

Variation in isotopic niche, digestive tract morphology, and mercury concentrations in two sympatric waterfowl species wintering in Atlantic Canada

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Abstract

Sympatric communities of organisms may exploit different ecological niches to avoid intra- and interspecific competition. We examined the isotopic niches of American black ducks (Anas rubripes) and mallards (A. platyrhynchos) wintering in coastal and urban areas of Atlantic Canada and compared isotopic niche with digestive tract morphologies and blood mercury (Hg) concentrations. Isotopic niche width (for δ^{13} C and δ^{15} N) varied between the three groups of ducks studied, with coastally foraging black ducks exhibiting the widest isotopic niche, followed by coastal mallards, while urban feeding black ducks had a narrow isotopic niche. These niche differences had physical and chemical consequences: coastal black ducks had longer digestive tracts, a larger range in gizzard sizes, and higher and more variable Hg concentrations than urban black ducks and coastal mallards. This plasticity in ecological niche may reduce competition among and within species, and subsequently explain why winter numbers of black ducks and mallards have increased in Atlantic Canada.

Key words: winter ecology, American black duck, Anas rubripes, Mallard, Anas platyrhynchos

Introduction

The concept of sympatric communities or populations exploiting different ecological niches to reduce competition is well-studied (MacArthur 1958; Toft 1980; Webb et al. 2002). Ecological niches are often quantified using stable isotope analysis (typically of δ^{13} C and δ^{15} N), as stable isotopes can describe the biotic and abiotic characteristics of an organism's environment such as diet, latitude, altitude, or its foraging habitat (i.e., marine or terrestrial; reviewed by Newsome et al. 2007; Hobson 2011). The resulting isotopic niche can be used to quantify differences in ecological niches within or among species (Newsome et al. 2007; Cloyed and Eason 2017; Pratte et al. 2017). Varying isotopic niches have been related to intra- and interspecific differences in traits such as in morphology (e.g., Cloyed and Eason 2017) or foraging behaviour (e.g., Pratte et al. 2017) as species exploit their differing ecological niches.

In birds, isotopes are often used as a proxy to study diet (e.g., Hobson and Clark 1992; Hobson 2011; English et al. 2017), and birds with diverse diets generally occupy wide isotopic niches

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(Eulaers et al. 2013; Pratte et al. 2017). In waterfowl, variation in diet is correlated with intra- and interspecific differences in digestive tract morphology (e.g., Miller 1975; Karasov and McWilliams 2005; Jónsson and Afton 2017). Longer guts are typically found in waterfowl with taxonomically diverse diets (Kehoe and Ankney 1985) or with high-energy or high-protein diets (van Gils et al. 2008; Olsen et al. 2011). Long guts increase food retention time in the gut to allow for a longer period of digestion and provide more surface area for absorption (van Gils et al. 2008; Olsen et al. 2011).

Since long guts increase absorption time, they may increase the opportunity to absorb contaminants from the diet (Kleinow and James 2001). In birds, contaminants are principally acquired through diet and thus are bioaccumulated from prey; birds that feed at high trophic levels generally have greater concentrations of contamination (Wolfe et al. 1998; Eulaers et al. 2013; Sebastiano et al. 2017). Mercury (Hg) has long been studied because of its known toxicity on birds and its prevalence in the environment from natural and anthropogenic sources, and because it biomagnifies in food webs (Wolfe et al. 1998; Whitney and Cristol 2018). Consequently, the ecological niche occupied by a bird species should strongly influence the contaminants they acquire (e.g., Colas et al. 2014), so measuring isotopic niche width may be a method to explain intra- and interspecific differences in Hg concentrations, especially for species at similar trophic levels. However, to the best of our knowledge, this interaction has not been explicitly studied in birds.

American black ducks, *Anas rubripes* (Brewster, 1902; hereafter black ducks) and mallards (*Anas platyrhynchos* (Linnaeus, 1758)) provide an interesting model to study the concept of isotopic niche affecting intra- and interspecific traits and competition. Both species are hunted, and harvest regulations are set under an Adaptive Harvest Management (AHM) framework that considers the competitive exclusion hypothesis, stating that black duck population growth is limited by competition for habitat by mallards (USFWS 2018). While mallards and black ducks do occupy similar habitats in eastern North America, there are studies that support (Ankney et al. 1987; Petrie et al. 2012) and reject (Morton 1998; Maisonneuve et al. 2006) the competitive exclusion hypothesis. More research on the competitive exclusion hypothesis is needed so it can be better incorporated into the black duck AHM framework, and understanding the winter ecology of black ducks is a priority identified by the Black Duck Joint Venture (Black Duck Joint Venture Management Board 2014).

Atlantic Canada is the northern limit of the black duck wintering range, an area where wintering black ducks and breeding mallards have increased over the last four decades (Robertson et al. 2017; Sauer et al. 2017). Black ducks wintering in Atlantic Canada exploit different ecological niches, ranging from coastal saltmarshes to urban areas and parks (McCorquodale and Knapton 2003; English et al. 2017). The diets of coastal and urban black ducks are different with coastal black ducks consuming a variety of animal (mainly marine invertebrates) and plant matter, whereas urban black ducks mostly consume anthropogenic subsidies (e.g., corn and bread; English et al. 2017). Sympatrically wintering mallards in coastal areas have diets consisting mostly of seeds and other vegetation and do not heavily forage on marine invertebrates (Drilling et al. 2002; English et al. 2017).

In this paper we extend the previous results of English et al. (2017) and specifically examine the isotopic niches of black ducks and mallards wintering in coastal and urban areas of Atlantic Canada. Further, we relate isotopic signatures and breadth of isotopic niches, as determined by δ^{13} C and δ^{15} N, to differences in digestive tract morphologies and blood mercury (Hg) concentrations. We chose Hg as a biomarker because of its known prevalence in prey of black ducks in the Bay of Fundy (English et al. 2015, 2017), and because only limited data on Hg concentrations in waterfowl have been reported in Atlantic Canada (see Braune and Malone 2006; Mallory et al. 2018). Hg bioaccumulation tends to be positively correlated with trophic level, notably in species that forage on invertebrate prey compared to plants (Wolfe et al. 1998). Consequently, in conjunction with isotopes its use may allow corroborating or enhanced ability to distinguish dietary or ecological patterns between



sympatric groups of birds. We expected the diverse diets of coastal black ducks would result in a wider isotopic niche, increased gut lengths, and higher blood Hg concentrations than urban black ducks. We did not manage to study urban mallards, but we expected the coastal mallards in our study to have values between coastal black ducks and urban black ducks.

Methods

Duck collection

Seventy-four American black ducks (38 males, 36 females) were collected for this study, with 37 collected opportunistically by hunters in coastal saltmarsh habitat of Nova Scotia and New Brunswick, Canada, from 26 November 2013 to 27 March 2014, and 37 collected and euthanized by cervical dislocation by Environment and Climate Change Canada employees in an urban freshwater pond in St. John's, Newfoundland and Labrador, from 11 February 2014 to 15 April 2014. Thirteen mallards (six males, seven females) were collected opportunistically by hunters in coastal saltmarsh habitat of Nova Scotia from 30 January 2014 to 25 March 2014. All ducks were sent to Acadia University, where they were stored frozen at -18 °C.

The collection of black ducks and mallards for this project was pre-approved by Acadia University (Animal Care Permit No. 02-14) and Environment and Climate Change Canada (Scientific Permit No. ST2785).

Isotopic niche analysis

Blood samples were taken from the heart of each frozen carcass, and stored whole at -18 °C. Additional blood samples were collected during winter banding operations in 2015. Live-trapped ducks were caught in a pond in Grand Manan, New Brunswick, using a baited single-wire funnel trap, and in a coastal saltmarsh in Cole Harbour, Nova Scotia, using a cannon net. Prior to drawing blood, the wing surface was sterilized with 95% ethanol, and then approximately 50 µL of blood were drawn from the brachial vein using a capillary tube. The sampled ducks were released where they were caught, and blood samples were stored whole at -18 °C.

Whole blood samples were dried at 60 °C for 48 h, ground into a powder, and sent to the Stable Isotopes in Nature Laboratory (SINLAB, University of New Brunswick, Fredericton, New Brunswick, Canada) for analysis of isotopes of carbon and nitrogen. Samples were combusted in an elemental analyzer, and gases were sent to the isotope-ratio mass spectrometer using a continuous flow interface. Data are reported as differences in isotopic ratios, for which the units are parts per thousand (or per mil; ‰), compared with Pee Dee Belemnite, for carbon, and atmospheric nitrogen, for nitrogen, according to the following equation:

$$\delta X = \left(\frac{R_{\text{sample}}}{R_{\text{std}}} - 1\right) \times 1000$$

where δX is the isotope of interest (either δ^{15} N or δ^{13} C, in ‰), *R* is the ratio of the abundance of the heavy to the light isotope (15 N/ 14 N or 13 C/ 12 C), with R_{sample} being the ratio within the sample, and R_{std} the ratio of heavy to light isotope within the international standard (Hobson and Clark 1992).

Due to the known effects of species and location on isotopic ratios in blood of the birds in this study (English et al. 2017), samples were divided in to three groups: coastal black ducks, urban black ducks, and coastal mallards. We used stable isotope Bayesian ellipses in R (SIBER) from the package "SIBER" and used a probabilistic method (Jackson et al. 2011) to assess differences in isotopic niches among the three groups of ducks following the posterior draws of Bayesian-simulated standard ellipse areas



(SEA_b; represented in $\%^2$). The SEA_b values were compared among the three groups using Bayesian inference as described in Jackson et al. (2011) (see Pettitt-Wade et al. 2015; Karlson et al. 2018 for examples). A Bayesian probability (*p*) of SEA_b size difference >0.6 was considered to be significant (e.g., Pettitt-Wade et al. 2015). Layman's metrics (Layman et al. 2007) were used to further quantify the trophic structure of the three groups of birds.

Digestive tract analysis

All 74 black ducks and three of the 13 mallards were sent to the Avian Energetics Laboratory (Long Point, Ontario), where full carcass processing on thawed birds occurred using standardized methods (e.g., Reinecke et al. 1982; Morton et al. 1990). To account for the effect of body size on digestive tract morphology, we extracted the first principal component (PC1) score of the correlation matrix of In-transformed tarsus length, culmen length, head length, wing chord length, and plucked body length as an index of overall body size (as per Kehoe et al. 1988; Jónsson and Afton 2017). The PC1 accounted for 58% of the total original variance. Four gut measurements were examined: empty gizzard mass, small intestine length, large intestine length, and the combined length of the first and second segments of the caeca. Gut morphology data for each variable were regressed on PC1 to adjust them for body size (all regressions were positive and significant (p < 0.05)). The residuals from each regression were used to calculate a new variable (y_i) corrected for body size of each digestive tract component as per the following formula:

$$y_i = y_{obs} - [a + b(PC1)] + \overline{y}_{obs}$$

where y_{obs} is the observed measurement of the particular digestive tract component, and a and b are the values from the regression of each digestive tract component on PC1.

Waterfowl gut morphology is strongly affected by the annual cycle (Drobney 1984; Moorman et al. 1992) so we only examined ducks collected during overlapping time periods. We excluded 24 coastal ducks collected at the end of November 2013 due to a lack of urban ducks collected near this time. For the black ducks, we used a multivariate analysis of covariance (ANCOVA) to assess the effects of the factors "sex" and "location" (coastal vs. urban) on each digestive tract component, with date and isotope (either δ^{15} N or δ^{13} C) as the covariate. The gut morphology of the small number of mallards (*n* = 3) was compared with black ducks using a separate multivariate analysis of variance (ANOVA).

Blood mercury analysis

From carcasses, we removed whole blood from clots in the heart, veins, or arteries. Samples (generally ~1–2 g) were freeze-dried, homogenized to a powder using a clean mortar and pestle, and transferred to 1.5 mL Eppendorf tubes and frozen until analysis. We analyzed for total Hg at the Center for Analytical Research on the Environment at Acadia University. Samples were analyzed on a Nippon Instruments MA-3000 Mercury Analysis System using thermal pyrolysis, gold amalgamation preconcentration, and atomic absorption spectroscopy detection. Samples were not blank corrected, as mean blanks were -0.01 ± 0.01 ng/g (n = 11) with the method detection limit (MDL) calculated as 3× the standard deviation of the blanks (MDL = 0.03 ng/g). All samples were well above detection limits, and repeated measurements on seven samples yielded similar values (mean difference 1.8%). Internal quality control included analytical blanks and certified reference material (DORM-4, National Research Council of Canada). The mean recovery for the certified reference material (n = 13) was 95.4% ± 4.4 % (SD) for total mercury (THg), so we did not recovery-correct THg values. THg concentrations are reported in parts per billion (ppb) dry weight.

Data on THg concentrations were not normally distributed (Shapiro–Wilk normality test: W = 0.86; p < 0.001), and were thus log₁₀-transformed. Only black ducks (n = 74) were analysed using

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ANCOVA. For black ducks, the factorial effects of sex and habitat (coastal vs. urban), and the covariates of date of sampling and isotope (either δ^{15} N or δ^{13} C), on blood Hg concentrations were investigated using ANCOVA. The data from mallards (n = 3) were compared with the coastal and urban black ducks using a separate ANOVA. All statistical analyses were performed using R version 3.4.3 (R Development Core Team 2015).

Results

Isotopic niche

Total convex hull area (as described by Layman et al. 2007) was greatest in coastal black ducks (48.22 $\%^2$), followed by coastal mallards (15.00 $\%^2$), and was the smallest in urban black ducks (8.26 $\%^2$). When the SEA_b were compared, isotopic niche varied significantly among the three groups (**Fig. 1**). Estimated SEA_b values for coastal black ducks were wider (mean SEA_b; 95% credible interval = 12.80; 9.41–16.12 $\%^2$) than urban black ducks (1.72; 1.18–2.28 $\%^2$; Bayesian p = 1.00, where p > 0.6 indicates a difference) and coastal mallards (8.18; 3.68–13.18 $\%^2$; Bayesian p = 0.94). Coastal mallards



Fig. 1. Isotopic niche areas based on the δ^{13} C and δ^{15} N values in samples of heart blood from American black ducks (ABDU; circles) and mallards (MALL; triangles). Top: Standard ellipse areas for black ducks and mallards showing the 40% credible interval. Bottom: Density box-plots of Bayesian estimates of standard ellipse area (SEA_b) showing the mean ellipse areas (black dot) and their credible intervals (50%, 75%, and 95%) obtained following a Bayesian approach of posterior estimate of simulated standard ellipse areas (Jackson et al. 2011). Letters above the boxes indicate significant differences in SEA_b.



occupied a wider isotopic niche than urban black ducks (Bayesian p = 1.00; Fig. 1). Coastal black ducks and mallards overlapped in their isotopic niches, but urban black ducks were isolated.

Digestive tract analysis

Fifty-three black ducks (31 males and 22 females) and three mallards (all males) were used in the digestive tract analysis. Means and standard deviations of the gut measurements are shown in **Table 1**. Sex and date had no effect on any of the gut measurements (all p > 0.05), so both sexes were pooled and only the effects of isotope and location are reported.

In black ducks, small intestine length and gizzard mass were significantly, positively correlated with δ^{15} N, and all gut measurements except gizzard mass were affected by location (**Table 2; Fig. 2**). For the gizzard mass, the interaction between location and δ^{15} N was significant ($F_{1,51} = 10.46$; p = 0.002). Gizzard mass in coastal black ducks tended to increase with increasing δ^{15} N ($\beta = 5.14 \pm 2.41$; $F_{1,14} = 4.56$, p = 0.051), whereas gizzard mass in urban black ducks decreased ($\beta = -3.08 \pm 1.24$; $F_{1,35} = 6.21$, p = 0.017) with increasing δ^{15} N (**Fig. 2**). Tukey's Honest Significant Difference (HSD) testing on the effect of location showed significant differences in the lengths of the small intestines but no differences in the lengths of the large intestines or caeca (**Table 1**).

The only gut measurement related to δ^{13} C was gizzard mass, and all gut measurements except gizzard mass were affected by location (Table 2). None of the interactions between δ^{13} C and location were significant (all p > 0.05). Tukey's HSD testing on the effect of location showed significant differences in the lengths of the small intestines, large intestines, and caeca between coastal and urban black ducks (Table 2).

When the covariates were removed and the effect of location alone was measured against gut morphology, location had a significant effect on small intestine, large intestine, and total caeca length

Table 1. Gut measurements (mean \pm SD; standardized to first principal component) for the coastal and urban black ducks and coastal mallards in this study.

	Small intestine length (mm)	Large intestine length (mm)	Total caeca length (mm)	Gizzard mass (g)
Coastal black ducks ($n = 16$)	156.92 ± 18.38	9.51 ± 1.59	32.67 ± 5.08	35.33 ± 11.66
Urban black ducks ($n = 37$)	135.75 ± 15.82	8.06 ± 2.08	27.00 ± 6.36	31.97 ± 4.25
Coastal mallards $(n = 3)$	130.58 ± 8.54	8.33 ± 0.98	24.62 ± 2.22	33.40 ± 13.10

Note: We only show data for ducks collected during overlapping time periods (January-April 2014).

Table 2. Multivariate analysis of covariance results for the δ^{15} N and δ^{13} C models of black ducks collected from January to April of 2014, with Tukey's HSD results used in post-hoc testing for pairwise comparisons between locations.

	δ	¹⁵ N		Location (δ^1	⁵ N model)	δ	¹³ C		Location $(\delta^1$	³ C model)
Gut measurement	F_1	Þ	F_1	Р	Tukey's HSD p	F_1	p	F_1	P	Tukey's HSD p
Small intestine length	6.37	0.015	21.08	<0.001	0.025	0.41	0.523	17.49	<0.001	<0.001
Large intestine length	2.18	0.147	6.30	0.016	0.211	3.11	0.084	5.72	0.021	0.021
Total caeca length	3.49	0.070	10.30	0.002	0.112	1.87	0.178	9.33	0.004	0.004
Gizzard mass	5.76	0.020	0.53	0.468	N/A	8.25	0.006	17.38	0.061	N/A

Note: All significant results are bolded. All degrees of freedom = 1.

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Fig. 2. Gut measurements (scaled to first principal component) measured against δ^{15} N ratios (‰) for the coastal (filled circles) and urban (open circles) black ducks collected during overlapping time periods in winter in Atlantic Canada. The lines represent linear regressions; shaded areas indicate 95% confidence intervals.

(multivariate ANOVA; all p < 0.05), but did not affect gizzard mass (p = 0.132). In the cases where location had a significant effect on gut morphology, coastal black ducks had consistently longer guts than urban black ducks (Tukey's HSD: all p < 0.05). Gizzard mass of coastal black ducks was almost $3 \times$ more variable than urban black ducks (Table 1).



In the separate multivariate ANOVA that included the mallards, the only significant difference for mallards was found in the small intestine length ($F_{2,53} = 10.04$, p < 0.001), where coastal mallards had shorter small intestines than the coastal black ducks (Tukey's HSD: p < 0.001). The gut morphologies of coastal mallards and urban black ducks did not differ (all p > 0.05).

Mercury concentrations

THg concentrations in blood samples did not vary between sexes of black ducks ($F_{1,73} = 0.15$; p = 0.702), so both sexes were pooled for the analysis. Both δ^{15} N ($F_{1,73} = 138.04$; p < 0.001) and location ($F_{1,73} = 26.73$; p < 0.001; Fig. 3) had a strong effect on THg concentrations, with coastal black ducks containing higher and a wider range of blood THg concentrations than urban black ducks (Table 3; Tukey's HSD: p < 0.001). THg was positively correlated with δ^{15} N in coastal ($\beta = 0.12$, $F_{1,38} = 25.36$, p < 0.001) and urban ($\beta = 0.27$, $F_{1,35} = 7.63$, p = 0.009) black ducks (Fig. 3). The interaction between δ^{15} N and location was not significant ($F_{1,74} = 3.13$; p = 0.081), and δ^{13} C ($F_{1,73} = 0.536$; p = 0.467) and date ($F_{1,73} = 0.133$, p = 0.716) did not have any effect on THg concentrations.

In the ANOVA that compared THg concentrations among the three groups of ducks in this study, the group variable was significant ($F_{2,74} = 10.57$, p < 0.001). Coastal mallards and urban black ducks had



Fig. 3. Blood mercury concentrations (log₁₀-transformed; ppb dry weight) measured against $\delta^{15}N$ (‰) for all coastal (filled circles) and urban (open circles) black ducks in this study. The lines represent linear regressions; shaded areas indicate 95% confidence intervals.

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 Table 3. Total mercury concentrations (ppb dry weight; not ln-transformed) in blood samples of American black ducks and mallards wintering in coastal and urban Atlantic Canada.

	Mean \pm SD	Range
Coastal black ducks ($n = 37$)	351.3 ± 204.1	40.6-1040.0
Urban black ducks $(n = 37)^a$	66.4 ± 55.1	9.0-256.0
Coastal mallards $(n = 3)^a$	71.6 ± 28.0	39.9-93.2

^aSignificantly different from coastal black ducks.

similar blood THg concentrations (Table 3; Tukey's HSD: p = 0.751) and coastal mallards had significantly lower blood THg concentrations than coastal black ducks (p = 0.004).

Discussion

Isotopic niche

Coastal and urban black ducks exploit different ecological niches at the northern limit of their winter range. When using isotopic niche as a proxy for measuring ecological niche, our data showed that coastal black ducks occupy a wider ecological niche than urban black ducks, and are generalists foraging on foods higher in δ^{15} N values compared with urban black ducks. While birds occupying wide isotopic niches generally have diverse diets, it should be noted that this may not be the case if the consumer is a specialist on prey with a high variance in isotopic signatures (Yeakel et al. 2016). Based on our previous work on winter black duck diets (English et al. 2017), isotopic research on wintering black ducks in Maine and New Jersey (Barboza and Jorde 2018), and our current study in which coastal black ducks had the greatest total convex hull area (an indicator of trophic diversity, Layman et al. 2007), coastal black ducks are not specialists. The urban black ducks in our study are likely not specialists in the classic sense, but rather have a limited diet due to exploiting a human-altered habitat in which a small variety of food items are available to them (English et al. 2017).

The isotopic niches of coastal and urban black ducks did not overlap, which may suggest this species can avoid intraspecific competition for wintering habitat by exploiting different ecological niches. Coastal saltmarsh is the traditional wintering habitat for black ducks (Longcore et al. 2000), and during winter at higher latitudes this habitat becomes restricted by ice cover (Barboza and Jorde 2018). By also wintering in urban parks, black ducks can avoid competing for saltmarsh habitat and can exploit an ample supply of anthropogenic food resources and survive winter in Atlantic Canada (English et al. 2017).

Coastal black ducks and coastal mallards overlapped in their isotopic niches, which may suggest that these ducks are competing for food resources in coastal areas. However, coastal black ducks occupied a wider isotopic niche that contained more marine signatures (i.e., higher δ^{15} N or δ^{13} C values; Hobson and Clark 1992) than the coastal mallards; we suspect that despite sharing wintering habitat in coastal areas, some black ducks and mallards are not competing for the same food resources. Black ducks evolved in coastal marine habitats (Longcore et al. 2000) and therefore may be better suited to exploit these habitats (or more resistant to competitive exclusion, but see Morton 1998) than mallards, whose traditional habitat is inland plains and wetlands where they may outcompete black ducks for habitat (Ankney et al. 1987; Drilling et al. 2002).



Gut morphology analysis

Gut morphology varied with isotopic niche. In general, coastal black ducks had longer guts than urban black ducks, and the small sample of mallards had gut morphologies similar to urban black ducks. Diet affects gut morphology in waterfowl in various ways (Miller 1975; Kehoe and Ankney 1985; Kehoe et al. 1988; Jónsson and Afton 2017), and the different gut morphologies of coastal and urban black ducks are likely a result of the different diets of these two groups (English et al. 2017). Longer guts are often associated with a higher diversity or quality of diet in waterfowl (van Gils et al. 2008). Consequently, we suspect the longer guts of the coastal black ducks reflect their more natural and diverse diets, as opposed to the shorter guts of the urban black ducks that mainly digest anthropogenic foods.

The urban black ducks in this study occupied a narrow isotopic niche and had relatively short guts, but there was still a high amount of variation in their gut length. Habitat explained much of the variation in digestive tract morphology in our study, but there is likely another factor contributing to the variation that we did not measure. We did not have reliable age data on the birds in this study, as age determination for black ducks in winter can be challenging (Ashley et al. 2006). Age is known to affect gut morphology in Lesser Snow Geese (*Anser caerulescens caerulescens* (Linnaeus, 1758); Jónsson and Afton 2017) and male mallards (Olsen et al. 2011), but the variation in gut morphology in these studies was better explained by differences in diet and habitat. Intermittent disruptions in food availability and cold stress may also affect gut morphology in black ducks (Barboza and Jorde 2002).

There was no difference in gizzard mass between coastal and urban black ducks. We predicted gizzard mass would be highest in coastal black ducks due to the need to digest hard-shelled prey but instead found their gizzard masses were more variable. Coastal black ducks exhibited the widest isotopic niche and had the most diverse diets of the groups of ducks in this study (English et al. 2017), and differences in gizzard mass may reflect individual differences in diets of coastal black ducks. Jónsson and Afton (2017) obtained a similar result by observing more variability in the gizzard masses of Snow Geese collected from coastal areas than from rice-prairies. Olsen et al. (2011) found that gizzard mass in male mallards was unresponsive to changes in diet through winter, despite these authors predicting the opposite. Waterfowl digestive tracts have a high degree of plasticity, and attributing differences in digestive tracts between groups to one factor is often challenging (Karasov and McWilliams 2005; Olsen et al. 2011).

The gut morphology of the coastal mallards we studied was similar to that of urban black ducks and, despite being sampled in very different winter habitats, is likely to be attributable to a similar reliance on vegetation in their diet through winter (English et al. 2017). A larger sample size of mallards may have introduced more variability in lengths of the digestive tracts, and the results from our small sample size should be cautiously interpreted.

Mercury analysis

Blood Hg concentrations varied with isotopic niche and δ^{15} N levels. In birds, Hg and δ^{15} N are often positively correlated in the blood, as Hg bioaccumulates through a food chain (Wolfe et al. 1998; Sebastiano et al. 2017), and this was the case in our study. As predicted, the coastal black ducks that occupied a wider isotopic niche had higher concentrations of blood Hg than the urban black ducks that occupied a narrow isotopic niche. Occupying a wide isotopic niche may increase an organism's exposure to contaminants in its environment. However, it should be noted that any consumer that feeds principally on a certain type of contaminated prey, irrespective of its trophic position, will be relatively more contaminated than conspecifics feeding on less contaminated food (e.g., Hindmarch and Elliott 2015).

Hg has been detected in marine biota and in coastal areas of the Bay of Fundy in Atlantic Canada (Elliott et al. 1992; Goodale et al. 2008; Sunderland et al. 2012), and concentrations of Hg ranging



from 0.01 to 0.04 ppm have been found in black duck prey from this region (English et al. 2015). Since the coastal black ducks in this study contained various marine invertebrates in their gizzards (English et al. 2017) it is likely the Hg accumulation in their blood was a result of eating more contaminated prey, while the urban black ducks were presumably consuming much less contaminated food items (anthropogenic foods such as bread or grains). Blood Hg concentrations in coastal black ducks were highly variable, and may again reflect individual differences in diet. Despite coastal black ducks having a mean Hg blood concentration almost $6\times$ higher than the urban black ducks, body condition was similar (English et al. 2018). The differences in gut morphology may also be contributing to the differences in the blood Hg concentrations, as the longer guts of coastal black ducks may provide more time for the uptake of contaminants (Kleinow and James 2001).

Our small, localized sample of mallards precludes any broad prediction of Hg concentrations in mallards across Atlantic Canada, but our results aligned with our prediction of lower blood Hg concentrations in coastal mallards when compared with coastal black ducks. Mallory et al. (2018) recently found ~4× higher blood Hg in mallards and one black duck collected in October in this region than each species had in the winter (this study), suggesting that both species may experience even higher Hg exposure in summer or premigration diets (or habitats). Moreover, the black duck had approximately $5\times$ higher blood Hg than the mallards (Mallory et al. 2018), consistent with our findings. The coastal mallards and black ducks in this study were collected in the same habitats, and despite overlapping isotopic niches, had very different blood Hg concentrations. Even though coastal mallards in Atlantic Canada forage on marine invertebrates (Drilling et al. 2002; English et al. 2017) it is possible that the three mallards in our study were foraging at a lower trophic level, not on the same invertebrate prey as the black ducks, or were foraging on species with atypical δ^{15} N/Hg relationships (e.g., polychaetes in the Bay of Fundy; Sizmur et al. 2013). The bioavailability of methylmercury is not consistent through saltmarshes in the Bay of Fundy (O'Driscoll et al. 2011), and depending where these mallards were foraging in the saltmarsh they may have been exposed to less Hg than the black ducks.

Recently, high Hg concentrations in the feathers and breast meat of black ducks were reported along the Penobscot River in Maine, an area contaminated by a chemical plant that operated along that river from 1967 to 2000 (Sullivan and Kopec 2018). The Hg concentrations in breast meat (mean 0.82 ± 0.21 ppm wet weight) were high enough for the State of Maine to prompt a warning against the consumption of black ducks harvested along the Penobscot River. Black ducks are one of the main species of waterfowl hunted in Atlantic Canada (Longcore et al. 2000), and the Canadian Food Inspection Agency does not list a Hg limit for the consumption of poultry or waterfowl (the only one listed is a 0.5 ppm limit for Hg in seafood products suitable for human consumption, CFIA 2018). Provencher et al. (2014) called for the continued monitoring of Hg contamination of marine birds because of the potential health implications on the wildlife and the people who consume these birds. Our research on two hunted and consumed species indicates the need for this continued monitoring, and it should be extended to other species of wildlife hunted and consumed in Atlantic Canada.

Conclusions

Species exploiting different ecological niches tend to exhibit different physical and behavioural adaptations (Cloyed and Eason 2017; Pratte et al. 2017), and black ducks and mallards wintering in Atlantic Canada are no exception. Our results provide further evidence of the flexibility and hardiness of black ducks at the northern limit of their winter range. The black ducks exploiting a wide ecological niche had a higher degree of Hg contamination, but this risk may be offset by the benefits of reducing competition for winter habitat. The increasing number of black ducks wintering in Atlantic Canada may be a result of their ecological niche plasticity.



Hg was a useful biomarker in our study. Hg has been used as a biomarker to explain differences in foraging behaviour and habitat use in studies spanning a wide range of taxa from marine mammals (Loseto et al. 2008) to fish (Desta et al. 2008) to birds (Eulaers et al. 2013). Combining a chemical biomarker with isotopic data allows for greater insights into a species' habitat use and intraspecific differences in diet. Therefore, chemical biomarkers such as Hg should be considered as a relevant method for ecologists studying relationships between inter- and intraspecific habitat use and foraging behaviour.

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Author contributions

MDE, GJR, NJOD, SJK, LEP, and MLM conceived and designed the study. MDE, GJR, NJOD, SJK, LEP, and MLM performed the experiments/collected the data. MDE, GJR, NJOD, SJK, LEP, and MLM analyzed and interpreted the data. MDE, GJR, NJOD, SJK, LEP, and MLM contributed resources. MDE, GJR, NJOD, SJK, LEP, and MLM drafted or revised the manuscript.

Competing interests

Mark L. Mallory is currently on the editorial board of this journal.

Data availability statement

All relevant data are within the paper.

References

Ankney CD, Dennis DG, and Bailey RC. 1987. Increasing mallards, decreasing American black ducks: coincidence or cause and effect? Journal of Wildlife Management, 51(3): 523–529. DOI: 10.2307/ 3801262

Ashley EP, North NR, Petrie SA, and Bailey RC. 2006. Age determination of American black ducks in winter and spring. Wildlife Society Bulletin, 34(5): 1401–1410. DOI: 10.2193/0091-7648(2006) 34[1401:ADOABD]2.0.CO;2

Barboza PS, and Jorde DG. 2002. Intermittent fasting during winter and spring affects body composition and reproduction of a migratory duck. Journal of Comparative Physiology B, 172(5): 419–434. PMID: 12122458 DOI: 10.1007/s00360-002-0267-y

Barboza PS, and Jorde DG. 2018. Monitoring responses to variation in food supply for a migratory waterfowl: American Black Duck (*Anas rubripes*) in winter. Journal of Comparative Physiology B, 188(5): 831–842. PMID: 29804127 DOI: 10.1007/s00360-018-1163-4

Black Duck Joint Venture Management Board. 2014. Black Duck Joint Venture Strategic Plan, 2014–2019. Black Duck Joint Venture Coordination Office, USFWS, Laurel, Maryland and Canadian Wildlife Service, Ottawa, Ontario.



Braune BM, and Malone BJ. 2006. Organochlorines and mercury in waterfowl harvested in Canada. Environmental Monitoring and Assessment, 114(1–3): 331–359. PMID: 16570226 DOI: 10.1007/s10661-006-4778-y

Canadian Food Inspection Agency (CFIA). 2018. Appendix 3—Canadian guidelines for chemical contaminants and toxins in fish and fish products [online]: Available from inspection.gc.ca/food/fish-andseafood/manuals/standards-and-methods/eng/1348608971859/1348609209602?chap=7#s20c7.

Cloyed CS, and Eason PK. 2017. Niche partitioning and the role of intraspecific niche variation in structuring a guild of generalist anurans. Royal Society Open Science, 4: 170060. PMID: 28405403 DOI: 10.1098/rsos.170060

Colas F, Vigneron A, Felten V, and Devin S. 2014. The contribution of a niche-based approach to ecological risk assessment: using macroinvertebrate species under multiple stressors. Environmental Pollution, 185: 24–34. PMID: 24212068 DOI: 10.1016/j.envpol.2013.09.033

Desta Z, Borgstrøm R, Gebremariam Z, and Rosseland BO. 2008. Habitat use and trophic position determine mercury concentration in the straight fin barb *Barbus paludinosus*, a small fish species in Lake Awassa, Ethiopia. Journal of Fish Biology, 73(3): 477–497. DOI: 10.1111/j.1095-8649.2008.01920.x

Drilling N, Titman RD, and McKinney F. 2002. Mallard (*Anas platyrhynchos*), version 1.0. *In* The birds of North America. *Edited by* AF Poole and FB Gill. Cornell Lab of Ornithology, Ithaca, New York. DOI: 10.2173/bna.658

Drobney RD. 1984. Effect of diet on visceral morphology of breeding wood ducks. The Auk, 101: 93–98. DOI: 10.1093/auk/101.1.93

Elliott JE, Scheuhammer AM, Leighton FA, and Pearce PA. 1992. Heavy metal and metallothionein concentrations in Atlantic Canadian seabirds. Archives of Environmental Contamination and Toxicology, 22(1): 63–73. PMID: 1554255 DOI: 10.1007/BF00213303

English MD, Robertson GJ, and Mallory ML. 2015. Trace element and stable isotope analysis of fourteen species of marine invertebrates from the Bay of Fundy, Canada. Marine Pollution Bulletin, 101(1): 466–472. PMID: 26490410 DOI: 10.1016/j.marpolbul.2015.09.046

English MD, Robertson GJ, Peck LE, and Mallory ML. 2017. Agricultural food resources and the foraging ecologies of American black ducks (*Anas rubripes*) and mallards (*Anas platyrhynchos*) at the northern limits of their winter ranges. Urban Ecosystems, 20(6): 1311–1318. DOI: 10.1007/s11252-017-0683-0

English MD, Robertson GJ, Peck LE, Pirie-Hay D, Roul S, and Mallory ML. 2018. Body condition of American Black Ducks (*Anas rubripes*) wintering in Atlantic Canada using carcass composition and a scaled mass index. Canadian Journal of Zoology, 96(10): 1137–1144. DOI: 10.1139/cjz-2017-0329

Eulaers I, Jaspers VLB, Bustnes JO, Covaci A, Johnsen TV, Halley DJ, et al. 2013. Ecological and spatial factors drive intra- and interspecific variation in exposure of subarctic predatory bird nestlings to persistent organic pollutants. Environment International, 57–58: 25–33. PMID: 32179324 DOI: 10.1016/j.envint.2013.03.009

Goodale MW, Evers DC, Mierzykowski SE, Bond AL, Burgess NM, Otorowski CI, et al. 2008. Marine foraging birds as bioindicators of mercury in the Gulf of Maine. EcoHealth, 5: 409–425. PMID: 19277786 DOI: 10.1007/s10393-009-0211-7



Hindmarch S, and Elliott JE. 2015. A specialist in the city: the diet of barn owls along a rural to urban gradient. Urban Ecosystems, 18(2): 477–488. DOI: 10.1007/s11252-014-0411-y

Hobson KA. 2011. Isotopic ornithology: a perspective. Journal of Ornithology, 152: 49–66. DOI: 10.1007/s10336-011-0653-x

Hobson KA, and Clark RG. 1992. Assessing avian diets using stable isotopes I: turnover of ¹³C in tissues. The Condor, 94(1): 181–188. DOI: 10.2307/1368807

Jackson AL, Inger R, Parnell AC, and Bearhop S. 2011. Comparing isotopic niche widths among and within communities: SIBER—Stable Isotope Bayesian Ellipses in R. Journal of Animal Ecology, 80: 595–602. PMID: 21401589 DOI: 10.1111/j.1365-2656.2011.01806.x

Jónsson JE, and Afton AD. 2017. Does organ and muscle plasticity vary by habitat or age in wintering Lesser Snow Geese *Anser caerulescens caerulescens*? Wildfowl, 67: 19–43.

Karasov WH, and McWilliams SR. 2005. Digestive constraints in mammalian and avian ecology. *In* Physiological and ecological adaptations to feeding in vertebrates. *Edited by* JM Starck and T Wang. Science Publishers, Enfield, New Hampshire. pp. 87–112.

Karlson AML, Reutgard M, Garbaras A, and Gorokhova E. 2018. Isotopic niche reflects stressinduced variability in physiological status. Royal Society Open Science, 5(2): 171398. PMID: 29515859 DOI: 10.1098/rsos.171398

Kehoe FP, and Ankney CD. 1985. Variation in digestive organ size among five species of diving ducks (*Aythya* spp.). Canadian Journal of Zoology, 63(10): 2339–2342. DOI: 10.1139/z85-346

Kehoe FP, Ankney CD, and Alisauskas RT. 1988. Effects of dietary fiber and diet diversity on digestive organs of captive Mallards (*Anas platyrhynchos*). Canadian Journal of Zoology, 66(7): 1597–1602. DOI: 10.1139/z88-233

Kleinow KM, and James MO. 2001. Response of the teleost gastrointestinal system to xenobiotics. *In* Target organ toxicity in marine and freshwater teleosts: organs. *Edited by* D Schlenk and WH Benson. CRC Press, London, UK. pp. 94. DOI: 10.1201/9781315109244

Layman CA, Arrington DA, Montaña CG, and Post DM. 2007. Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology, 88(1): 42–48. PMID: 17489452 DOI: 10.1890/0012-9658(2007)88[42:CSIRPF]2.0.CO;2

Longcore JR, McAuley DG, Hepp GR, and Rhymer JM. 2000. American Black Duck (*Anas rubripes*), version 2.0. *In* The birds of North America. *Edited by* AF Poole and FB Gill. Cornell Lab of Ornithology, Ithaca, New York. DOI: 10.2173/bna.481

Loseto LL, Stern GA, Deibel D, Connelly TL, Prokopowic A, Lean DRS, et al. 2008. Linking mercury exposure to habitat and feeding behaviour in Beaufort Sea beluga whales. Journal of Marine Systems, 74(3–4): 1012–1024. DOI: 10.1016/j.jmarsys.2007.10.004

MacArthur RH. 1958. Population ecology of some warblers of northeastern coniferous forests. Ecology, 39(4): 599–619. DOI: 10.2307/1931600

Maisonneuve C, Bélanger L, Bordage D, Jobin B, Grenier M, Beaulieu J, et al. 2006. American black duck and mallard breeding distribution and habitat relationships along a forest-agriculture gradient



in Southern Québec. The Journal of Wildlife Management, 70(2): 450-459. DOI: 10.2193/0022-541X(2006)70[450:ABDAMB]2.0.CO;2

Mallory ML, Provencher JF, Robertson GJ, Holland ER, Klapstein SJ, Stevens K, et al. 2018. Mercury concentrations in blood, brain and muscle tissues of coastal and pelagic birds from northeastern Canada. Ecotoxicology and Environmental Safety, 157: 424–430. PMID: 29655158 DOI: 10.1016/j.ecoenv.2018.04.004

McCorquodale DB, and Knapton RW. 2003. Changes in numbers of wintering American black ducks and Mallards in urban Cape Breton Island, Nova Scotia. Northeastern Naturalist, 10: 297–304. DOI: 10.1656/1092-6194(2003)010[0297:CINOWA]2.0.CO;2

Miller MR. 1975. Gut morphology of Mallards in relation to diet quality. Journal of Wildlife Management, 39(1): 168–173. DOI: 10.2307/3800481

Moorman TE, Baldassarre GA, and Richard DM. 1992. Carcass mass, composition and gut morphology dynamics of Mottled Ducks in fall and winter in Louisiana. The Condor, 94(2): 407–417. DOI: 10.2307/1369213

Morton ES. 1998. Pairing in mallards and American black ducks: a new view on population decline in American black ducks. Animal Conservation, 1(4): 239–244. DOI: 10.1111/j.1469-1795. 1998.tb00034.x

Morton JM, Kirkpatrick RL, and Vaughan MR. 1990. Changes in body composition of American black ducks wintering at Chincoteague, Virginia. The Condor, 92(3): 598–605. DOI: 10.2307/1368679

Newsome SD, Martinez del Rio C, Bearhop S, and Phillips DL. 2007. A niche for isotopic ecology. Frontiers in Ecology and the Environment, 5(8): 429–436. DOI: 10.1890/060150.1

O'Driscoll NJ, Canário J, Crowell N, and Webster T. 2011. Mercury speciation and distribution in coastal wetlands and tidal mudflats: relationships with sulphur speciation and organic carbon. Water, Air, & Soil Pollution, 220(1–4): 313–326. PMID: 21949450 DOI: 10.1007/s11270-011-0756-2

Olsen RE, Cox RR, Afton AD, and Ankney CD. 2011. Diet and gut morphology of male Mallards during winter in North Dakota. Waterbirds, 34(1): 59–69. DOI: 10.1675/063.034.0107

Petrie MJ, Drobney RD, Sears DT, and Armstrong LM. 2012. Evidence for Mallard *Anas platyrhynchos* and American Black Duck *Anas rubripes* competition in western New Brunswick, Canada. Wildfowl, 62: 146–164.

Pettitt-Wade H, Wellband KW, Heath DD, and Fisk AT. 2015. Niche plasticity in invasive fishes in the Great Lakes. Biological Invasions, 17(9): 2565–2580. DOI: 10.1007/s10530-015-0894-3

Pratte I, Boadway KA, Davis SE, Maftei M, and Mallory ML. 2017. Diet dichotomy between two migrant seabirds breeding near a high Arctic polynya. Royal Society Open Science, 4: 160982. PMID: 28405388 DOI: 10.1098/rsos.160982

Provencher JF, Mallory ML, Braune BM, Forbes MR, and Gilchrist HG. 2014. Mercury and marine birds in Arctic Canada: effects, current trends, and why we should be paying closer attention. Environmental Reviews, 22(3): 244–255. DOI: 10.1139/er-2013-0072

R Development Core Team. 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria [online]: Available from R-project.org/.



Reinecke KJ, Stone TL, and Owen RB. 1982. Seasonal carcass composition and energy balance of female black ducks in Maine. The Condor, 84: 420–426. DOI: 10.2307/1367447

Robertson GJ, Tomlik M, Milton GR, Parsons GJ, and Mallory ML. 2017. Increases in the number of American black ducks wintering in Nova Scotia, 1970–2015. Journal of Fish and Wildlife Management, 8(2): 669–675. DOI: 10.3996/032017-JFWM-031

Sauer JR, Niven DK, Hines JE, Ziolkowski DJ Jr, Pardieck KL, Fallon JE, et al. 2017. The North American Breeding Bird Survey, Results and Analysis 1966–2015. Version 2.07.2017. USGS Patuxent Wildlife Research Center, Laurel, Maryland [online]: Available from mbr-pwrc.usgs.gov/bbs/specl15.html.

Sebastiano M, Bustamante P, Eulaers I, Malarvannan G, Mendez-Fernandez P, Churlaud C, et al. 2017. Trophic ecology drives contaminant concentrations within a tropical seabird community. Environmental Pollution, 227: 183–193. PMID: 28460236 DOI: 10.1016/j.envpol.2017.04.040

Sizmur T, Canário J, Gerwing TG, Mallory ML, and O'Driscoll NJ. 2013. Mercury and methylmercury bioaccumulation by polychaete worms is governed by both feeding ecology and mercury bioavailability in coastal mudflats. Environmental Pollution, 176: 18–25. PMID: 23395989 DOI: 10.1016/ j.envpol.2013.01.008

Sullivan KM, and Kopec AD. 2018. Mercury in wintering American black ducks (*Anas rubripes*) downstream from a point-source on the lower Penobscot River, Maine, USA. Science of the Total Environment, 612: 1187–1199. PMID: 28892863 DOI: 10.1016/j.scitotenv.2017.08.146

Sunderland EM, Amirbahman A, Burgess NM, Dalziel J, Harding G, Jones SH, et al. 2012. Mercury sources and fate in the Gulf of Maine. Environmental Research, 119: 27–41. PMID: 22572623 DOI: 10.1016/j.envres.2012.03.011

Toft CA. 1980. Feeding ecology of thirteen syntopic species of anurans in a seasonal tropical environment. Oecologia, 45(1): 131–141. PMID: 28310947 DOI: 10.1007/BF00346717

U.S. Fish and Wildlife Service (USFWS). 2018. Adaptive harvest management: 2019 hunting season. U.S. Department of Interior, Washington, D.C.

van Gils JA, Beekman JH, Coehoorn P, Corporaal E, Dekkers T, Klaassen M, et al. 2008. Longer guts and higher food quality increase energy intake in migratory swans. Journal of Animal Ecology, 77(6): 1234–1241. PMID: 18662243 DOI: 10.1111/j.1365-2656.2008.01452.x

Webb CO, Ackerly DD, McPeek MA, and Donoghue MJ. 2002. Phylogenies and community ecology. Annual Review of Ecology and Systematics, 33: 475–505. DOI: 10.1146/annurev.ecolsys. 33.010802.150448

Whitney MC, and Cristol DA. 2018. Impacts of sublethal mercury exposure on birds: a detailed review. Reviews of Environmental Contamination and Toxicology, 244: 113–163. PMID: 28710647 DOI: 10.1007/398_2017_4

Wolfe MF, Schwarzbach S, and Sulaiman RA. 1998. Effects of mercury on wildlife: a comprehensive review. Environmental Toxicology and Chemistry, 17(2): 146–160. DOI: 10.1002/etc.5620170203

Yeakel JD, Bhat U, Elliott Smith EA, and Newsome SD. 2016. Exploring the isotopic niche: isotopic variance, physiological incorporation, and the temporal dynamics of foraging. Frontiers in Ecology and Evolution, 4: 1. DOI: 10.3389/fevo.2016.00001