet | 13.4‰ | 13.612 ‰ | 0.223 |
| $k_{bloodN}$ | Blood turnover rate (vs. feather growth) | 0.036 ‰/mm | 0.0263 ‰/mm | 0.00272 |
| $k_{clawN}$ | Claw turnover rate (vs. feather growth) | 0.036 ‰/mm | 0.0475 ‰/mm | 0.0175 |
| $k_{bloodN}$ | Blood turnover rate (vs. time = 3.87 mm/day) | | 0.1018 ‰/day | 0.0105 |
| $k_{clawN}$ | Claw turnover rate (vs. time) | | 0.1838 ‰/day | 0.0677 |
| Lag | Time (in feather growth) required for new claw material to migrate to tip | 20 mm | 36.129 mm | 7.859 |
| $C_{new}$ | Blood (and adjusted claw) at equilibrium with new diet | -13.225 ‰ | -13.797 | 0.281 |
| $k_{bloodC}$ | Blood turnover rate (vs. feather growth) | 0.0225 ‰/mm | 0.0341 ‰/mm | 0.00274 |
| $k_{clawC}$ | Claw turnover rate (vs. feather growth) | 0.0225 ‰/mm | 0.0214 ‰/mm | 0.0120 |
| $k_{bloodC}$ | Blood turnover rate (vs. time = 3.87 mm/day) | | 0.1320 ‰/day | 0.00958 |
| $k_{clawC}$ | Claw turnover rate (vs. time) | | 0.0828 ‰/day | 0.0421 |

*All variables were estimated as the mean for the entire sample population.

The linear lag and exponential lag models converged for the hypothetical, but not for the actual (observed) data. Thus, a dual-tissue model with claws experiencing a lag, followed by isotopic signature turnover as a exponential decay curve, seemed to best fit these data. Therefore, I used the dual-tissue, exponential decay with lag model to obtain estimates of blood and claw turnover rates (Table 4.4). To back-calculate to breeding ground ($N_{old}$) isotopes, I used only the blood isotope values ($N_{tb}$) and the model estimates of blood turnover rate ($k_b$) and new blood signature ($N_{new}$):

$$N_{old} = \frac{N_{tb}}{e^{-k_b t}} + N_{new} \quad (8)$$

Back-calculated values using blood isotope signatures and turnover rates are shown in Figure 4.7. Four individuals, whose primary feather lengths indicated that they had been on the wintering grounds long enough for both blood and claws to equilibrate to the new diet produce estimations that were not realistic, due to the model not having enough information to perform a back-calculation. Once those individuals were

excluded, $N_{old}$ ranged from -22 to 19‰ and $C_{old}$ from -39.3 to -14.0‰. The observed turnover curves for the difference between claw and blood signatures over time seemed to match the expected (hypothetical) curves well (Figure 4.7).

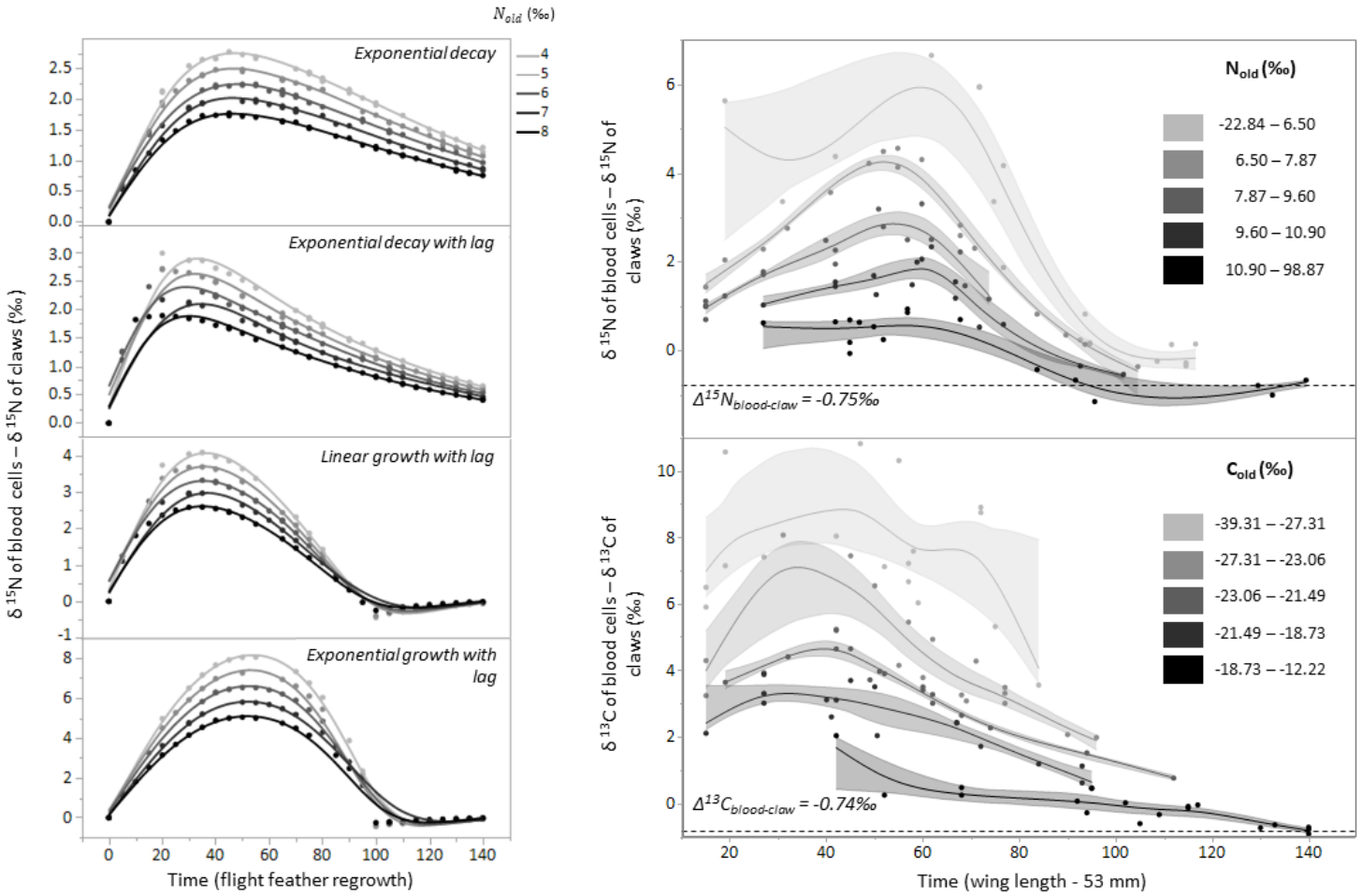


**Figure 4.7 Hypothetical (left) and actual (right) values of the difference in isotope values between blood cells and claws over time (or wing growth).**

Note: Values of $N_{old}$ and $C_{old}$ in right-hand panel have been back-calculated using a dual-tissue turnover model using an exponential decay model, with a time lag for claws, for both blood cells and claws (equations 8a and 8b).

## 4.4. Discussion

Here, I suggest a novel method of utilizing dual-tissue, single-isotope turnover modeling to enable a back-calculated estimate of the stable isotope signature of diet prior to migration. I describe three theoretical turnover curves to explain the incorporation of a new isotopic signature into avian claws and provide an initial test of these three models using field-collected data in wintering harlequin ducks. I also show that primary feather growth in molting harlequin ducks appears to provide a reliable estimate of time since arrival.

Many studies have been conducted with the hopes of being able to use claw stable isotope signatures in order to make inferences regarding pre-migration habitat, only to lament the rapid incorporation of the new, post-migratory diet. Indeed, the results here show that claws had already begun to incorporate the new dietary signature in most of the individuals that we captured. As such, studies looking to utilize claw isotopes as an inert record of dietary signature must account for this incorporation rate, or sample birds within a very small window of time upon their arrival. For example, the dual-tissue model estimated that claws experienced an inert "lag" of only 36.129 mm of wing growth before beginning to incorporate marine signatures (Table 4.4). This corresponds to a period of approximately 10.32 days, if wing growth is estimated to be approximately 3.87 mm/day (G. J. Robertson et al., 1997).

However, it is also likely that longer-clawed species, such as passerines, will provide a longer window of opportunity to sample claw tips that are purely reflective of the pre-migratory diet. In addition to having longer claws overall, passerines also experience slower claw growth (0.03-0.05 mm/day) than waterfowl (0.068-0.076 mm/day) (Hahn et al., 2014). Bearhop et al. (2003) found claw growth rate to range from 0.02-0.06 mm/day across several species of passerines (mean = 0.04 ± 0.01 mm/day, n = 43 individuals). Regardless, I show here that an alternative to this short temporal window is comparing blood cell and claw signatures to provide a more accurate estimate of diet prior to migration.

Most studies model claw isotope turnover as an exponential decay function, similar to blood, liver, brain, and other tissues that continuously regenerate (Boecklen et al., 2011; Lourenço et al., 2015). Indeed, claw isotopes often appear to turn over

following an exponential decay function (e.g. Hopkins III et al., 2013), but this is likely more because individuals are coming from across a wide range habitats and, accordingly, isotopic signatures, to a much smaller range. Due to the fact that field studies often capture individuals within a small geographic area, it is quite likely that these individuals will show less variation in their current diet than in their pre-migration diets. As such, the sample population will experience a reduction in isotope variation over time as they all equilibrate towards a new, similar isotope signature, resembling an exponential decay turnover curve. In actuality, it would be difficult to distinguish whether that same pattern was instead due to a population of individuals whose claws are all experiencing linear or exponential isotopic turnover over time (Figure 4.3).

At least one other study has modeled claw isotope turnover as linear (Fraser et al., 2008). To my knowledge, no other study has proposed an exponential growth model for avian claws, though Hahn et al. (2014) strongly stressed that claw growth is conical in birds, rather than longitudinal, as it is in mammals. Fraser et al. (2008) stress that remarkably little is known about avian claw growth. It is thought, however, that material is deposited from both the nail bed, and from the inside of nail itself (Bearhop et al. 2003).

My single tissue and dual tissue models did not agree in the shape of the claw turnover curve. In the single tissue models, claws did not fit an exponential decay curve (Figure 4.5). The only way the single tissue model would converge for claws was if $N_{new}$ and $C_{new}$ were constrained to a maximum value. Even when the model converged (under constraint), the resulting curve did not fit the data well; the trendline did not turn over as fast as the data (Figure 4.5; panels B and D). In the dual tissue models, only the exponential decay model converged, suggesting that claw turnover is indeed similar to that of blood, liver, and other continuously regenerating tissues. However, the model was also inconsistent in its estimation of Lag. The amount of time Lag before new claw material has begun migrating to the tip of the claw was different for $\delta^{15}N$ (61.3 mm, i.e. ~17.5 days) and $\delta^{13}C$ (36.1 mm, i.e. 10.3 days) (Table 4.4). I expected the estimate of Lag to be similar for both isotopes (Figure 4.3; panels B-D), if claw turnover is due solely to physical growth. However, it is possible that the turnover of blood components, or isotopic routing, could explain the difference in claw turnover rates between isotopes. The inconsistent results between the single and dual tissue models indicate that more work and testing are needed. In either case, the model indicated that there was a lag of

36-67 mm of wing growth (equal to approximately 10-19 days). This concept of lag should be considered for inclusion in any future studies of isotope turnover in avian claw tips.

Blood plasma values showed little to no change over time (Figure 4.5). Plasma is mainly water, but also contains many important substances such as proteins (albumin, clotting factors, antibodies, enzymes, and hormones), sugars (glucose), and fat particles. Plasma generally turns over within a matter of days (Boecklen et al., 2011); thus, the blood plasma signatures observed here are likely representative of the new, wintering diet. Blood and claw values of individuals with the longest wings seem to have reached the same signature as plasma, and little change is seen in any of the tissues after 120-130 mm of wing growth (~34.3-37.1 days) on the wintering grounds. Thus, these four individuals seemed to have reached equilibrium with the new diet. They had the longest wing lengths of all those sampled, and all four had claw isotope values that exceeded their respective blood cell isotope values (Figure 4.5).

This is consistent with previous findings that keratin-based tissues experience greater diet-tissue discrimination than blood. The specific amino acid make-up of claws, and other keratin-based tissues, is thought to contribute to this pattern (Martínez del Rio et al., 2009; Wolf et al., 2009). Additionally, blood contains lipids, which are $\delta^{13}C$-depleted relative to proteins (Tieszen et al., 1983), while feathers are lipid-poor and claws contain no lipids at all (Cherel et al., 2005). A review of 27 populations and 22 avian species found that feathers were consistently enriched in $\delta^{13}C$ compared with blood (Cherel et al., 2014). The same study found that feathers were significantly enriched in $\delta^{15}N$ compared with blood in most populations and species; however, four species exhibited no difference in $\delta^{15}N$ values between feathers and blood, while two additional species actually showed the opposite (Cherel et al., 2014).

Using just the four individuals that seemed to be at equilibrium with the new diet, claws appeared enriched in $\delta^{13}C$ and $\delta^{15}N$ by 0.748 ± 0.148‰ (mean ± SD) and 0.743 ± 0.101‰, respectively, relative to blood cells (Table 4.2). Despite the low sample size, these findings were consistent with previous findings for feathers and claws, relative to blood. Lourenço et al., 2015 found the discrimination factor for $\delta^{15}N$ in in dunlin, *Calidris alpina*, claws to be approximately 1.76‰ higher than in blood cells, and $\delta^{13}C$ to be approximately 1.8‰ higher. Both values are higher than my estimates of 0.748‰ and

0.743‰ but are consistent in the direction of the difference. The only other study that I am aware of to compare discrimination factors between avian claws and another tissue actually found the opposite in both $\delta^{13}C$ and $\delta^{15}N$; out of claws, blood, plasma, and blood cells, claws had the lowest discrimination factor (Barquete et al., 2013). A review of differences in stable isotope signatures between feathers (another keratin-based tissue) and blood in captive and wild caught birds revealed differences of -0.6-1.5‰ for $\delta^{15}N$ and 0.1-3.1‰ for $\delta^{13}C$ in captive birds, and 0.3-1.5‰ for $\delta^{15}N$ and 0.7-1.7‰ for $\delta^{13}C$ in wild birds (Cherel et al., 2014). Meanwhile, Bearhop et al. (2003) found that $\delta^{13}C$ and $\delta^{15}N$ in feathers and claws were comparable, with claws being only 0.3-0.4‰ lower than feathers, for both isotopes. Bearhop et al. (2002) found feathers to be 0.8-1.8‰ higher than blood (average = 1.3‰). Taken together, claw isotopes may only be expected to be 0.9-1.0‰ higher than blood, which is consistent with the estimates I found here.

My single tissue model estimated that blood $\delta^{15}N$ turns over faster (0.142‰/day) than blood $\delta^{13}C$ (0.0871‰/day) (Table 4.3). This is consistent with some studies of avian blood isotope turnover (Stuart Bearhop et al., 2002), but the opposite of at least one (Carleton & Rio, 2005). This could be due to the larger difference between freshwater and marine $\delta^{13}C$ signatures in relative to the smaller difference between freshwater and marine $\delta^{15}N$ signatures. The larger difference between the $\delta^{13}C$ signature in freshwater environments versus marine environments could “mask” the actual turnover rate for $\delta^{13}C$, making it appear smaller than it actually is; in other words, the $C_{old}$-$C_{new}$ portion of the turnover equation (Figure 4.3) might overshadow the k (turnover rate) portion of the equation. Alternatively, the opposite may occur for blood $\delta^{15}N$; the smaller difference in $\delta^{15}N$ signatures between freshwater and marine environments relative to the difference in $\delta^{13}C$ signatures could mean that the turnover rate for $\delta^{15}N$ is overestimated.

In contrast, the dual tissue model estimated that blood $\delta^{15}N$ turned over slower (0.1018‰/day) than $\delta^{13}C$ (0.1320‰/day) (Table 4.4). These results are similar to estimates of turnover rates for blood components in other avian species. For example, Hobson and Clark (1992) found the fractional turnover rate of $\delta^{13}C$ in whole blood of captive Japanese Quail, *Coturnix japonica*, to be 0.062 $day^{-1}$. Carleton and Rio (2005) found $\delta^{13}C$ to be incorporated into house sparrow blood at a rate approximately 1.5 times faster than $\delta^{15}N$ (n = 26). However, (Stuart Bearhop et al., 2002) found no significant difference in whole blood C and N turnover rates (0.058‰ $day^{-1}$ and 0.046‰ $day^{-1}$, respectively; n = 9) in great skuas, *Catharacta skua*, following a dietary switch. As

such, the half-lives of each isotope were also similar (15.7 ± 2.1 d and 14 ± 4 d, respectively). Prior to the (Stuart Bearhop et al., 2002) study, only one other study had looked at $\delta^{13}C$ and $\delta^{15}N$ turnover rates within the same tissue; (Hilderbrand et al., 1996) found that $\delta^{13}C$ and $\delta^{15}N$ turnover rates in American black bear, *Ursus americanus*, blood plasma (0.15‰ $day^{-1}$ and 0.2‰ $day^{-1}$, respectively) and blood cells (0.02‰ $day^{-1}$ and 0.031‰ $day^{-1}$, respectively) were comparable, but that $\delta^{13}C$ turned over approximately 1.5 times slower than $\delta^{15}N$ in both tissues (the opposite of what was found in sparrows). However, (Hilderbrand et al., 1996) only compared $\delta^{13}C$ and $\delta^{15}N$ simultaneously using the same subjects for the blood plasma; the turnover rates for $\delta^{13}C$ in blood cells were compared using different individuals as well as a different dietary shift (salmon to apples vs. 35% herring : 65% vegetation to mule deer) than for $\delta^{15}N$.

Claw isotope turnover rate likely relies on the way the claw is grown and how long it takes for it to be replaced (Hahn et al., 2014). Hopkins et al. (2013) found that stable isotopes ($\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$) in 1 mm claw tips of adult female Lesser Scaup, *Aythya affinis*, equilibrated to a new diet within 3-3.5 months. Lourenço et al. (2015) found that 2 mm claw tips of captive dunlins, *Calidris alpina*, equilibrated with a new diet within 100-120 days, which is also the approximate amount of time required to replace the claw. $\delta^{13}C$ in dunlins turned over at a rate of 0.020 $day^{-1}$ and $\delta^{15}N$ at a rate of 0.026 $day^{-1}$ (Lourenço et al., 2015). The amount of time for claw replacement is quite similar across passerines, penguins, and shorebirds (Lourenço et al., 2015), and seems to be similar in ducks as well (e.g. Hopkins III et al., 2013). However, harlequin ducks wear their claws down much more than shorebirds and passerines; thus, the turnover rates in harlequin claws is likely to appear much faster (or lag time shorter) than in longer-clawed species, since the claw tip being sampled is more recently grown. For example, the captive dunlins observed by (Lourenço et al., 2015) had a mean claw length of 4.9 ± 0.4 mm (n = 29), while the harlequin ducks we sampled had claws no longer than 3-4 mm (pers. obs.).

Harlequin duck claws appeared to reach equilibrium with the new diet by 100-120 mm of wing growth (Figure 4.5). This is equivalent to approximately 28.6-34.3 days. As predicted, this is fast relatively to other species. For example, Lourenço et al. (2015) found that the half-lives of $\delta^{13}C$ and $\delta^{15}N$ in the claws of captive dunlins to be 27 (turnover rate = 0.026 $day^{-1}$) and 35 days (turnover rate = 0.020 $day^{-1}$), respectively. If

isotope turnover in claw is related to growth rate and claw replacement, then harlequin duck claws turnover roughly twice as fast as dunlins.

Regardless of the shape of the turnover curves, individuals should retain their relative positions over time, with respect to stable isotope signatures (Figure 4.6). In other words, individuals within a group exhibiting the same amount of wing feather growth (i.e. experiencing the same amount of time since arrival on the wintering grounds) should exhibit higher or lower stable isotope signatures relative to each other based on the relative isotope signatures of their respective breeding grounds. Individuals that bred on breeding grounds with a relatively high stable isotope signature should tend to retain a higher signature for that isotope throughout the turnover period, within each tissue type, relative to others who have been on the wintering grounds for the same amount of time as them.

### 4.4.1. Conclusions

Claws appear to offer some unique potential avenues for ecological investigation using stable isotopes to make inferences about migratory species. Claws are inert once they are built, but also capture a temporal gradient along their length as they grow. This could allow them to be used as a complementary tissue for stable isotope analysis, but with an even slower turnover rate with which to compare between tissues. This slower turnover rate allows for a longer window of investigation following dietary switch. A slower turnover rate means one can more easily distinguish between individuals coming from breeding grounds with differing signatures, for a longer amount of time. The longer the amount of time since dietary switch, and the closer the tissue signature is to the new diet, the less information the model can give in terms of projecting back to pre-migratory signatures.

My single and dual tissue models disagreed on the shape of claw turnover curves. However, the hypothetical data in Figure 6 show that the relative position of individuals over time does not change much between curve shapes. In fact, the back-calculation estimates were relatively robust to a range estimates of $N_{new}$, $C_{new}$, k, eq and lag. Furthermore, these estimates were in the known range for breeding habitat isotopes (see Chapter 5). The accuracy of each individual back-calculation remains to be explicitly tested. However, these models seem to offer similar estimates, within a sample

population of individuals, of the range of pre-migratory isotopes inhabited, and their relative proportions within the population.

As suggested by (Langin et al., 2007), the assignment of pre-migratory habitat to specific individuals may be difficult, due to noisiness in the data caused by multiple factors across multiple temporal periods affecting isotopic signatures within an individual. This methodology may indeed be most useful in addressing the very problem it sought to solve; elucidating broad, range-wide population trends, rather than behaviour of individuals, in migratory species.

### 4.4.2. Future studies

Future studies could improve upon the field methodology described here by taking successive claw clippings (either all at once, or over time) and use the change in stable isotope signatures along the length of the claw to model turnover. Alternatively, whole claws from museum specimens could be used for this purpose as well. The difficulty with this museum specimen approach would be identifying those individuals that had recently experienced a dietary shift. An adult harlequin duck that perished during wing molt would, however, be a good candidate for this. In fact, the length of the primary feathers on that individual would provide additional information with respect to time since dietary switch.

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# Chapter 5. Harlequin duck breeding habitat associations: insights from stable isotopes in claws and blood cells

## 5.1. Introduction

An organism's reproductive fitness is ultimately a cumulative result of conditions it encounters over the course of its life, during which, its strategies, behaviours, health status, reproductive success, and habitats may all change. Furthermore, conditions affecting individual or group behaviours in one part of the annual cycle can have consequences for the success of an individual in subsequent seasons. These carryover effects, such as timing of arrival, can have strong implications for breeding success and fitness. For example, early breeding ground arrival is usually associated with higher breeding success (Aebischer et al., 1996; Currie et al., 2000; McKellar et al., 2013; Norris et al., 2004; Rockwell et al., 2012; Saino et al., 2004; Sergio et al., 2007) which can ultimately lead to consequences on population dynamics (Mazerolle & Hobson, 2005; Newton, 2006).

Due to the interrelated nature of behaviours and decisions made throughout the annual cycle, much effort has been made to account for carryover effects when interpreting current behaviours, distributions, and habitat selection decisions. However, the logistical task of determining habitat usage, physiological condition or behaviours in previous seasons can be quite difficult, especially in highly vagile migratory species which travel vast distances, often too quickly for humans to easily track. This problem is further exacerbated by the fact that these highly migratory species can experience different degrees of migratory connectivity, the extent to which geographic locations of habitats used in one season (e.g. breeding) are linked (or not) to those used in another season (e.g. winter).

Stable isotopes offer a means of obtaining a glimpse into the main events or conditions experienced in an organism's past. They can also help to identify the extent of migratory connectivity within a species. Stable isotopes provide body tissue records that can reveal the how effects of events or environmental conditions at various parts of the annual cycle influence behavioural strategies and, ultimately, reproductive fitness. In migratory species, such as the harlequin duck, these habitats and, thus, behavioural

contexts include wintering, breeding, migratory and stop over habitats. For example, studies of feather and blood isotopes have revealed that the earliest individuals to arrive on the breeding grounds came from the highest quality wintering grounds (Stuart Bearhop et al., 2004, p. 2004; Gill et al., 2001; Gunnarsson et al., 2005), were in better condition at stopover sites (Stuart Bearhop et al., 2004) and also when they arrived on the breeding grounds (Marra et al., 1998), and these early arrivals continued to use the highest quality habitat during the breeding season (Gunnarsson et al., 2005).

Stable isotopes are often used on tissues that regenerate continuously and quickly, such as blood and liver (Boecklen et al., 2011). This focus on quickly regenerating tissues can provide a indication of current dietary items, but turns over too quickly to be used for the identification of previous dietary items. In contrast, stable isotopes records in inert tissues, such as feathers and hair, provide a stable record that reflects the diet at the time of tissue formation, as the stable isotopes of inert tissues do not decay or turn over after they has been formed (with the exception of some hydrogen exchange with moisture in the air) (Hopkins III et al., 2013). Continuously growing tissues such as a claws and hair fall in between these two ends of the continuum. While the isotopic signature is inert once the hair or claw tissue is produced, these tissues continuously incorporate the current dietary signature, including any changes, as they grow. As such, many studies have been conducted with the intention of using claw stable isotope signatures in order to make inferences about pre-migration habitat, only to lament the rapid incorporation of isotopic signatures from the new, post-migratory diet (e.g., S. Bearhop et al., 2003; Hahn et al., 2014; Mazerolle & Hobson, 2005). Indeed, the results in Chapter 4 showed that harlequin duck claws from individuals caught during wing molt had begun to incorporate the new marine (winter) diet within 10-19 days of arrival. As such, studies looking to utilize claw isotopes as an inert record of dietary signature must account for this incorporation rate using isotope turnover models. Alternatively, claw samples would need to be taken within a very small window of time upon arrival, before isotopes from the diet in the new habitat become incorporated.

A potential application of isotope turnover models is to use isotope turnover curves to obtain a back-calculated estimate of pre-migratory habitat isotope signatures. This approach could mitigate the problem of the incorporation of new dietary isotopes into inert but continuously growing tissues by modelling isotopic turnover to account for the incorporation of new dietary isotopes directly. Here, I used population-level turnover

curves (see Chapter 4 for full details on this methodology) to obtain back-calculated estimates of the breeding ground isotope signatures of harlequin ducks caught after recently arriving on the wintering grounds. I then compared these back-calculated estimates to the signatures found in invertebrate prey collected from across the harlequin duck breeding range in southern British Columbia (BC) and Alberta (AB), Canada.

Harlequins disperse over a large geographic region during the breeding season and nest at low densities within watersheds. Freshwater invertebrates are the main food source for harlequin ducks during the breeding season and have been linked to harlequin duck breeding propensity, productivity, and abundance (J. C. Bond et al., 2008; LeBourdais, 2006; MacCallum et al., 2016). The isotopes of freshwater invertebrates during the breeding season are incorporated into harlequin blood and eggs (J. C. Bond et al., 2007). Given the importance of this food item and its incorporation into harlequin duck body tissues during the breeding season, invertebrate isotope signatures should provide a reasonable indication of the isotopic signatures that ducks are incorporating into their body tissues across various environmental gradients on the breeding grounds.

I collected harlequin duck blood cell and claw tip samples from recent arrivals returning to the wintering grounds. I used the isotope signatures recorded in these tissues to back-calculate estimates of each individual's at-equilibrium $\delta^{13}C$ and $\delta^{15}N$ signatures on the breeding grounds using the dual-tissue turnover model developed in Chapter 4. I then looked at how invertebrate isotopes varied with environmental variables of potential importance to harlequin ducks (Chapters 2 and 3) using samples collected from across the western harlequin duck breeding range to determine the feasibility of making inferences about harlequin duck breeding habitat usage, preferences, or migratory behaviour based on stable isotope signatures reflective of their breeding ground diets. I conclude by suggesting several research avenues and possible methodologies future researchers could use to continue to tackle this important problem.

## 5.2. Methods

As harlequins migrate from their inland freshwater breeding habitat to their marine wintering habitat, they experience a non-overlapping dietary shift in both $\delta^{13}C$

and $\delta^{15}N$ signatures (J. C. Bond et al., 2007) (Figure 5.1). This occurs because marine environments tend to be enriched in $\delta^{13}C$ and $\delta^{15}N$ relative to freshwater and terrestrial habitats. As soon as harlequin adult females arrive on the marine wintering grounds, undergoing this dietary shift, they also experience a complete flight feather molt, rendering them flightless for a period of 20-40 days (Palmer, 1976). Their feathers steadily grow over the next month (G. J. Robertson et al., 1997). I was able to use this steady regrowth of feathers to my advantage, as a measure of time since arrival on the wintering ground in my models (see Chapter 4 for full details).

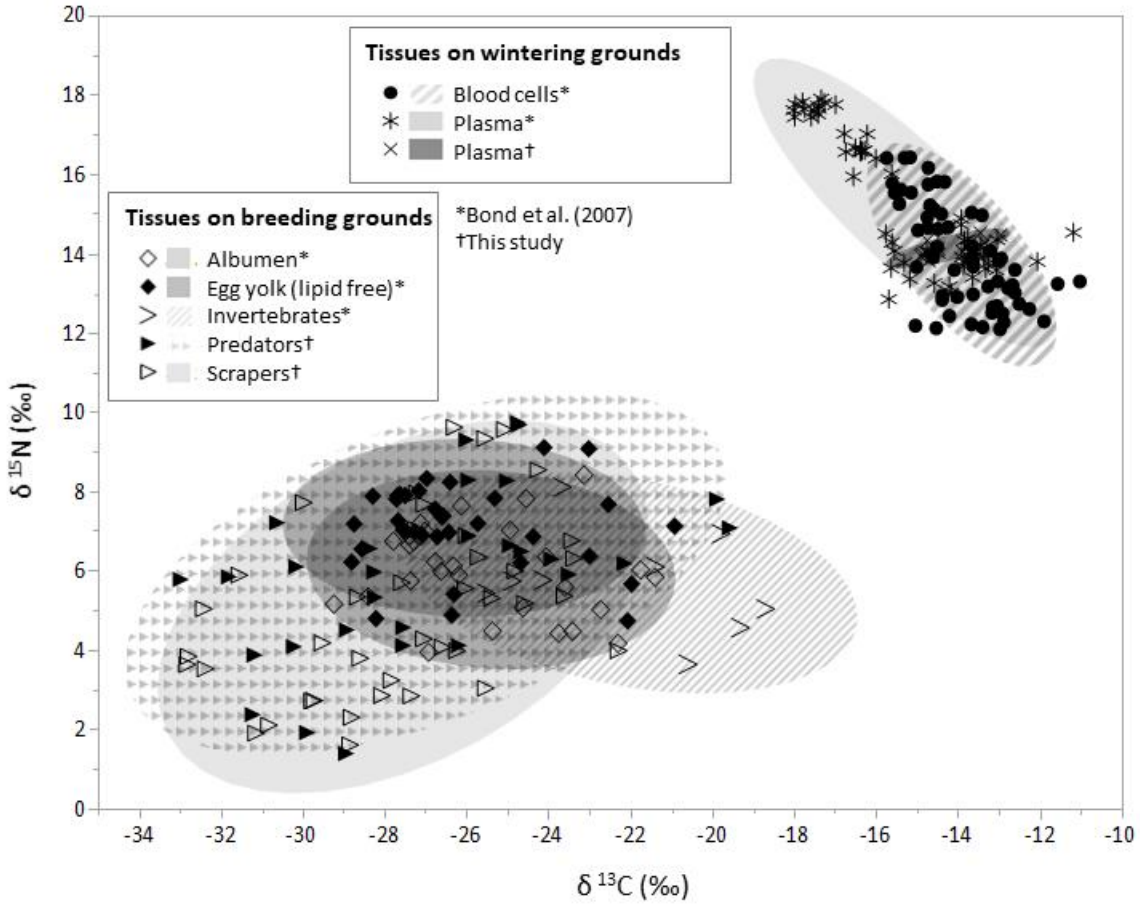


**Figure 5.1 Biplot of $\delta^{13}C$ and $\delta^{15}N$ signatures in various harlequin duck body tissues and prey items throughout the annual cycle.**

Notes: Samples from the breeding season are distinctly depleted in both isotopes (lower left quadrant) while wintering ground marine isotopes are distinctly enriched (top right quadrant). Invertebrate (food) $\delta^{15}N$ values have been increased by 3.5‰ in order to make them directly comparable to harlequin ducks, assuming ducks are one trophic level higher than their invertebrate prey.

### 5.2.1. Capture and sampling

Harlequin ducks were captured at five sites on Hornby Island, BC, in the Strait of Georgia from September 7-13, 2012. Ducks undergoing wing molt were captured by corralling them with kayaks and inflatable zodiacs into drive traps (Gregory J. Robertson et al., 1998), while flighted individuals were caught by either standard or floating mist-nets (Kaiser et al., 1995). I drew blood samples, clipped claw tips, and collected fecal samples from these individuals. See Chapter 4 for full details on capture, measurements, sampling, and storage methodology.

To obtain stable isotope signatures of harlequin duck dietary items on the breeding grounds, I took subsamples of the predatory and scraping invertebrates from the invertebrate samples I collected for Chapter 2. I selected at least one sample containing a predatory taxon and one sample containing a scraping taxon from each river section. See Chapter 2 for detailed methodology on site selection, field collection, and storage and identification of invertebrates.

Duck blood cell, plasma and fecal samples and invertebrate samples selected for stable isotope analysis were dried at 60°C for 48 h, then left to equilibrate with the ambient humidity for at least 24 h. Dried samples were then homogenized using a mortar and pestle. Duck claw and invertebrate samples were rinsed with a 2:1 chloroform:methanol solution to remove surface contaminants, and then dried in a fume hood for at least 24 hours. Claws were prepared whole for stable isotope analysis. Large invertebrates were homogenized using a mortar and pestle, while small invertebrates were prepared whole for stable isotope analysis. Occasionally, many invertebrates needed to be combined to achieve minimum target sample weights for stable isotope analysis.

Between 0.3-0.4 mg of homogenized plasma, 0.8-1.8 mg of homogenized blood cells, 1.1-2.2 mg of homogenized fecal samples, 0.2-2.2 mg of whole claw tip samples, and 0.2-2.1 mg of homogenized or whole invertebrate samples, respectively, were weighed into tin cups for stable carbon and nitrogen isotope analysis. Samples were then sent for dual $\delta^{13}C$ and $\delta^{15}N$ isotope analysis at either (1) the Stable Isotope Facility at the University of California (Davis, CA, USA), using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer

(Sercon Ltd., Cheshire, UK); or, (2) the Environmental Isotope Laboratory at the University of Waterloo (Waterloo, ON, Canada), using a 4010 Elemental Analyzer (Costech Instruments, Italy) coupled to a Delta Plus XL (Thermo-Finnigan, Germany) continuous flow isotope ratio mass spectrometer (CFIRMS).

### 5.2.2. Isotope turnover modeling

I modified the dual-tissue isotope turnover model developed in Chapter 4 to estimate the mean population level turnover rates for $\delta^{13}C$ and $\delta^{15}N$ in harlequin duck blood cells and claw tips. In Chapter 4, blood and claw turnover rates were estimated using the equations:

When $t < lag$:

$$N_{tb} = N_{new} + (N_{tc} - N_{new})e^{-k_b t} \quad (1)$$

When $t \geq lag$:

$$N_{tb} = N_{new} + (N_{tc} - N_{new})\frac{e^{-k_b t}}{e^{-k_c(t-lag)}} \quad (2)$$

Here, I further assumed that the proportion of $\delta^{13}C$ that has not yet turned over in claws ($e^{-kc(t-lag)}$) should be equal to the proportion of $\delta^{15}N$ in claws that has not yet turned over. This is due to the assumption that stable isotope turnover in claws is mainly related to physical growth rate, rather than a strict half-life related to exponential decay, such as that observed in blood or liver cells. As such, the proportion of both $\delta^{13}C$ and $\delta^{15}N$ that has not yet turned over should be equal to the proportion of "old" pre-migratory claw material vs. newly grown claw material. In other words, the proportion of claw material that has not yet turned over (prop(claw)) should be equal to:

$$prop(claw) = \frac{N_{tc}-N_{new}}{N_{tb}-N_{new}} \cdot e^{-k_{bN}t} = \frac{C_{tc}-C_{new}}{C_{tb}-C_{new}} \cdot e^{-k_{bC}t} \quad (3)$$

Rearranging equation 3 allows one to solve for either $N_{tb}$ or $C_{tb}$:

$$N_{tb} = \frac{(N_{tc}-N_{new})\cdot(C_{tb}-C_{new})}{(C_{tc}-C_{new})} \cdot \frac{e^{-k_{bN}t}}{e^{-k_{bC}t}} + N_{new} \quad (4)$$

$$C_{tb} = \frac{(C_{tc} - C_{new}) \cdot (N_{tb} - N_{new})}{(N_{tc} - N_{new})} \cdot \frac{e^{-k_{bC}t}}{e^{-k_{bN}t}} + C_{new} \quad (5)$$

Using equations 4 and 5 in a non-linear regression estimates the turnover rates for both blood-N ($k_{bN}$) and blood-C ($k_{bC}$) using all four isotope values ($\delta^{13}C$ and $\delta^{15}N$ in both blood and claws), without a need to account for any "lag" in claw isotope values. Allowing the non-linear regression to also adjust the time (t) allows a finer pinpointing of the time since dietary switch (i.e. time = 0).

Breeding ground (old) isotopes, were then back-calculated using only the current blood isotope values ($N_{tb}$) and the model estimates of blood turnover rate for $\delta^{13}C$ ($k_{bC}$) and $\delta^{15}N$ ($k_{bN}$), the new blood signature ($N_{new}$), as well as a slightly adjusted time since dietary switch:

$$N_{old} = \frac{N_{tb}}{e^{-k_{bN}t}} + N_{new} \quad (6)$$

$$C_{old} = \frac{C_{tb}}{e^{-k_{bC}t}} + C_{new} \quad (7)$$

### 5.2.3. Stable isotope signatures along environmental gradients

$\delta^{13}C$ and $\delta^{15}N$ are often correlated and can be affected by the same environmental variables. For these reasons, and due to the large number of environmental predictor variables, I used partial least squares to investigate how $\delta^{13}C$ and $\delta^{15}N$ in the invertebrate samples varied over the environmental variables identified as being potentially important to invertebrates in Chapter 2, and to harlequin ducks in Chapters 2 and 3. I was then able to identify which suites of environmental variables appeared to be important for harlequin duck breeding habitat selection and may also be recorded in the stable isotope signatures of their body tissues.

While partial least squares analysis was appropriate for this set of interrelated isotope signatures and environmental data, it was unable to account for the nested river within watershed (sub-drainage) study design. Therefore, as a complementary analysis, I also competed suites of environmental variables to determine which individual environmental variables, or combination thereof, best explained the observed variation in invertebrate $\delta^{13}C$ and $\delta^{15}N$ signatures. I did this using the same information theoretic framework and sets of biogeoclimatic hypotheses (terrain, climate, vegetation, human

disturbance, invertebrates) described in Chapter 2. I used linear mixed models to compete different suites of variables, while nesting the random effect of river reach within that of sub-drainage, crossed with the random effect of sampling year. Due to the high number of environmental variables, hypotheses were tested using a staged approach (e.g. English et al., 2017; Maccallum et al., 2016; Murray et al., 2015). Each hypothesis was tested by competing a suite of biogeoclimatic variables related to that hypothesis within an information theoretic framework, using AICc (Hurvich & Tsai, 1989). Prior to model selection, all independent variables were rescaled by subtracting the mean and dividing by two standard deviations (Gelman, 2008). Due to the high level of intercorrelation between many environmental variables, variable combinations were only included in the same model when they had a Pearson correlation coefficient of less than 0.6 ($\alpha = 0.05$). Variables from the most parsimonious, strongest supported model(s) were then competed using a final combined model. Models within $\Delta$AICc = 2 of the best supported model (lowest AICc value) were considered to have similar support. Smaller models that were nested inside larger models with similar support ($\Delta$AICc < 2) were considered most parsimonious; the additional term in the larger model was considered uninformative, as its inclusion makes no difference to AICc (Arnold, 2010; Burnham & Anderson, 2002).

Finally, I made inferences about the types of breeding ground habitats likely used by the individuals we captured, prior to their migration to the wintering grounds, by using their back-calculated breeding ground isotope signature estimates to infer the type of habitat used during the breeding season.

Statistical analyses were performed using JMP (version 13.1.0, SAS Institute Inc.) and R (version 3.5.1, the R Foundation for Statistical Computing).

## 5.3. Results

The non-linear regression estimated the rate of blood cell $\delta^{15}N$ turnover to be 0.0152 ± 0 ‰/mm and $\delta^{13}C$ turnover to be 0.0242 ± 0.003 ‰/mm (parameter estimates ± approximate standard error) (n = 65). The model estimated that $\delta^{15}N$ in tissues at equilibrium with the new (winter) diet ($N_{new}$) had a mean of 15.256 ± 0.53 ‰, while $C_{new}$ was estimated at a mean of -13.762 ± 0.31 ‰ (Figure 2). The model estimated that the true time of dietary switch (time = 0) occurred 14.948 ± 12.77 mm of feather growth

sooner than initially estimated. As such, 14.948 mm was added to all wing measurements. Thus, when back-calculating the estimates of $N_{old}$ and $C_{old}$ (breeding ground signatures), the resulting estimates would be lower than they would have otherwise been calculated, all other variables being held constant. However, the dual-tissue model also yielded relatively low estimates of blood $\delta^{13}C$ and $\delta^{15}N$ turnover relative to the single tissue model, which, as a result, tended to counteract the effects of additional time on the back-calculation to breeding ground isotope signatures. The single tissue models estimated $\delta^{15}N$ turnover at 0.0369 ‰/mm and $\delta^{13}C$ turnover at 0.0235 ‰/mm (see Chapter 4 for full details). Here, the model estimated the $\delta^{15}N$ turnover rate at less than half the rate estimated by the single tissue model. The model presented here was also the only model to yield a higher turnover rate for blood $\delta^{13}C$ than $\delta^{15}N$, which fits the data well given that blood $\delta^{15}N$ values (range = 6.5 – 14.2 ‰) spanned 80% of the full range of claw $\delta^{15}N$ values (4.7 – 13.6 %) while $\delta^{13}C$ blood values (range = -21.2 – -13.4‰) only spanned the top two-thirds of the claw $\delta^{13}C$ values (range = -29.4 – -13.9‰). As such, it appeared that blood $\delta^{13}C$ had turned over at a faster rate than blood $\delta^{15}N$, which agrees with the parameter estimates of the dual tissue model.

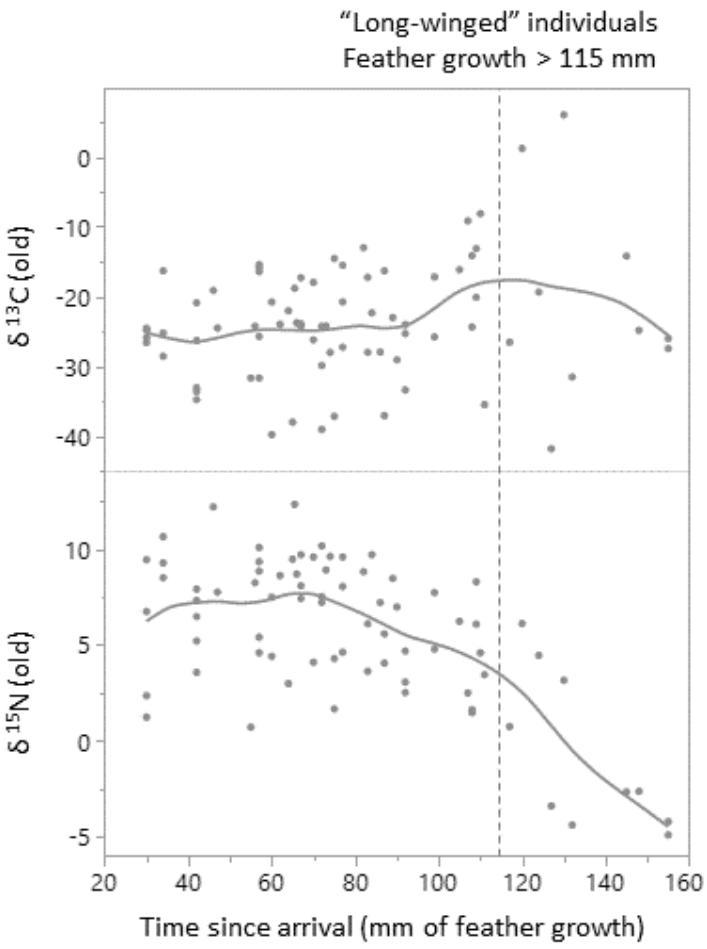


**Figure 5.2 Back-calculated estimates of $\delta^{13}C$ and $\delta^{15}N$ by time since arrival (measured as feather growth).**

Here, feather growth has had an additional 14.948 mm added to each measurement, as suggested by the dual-tissue turnover model.

Back-calculated estimates of $\delta^{13}C$ and $\delta^{15}N$ appeared to become more variable the longer individuals had been on the wintering grounds (Figure 5.2). Estimates of $\delta^{15}N$ signatures on the breeding grounds tended to decrease the longer an individual had been on the wintering grounds (Figure 5.2). This possibly suggests a bias in the model wherein the turnover rate of N has been overestimated, leading to underestimated $N_{old}$ values. However, this trend was eliminated when "long-winged" individuals, those with feather regrowth greater than 115 mm (n = 10), were removed. Additionally, forcing the model to take on a smaller value of $\delta^{15}N$ led to $N_{old}$ estimates that were higher than observed claw values, which is not realistic. Claws should provide a minimum starting estimate of $N_{old}$ for each individual, and most $N_{old}$ estimates should be lower than what

was measured in claws. Thus, it appeared that the $\delta^{15}N$ turnover rate provided a reasonable estimate, and that long-winged individuals are difficult to back-calculate for either isotope, due to the amount of time since dietary switch – measurement errors are amplified more back in time, plus differences (proportions of tissue remaining to turn over) are smaller, meaning measurement errors in differences between isotope ratios are amplified.

The back-calculated estimates of $N_{old}$ ranged from 0.725 – 12.35‰ when "long-winged" (feather growth greater than 115 mm) individuals were excluded (Figure 3). This range fit well with the range of $\delta^{15}N$ values found in invertebrate samples which, when corrected +3.5‰ to account for the higher trophic level of ducks, was estimated at $\delta^{15}N$ = 1.00 – 10.0‰. The $N_{old}$ estimates were all within the expected breeding ground estimates and rarely overlapped with observed winter ground isotope signatures (Figure 3). Even the long-winged individuals yielded reasonable estimates ($\delta^{15}N$ = -4.89 – 6.14‰), given their blood was almost completely turned over, making it difficult to reliably back-calculate to breeding ground signature. However, back-calculated estimates from long-winged individuals were removed from interpretations of breeding ground habitat use, given their tendency to fluctuate with small changes to parameter estimates.

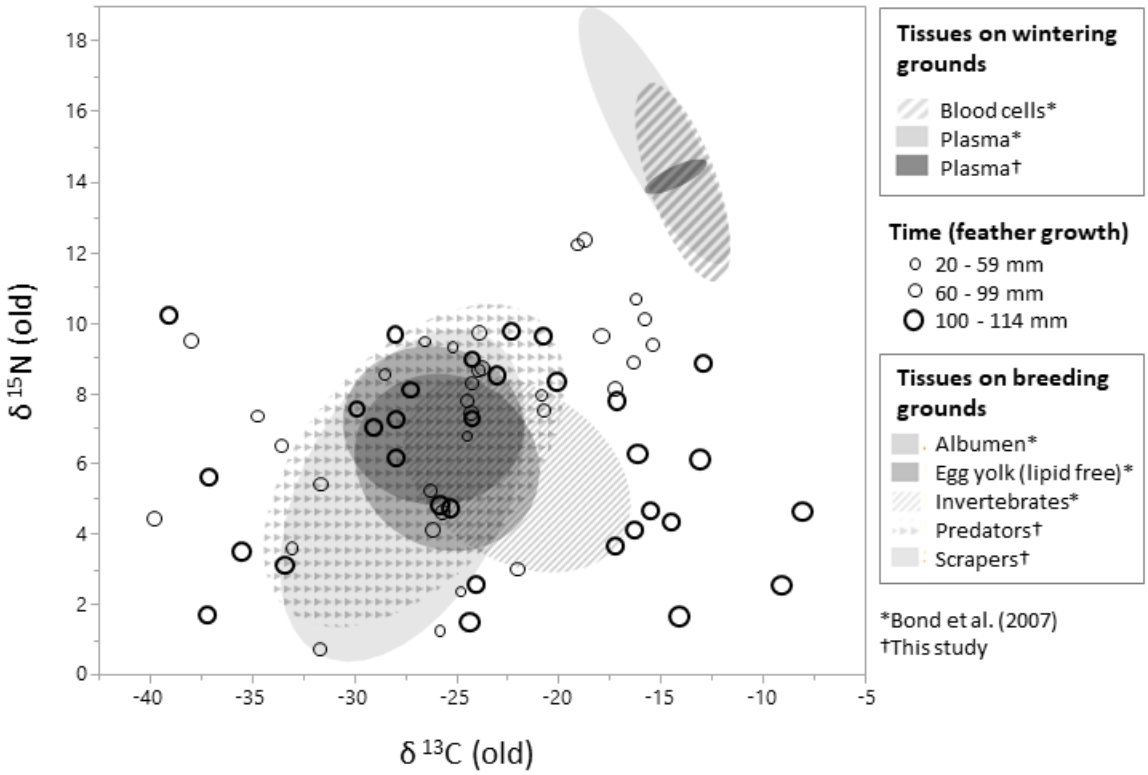


**Figure 5.3 Back-calculated estimates of $\delta^{13}C$ and $\delta^{15}N$ signatures in duck blood cells at equilibrium with the pre-migratory, breeding ground habitat (n = 65).**

Notes: Only estimates from "short-winged" individuals (feather growth < 115 mm) are shown here. Shading indicates the expected marine and breeding ground signatures based on a previous study of isotopes in harlequin duck body tissues and stream invertebrates (J. C. Bond et al., 2007), along with the invertebrate isotopes I collected during harlequin duck breeding stream surveys.

With long-winged individuals excluded, the back-calculated $\delta^{13}C$ breeding ground estimates showed a larger range than the back-calculated $\delta^{15}N$ estimates and, unlike the $\delta^{15}N$ estimates, exceeded the observed ranges of both breeding and wintering ground isotope signatures (Figure 3). Breeding ground $\delta^{13}C$ estimates ranged from -39.7 – -8.0‰ which was greater than expected, given the range of isotope values observed in invertebrates and duck body tissues on the breeding grounds (-33‰ to -18‰; Figure 5.2).

## 5.3.1. Isotopic signatures over environmental gradients

Partial least squares analysis was conducted to investigate the effects of environmental variables of interest from Chapters 2 and 3 on $\delta^{13}C$ and $\delta^{15}N$ isotope

signatures in freshwater invertebrates from across the western harlequin ducks breeding range (Figure 5.4). The partial least squares analysis reduced the environmental data to 7 factors (Figure 5.4). These factors were similar to some of the seven environmental factors identified for a larger dataset that included this partial dataset, in Chapter 2, but did not consider $\delta^{13}C$ and $\delta^{15}N$ at the same time.

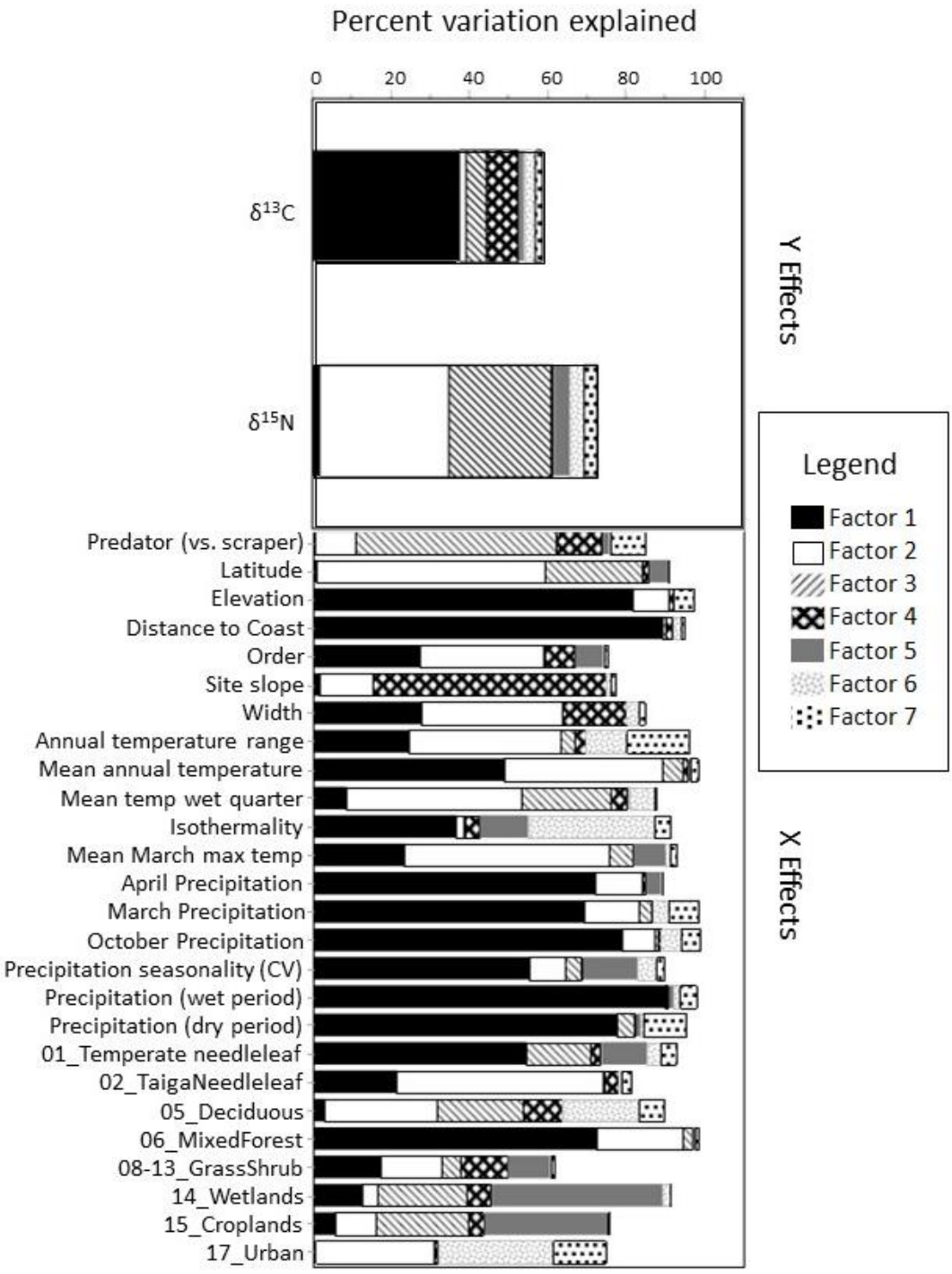


**Figure 5.4** **Percent of variation explained by the seven latent factors identified by partial least squares analysis as underlying variation in Y effects: $\delta^{13}C$ and $\delta^{15}N$ (top panel); and, in X effects: terrain, climate, vegetation, and land use (bottom panel).**

| Coefficient | $\delta^{13}C$ | $\delta^{15}N$ |
|---|---|---|
| Intercept | 0.0000 | 0.0000 |
| Predator (vs. scraper) | 0.2385 | 0.4682 |
| Latitude | 0.0812 | -0.0408 |
| Elevation | 0.1521 | 0.2479 |
| Distance to Coast | -0.1767 | -0.0944 |
| Order | 0.1035 | 0.3538 |
| Site slope | 0.2494 | -0.0064 |
| Width | 0.1813 | 0.3482 |
| Annual temperature range | -0.1439 | -0.0402 |
| Mean annual temperature | -0.1384 | -0.1916 |
| Mean temp wet quarter | -0.1695 | -0.2230 |
| Isothermality | -0.2854 | -0.1893 |
| Mean March max temp | -0.1968 | -0.1816 |
| April Precipitation | -0.0381 | -0.0510 |
| March Precipitation | 0.1406 | 0.1100 |
| October Precipitation | 0.1719 | 0.1519 |
| Precipitation seasonality (CV) | 0.0665 | -0.1033 |
| Precipitation (wet period) | 0.1768 | 0.1597 |
| Precipitation (dry period) | 0.2094 | 0.4298 |
| 01_Temperate needleleaf | 0.0335 | -0.0779 |
| 02_TaigaNeedleleaf | 0.1329 | 0.2102 |
| 05_Deciduous | 0.0293 | 0.1397 |
| 06_MixedForest | 0.1611 | 0.1671 |
| 08-13_GrassShrub | -0.1085 | -0.2110 |
| 14_Wetlands | -0.0217 | -0.1155 |
| 15_Croplands | 0.0689 | 0.0157 |
| 17_Urban | 0.2098 | 0.1787 |

**Figure 5.5 Coefficient estimates from a partial squares analysis of the effect of environmental factors on $\delta^{13}C$ and $\delta^{15}N$ in invertebrates sampled from across the harlequin duck breeding range.**

While partial least squares analysis was appropriate for this interrelated dataset, it was unable to account for the nested river within watershed (sub-drainage) study design. Therefore, as a complementary analysis, I also competed suites of environmental variables to determine which individual environmental variables, or combination of individual environmental variables, best explained the observed variation in invertebrate $\delta^{13}C$ (Table 5.1) and $\delta^{15}N$ (Table 5.2) signatures. I competed these suites of environmental variables using the same information theoretic framework and sets of biogeoclimatic hypotheses (terrain, climate, vegetation, human disturbance,

invertebrates) described in Chapter 2. I used linear mixed models to compete different suites of variables, while nesting the random effect of river reach within that of sub-drainage, crossed with the random effect of sampling year. The results of this analysis were used to further confirm the habitat-isotope associations, as most of the effects of the individual environmental variables on $\delta^{13}C$ and $\delta^{15}N$ in the partial least squares analysis were in a direction consistent with that predicted by their underlying associations with other environmental variables.

**Table 5.1 Linear mixed models of environmental variables affecting $\delta^{13}C$ in invertebrate prey found in potential harlequin duck breeding habitat.**

| Model | K | logLik | AICc | ΔAICc | wt |
|---|---|---|---|---|---|
| Climate (models = 67) | | | | | |
| $\delta^{13}C$ ~ Mean November Precipitation + | 9 | -133.7 | 288.5 | 0 | 0.327 |
| $\delta^{13}C$ ~ Precipitation during the cold quarter + FFG | 9 | -133.9 | 288.9 | 0.43 | 0.264 |
| $\delta^{13}C$ ~ Mean October Precipitation | 9 | -134.1 | 289.4 | 0.89 | 0.209 |
| $\delta^{13}C$ ~ Mean November Precipitation | 9 | -134.2 | 289.5 | 0.98 | 0.200 |
| (Null) Functional Feeding Group (FFG) + DegreeDays | 7 | -145.0 | 305.9 | 17.36 | 0 |
| Terrain and physical attributes (models = 17) | | | | | |
| $\delta^{13}C$ ~ Distance to Coast | 9 | -135.0 | 291.1 | 0 | 0.999 |
| (Null) FFG + DegreeDays | 7 | -145.0 | 305.9 | 14.73 | 0.001 |
| Land Cover (models = 70) | | | | | |
| $\delta^{13}C$ ~ PercentMixedForest | 9 | -133.5 | 288.1 | 0 | 1 |
| (Null) FFG + DegreeDays | 7 | -145.0 | 305.9 | 17.76 | 0 |
| Human Disturbance (models = 10) | | | | | |
| (Null) FFG + DegreeDays | 7 | -145.0 | 305.9 | 0 | 1 |
| Invertebrates (food) (models = 151) | | | | | |
| $\delta^{13}C$ ~ sqrt(SmallPupaCount) + sqrt(ScraperBiomass) | 9 | -140.6 | 302.4 | 0 | 0.850 |
| (Null) FFG + DegreeDays | 7 | -145.0 | 305.9 | 3.48 | 0.150 |
| Final combined models (models = 48) | | | | | |
| $\delta^{13}C$ ~ Mean October Precipitation + sqrt(ScraperBiomass) | 9 | -139.4 | 299.9 | 0 | 0.247 |
| $\delta^{13}C$ ~ Mean December Precipitation + sqrt(ScraperBiomass) | 9 | -139.5 | 300.3 | 0.36 | 0.206 |
| $\delta^{13}C$ ~ Distance to Coast | 8 | -141.2 | 300.9 | 1.04 | 0.147 |
| $\delta^{13}C$ ~ Distance to Coast + Fish | 9 | -139.9 | 301.1 | 1.16 | 0.138 |
| $\delta^{13}C$ ~ Precipitation during cold quarter + sqrt(ScraperBiomass) | 9 | -140.2 | 301.6 | 1.74 | 0.103 |
| (Null) FFG + DegreeDays | 7 | -145.0 | 305.9 | 5.98 | 0.012 |

Only models with strong support (ΔAICc < 2) are shown, plus the null model for comparison.
Control variables for each response variable are only listed next to the null model for simplicity, but were included in all models.
Smaller models nested within larger models with similar support (ΔAICc < 2) were considered to be most parsimonious; thus, models containing additional, non-explanatory variables are not shown.
Functional feeding group (FFG) was either predator or scraper.

**Table 5.2 Linear mixed models of environmental variables affecting $\delta^{15}N$ in invertebrate prey found in potential harlequin duck breeding habitat.**

| Model | K | logLik | AICc | ΔAICc | wt |
|---|---|---|---|---|---|
| Climate (models = 67) | | | | | |
| $\delta^{15}N$ ~ Total growing season precipitation | 9 | -106.3 | 233.9 | 0 | 0.299 |
| $\delta^{15}N$ ~ October precipitation | 9 | -106.5 | 234.2 | 0.37 | 0.248 |
| (Null) Functional feeding group (FFG) + PercentGrowSeason + PercentGrowSeason$^2$ | 8 | -109.1 | 236.7 | 2.87 | 0.071 |
| Terrain and physical attributes (models = 17) | | | | | |
| $\delta^{15}N$ ~ Order | 9 | -106.4 | 234 | 0 | 0.481 |
| $\delta^{15}N$ ~ Width | 9 | -106.6 | 234.4 | 0.4 | 0.394 |
| (Null) FFG + PercentGrowSeason + PercentGrowSeason$^2$ | 8 | -109.1 | 236.7 | 2.7 | 0.125 |
| Land Cover (models = 70) | | | | | |
| (Null) FFG + PercentGrowSeason + PercentGrowSeason$^2$ | 8 | -109 | 236.7 | 0 | 1 |
| Human Disturbance (models = 10) | | | | | |
| (Null) FFG + PercentGrowSeason + PercentGrowSeason$^2$ | 8 | -109 | 236.7 | 0 | 1 |
| Invertebrates (food) (models = 151) | | | | | |
| $\delta^{15}N$ ~ Small filterer-collector abundance | 6 | -109.8 | 233 | 0 | 0.984 |
| (Null) FFG + PercentGrowSeason + PercentGrowSeason$^2$ | 5 | -115.1 | 241.2 | 8.27 | 0.016 |
| Final combined models (models = 48) | | | | | |
| $\delta^{15}N$ ~ Small filterer-collector abundance | 9 | -133.7 | 288.5 | 0 | 0.327 |
| $\delta^{15}N$ ~ Total growing season precipitation | 9 | -133.9 | 288.9 | 0.43 | 0.264 |
| $\delta^{15}N$ ~ Order | 9 | -133.9 | 288.9 | 0.43 | 0.264 |
| $\delta^{15}N$ ~ October precipitation | 9 | -134.1 | 289.4 | 0.89 | 0.209 |
| $\delta^{15}N$ ~ Width | 9 | -134.2 | 289.5 | 0.98 | 0.200 |
| (Null) FFG + PercentGrowSeason + PercentGrowSeason$^2$ | 7 | -145.0 | 305.9 | 17.36 | 0 |

Only models with strong support (ΔAICc < 2) are shown, plus the null model for comparison.
Control variables for each response variable are only listed next to the null model for simplicity, but were included in all models.
Smaller models nested within larger models with similar support (ΔAICc < 2) were considered to be most parsimonious; thus, models containing additional, non-explanatory variables are not shown.
Functional feeding group (FFG) was either predator or scraper.

Variation in invertebrate $\delta^{13}C$ signatures were mostly associated with variation in Factor 1 (Figure 5.4), a suite of variables related to the way in which some aspects of precipitation and associated vegetation (particularly temperate needleleaf and mixed forest) change with elevation and distance to coast. Similarly, $\delta^{13}C$ was negatively associated with distance to coast and positively associated with some precipitation variables (Figure 5.6).

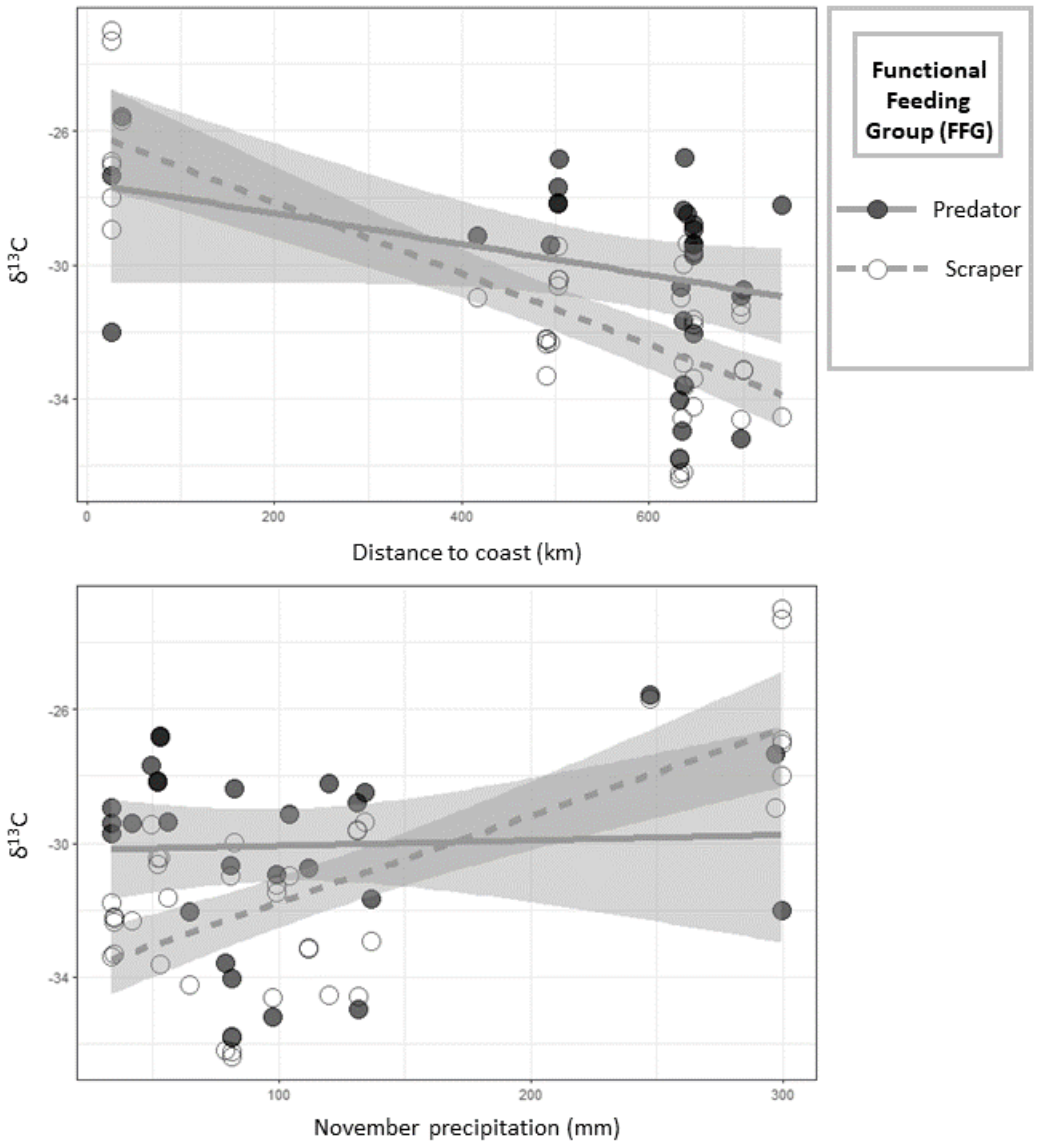


**Figure 5.6 Effects of (1) distance to coast and (2) November precipitation on $\delta^{13}C$ signatures in freshwater invertebrates found in potential harlequin duck breeding habitat.**

The variation in invertebrate $\delta^{15}N$ signatures mostly determined by river order or width (size) with larger rivers bearing invertebrates with elevated $\delta^{15}N$ signatures (Figure 5.7). $\delta^{15}N$ was affected by environmental variables relating to Factors 2 and 3 and signatures were elevated in high latitude sites, such as Jasper National Park, where large order rivers with warm wet periods flow through a relatively high proportion of taiga needleleaf habitat and low amounts of deciduous and mixed forests. In this way, Factor 2 is quite similar to the environmental variable identified in the factor analysis in Chapter 2. Factor 3 related to low latitude sites with lots of grass, shrubs, wetlands and croplands and were associated with smaller rivers and lower $\delta^{15}N$. All of these diverse habitat types had one common set of variables: river size. $\delta^{15}N$ signatures increased with river order and width (size) and decreased with site slope. The latter is consistent with the first two variables; sites with steep slopes are usually higher up in watersheds, where river order and width are lower. The staged analysis also identified order and width, individually, as explaining the most $\delta^{15}N$ variation of the independent variables examined, along with two precipitation variables, and the abundance of small filterer-collectors (Table 5.2). Order and width are therefore considered to be equivalent in their explanatory power and were also consistently identified as being important factors in determining invertebrate size and abundance (Chapter 2), harlequin duck abundance (Chapters 2 and 3) and now $\delta^{15}N$ as well.

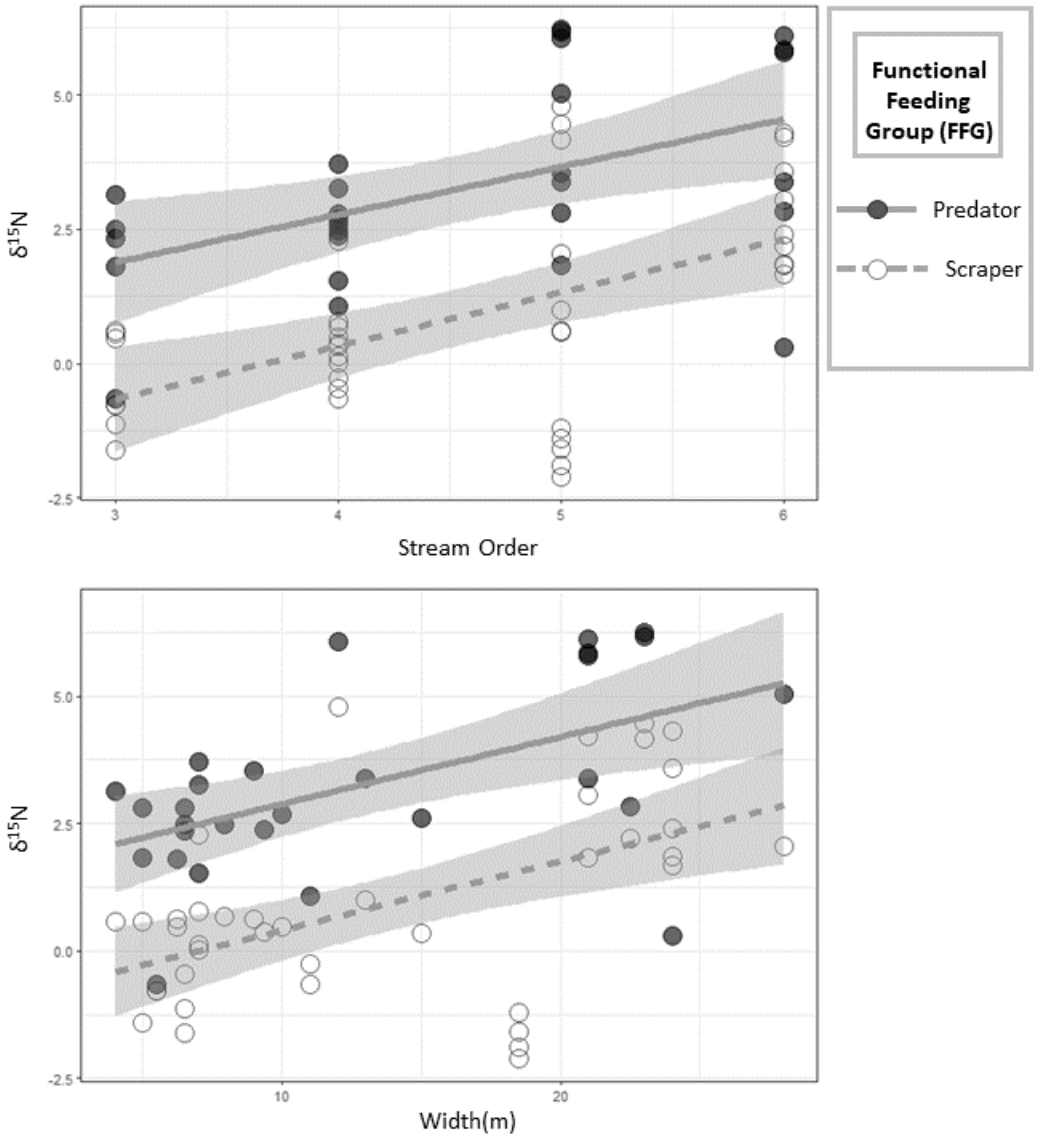


**Figure 5.7 Effects of (1) river order and (2) width on $\delta^{15}N$ signatures in freshwater invertebrates found in potential harlequin duck breeding habitat.**

Back-calculated estimates of $\delta^{13}C$ and $\delta^{15}N$ on the breeding grounds were not significantly related to duck body mass, size (tarsus and culmen lengths), or body condition (residuals of a regression of mass onto tarsal length).

## 5.4. Discussion

Harlequin ducks are notoriously hard to study during the breeding season due to their propensity to disperse over a large geographic region and nest at low densities

within watersheds. In this final data chapter, I show a possible way to tackle this difficult problem to gain insights into breeding habitat usage. Specifically, here I combined key findings from my other data chapters and, using stable isotope turnover models, was able to make inferences about harlequin duck breeding habitat usage. This approach was made possible because of three key factors: first, harlequin ducks main change what they are foraging on between the breeding season and the winter, resulting in a non-overlapping dietary shift in both $\delta^{13}C$ and $\delta^{15}N$ signatures. Second, because females complete a full flight feather molt as soon as they arrive on the wintering grounds, I was able to both capture the ducks and estimate how much time had passed since they had arrived. This gave me a time stamp to use in my models for back-calculations. Third, harlequin duck isotope signatures vary depending on the type of tissue and the amount of time they have been consuming the new diet. Blood cells regenerate quickly and continuously, such that blood cell isotopic signatures reflect the duck's current diet within a few days to weeks. Claws grow continuously, such that while the isotopic signature is inert once the claw tissue is produced, as it grows the tissue continuously incorporates the current dietary signature. Here I utilized these differences to obtain estimates of pre-migratory, breeding habitat isotope signatures. I was then able to use the dual-tissue model to back-calculate isotope signature estimates in the ducks' breeding ground body tissues. I then looked at how invertebrate isotopes varied with environmental variables of potential importance to harlequin ducks (Chapters 2 and 3) using samples collected from across the western harlequin duck breeding range. Below I discuss the feasibility of making inferences about harlequin duck breeding habitat usage, preferences, or migratory behaviour based on stable isotope signatures reflective of their breeding ground diets. I conclude by suggesting several research avenues and possible methodologies future researchers could use to continue to tackle this important problem.

There appeared to be some potential for stable isotope signatures to reveal ecologically important habitat associations in breeding harlequin ducks. Some of the key trends found in the partial least squares analysis mirror the latent variables underlying variation in the interrelated environmental variables in the breeding habitat survey analysis in Chapter 2. Both $\delta^{13}C$ and $\delta^{15}N$ invertebrate signatures tended to be affected by "clumps" of environmental variables, usually in a direction consistent with the inherent associations within those environmental variables themselves. For example, in Chapter 2, the latent environmental variable that explained the most overall variation in the

measured environmental variables was associated with wet coastal habitats on one end and higher elevation, drier habitats in the continental interior at the other. Similarly, $\delta^{13}C$ tended to be positively associated with wet, coastal habitats, and decreased as sites moved further inland. Inland sites tended to be higher elevation than coastal sites, confounding the effects of elevation and distance to coast. However, elevation was not identified as a strongly explanatory variable in the staged analysis of how $\delta^{13}C$ and $\delta^{15}N$ change over environmental gradients (Table 5.1 and Table 5.2). As such, $\delta^{13}C$ signatures appeared more driven by the distance from marine inputs than the associated change in elevation. Previous studies have found $\delta^{13}C$ in freshwater invertebrates to be associated with marine inputs. For example, streams with higher salmon spawning densities have enriched $\delta^{13}C$ and $\delta^{15}N$ signatures (Bilby et al., 1996; Hicks et al., 2005). Temperate coastal streams had warmer climates, which is also associated with $\delta^{13}C$ and $\delta^{15}N$ enrichment (Allan & Castillo, 2007; Finlay, 2001; Friberg et al., 2009). Harlequins also appear to pay some attention to this latent environmental factor; in Chapter 2, harlequin counts were higher on the coast than on equivalently sized rivers at higher elevation sites in the interior. Similarly, the MaxEnt analysis in Chapter 3 found habitat suitability decreased for harlequin ducks with increasing distance from the coast (wintering habitat) in publicly available sightings. However, the invertebrate isotopes examined here were obtained from a limited number of sampling sites that were mainly coastal, or far inland. It would be important to obtain isotope signatures from sites all along at a variety of distances from the coast to determine whether this pattern persists all along the gradient of coastal to inland habitat or is instead being driven mainly by a few coastal sites with elevated $\delta^{13}C$ signatures (Figure 5.6).

$\delta^{15}N$ was most associated with river order or width, which has repeatedly been found to be a variable of interest for harlequin duck breeding habitat site selection (e.g. J. Bond et al., 2007; Crowley, 1993; MacCallum et al., 2016). $\delta^{15}N$ tended to increase with stream size, which could be due to greater invertebrate species diversity in larger rivers leading to longer food chains and increased numbers of trophic levels. Since $\delta^{15}N$ increases with each trophic level, larger rivers with more trophic levels could have higher mean $\delta^{15}N$ signatures within their invertebrate communities (e.g. Harding et al., 2014; McHugh et al., 2010; Thompson & Townsend, 2005). $\delta^{15}N$ increase with stream size could also be due to increased proportions of higher trophic level organisms, such as a higher proportion of predators, in larger rivers. However, the only invertebrate

community association that I observed was a tendency for $\delta^{15}N$ to increase with the abundance of small filtering-collectors. Rather than representing a trophic enrichment per se, these were most likely chironomid larvae, which tend to be fairly tolerant to disturbance, warm waters and associated low oxygen levels. As such, higher $\delta^{15}N$ where increased counts of filterer-collectors were observed could be due to an association between open canopy, large, warm rivers (with associated longer food chains) and chironomid larvae. A linear regression revealed that filterer-collector abundance increased with river width and order, consistent with abundance of filterer-collectors being a signal of river size, rather than a mechanism contributing directly to $\delta^{15}N$ enrichment.

A few other factors affected $\delta^{13}C$ and $\delta^{15}N$ as well. Urban buildup, which includes highways and roads, in addition to cities, was found to have a positive effect on both $\delta^{13}C$ and $\delta^{15}N$, but only in the partial least squares analysis, and not in the staged analysis. Croplands were only found to have a small positive association with $\delta^{13}C$ and no association with $\delta^{15}N$. Often, point-source pollution in urban areas, including sewage, and agricultural fertilizers in rural areas will increase $\delta^{15}N$ signatures. However, many of the sites I sampled were in relatively remote and/or high elevation areas, where both buildup and agriculture can be present, but not at the intensities experienced at lower elevations or in large urban areas. Most of these sites were near provincial or federal parks and some were only accessible by foot. Even the most urban sites, such as those along the Salmo river, were only surrounded by small towns.

$\delta^{13}C$ appears to provide a signal of distance to coast while $\delta^{15}N$ appears to provide an estimate of river size. Both of these habitat characteristics also appear to be of potential importance for harlequin duck breeding habitat suitability. Therefore, it may be possible to make some broad inferences regarding breeding habitat usage using $\delta^{13}C$ and $\delta^{15}N$ signatures in harlequin duck claws and blood. However, none of the measures of duck size or condition (mass, tarsus and culmen length) were significantly related to either $\delta^{13}C$ nor $\delta^{15}N$ breeding ground signatures. This finding is not surprising given harlequin ducks, particularly females, display a relatively high degree of natal philopatry and many pairs reuse the same reaches and even feeding sites, year after year (Gregory James Robertson & Goudie, 1999). Harlequins are relatively k-selected for a duck (Goudie et al., 1994), live long, slow lives, often mate for life, take several years to reach breeding maturity, and often defer breeding in unfavourable years (Gregory James

Robertson & Goudie, 1999). Therefore, it may be oversimplistic to expect that a snapshot measurement of body size or mass would also be related to a long-term breeding habitat association based partly on natal origin and sex, but also dependent upon social interactions, pair-bonding, and natal origin of the selected mate. However, it may also have been reasonable to expect individuals migrating from less preferred habitat to also be in poorer condition as a result, or for individuals migrating from preferred habitats to arrive earlier.

Back-calculated estimates of $\delta^{15}N$ tended to decrease with time since arrival (Figure 5.2). This possibly suggests a bias in the turnover model where the turnover rate of N has been overestimated, leading to back-calculated estimates that are lower than they should be. However, this trend was eliminated when “long-winged” individuals, those with feather regrowth greater than 115 mm (n = 10), were removed. Thus, while it is possible that the $\delta^{15}N$ turnover rate was overestimated, the estimates were obtained from long-winged individuals, whose differences between blood and claw isotopes were relatively small. As a result, the back-calculations are more susceptible to measurement error than in individuals whose tissues are just starting to turn over and whose isotope signatures need not be projected as far back in time.

### 5.4.1. Future Studies

The trend for $\delta^{13}C$ to decrease with distance to coast makes sense given the influence of marine inputs on stream isotope signatures. Use of this pattern could yield interesting insights into migratory costs and associated behaviours in the breeding and wintering seasons. For example, since harlequin ducks are thought to migrate very quickly from coast to interior, often over land and sometimes even non-stop, $\delta^{13}C$ could provide an indirect measure (distance from coast to breeding habitat) of the relative costs of migration for each individual.

Future studies could benefit greatly from the inclusion of isotope maps for terrestrial and aquatic systems. Such isotope maps could then serve as environmental layers in algorithms such as MaxEnt (see Chapter 3) to compare the observed distribution of isotope values across animals migrating from a range of habitats (here, using back-calculation of isotopes in claws and blood) to the expected distributions based on availability of habitat. In this case, availability of habitat would be described in

terms of stable isotope gradients over geographic space rather than environmental measurements such as terrain and climate. Further, rather than using publicly available sightings in geographic space, the “presence” points would be pairs or groups of stable isotope signatures, for example, $^{13}C$, $^{15}N$, $^{2}H$, $^{18}O$, $^{34}S$, as may be most biologically relevant for the species of interest. The isotope map would serve as the model’s prior, with uniform distribution expected across isotope space should habitat selection occur without association to isotope signatures or, rather, preferences for certain habitat characteristics which also happen to be associated with a predictable stable isotope signature pattern. Deviations from this expectation would then reveal habitat preferences or, at least, behavioural associations with environmental factors or habitat types, which could inform studies of migration ecology, food web interactions, energy storage and usage, population structure, and identification of critical habitat for species of conservation concern.

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