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*Letters*

# Cross-Seasonal Association Between Winter Trophic Status and Breeding Ground Selenium Levels in Boreal White-Winged Scoters

# Relations intersaisonnières entre le statut trophique durant l'hiver et les taux de sélénium de Macreuses brunes sur leurs aires de nidification boréales

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ABSTRACT. The effect of cross-seasonal interactions on reproduction and fitness in migratory species is of increasing interest to ecologists, particularly because of the conservation implications of habitat change. Variation in contaminant levels that can affect reproduction in migratory species may reflect differing exposure across seasons. We examined the relationship between concentrations of liver selenium, a known teratogen, and winter trophic level in breeding White-winged Scoters (*Melanitta fusca*) using claw $\delta^{15}N$ values as an index of winter trophic level. Claw $\delta^{15}N$ was a significant predictor of variation in breeding ground selenium levels ($r = 0.32$), and liver selenium increased by approximately 12 ± 5 SE $\mu g \cdot g^{-1}$ with one trophic level increase in $\delta^{15}N$ ($\Delta 3‰$). This relationship demonstrates that contaminant exposure from wintering or staging areas can result in higher levels in birds on breeding grounds, where some contaminants are more likely to have impacts.

RÉSUMÉ. L'effet des interactions intersaisonnières sur la reproduction et l'adaptation des espèces migratrices revêt un intérêt grandissant chez les écologistes, notamment en raison de la problématique de conservation associée au changement des habitats. La variation du taux des contaminants pouvant avoir un effet sur la reproduction des espèces migratrices peut refléter des expositions différentes selon les saisons. Nous avons étudié la relation entre la concentration du sélénium hépatique, un élément tératogène connu, et le niveau trophique hivernal de Macreuses brunes (*Melanitta fusca*) reproductrices, en utilisant les valeurs de $\delta^{15}N$ de leurs griffes comme indice du niveau trophique en hiver. Le $\delta^{15}N$ des griffes était une variable explicative significative de la variation du taux de sélénium sur les aires de reproduction ($r = 0.32$), tandis que le sélénium hépatique augmentait d'environ 12 ± 5 $\mu g \cdot g^{-1}$ avec la hausse d'un niveau trophique de $\delta^{15}N$ ($\Delta 3‰$). Cette relation montre que l'exposition à des contaminants sur les aires d'hivernage ou de repos peut entraîner des taux de contamination plus élevés chez les oiseaux sur les aires de nidification, où certains contaminants sont davantage susceptibles d'avoir des effets.

Key Words: *contaminants; cross-seasonal effects; scoters; selenium; stable-isotopes; trophic levels.*

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## INTRODUCTION

In migratory species, survival and reproductive success can be strongly affected by events occurring away from the breeding grounds (Heitmeyer and Fredrickson 1981, Webster and Marra 2005). Use of poor quality habitat on wintering and staging areas has been linked to reduced fitness in poor quality individuals and population level effects (Gunnarsson et al. 2005, Norris 2005). Contaminants acquired on wintering and staging areas may also be retained and carried to relatively pristine breeding grounds where they may affect reproductive success and concomitantly population growth (Blais et al. 2005). Therefore, understanding whether there are carry-over effects from sources of variation in contaminant exposure may help to quantify the potential impact on fitness of nonbreeding habitat change and to develop appropriate conservation strategies.

Selenium (Se) is an essential micronutrient required in small quantities for normal biological function, but is toxic to vertebrates at concentrations slightly over essential levels, which are thought to range from 4 to 10 $\mu g \cdot g^{-1}$ (Heinz et al. 1989, Ohlendorf 2003). Selenium occurs naturally in the environment, but can be anthropogenically enriched primarily through burning of fossil fuels, irrigation of seleniferous soils, and mining and smelting of ores containing Se (Ohlendorf 2003). Selenium is also known to bioaccumulate with increasing trophic level within a food chain (Dobbs et al. 1996, Stuart et al. 2004), and concentrations are generally higher in marine than in freshwater environments (Haygarth 1994, Ohlendorf 2003). Although Se can be toxic to adult birds, developing embryos are considered far more sensitive, and ecotoxicologists should primarily be concerned with potential Se induced reproductive impairment (Heinz 1996). Embryos are exposed to Se by maternal transfer of organoselenium accumulated by the female through her diet, and hatching failure has been observed at dietary concentrations that are only slightly greater than background levels (Heinz et al. 1989, Stanley et al. 1994). But, Se is also eliminated from the body through natural metabolic processes and excretion, though the rate of elimination depends on tissue metabolism (Heinz et al. 1990, Ohlendorf 2003). Therefore, variation in Se exposure within a species on the wintering grounds may not be detected on the breeding grounds if Se is eliminated rapidly.

Stable-isotope analysis has emerged as a useful tool for studying cross-seasonal processes (Hobson 2005). Stable-carbon isotopes differ among marine-offshore, inshore, and freshwater systems, each respectively more depleted in $\delta^{13}C$ than the next, which can serve to identify large-scale patterns of habitat use (Smith et al. 1996, Hobson 1999). Stable-nitrogen isotopes reflect relative trophic status of organisms within a food web, in which a difference of *ca.* 3‰ for $\delta^{15}N$ normally represents one trophic level (DeNiro and Epstein 1981, Kelly 2000). Moreover, this bioindicator, $\delta^{15}N$, has been used to explain variation in contaminant levels within food chains (Kidd et al. 1995, Quinn et al. 2003). However, to date, no studies have reported on cross-seasonal sources of variation in Se concentrations in breeding birds.

Metabolically inert tissues, such as feathers or claws, represent the assimilated diet of an organism at the time those tissues were formed (Bearhop et al. 2003, Hobson et al. 2006). Due to their slow growth, the distal portion of claws is thought to reflect growth that occurred 2 to 5 mo prior to sampling (Bearhop et al. 2003, Hobson et al. 2006, RGC, *unpublished data*). Therefore, we examined whether there was a significant relationship between liver Se concentrations and claw tip $\delta^{15}N$ values in female White-winged Scoters (*Melanitta fusca*; hereafter scoters) collected from boreal breeding grounds. Scoters winter in marine and estuarine habitats yet breed in freshwater ecosystems in the boreal forest (Brown and Fredrickson 1997). We also examined whether variation in liver Se could be explained by claw tip $\delta^{13}C$, which could indicate differences in marine or estuarine wintering habitats. Given that Se bioaccumulates with increasing trophic level and is more enriched in marine than freshwater habitats, we tested the hypothesis that variation in Se levels in scoters on breeding grounds can be attributed to wintering ground trophic status or habitat preferences. Specifically, we predicted that liver Se concentrations of scoters on the breeding grounds would be positively related to claw tip $\delta^{13}C$ and $\delta^{15}N$ values.

## METHODS

We collected 49 female scoters near Inuvik, NT ($67^0$N, $133^0$W), over a 2-wk period in 2003 (n = 14) and 2004 (n = 35). Birds were approached from

shore or by canoe and when possible shot on the water to avoid excessive damage from shot. We did not use decoys to avoid condition bias (Pace and Afton 1999). Birds were individually bagged and stored under snow cover or in permafrost for up to 6 d before being frozen at -18°C and transported to the Prairie and Northern Wildlife Research Center, Saskatoon, Saskatchewan, for dissection and analysis.

Claws from the third toe of the right foot were removed from each bird and placed individually in 20 ml scintillation vials. Claws were soaked in a 2:1 choroform:methanol solution for a minimum 24 h, then drained and rinsed with new solution to remove surface contamination. Claws were air dried again for 24 h before a 0.95–1.05 mg sample (~3mm from a claw 8–10 mm in length) from the claw tip was removed for nitrogen isotope analysis. Samples were combusted using pyrolytic continuous-flow isotope-ratio mass spectrometry (CFIRMS) to determine carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotope ratios at the University of Saskatchewan, Saskatoon, Saskatchewan, Canada. All stable isotope ratio results are reported in delta notation (δ), in units of parts per thousand, i.e., parts per mil, (‰) and normalized to international standards ($\delta^{13}C$ – PeeDee Belemnite; $\delta^{15}N$ – air; http://www.canadianarchaeology.ca/radiocarbon/card/normal.htm ).

Measurement error (95% CI) based on results from reference materials, i.e., egg albumen, analyzed every eight samples was ± 0.35‰. Due to variation in baseline $\delta^{15}N$ among food webs, interpretation of this biomarker as relative trophic level should only be done for a food web within an ecosystem (Cabana and Rasmussen 1996). However, scoters overwinter on both the East and West coasts, and occasionally in freshwater habitats (Brown and Fredrickson 1997). Therefore, we used discriminant function analysis based on $\delta^{13}C$ and $\delta^{15}N$ from scoters wintering on the Canadian Atlantic and Pacific coasts to identify wintering origin of our collected birds (Swoboda 2007); all birds were identified as wintering in marine areas along the Pacific coast.

We also analyzed developing follicles for $\delta^{15}N$ to test whether claw samples or liver Se were derived from the breeding grounds, as scoters use dietary protein for egg formation (Dobush 1986, DeVink 2007. A lack of relationship between liver Se and follicle protein $\delta^{15}N$ would indicate that liver Se did not originate from the breeding grounds. Likewise, a lack of relationship between claw $\delta^{15}N$ and follicle protein $\delta^{15}N$ would be consistent with the assumption that claw tissues we sampled were not produced on the breeding ground. Large yolky follicles were oven dried at 80°C to a constant weight. Then, they were soaked, rinsed, and dried using the above method to remove lipids. Lean, dry follicles were sampled and analysed for $\delta^{15}N$ using the above methods.

Livers were removed from carcasses, placed individually in acid-washed glassware, and sent to the National Wildlife Research Center in Ottawa, Ontario, for Se analysis. Tissue samples were homogenized and approximately 0.5 g placed into preweighed, acid-washed test tubes, freeze-dried, and their dry masses recorded. Deionzed $H_2O$ (0.5 ml) and $HNO_3$ (either 0.5 ml or 1.0 ml) were added to each test tube, and samples were allowed to sit overnight at room temperature. The following day they were heated at 100°C in dry baths for 6 h. Samples were allowed to cool overnight, and volumes were then adjusted to 4.0 ml with deionized $H_2O$. Se was analyzed by graphite furnace atomic absorption spectrometry using the Perkin-Elmer 3030b equipped with a deuterium background corrector, HGA-300 graphite furnace, and AS-40 autosampler. All concentrations are reported on a dry weight basis. Standard reference materials (Tort-2, Dorm-2 and Dolt-2 from the National Research Council, Ottawa) were analyzed for quality assurance, and all samples were within certified limits. Five true duplicates were also analyzed and sample recoveries ranged from 0.3 to 11.3% relative standard deviation.

We used ANCOVA to test for effects of collection day, year, claw $\delta^{13}C$, claw $\delta^{15}N$, and year*collection day interaction on liver Se. In the absence of a significant year or year*collection day interaction effect, we removed the covariate year to reduce model complexity and used multiple regression analysis (Norušis 1990). We used ANCOVA to test for a relationship between liver Se concentration and follicle protein $\delta^{15}N$ levels while controlling for effects of collection date, and linear regression to test for a relationship between claw and follicle protein $\delta^{15}N$ values.

## RESULTS

Liver selenium (Se) concentration (ANCOVA; $F_{16}$ = 0.026, $P$ = 0.83) and claw $\delta^{15}N$ (linear regression; $F_{16}$ = 0.08, $P$ = 0.78) were not associated with follicle protein $\delta^{15}N$. There was no effect of year or year*collection day on liver Se (ANCOVA; $F_{1,44}$ < 0.12, $P$s > 0.73), so we reduced our model to include only claw $\delta^{15}N$, $\delta^{13}C$, and collection date as predictors of liver Se concentrations. Claw $\delta^{15}N$ values ranged from 12.9 to 17.0‰, spanning almost two trophic levels (DeNiro and Epstein 1981, Kelly 2000). Claw $\delta^{13}C$ ranged from -18.3 to -15.1‰ Se concentrations varied from 3.9 to 75.1 $\mu g \cdot g^{-1}$, and 31 of the 49 females had concentrations in excess of 33 $\mu g \cdot g^{-1}$, the threshold for physiological impairment for captive adult mallards (Heinz 1996). After controlling for collection date, there was a significant positive relationship between claw $\delta^{15}N$ and liver Se (multiple regression; $\beta_{clawN}$ = 3.9 ± 1.7 SE; $t_{1,46}$ = 2.258, $P$ = 0.029, $r$ = 0.32; Fig. 1), but not with $\delta^{13}C$ ($\beta_{clawC}$ = 1.4 ± 3.3 SE; $t_{1,46}$ = 0.42, $P$ = 0.68, $r$ = 0.06). There was a negative relationship ($\beta_{colldate}$ = -1.12 ± 0.39; $t_{1,46}$ = -2.844, $P$ = 0.007, $r$ = -0.39) between liver Se and collection date.

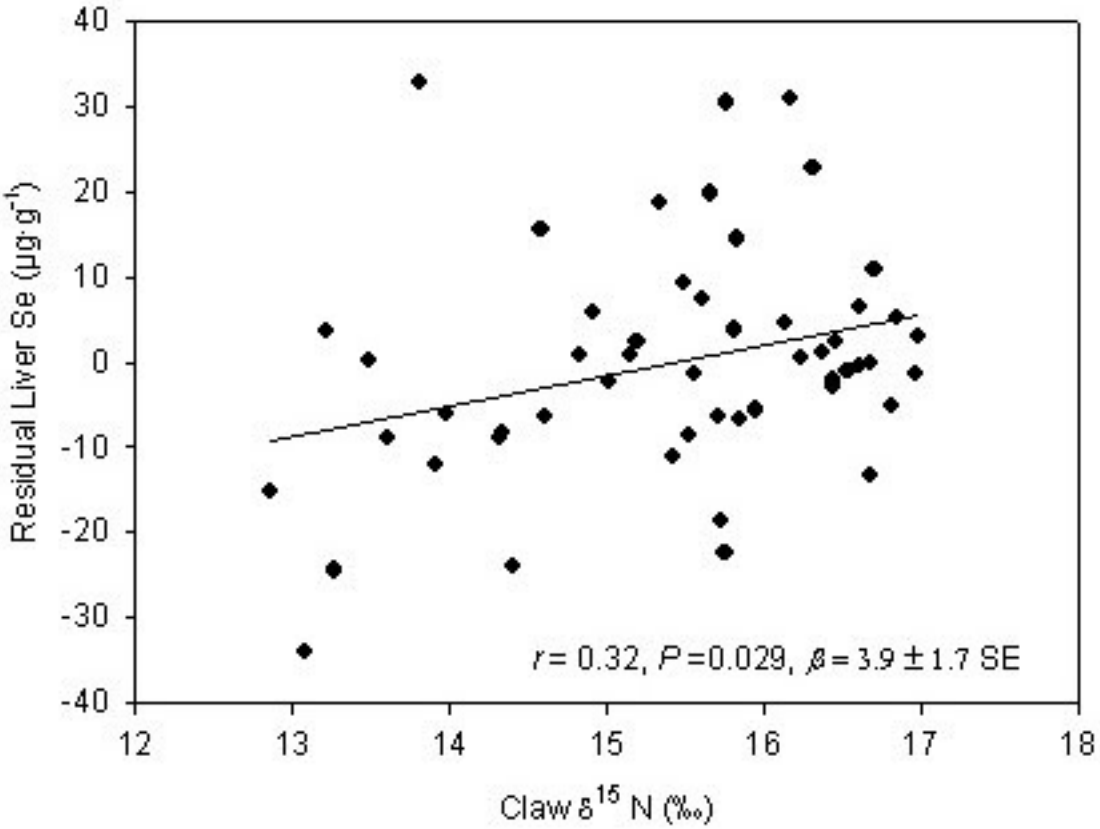
## DISCUSSION

The level of variation in both claw $\delta^{15}N$ and liver selenium (Se) provided adequate variation to detect an effect of winter trophic position on breeding ground liver Se concentrations. Based on this parameter estimate for claw $\delta^{15}N$, females feeding at approximately one trophic level ($\Delta$3‰ $\delta^{15}N$) higher on wintering grounds had 12 ± 5 $\mu g \cdot g^{-1}$ more liver Se at the time of collection (Fig. 1). Although wintering and migrant scoters are known to feed in both marine and estuarine habitats, which we assume would have lower Se concentrations due to freshwater inputs, we found no relationship between claw $\delta^{13}C$ and liver Se. Given that the range in claw $\delta^{13}C$ values (-18.3 to -15.1‰) were consistent with marine signals, this suggests that the scoters we sampled did not use estuarine systems extensively at the time claws were grown or when Se was acquired. The negative relationship between liver Se and collection date suggests that Se was likely depurated over time since leaving the marine wintering grounds (see also DeVink et al. 2007), but not to an extent that prevented the detection of a correlation between apparent winter trophic level and liver Se concentrations. This demonstrates that in migratory species, differences in individual exposure to Se on wintering and staging areas can carry over onto breeding grounds. These results are in agreement with those of other studies, which concluded that Se in tissues of Eider ducks during the breeding period was mainly derived from their diet while at sea during late winter and early spring (Grand et al. 2002, Wilson et al. 2004). High dietary exposure to Se in marine wintering and staging areas could pose a risk to species that rely on endogenous protein for egg formation, because embryonic development is a sensitive endpoint for Se toxicity in birds and Se deposition into eggs is likely in the form of amino acids (Heinz 1996).

One potential source of uncontrolled variation in Se exposure is the uncertainty about wintering population origins along the Pacific coast. Though we identified all our birds as wintering on the West coast, it is possible that they fed in locations with different baseline $\delta^{15}N$ values. Though this would cause differences in claw $\delta^{15}N$ (Cabana and Rasmussen 1996), it would not likely cause the relationship we observed between claw $\delta^{15}N$ and liver Se. Indeed, scoters primarily use protein acquired from dietary sources on breeding grounds to produce eggs (Dobush 1986, DeVink 2007). Therefore, the lack of relationship between follicle protein $\delta^{15}N$ and either claw $\delta^{15}N$ or liver Se, and the claw $\delta^{13}C$ values indicative of marine sources validated our assumption that liver Se was not acquired on the breeding grounds, and that the portion of claw we sampled did not represent a breeding ground diet signal. Furthermore, growth rates of the clipped middle claws of captive Lesser Scaup (*Aythya affinis*) held outdoors averaged 0.026 mm/d (95% confidence interval, 0.011 to 0.042 mm/d), suggesting that as much as 2.5–3.8 mm of claw could be replaced in 60–90 d (RGC, *unpublished data*). Given that these claws are typically 8–10 mm long, the 3 mm portion that we used should represent the isotopic signals acquired on wintering areas. Further investigation of claw growth rates in captive and wild birds would be informative.

There is interspecific variation in Se tolerance among birds, and species have likely adapted to different levels of Se exposure at the natural concentrations of their habitats (Skorupa 1998). The cross-seasonal relationship that we observed in somatic Se may have greater implications for species that winter or stage in habitats anthropogenically enriched in Se, e.g., the Great Lakes, where exposure may have increased above historical levels, and particularly those that use

**Fig. 1.** Plot of claw tip $\delta^{15}N$ vs. residuals of liver selenium (Se) concentration, corrected for effect of collection date, and partial correlation statistics.




endogenous nutrients for egg formation, e.g., Lesser Scaup (Esler et al. 2001), Northern Pintail (*Anas acuta*, Mann and Sedinger 1993). These birds may then accumulate higher Se burdens than normally experienced, and increased transfer to eggs may lead to reproductive failure through teratogenesis (Heinz et al. 1989) and subsequently to population level changes (Skorupa 1998). Cross-seasonal effects of wintering habitat quality on reproductive fitness have been demonstrated in migratory species (Norris 2005). Habitat quality on the wintering grounds may influence fitness through effects on timing of departure from wintering grounds (Marra and Holmes 2001) or body condition upon arrival (Heitmeyer and Fredrickson 1981, Gunnarsson et al. 2005). Our study demonstrates that wintering ground exposure to contaminants known to cause reproductive failure may result in high concentrations in females nesting in relatively pristine habitats. Scoters use exogenous protein to form eggs thus avoiding the deposition of potentially toxic levels of Se into eggs (Dobush 1986, DeVink 2007). However, in species that do use endogenous nutrients high in Se content, transfer to eggs and subsequent reproductive failure could impact populations if exposure was widespread.

*Responses to this article can be read online at:*
*http://www.ace-eco.org/vol3/iss1/art3/responses/*

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