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Detecting silent stressors: Trace element effects on nutritional status of declining scoter ducks of Puget Sound, USA

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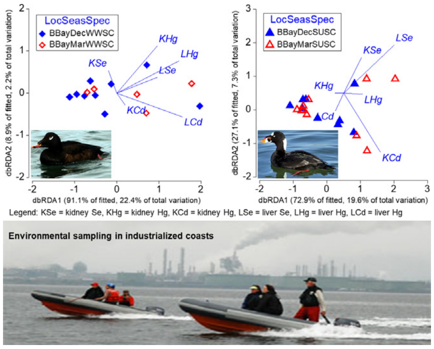
HIGHLIGHTS

- Wintering scoter ducks obtained by boat had sublethal levels of Cd, Hg, and Se.
- Health effects of contaminants can involve a suite of different physiological effects.
- Multivariate statistics can consider both multiple stressors and multiple responses.
- Multivariate methods explained 14 to 27% of winter declines in nutritional status.
- Integrated pollutant effects per seasonality can better inform population dynamics.

GRAPHICAL ABSTRACT



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ABSTRACT

White-winged scoters (*Melanitta fusca*; WWSC) and surf scoters (*M. perspicillata*; SUSC) have declined by over 60% in recent decades. Identifying contributing factors from within a mosaic of sublethal, multiple stressors is challenging. In urbanized Puget Sound, Washington, USA where scoters winter, changes in prey availability explained only a portion of local declines, suggesting that other "silent stressors" such as sublethal contaminants might play a role. Past studies of pollutant effects on scoters used Fisherian statistics that often revealed few correlates; however, novel statistical approaches could detect and provide more insights about sublethal impacts. Our objectives were to (1) relate pollutant accumulation to health of the birds, and (2) compare permutational multivariate statistics with traditional approaches in identifying sublethal health effects. We collected scoters from three locations in Puget Sound in December 2005 and March 2006, and measured cadmium (Cd), mercury (Hg), and selenium (Se) levels in livers and kidneys. To assess impacts of low contaminants levels in tissues on nutritional status (whole-body mass, lipid, and protein; and triglycerides, β-hydroxybutyrate, and uric acid in blood), we compared statistical methods. Permutational multivariate methods use Monte Carlo techniques to assess how an integrated matrix of physiological responses in each animal respond to contaminants. Univariate regressions revealed very few and inconsistent relationships. In contrast, multivariate models showed that liver Hg and Se explained 25% of the variance in nutritional status of white-winged scoters; and in surf scoters, Cd, Hg, and Se in tissues explained 14 to 27% of nutritional status depending on site. The influence of these factors equals

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other aspects of habitat such as foraging conditions. Our study indicates that permutational multivariate statistics can be a powerful tool for identifying sublethal contaminant associations that, with non-contaminant stressors, can influence nutritional status and thus, contribute to population dynamics.

1. Introduction

Across the planet, wild populations are declining in response to multiple anthropogenic stressors such as habitat destruction, changes in food webs, and chronic pollution (Dirzo et al., 2014; WWF, 2016). For example, for scoter ducks (*Melanitta* spp.), which winter at a number of sites from the Beaufort Sea to Puget Sound on the Pacific Coast of North America, counts have decreased by >60% over the past 25 to 50 years from a number of interacting factors, many of which remain unidentified (Dickson and Gilchrist, 2002; Nysewander et al., 2005; Anderson et al., 2020). Thus, for scoters as for many other species, a preeminent challenge in ecology is to quantify the role of multiple, sublethal stressors in their declines (Trust et al., 2000; Barjaktarovic et al., 2002; Lovvorn et al., 2013).

For white-winged scoters (*M. fusca*) and surf scoters (*M. perspicillata*) in Puget Sound (Fig. 1), Anderson and Lovvorn (2011) evaluated the effects of foraging conditions on nutritional status, which included seasonal differences in body composition (body mass, lipid, and protein) and plasma biomarkers (triglycerides, β-hydroxybutyrate, and uric acid). However, in that study and others, prey availability did not entirely explain variation in scoter nutritional status or spatial distributions (Lewis et al., 2007; De La Cruz et al., 2009; Lok et al., 2011). Waterbirds such as scoters that winter in urbanized coastal habitats, including Puget Sound, often carry substantial contaminant burdens (Ohlendorf and Fleming, 1988; Henny et al., 1990; Henny et al., 1991; Barjaktarovic et al., 2002). However, it has been difficult to assess sublethal contaminant impacts on the nutritional status of birds independently of foraging conditions or other factors affecting energy balance (Takekawa et al., 2002; Lovvorn et al., 2013).

Varying resource availability in winter is potentially important to effects of contaminants on organismal health. Past studies of contaminant exposure in scoters examined total pollutant uptake during brief periods in mid-winter, or broadly assessed differences among populations on marine wintering areas versus breeding areas (Elliott et al., 2007; DeVink et al., 2008; Gurney et al., 2014). However, few studies have investigated variations in contaminant levels among wintering subpopulations in the same region from early winter to pre-migration when prey can become limited.

Cadmium (Cd), mercury (Hg), and selenium (Se) are common contaminants in urbanized embayments, and occur in bivalves that are often the main prey of scoters in Puget Sound (USFWS, 1994; Johnson, 2000; E.M. Anderson et al., 2008). Even at sublethal levels, these contaminants have been linked to oxidative stress (Hoffman and Heinz, 1998) or to lower body mass in sea ducks (Henny et al., 1991), providing plausible support for a role of contaminants in scoter population declines. In long-lived species such as sea ducks, Cd that can inhibit spermatogenesis (Lofts and Murton, 1967; White et al., 1978) may be of particular concern due to its long biological half-life of from 3 to 12 months (Anderson and Van Hook Jr, 1973; Blomqvist et al., 1987). Mercury is a well-known neurotoxin that can affect endocrine levels (Adams et al., 2009; Tartu et al., 2014), behavior, and reproduction (Scheuhammer, 1987; Tartu et al., 2013). High levels of Se typically cause severe developmental and reproductive defects in freshwater waterfowl (Ohlendorf and Fleming, 1988; Heinz et al., 1989).

In this study, we use the nutritional status of scoters as our response variable because all means by which organisms cope with contaminants require redirection of energy from growth and reproduction (Newman and Clements, 2009). Organisms mitigate the effects of contaminants in

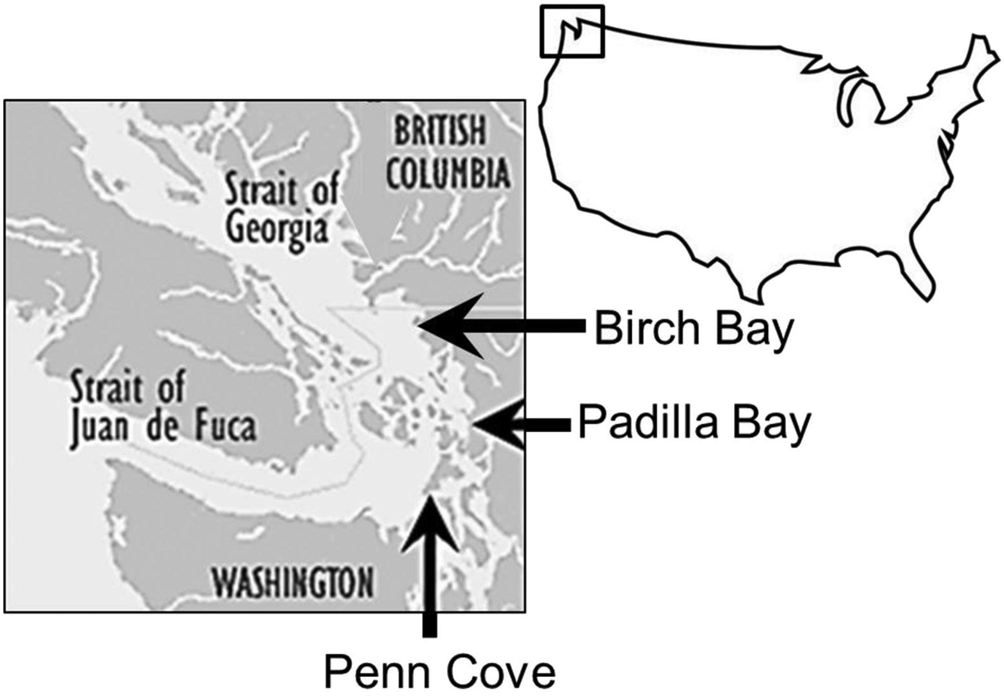

Fig. 1. Principal sample collection sites within Puget Sound, Washington, USA: Birch Bay, Padilla Bay, and Penn Cove.

five important ways: behavioral avoidance, sequestration, detoxification, elimination, and cellular repair of damage caused by toxicants. This dysfunctional redirection of energy or other resources away from desirable allocation links contaminant exposure to declines in nutritional status (Wayland et al., 2002; Elliott et al., 2007; Hargreaves et al., 2010; Sokolova et al., 2012). For example, studies on wintering surf scoters and greater scaup (*Aythya marila*) in San Francisco Bay showed that body mass decreased with increasing concentrations of hepatic Hg (Ohlendorf et al., 1991; Hoffman et al., 1998).

Accordingly, our study examined body composition and biomarkers that reflect nutritional status to assess how low tissue levels of contaminants potentially alter energy allocation in scoters (Adams et al., 1992; De Coen and Janssen, 2003; Ferreyra et al., 2015; Provencher et al., 2016). Body composition reflects a long timeframe of energy balance, and plasma biomarkers reveal changes in nutrient metabolism over the span of hours. Levels of plasma triglycerides are positively correlated with rates of fat synthesis and deposition, especially over the previous few hours (Ramenofsky, 1990; Jenni and Jenni-Eiermann, 1996; Seaman et al., 2006). In contrast, higher levels of plasma β-hydroxybutyrate (hereafter butyrate) reflect fat catabolism or loss (Anteau and Afton, 2008). Elevated uric acid indicates protein catabolism (Jenni-Eiermann and Jenni, 1991; Jenni-Eiermann and Jenni, 1996). In mallards (*Anas platyrhynchos*) dietary selenite increased plasma uric acid by 4-fold but a direct link with hepatic Se was not made (Hoffman and Heinz, 1998).

Assessing effects of sublethal levels of contaminants is exceptionally complicated, however, by many factors including the time course of response, varying physiological responses to different contaminants (Harding, 2008), and interactions among different contaminants (Möller, 1996). Contaminant levels might be too low to invoke a strong response, or individual vulnerability may be affected by energy reserves. Finally, mixtures of contaminant and non-contaminant stressors can have additive, synergistic, or antagonistic interactions (Jackson et al., 2016).

Fig. 2 shows that the general adaptation to any combination of chronic stress initially depresses and then triggers elevated metabolism during an alarm phase followed by a resistance period of sustained higher metabolism. Thus, for low exposure levels, homeostasis might regulate toxic effects without affecting survival or reproduction as long as energy reserves are adequate (Kooijman, 2000). Prolonged energy demands can shift the animal into exhaustion, during which energy demands exceed homeostasis and physiological performance plummets. (Selye, 1936; Selye, 1946; Beyers et al., 1999; Sokolova et al., 2012).

In the past, tissue concentrations of contaminants were related to individual variables such as body mass or biomarkers as single dependent variables, but results were sometimes contradictory. For example, in San Francisco Bay, Takekawa et al. (2002) reported decreased pancreatic mass and total body protein with increasing liver Se and Hg in canvasback ducks (*Aythya valisineria*), implying that coping with trace elements decreased the energy or nutrients available for building pancreatic or muscle tissue. However, at the same time, heart mass was positively related to liver Hg, and total carcass fat increased with higher Cd concentrations in kidney. Considering these complications, it is perhaps unrealistic to expect that a sublethal level of one element in one tissue will produce a statistically detectable effect on any single component of body composition or plasma metabolites. The key is to integrate the complex physiology of sublethal responses to suites of contaminant exposure. Using this approach, we can identify sublethal effects that might become important to energy balance when interacting with other stressors.

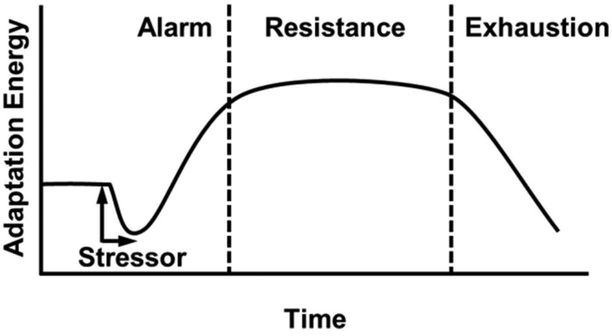

Fig. 2. The General Adaptation Syndrome presenting the three stages of energy demands as a function of time in animals challenged by stressors (after Selye, 1956).

1.1. Study objectives and hypotheses

We compared inference from traditional regression approaches that associate tissue accumulation of trace elements to single aspects of nutritional status to permutational, multivariate approaches. The multivariate approaches subsume complex physiological responses into a unique signature for each animal and can then be evaluated relative to simultaneous exposure to multiple trace elements. We compared these methods while testing two hypotheses for surf and white-winged scoters in Puget Sound: (1) levels of Cd, Hg, and Se in liver and kidney differ among seasons, locations, and species; and (2) detected differences are related to appreciable variations in nutritional status.

2. Materials and methods

2.1. Study area and sample collection

Surf scoters and white-winged scoters were collected by shotgun with steel shot ammunition from 12 to 13 March 2005, 10–14 December 2005, and 17–19 and 27 March 2006 (Anderson and Lovvorn, 2011). Collections occurred at three sites in Puget Sound: Birch Bay (48.9° N, 122.8° W), Padilla Bay (48.5° N 122.5° W), and Penn Cove (48.2° N, 122.7° W) (Fig. 1). These sites have distinct benthic habitat types that typify those available to scoters in Puget Sound, and supported appreciable numbers of scoters. Penn Cove has maximum depth at mean lower low water (MLLW) of ~25 m; it contains little vegetation, and the dominant prey items for surf scoters are mollusks in the intertidal zone and polychaete worms in the subtidal zone (E.M. Anderson et al., 2008). Padilla Bay contrasts sharply with Penn Cove in having mainly intertidal habitat with maximum depth at MLLW of ~2 m. Padilla Bay has one of the largest contiguous seagrass beds on the North American Pacific coast, supporting diverse epifaunal invertebrate prey consumed by surf scoters including shrimp, isopods, and snails. Birch Bay has maximum depth at MLLW of ~12 m with extensive sandy intertidal habitat and seagrass beds that increase in prevalence at lower intertidal to subtidal elevations. In Birch Bay, white-winged scoters consume bivalves and surf scoters have diverse diets (including especially polychaete worms, brittle stars, and bivalves), except in early spring when both species consume mainly spawn of Pacific herring (*Clupea pallasi*) during its brief period of availability (E.M. Anderson et al., 2008).

Fifty-one adult (after hatch year) male surf scoters (14 at Padilla Bay, 18 at Penn Cove, and 19 at Birch Bay) and 14 adult male white-winged scoters (all at Birch Bay) were collected. Females were excluded from all analysis due to a small collection sample ($n = 4$). Trace element data for females are presented in the Supplementary Information (SI) Table S1. We collected adult males to reduce impacts on population processes as much as possible. In this area, sex ratios in scoters in winter are skewed appreciably toward males because females tend to winter further south. Moreover, scoters captured along the Pacific Coast, including this study area, showed little evidence that overwinter patterns in body mass differ between the sexes (Anderson et al., 2009). After collection, liver and kidney tissues were placed into Whirl-Pak bags, and stored at −20 °C until analyzed for Cd, Hg, and Se. Whole-body proximate analysis and analyses of metabolites in blood plasma are described in Anderson and Lovvorn (2011), and the published data are included for reference in SI Table S2.

2.2. Contaminant analyses

Detailed descriptions of sample preparation and contaminant analyses are provided in the supplementary online information (SI Appendix S1). In brief, all frozen liver and kidney samples were freeze-dried to dry masses of 0.1 to 0.5 g, and then microwave digested (MARS Microwave Reactions System, CEM Corp). Using a Varian (now Agilent) 240FS Flame Atomic Absorption Spectrophotometer with an integrated Vapor Gas Accessory, samples were analyzed for Se by hydride-generation atomic absorption spectroscopy (Brumbaugh and Walther, 1991), and for total Hg analyses, tissues were analyzed using cold vapor atomic absorption spectroscopy (APHA et al., 2005). For cold vapor analyses, the reductant was a solution of stannous chloride with a carrier matrix of 18 MΩ water. For Cd analyses, tissues were analyzed on a Varian (now Agilent) 240/280 Zeeman Graphite Furnace Atomic Absorption Spectrophotometer with GTA Accessory.

Our quality assurance and quality check protocols required that all calibration verifications and quality checks fell within ±20% of the expected values or the sample analysis was repeated. Standards of sodium selenite (Na2SeO3; MP Biomedicals®), Hg, and Cd (Fisher Scientific®), were prepared using trace metal grade (TMG) hydrochloric acid (HCl; Se and Hg) or TMG nitric acid (HNO_3; Cd) and ultrapure 18 MΩ water. Calibration verifications included standard blanks, a mid-range standard, a randomly selected sample duplicate, and a spike for matrix effects (50% tissue sample with 50% EQC) that were run every 10 samples. External quality checks (EQCs) included NIST certified standard reference material® Bovine Liver 1577c (Fazio et al., 1993) as well as reference standards (8.5 and 20 μg SeO3/L; Fluka® Reference Standard for Se, or 25 μg Cd/L, and 25 μg Cd/L; Spex CertiPrep® for Cd and Hg). Both EQCs were analyzed at the beginning and end of each analytical run.

2.3. Data analyses

The 6 dependent variables for nutritional status were body composition (mass, lipid, and protein), and plasma metabolites (triglycerides, butyrate, and uric acid). Trace element concentrations (Se, Hg, Cd) in liver and kidney were the 6 independent variables. We describe statistical analyses in detail in the supplementary online material (SI Appendix S2). Briefly, we first conducted a principal components analysis (PCA) to assess whether structural size affected body composition (Lovvorn et al., 2003) using the statistical package R (R Core Team, 2014). We performed PCA analyses on common measurements of structural size: culmen length (The upper margin of the bill), 9th primary feather length (the stiff, penultimate flight feather on each wing), and tarsus length (leg bone above webbed foot), and then regressed eigenvalues for the first principle component (PC1) against the components of body composition, which were body mass, lipid, or protein. PC1 eigenvalues were not correlated with any of the three, so these variables were not corrected for structural size.

Descriptive summaries of trace element concentrations are presented as averages and geometric means (the nth root of the product of n samples). Outliers are defined as values more than two standard deviations from the arithmetic mean. We excluded them from the arithmetic means and two regression analyses but otherwise included them in all statistical analyses, The extreme values represented by outliers are discussed throughout Results and shown in Fig. 3.

2.3.1. Data analyses for hypothesis 1: hypothesis testing for differences in trace element concentrations among species, seasons, and locations

To test Hypothesis 1 for differences in trace element levels between species, site, and season, we used parametric analyses of variance (ANOVA) with Tukey's HSD post hoc tests to account for unequal variances (R Core Team, 2014). To test Hypothesis 1 with nonparametric, multivariate statistics, we used PRIMER ver.7 and PERMANOVA+ (PRIMER-e, Plymouth, UK). In the latter case, we first generated shade plots of both nutritional status and element levels in tissues. Shade plots are visual representations of the data that indicate whether one or two variables exert excessive influence due to greater absolute values and high variances. Given that the absolute values of our biological data spanned five orders of magnitude, shade plots indicated the need for an inter-quartile transform. The inter-quartile transform, which divides values by the interquartile range, is a relatively mild transform that makes widely differing variances among variables more similar without losing information provided by a particular factor. For a full discussion of transforms see (M. Anderson et al., 2008; Clarke et al., 2014; Clarke and Gorley, 2015) and references therein.

For permutational multivariate analyses, the six dependent variables or the six independent variables for each individual were evaluated simultaneously as distinct "signatures" centroids using Huygens theorem. We next generated resemblance matrices of nutritional status or tissue burdens from the Euclidean distances between each individual animal. The resemblance matrix for each group in our study was then used in hypothesis testing and inference analyses.

For hypothesis testing, we conducted permutational multivariate analysis of variance (PERMANOVA) to compare groups and seasons (for example, trace element concentrations in surf scoters from Birch Bay in December) (Anderson and Ter Braak, 2003). Permutation analyses take a similar approach to Monte Carlo simulations, which converge on patterns by repeated randomized sampling from a distribution (Mantel, 1967; Hope, 1968). Anderson and Robinson (2003) discuss the relationship between Monte Carlo and permutation tests in detail. Rather than sampling from a distribution, permutation analyses make comparisons by repeated sampling within the resemblance matrix of *assigned* groups versus *randomly assigned matrices* repeated 999 times (999 permutations) to produce familiar F statistics, *p*-values, and R^2 measures (Anderson and Walsh, 2013). For trace element levels, PERMANOVA analyses included main-effects tests for season or location, followed by independent pairwise *t*-tests to investigate differences between field sites within the same season, or within field sites between seasons or species (the latter possible only at Birch Bay).

To evaluate the potential for Type 1 errors (finding a difference when none exists) in PERMANOVA analyses, we conducted PERMDISP analyses (Tables S4, S5) to test for homogeneity of variance, and examined the stress values generated by unconstrained ordination of the data in non-metric multidimensional scaling (MDS) (Figs. S2, S3) (Clarke et al., 2014). Homogeneity of variance was supported in all but two comparisons (surf scoters, tissue accumulations in December vs March at Padilla Bay and Penn Cove). However, neither PERMANOVA pairwise comparison was significantly different (e.g. no Type 1 error occurred). Stress values in MDS were less than 0.2, which indicates separation in two-dimensional space and homogeneous dispersion. Finally, Type 1 errors are unlikely for relatively low sample sizes because increasingly large sample sizes cause p-values to converge on the a priori significance level (α), and lead to Type 1 errors. Given our small sample sizes, results of PERMDISP, and low stress values in MDS, the probability of Type 1 errors is highly unlikely in PERMANOVA analyses.

For interest relative to nutritional status, we conducted PERMANOVA analyses on location, season, and interspecific distinctions including PERMDISP assessments. Those results that support findings by Anderson and Lovvorn (2011) (SI Appendix S3, Tables S6, S7, S8, S9).

2.3.2. Data analyses for hypothesis 2: inferences of trace element effects on nutritional status

We next compared inference from univariate parametric regressions (single dependent variable, single or multiple independent variables) versus multivariate permutational methods. Using linear models in the statistical software R for both simple and multiple regression,

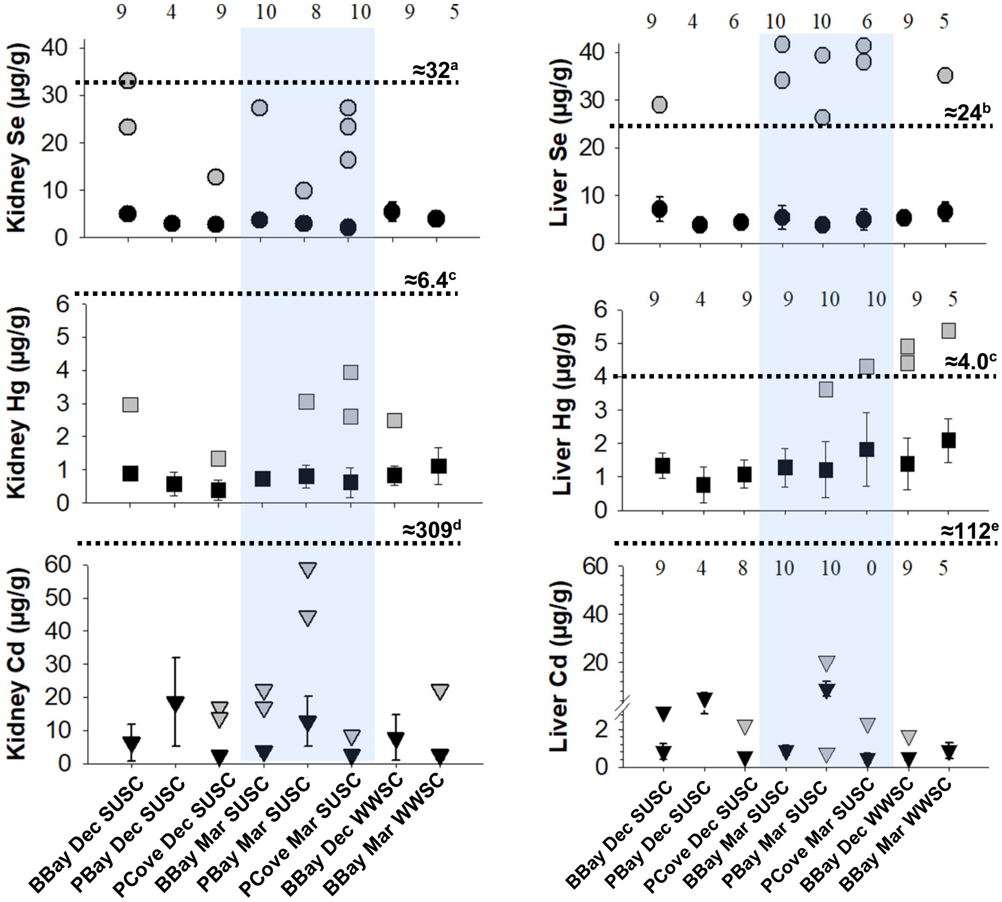

Fig. 3. Arithmetic means ± SD (black symbols, some SDs are covered by the symbol) and outliers (gray symbols) for trace element concentrations in kidney and liver among scoters at different sites in Puget Sound during December 2005 and March 2006. Shaded regions denote SUSC during March, which perhaps included incoming migrants. Values at the top indicate sample sizes. X-axis labels are organized by location, season, and species. BBay = Birch Bay, PBay = Padilla Bay, PCove = Penn Cove, Dec = December, Mar = March, SUSC = surf scoter, WWSC = white-winged scoter. Outliers were more than two standard deviations from means. For results of statistical tests, see Tables 1 and 2. Dashed lines and labels represent effect thresholds in captive mallards, converted from fresh mass to dry mass with Eqs. (1) and (2), from: [a](O'Toole and Raisbeck, 1997), [b](Heinz et al., 1989), [c](Heinz, 1979), [d](White et al., 1978), and [e](Cain et al., 1983).

adding a second-order polynomial when appropriate (R Core Team, 2014), we regressed each of the 6 dependent response variables on the 6 independent variables for the 8 separate sites or seasons in our data set. We chose least-squares linear models over generalized linear models because the latter are prone to more Type I errors with small sample sizes, especially if there is more than one independent variable (Ives, 2015). Thus, 288 linear regressions were potentially significant (e.g. for Birch Bay: 2 scoter species × 2 seasons × 6 dependent variables × 6 independent variables = 144). For surf scoters only at the 2 other field sites: 2 locations × 2 seasons × 6 dependent variables × 6 independent variables = 144. We also conducted multiple regression analyses of nutritional status among all 8 datasets, using both stepwise and best inclusion methods to evaluate the 56 potentially significant regressions.

For multivariate analyses of inference, we conducted distance-based redundancy analyses (dbRDA) to determine each individual's centroid signature in non-metric multidimensional space, followed by distance-based linear models (DistLMs), which are multivariate-multilinear regressions (Anderson et al., 2004). This approach can evaluate subtle distinctions in complex systems (Kraft et al., 2011). The dbRDA visually portrays nutritional status as centroids for each surf scoter and white-winged scoter. Radial vectors show the magnitude and direction of tissue levels of elements relative to nutritional status. DistLMs test for overall significance of a particular element, and then quantify how much variance in scoter condition is explained by the tissue accumulation of each element (the R^2 of each added element). To avoid over-fitting, we used Akaike information criteria. In this case, we used AICc, which corrects for small sample sizes. We chose the best AICc model depending on whether each added variable was significant or trending toward significance in its own right (i.e. $p\text{-value} \leq 0.10$) (Burnham and Anderson, 2002).

To compare our findings to levels of trace elements in the literature that others expressed as mg kg^{-1} wet mass, we developed regression relationships between our direct measures of wet masses versus freeze-dried masses of 64 kidneys and 63 livers. Converted values are denoted herein by the ≈ symbol. The equations for converting wet to dry masses were:

$$\text{dry kidney mass}\left(\text{mg kg}^{-1}\right) = 0.0096 + 0.240 \times \text{wet kidney mass}\left(\text{mg kg}^{-1}\right) \tag{1}$$

$$\text{dry liver mass}\left(\text{mg kg}^{-1}\right) = 0.152 + 0.224 \times \text{wet liver mass}\left(\text{mg kg}^{-1}\right) \tag{2}$$

Associated R^2 values were 0.83 for kidney and 0.81 for liver, both $p \leq 0.003$.

3. Results

Aside from a few individual outliers that we report and included in statistical analyses, average tissue levels of Cd, Hg, and Se were low compared to those in some other wild populations. Moreover, aside from single elements in a few individuals (see outliers on Fig. 3), values fell far below levels in laboratory studies that caused profound impairments such as mortality, deformity, or reproductive failure (Tables 1, 2; SI Table S3). Compared to studies on captive mallards, the arithmetic means of Cd herein were 0.6 to 4% of the kidney (540.7 mg kg^{-1} d.m.) and 0.1 to 2% of the liver (435.8 mg kg^{-1} d.m.) concentrations associated with protein breakdown and impaired carbohydrate metabolism (e.g. higher uric acid, lower body weight, lower aldolase activity) (Digiulio and Scanlon, 1984). Similarly, kidney Cd herein was 1 to 7% of the 72.2 mg Cd/kg w.m. (≈309.2 mg kg^{-1} d.m.) associated with testes necrosis and aspermatogenesis (White et al., 1978). While not directly comparable, levels herein were 0.6 to 8% of liver Cd in ducklings (42.21 mg kg^{-1} w.m.; ≈112 mg kg^{-1} d.m.) associated with low serum glutamic pyruvic transaminase and lower hemoglobin levels (Cain et al., 1983). Even our highest observed tissue kidney or liver Cd concentrations were 20 to 5% of toxic thresholds.

Apparently, the only captive study evaluating toxicosis relative to kidney Hg was conducted on female mallards, and thus, not directly comparable. Kidney Hg in male surf and white-winged scoters herein were 8 to 17% of the level (1.60 mg kg^{-1} w.m.; ≈6.41 mg kg^{-1} d.m.) causing reproductive impairment in captive female mallards fed Hg. Compared to liver thresholds of toxicity in male mallards, means herein were 19 to 69% of toxic levels (1.49 mg kg^{-1} w.m.; ≈3.96 mg kg^{-1} d.m.) (Heinz, 1979). That threshold in liver Hg was exceeded in one surf scoter (4.3 mg kg^{-1} d.m.), and three white-winged scoters (4.4, 4.9, and 5.4 mg kg^{-1} d.m.).

For kidney Se, captive male mallards developed alopecia and epithelial necrosis at kidney levels with an arithmetic average of 8 mg kg^{-1} w.m. (≈32 mg kg^{-1} d.m.) (O'Toole and Raisbeck, 1997). Groups in this study were 9 to 31% of that mean. For liver Se, several individuals (39, 41, and 41 mg kg^{-1} d.m.) exceeded the arithmetic mean of 9.1 mg kg^{-1} w.m. (≈24.2 mg kg^{-1} d.m.) associated with mallard reproductive impairment (Heinz, 1996). However, arithmetic means of the groups herein were 16 to 51% lower than those toxic tissue levels.

Anderson and Lovvorn (2011) reported that for both scoter species almost every aspect of nutritional status trended toward poorer health in spring. Our separate PERMANOVA analyses and results support their findings (SI Appendix S3, Tables S6 and S7).

3.1. Hypothesis 1. Trace element concentrations differ by location, season, and species

Parametric approaches partly supported hypothesis 1 (Tables 1, 2). Recalling that only surf scoters were collected at all three field sites, parametric results using two-way ANOVA indicated higher Cd levels in both kidney and liver at Padilla Bay compared to Birch Bay or Penn Cove in December and March. Within each field site, tissue levels did not differ seasonally in surf scoters at any field site. Similarly, trace element levels did not differ seasonally in white-winged scoters. For interspecific comparisons at Birch Bay, white-winged scoters had higher liver Hg levels than surf scoters only in March. No other contaminant levels differed significantly between the two species.

Regarding distinctions among locations, both main-effects PERMANOVA analyses ($p = 0.001$, Table 3a) and pairwise PERMANOVA *t*-tests ($p \leq 0.005$, Table 4a) supported differences in tissue levels in surf scoters with the single exception that concentrations did not differ between Birch Bay versus Penn Cove in March ($p = 0.81$, Table 4a). Seasonal changes in tissue levels between December and March were not significant in either surf scoters ($p = 0.18$) or white-winged scoters ($p = 0.20$) according to either main effects PERMANOVA or pairwise t-tests in PERMANOVA ($p \geq 0.14$, Table 4b and c). As with parametric tests, PERMANOVA did not indicate interspecific differences in element levels at Birch Bay (Table 3c, $p \geq 0.16$), and this pattern held true regardless of season (Table 4c, $p \geq 0.17$).

3.2. Hypothesis 2. Higher trace element concentrations correlate with seasonal declines in nutritional status.

Using parametric approaches with $\alpha = 0.10$, six univariate regression models were significant out of the 288 possible comparisons between individual elements and single aspects of nutritional status (Fig. 4). For surf scoters from Penn Cove, no linear regressions were

Table 1

Kidney concentrations (mg kg^{-1} dry mass, arithmetic mean ± SD, and geometric mean and range in parentheses in second line) of Cd, Hg, and Se for male surf scoters (SUSC) and white-winged scoters (WWSC) in Puget Sound in 2005 to 2006. Within species and trace element, means sharing the same letter do not differ between sites (two-way ANOVA with Tukey HSD post hoc tests on log-transformed contaminant values, $p < 0.05$).

Subpopulation	*n*	[Cd] mg kg^{-1} arith mean ± SD geom mean (range)	*n*	[Hg] mg kg^{-1} arith mean ± SD geom mean (range)	*n*	[Se] mg kg^{-1} arith mean ± SD geom mean (range)
SUSC Dec 2005						
Birch Bay	9	**A** 6.23 ± 5.49 4.05 (1.13–15.71)	9	**A** 0.88 ± 0.22 0.86 (0.67–1.41)	9	**A** 10.08 ± 10.57 7.09 (3.70–33.03)
Padilla Bay	4	**B** 18.55 ± 13.44 23.99 (4.11–33.25)	4	**A** 0.57 ± 0.35 0.49 (0.26–1.03)	4	**A** 2.85 ± 0.91 2.73 (1.84–3.98)
Penn Cove	9	**A** 5.17 ± 5.88 3.11 (0.55–16.85)	7	**A** 0.48 ± 0.43 0.50 (0.13–1.34)	9	**A** 3.85 ± 3.42 3.13 (1.47–12.73)
SUSC Mar 2006						
Birch Bay	10	**A** 6.81 ± 6.95 4.78 (1.70–22.22)	10	**A** 0.66 ± 0.27 0.72 (0.52–0.95)	10	**A** 5.95 ± 7.60 4.13 (2.08–27.26)
Padilla Bay	8	**B** 22.44 ± 19.54 15.82 (5.34–58.88)	8	**A** 0.99 ± 0.93 0.92 (0.49–3.06)	8	**A** 3.80 ± 2.52 3.34 (2.22–9.81)
Penn Cove	10	**A** 3.34 ± 2.15 2.89 (1.45–8.59)	9	**A** 1.08 ± 1.27 0.72 (0.13–3.94)	10	**A** 8.11 ± 10.15 4.07 (1.39–27.29)
WWSC Dec 2005[a]						
Birch Bay	9	**A** 7.79 ± 6.93 5.21 (1.71–17.68)	9	**A** 1.02 ± 0.62 0.89 (0.51–2.50)	9	**A** 5.39 ± 2.03 5.00 (2.47–7.81)
WWSC Mar 2006[a]						
Birch Bay	5	**A** 6.52 ± 8.89 3.46 (0.75–22.25)	5	**A** 1.11 ± 0.56 1.00 (0.54–1.95)	5	**A** 3.88 ± 1.47 3.62 (1.90–5.55)

[a] For WWSC, letters indicate comparisons only with the SUSC at Birch Bay, and not comparisons with all SUSC.

Table 2
Liver concentrations (mg kg^{-1} dry mass, arithmetic mean ± SD, and geometric mean and range in parentheses in second line) of Cd, Hg, and Se for male surf scoters (SUSC) and white-winged scoters (WWSC) in Puget Sound in 2005 to 2006. Within species and trace element, means sharing the same letter do not differ between sites (two-way ANOVA with Tukey HSD post hoc tests on log-transformed contaminant values, $p < 0.05$).

Subpopulation	*n*	[Cd] mg kg^{-1} arith mean ± SD geom mean (range)	*n*	[Hg] mg kg^{-1} arith mean ± SD geom mean (range)	*n*	[Se] mg kg^{-1} arith mean ± SD geom mean (range)
SUSC Dec 2005						
Birch Bay	9	**A** 1.07 ± 0.81 0.83 (0.26–2.96)	9	**A** 1.33 ± 0.38 1.29 (0.88–1.90)	9	**A** 9.48 ± 7.66 7.72 (2.72–28.87)
Padilla Bay	4	**B** 5.07 ± 2.26 4.53 (1.87–7.17)	4	**A** 0.76 ± 0.54 0.65 (0.45–1.57)	4	**A** 3.81 ± 0.88 3.74 (2.89–4.91)
Penn Cove	6	**A** 0.78 ± 0.61 0.67 (0.37–2.26)	6	**A** 1.09 ± 0.43 0.98 (0.30–1.48)	9	**A** 4.31 ± 1.26 4.14 (2.47–6.21)
SUSC Mar 2006						
Birch Bay	10	**A** 0.89 ± 0.25 0.86 (0.61–1.33)	10	**A** 1.28 ± 0.58 1.17 (0.47–2.69)	10	**A** 11.89 ± 13.95 6.30 (3.06–41.55)
Padilla Bay	10	**B** 9.10 ± 5.42 6.96 (0.76–20.29)	10	**A** 1.46 ± 1.10 1.15 (0.43–3.63)	10	**A** 9.62 ± 12.61 5.80 (2.78–39.31)
Penn Cove	10	**A** 0.67 ± 0.64 0.49 (0.10–2.34)	6	**A** 2.24 ± 1.41 1.80 (0.47–4.30)	10	**A** 11.87 ± 14.76 7.01 (2.69–41.26)
WWSC Dec 2005[a]						
Birch Bay	9	**A** 0.65 ± 0.42 0.57 (0.33–1.67)	9	**AB** 2.11 ± 1.59 1.64 (0.56–4.90)	9	**A** 5.27 ± 1.40 5.12 (3.72–8.45)
WWSC Mar 2006[a]						
Birch Bay	5	**A** 0.89 ± 0.42 0.80 (0.45–1.34)	5	**B** 2.75 ± 1.57 2.46 (1.41–5.37)	5	**A** 12.27 ± 12.86 7.58 (3.74–35.07)

[a] For WWSC, letters indicate comparisons only with the SUSC at Birch Bay, and not comparisons with all SUSC.

significant. At other locations, regression slopes were sometimes positive or sometimes negative. For example, at Birch Bay in December, regression models indicated that increased liver Se explained 54% of the decline in body mass of surf scoters, 55% lower protein in surf scoters, and 34% in white-winged scoters. Higher liver Hg explained 40% of lower lipid content in white-winged scoters in December at Birch Bay. In contrast according to regression models, higher Hg in the kidney explained 65% of increasing body mass and 92% of protein increases in surf scoters in March at Padilla Bay. Neither kidney Cd nor liver Cd explained an aspect of nutritional status. None of the 56 potential combinations of multiple regression analyses of nutritional status among all 8 datasets were significant.

Permutational dbRDA and DistLM analyses simultaneously evaluate the matrix of dependent variables that comprise nutritional status relative to multiple trace elements. For surf scoters at Padilla Bay, the best AICc model indicated that liver Hg explained 19% of their decline in nutritional status. For surf scoters at Penn Cove, Cd levels in the kidney explained 14% of the variation in their nutritional status. Among surf scoters at Birch Bay, the DistLM model indicated that liver Se and kidney Cd explained up to 27% of the change in nutritional status of surf scoters in winter. For white-winged scoters at Birch Bay, the best AICc model indicated that Hg and Cd levels in livers explained 24% of changes in nutritional status (Table 5, Fig. 5).

Table 3
Results of PERMANOVA main-effects comparisons of trace element levels in tissues of (a) surf scoters (SUSC) and (b) white-winged scoters (WWSC) in Puget Sound.

Source	df	SS	MS	F	*p*
a) SUSC tissue levels by location or season					
Location	2	363.06	181.53	9.38	0.001
Season	1	29.74	29.74	1.54	0.178
Location × season	2	28.53	14.26	0.74	0.594
Residual	45	870.71	19.35		
Total	50	1292.00			
b) WWSC tissue levels by season					
Season	1	6.64	6.64	1.47	0.202
Residual	12	54.14	4.51		
Total	13	60.78			
c) WWSC vs. SUSC tissue levels at Birch Bay only					
Species	1	27.07	27.07	1.7131	0.158
Residual	31	489.86	15.802		
Total	32	516.93			

Regarding bioaccumulation of Se and Hg, their molar ratios averaged 18.2:1 in liver and 19.8:1 in kidney tissues (SI Fig. S1).

4. Discussion

4.1. Challenges in detecting sublethal effects of contaminants

Detecting impacts of sublethal stressors on wild, mobile populations such as scoters is a profound challenge for many reasons. These reasons

Table 4
Results of PERMANOVA pairwise t-tests of trace elements in tissues of: (a) surf scoters (SUSC) within collection months among field sites; (b) SUSC and white-winged scoters (WWSC) within field sites between collection months; and (c) inter-specific differences between SUSC and WWSC collected only at Birch Bay.

Comparison	DF	t	*p*
a) SUSC levels in December			
Birch Bay vs. Padilla Bay	10	2.39	0.005
Birch Bay vs. Penn Cove	16	1.82	0.005
Padilla Bay vs. Penn Cove	10	4.62	0.001
a) SUSC levels in March			
Birch Bay vs. Padilla Bay	18	2.74	0.001
Birch Bay vs. Penn Cove	18	0.81	0.805
Padilla Bay vs. Penn Cove	18	2.64	0.001
b) SUSC levels between months			
Birch Bay Dec vs. Mar	17	0.64	0.651
Padilla Bay Dec vs. Mar	11	0.79	0.624
Penn Cove Dec vs. Mar	17	1.42	0.138
b) WWSC levels between months			
Dec vs. Mar	12	1.47	0.202
c) SUSC vs. WWSC levels at Birch Bay			
December	16	1.28	0.167
March	15	0.89	0.508

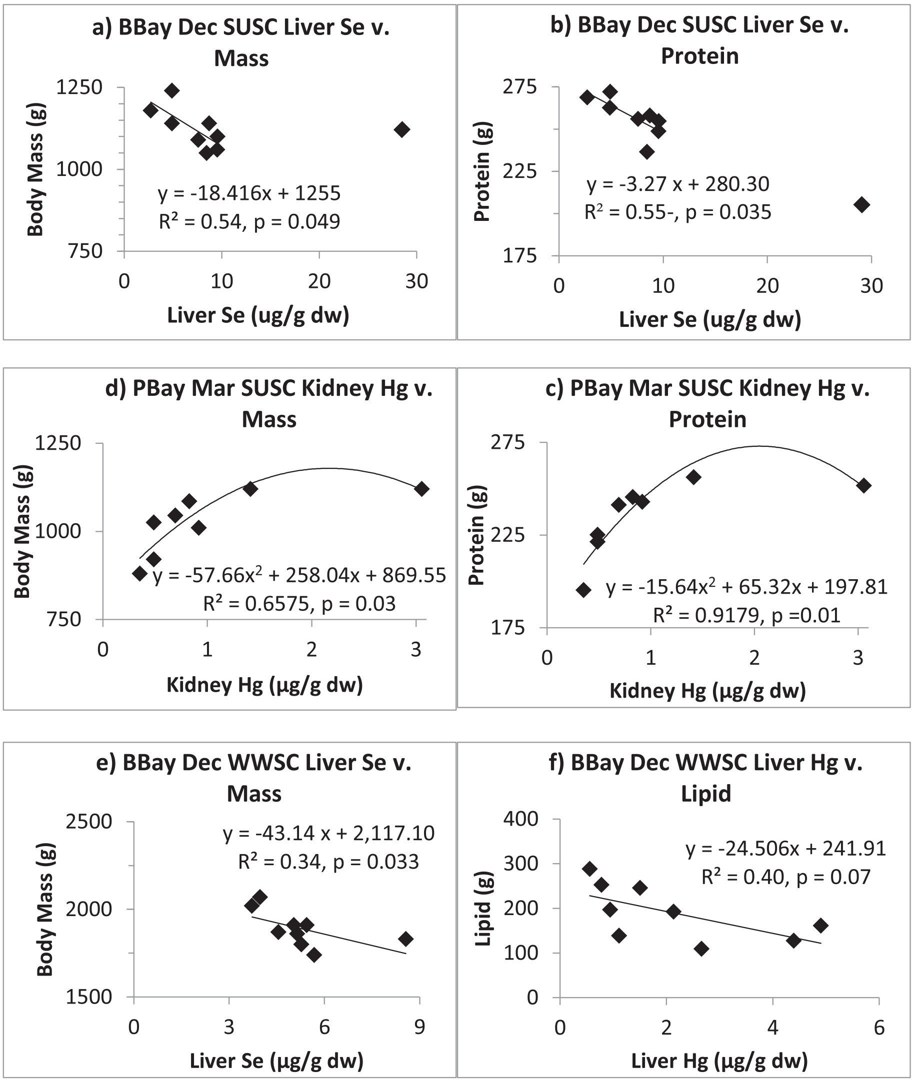

Fig. 4. Simple regressions of surf scoters (SUSC) collected at Birch Bay (BBay) in December 2005 (panels a and b) and at Padilla Bay (PBay) in March 2006 (panels c and d), and of white-winged scoters (WWSC) collected at Birch Bay in December 2005 (panels e and f). Outliers are not included in panels a and b. Of the 288 possible comparisons, no other univariate regression relationships between levels of trace elements and nutritional status had $p < 0.10$.

include high variances and low levels of any single pollutant in tissues (Ohlendorf et al., 1986; Barjaktarovic et al., 2002; Elliott et al., 2007), and variable responses of different physiological processes to different combinations of metals (Kamunde and MacPhail, 2011). Low contaminant exposure can actually stimulate metabolism, as opposed to impairing physiological function at higher doses (a phenomenon known as hormesis). In red-winged blackbirds (*Agelaius phoeniceus*), mild stress from low Se exposure was stimulatory: low Se exposures increased egg hatchability, which then declined if egg Se concentrations increased above 10 mg kg^{-1} dry mass (Harding, 2008). Moreover, energy reserves or ample food availability can alter the positive hormetic response or the duration of homeostasis (Kooijman, 2000). Takekawa et al. (2002) suggested that for ducks arriving at a polluted site with high energy reserves, their exposure to contaminants triggered higher metabolic rates and faster growth. Contaminant levels might also be too low to induce a statistically significant effect because the insult barely exceeds homeostatic control. Finally, in wild sea ducks (tribe Mergini), effects of chronic exposure to trace elements may be minimal because the birds apparently tolerate tissue levels that are often far higher than would severely affect or kill mainly freshwater duck species (Franson et al., 2007; Wilson et al., 2007; Lovvorn et al., 2013; Miller et al., 2016). Detecting the net sublethal effects of these complex factors requires considering a suite of different but covarying health responses to multiple interacting elements.

In contrast to assessing contaminant levels relative to thresholds for acute toxicity (Eisler, 2000; Fox et al., 2005), the goal of our study was to examine contaminant effects on physiological variables that reflect energy and nutrient balance, and to compare them among wintering populations. The question of interest is whether sublethal trace element levels in scoter tissues can exacerbate the seasonal loss of energy reserves by constituting a chronic physiological burden. In fact, levels of single trace elements in scoters were well below thresholds of overt physiological impairment determined for captive mallards. Different individual scoters had high concentrations of different elements, with no individual having high levels of all three elements. Thus, analyzing single inorganic contaminants in isolation from other contaminants would have limited our understanding of overall health effects and their ecological repercussions (Möller, 1996).

4.2. Comparing statistical approaches: differences among sites and seasons

Our results for Hypothesis 1 highlight some distinctions between the two statistical approaches. Between field sites, parametric ANOVAs indicated that surf scoters collected a Padilla Bay had higher kidney and

Table 5

(a) Distance-based multivariate model (DistLM) and (b) AICc results for surf scoters and white-winged scoters at each location in Puget Sound. Marginal tests show the proportion of variance in nutritional status explained by individual trace elements in each organ. Bold fonts indicate marginal relationships with $p < 0.10$, and the corresponding AIC_c model that includes only those factors. Key: KSe = kidney Se, KCd = kidney Cd, LSe = liver Se, LHg = liver Hg, LCd = liver Cd, RSS = relative sum of squared deviations.

	(a) DistLM marginal tests					(b) AICc models			
	Factors	SS (trace)	Pseudo-F	p	Prop.	AICc	RSS	Factors	R^2
Surf scoters at Birch Bay									
KSe	1	3.76	0.88	0.465	0.049	28.37	65.86	4	0.137
KHg	2	4.76	1.13	0.382	0.062	**28.07**	**55.80**	**3,4**	**0.269**
KCd	3	8.19	2.04	**0.078**	0.107	29.46	50.59	2–4	0.337
LSe	4	10.50	2.71	**0.033**	0.137	31.98	47.39	2–4,6	0.379
LHg	5	4.46	1.05	0.379	0.058	35.24	44.67	1–4,6	0.415
LCd	6	4.05	0.95	0.461	0.053	39.49	42.53	All	0.443
Surf scoters at Padilla Bay									
KSe	1	2.39	0.58	0.700	0.050	**19.38**	**38.69**	**5**	**0.194**
KHg	2	4.43	1.12	0.349	0.092	21.25	34.23	2,5	0.287
KCd	3	5.01	1.28	0.264	0.104	24.24	30.87	2,4,5	0.357
LSe	4	3.63	0.90	0.471	0.076	29.07	29.14	1,2,4,5	0.393
LHg	5	9.33	2.65	**0.042**	0.194	35.86	27.76	1,2,4–6	0.422
LCd	6	2.68	0.65	0.620	0.056	45.54	26.25	All	0.453
Surf scoters at Penn Cove									
KSe	1	9.84	1.03	0.377	0.057	**43.76**	**148.07**	**3**	**0.138**
KHg	2	5.85	0.60	0.597	0.034	44.85	134.92	1,3	0.215
KCd	3	23.76	2.73	**0.079**	0.138	47.25	129.03	1,3,6	0.249
LSe	4	8.17	0.85	0.447	0.048	50.12	123.09	1,3,5,6	0.284
LHg	5	5.01	0.51	0.666	0.029	53.61	117.43	1,3–6	0.317
LCd	6	4.58	0.47	0.691	0.027	58.66	116.64	All	0.321
White-winged scoters at Birch Bay									
KSe	1	3.64	0.84	0.487	0.065	**23.81**	**42.10**	**5,6**	**0.245**
KHg	2	3.40	0.78	0.538	0.061	26.40	37.93	2,5,6	0.320
KCd	3	1.91	0.43	0.808	0.034	29.41	32.77	2,3,5,6	0.413
LSe	4	3.36	0.77	0.509	0.060	33.80	28.19	1–3,5,6	0.494
LHg	5	10.11	2.65	**0.053**	0.181	41.86	26.99	All	0.516
LCd	6	11.20	3.01	**0.035**	0.201				

liver Cd levels than other locations in both December and March but revealed no other differences. By assessing contaminant levels in concert, PERMANOVA yielded potentially greater insight into effects of location by demonstrating that tissue levels differed among all field sites in both seasons with the single exception of Birch Bay versus Padilla Bay in March. As for interspecific distinctions, PERMANOVA showed no differences in tissue levels, whereas parametric ANOVA detected higher hepatic Hg in white-winged scoters than in surf scoters in March. Neither method indicated seasonal differences in contaminant levels, meaning chronic burdens of trace elements were superimposed on regular declines in nutritional status of scoters from December to March (Anderson and Lovvorn, 2011).

4.3. Comparing statistical approaches: contaminants and nutritional status

In our study, the rare and often conflicting correlations of simple linear regressions illustrate the complications of investigating sublethal effects. Of the 288 possible comparisons, only 6 regression relationships were statistically significant ($p < 0.10$). Two regression models indicated that liver Se was negatively correlated with body mass and protein in surf scoters and body mass in white-winged scoters collected soon after arrival in December. Liver Hg was higher in white-winged scoters arriving with lower lipid levels at Birch Bay. Ecologically, negative slopes are consistent with the exhaustion phase of stress adaptation and some level of impairment. In contrast, for surf scoters at Padilla Bay in March, regression models indicated that sea ducks with higher body mass and protein levels had higher levels of kidney Hg, potentially indicating stimulatory growth relative to tissue burdens over winter (Fig. 2, alarm phase). However, the more plausible explanation is likely greater Hg accumulation by older, larger birds. Such conflicting results are similar to those reported for scaup wintering in San Francisco Bay, migrating through the mid-continent of North America, or breeding in Alaska (Takekawa et al., 2002; Anteau et al., 2007; Badzinski et al., 2009). Takekawa et al. (2002) suggested that for animals arriving at a polluted site with high energy reserves, their exposure to contaminants triggered higher metabolic rates and faster growth. Nonetheless, inconsistent effects for single dependent variables make it difficult to characterize overall patterns. Moreover, contaminants may shift scoters from the resistance phase of Fig. 2 to the exhaustion phase when this burden is superimposed on regular declines in nutritional status of scoters from December to March (Anderson and Lovvorn, 2011).

In contrast, for birds collected in Birch Bay from December to March, DistLM models of the apparent effects of trace elements are consistent with findings from Anderson and Lovvorn (2011) of declining nutritional status over winter. While the only statistically significant seasonal change in body composition or plasma metabolites was a loss in total body lipids, losses were considerable. On average, lipids in surf scoters declined by 28% in spring, and 20% in white-winged scoters. Fig. 5 depicts the complex physiological changes in nutritional status across winter in multivariate space. Based on the degree of uncertainty described above in Section 4.1, we do not speculate about specific mechanisms. Despite the challenges of characterizing the suite of interactions among pollutants with different relative concentrations and effects, DistLM models indicated that kidney Cd and liver Hg explained 27% of nutritional status in surf scoters. For white-winged scoters, hepatic Hg and Cd levels explained 24% of nutritional status.

Between December and March, sea ducks at Padilla Bay and Penn Cove showed significant declines in body mass (6 and 13%), total lipid (48 and 44%), and plasma triglycerides (19 and 50%). Triglyceride levels indicate fat catabolism, and thus, were consistent with weight loss. Among surf scoters at Padilla Bay, liver Hg explained 19% of their nutritional status across the wintering period. For surf scoters collected at Penn Cove, DistLM models indicated that kidney Se explained 14% of overall nutritional status.

We recognize that correlation may not be causation or fully characterize relationships between contaminant levels and seasonal declines

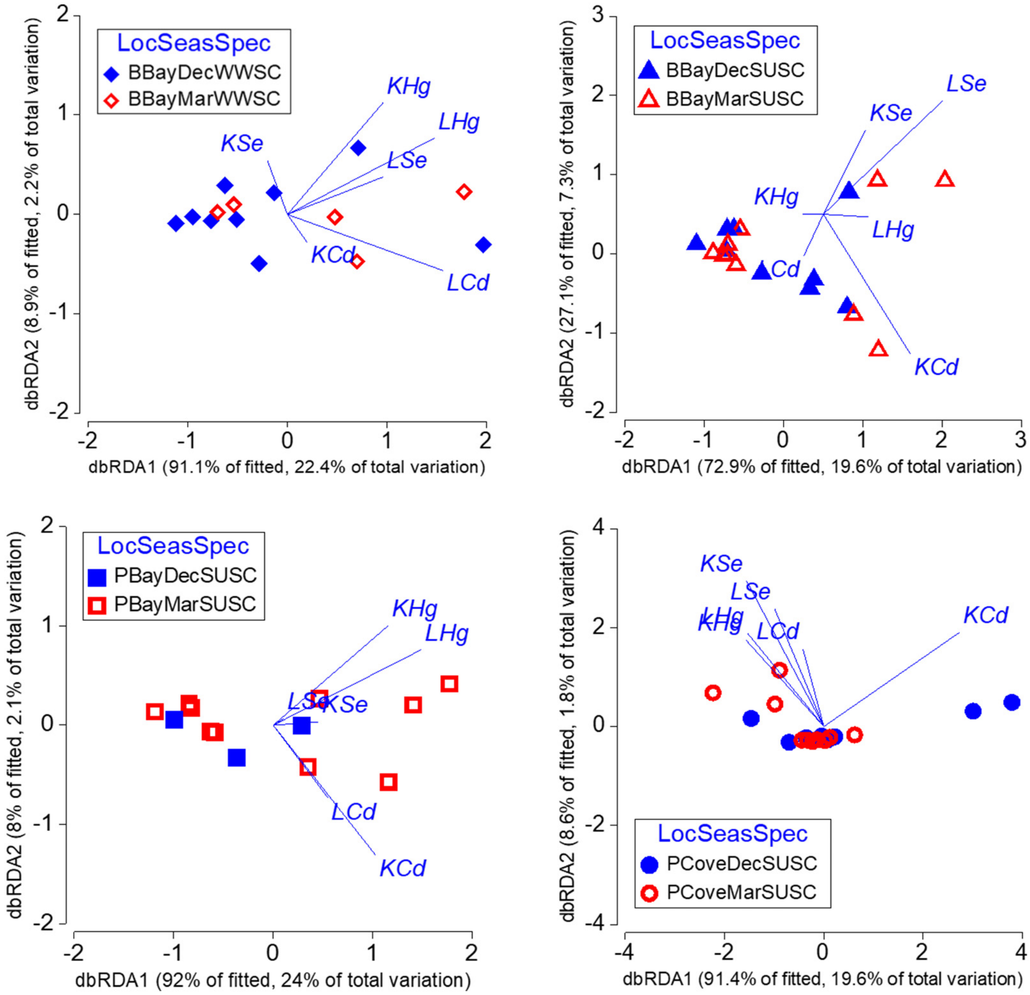

Fig. 5. Distance-based redundancy analysis (dbRDA) plots for all individual scoters within each location (Loc) by season (Seas) by species (Spec). Symbols represent centroids for each individual of nutritional status (body mass, protein, and lipid; plasma triglycerides, butyrate, and uric acid). Radial vectors depict the magnitude and direction of independent variables relative to nutritional status. Axis labels show percentage contribution to the ordination. Legends are organized by location, season, and species. BBay = Birch Bay, PBay = Padilla Bay, PCove = Penn Cove, SUSC = surf scoter, WWSC = white-winged scoter, Dec = December, Mar = March, KSe = kidney Se, KHg = kidney Hg, KCd = kidney Hg, LSe = liver Se, LHg = liver Hg, LCd = liver Hg.

in nutritional status. These highly mobile birds shift locations and habitat types over time during winter (Anderson and Lovvorn, 2011) depending on food availability; thus, it is unclear where the individuals sampled accumulated (or depurated) trace elements over winter at various urban and industrialized sites in the Puget Sound region. For example, despite low contaminant levels, we expected stronger relationships between trace elements and nutritional status in sea ducks at Penn Cove because if food resources are plentiful, then the thresholds for effects might be higher (Kooijman, 2000). Based on changing bird counts among the field sites between December and March, Anderson and Lovvorn (2011) concluded that surf scoters mainly left Penn Cove as food resources became limited, while increasing in numbers at Birch Bay and Padilla Bay. Unchanged or increased wet mass of bivalve prey in scoter esophagi and gizzards indicated that in Birch Bay regardless of season both species were well provisioned (E.M. Anderson et al., 2008). Although the elements we evaluated are often the most available and potentially toxic, consideration of other metals as well as organic pollutants might yield further insights.

4.4. Potential role of contaminants in population dynamics

An important issue, particularly in the context of other stressors, is the degree to which sublethal trace elements associated with poor nutritional status in sea ducks affect survival, reproductive success, and resulting population dynamics. Aside from the effects of contaminants, several studies conducted only on freshwater dabbling ducks (Anatini) demonstrate strong effects of body mass and condition on annual survival (Davis et al., 2011 and references therein). For wild populations, the pre-breeding body mass and lipid reserves clearly affect the breeding propensity, clutch size, nest success, and brood attendance of common eiders that, like white-winged scoters, depend heavily on stored reserves during one or all of these periods (Korschgen, 1977; Coulson, 1984; Dobush, 1986; Erikstad et al., 1993; Bustnes et al., 2002; Oosterhuis and van Dijk, 2002; Coulson, 2010).

Evaluating effects of sublethal contaminants on population dynamics is challenging. A study of Cd, Hg, Se, and lead (Pb) in the blood of white-winged scoters showed that elevated levels of trace elements did not translate into poor reproductive success. White-winged scoters wintering on the eastern seaboard had higher Cd, Pb, and Se levels at the breeding grounds in Saskatchewan (Hg did not differ among populations). However, there was no difference in nest success or clutch size (Gurney et al., 2014), possibly because blood levels reflect exposure over a short period during which body condition might not suffer, or as stated above, wild sea ducks tolerate higher levels of trace elements and those thresholds were not exceeded.

While conclusive links remain indirect, some studies have shown population-level effects of interactions among contaminant and noncontaminant stressors. For example, Bustnes et al. (2015) concluded that the detrimental effects of organic pollutants on growth of great skua (*Stercorarius skua*) offspring differed among populations depending on food availability and habitat quality. In common eiders, high levels of Se in blood decreased clutch size via egg inviability, although the authors considered this effect correlative and urged consideration of other factors not included in the study (Wilson et al., 2007). Models by Bardsen et al. (2018) indicated that pollutants played a role in population viability (i.e., extinction risk) in common eiders, but only when compounded by climate warming and egg predation.

Although low, blood levels of lead (Pb) in common eiders correlated negatively with arrival date and body condition, two factors that affect reproductive success (Provencher et al., 2016). In south polar skua (*Stercorarius maccormicki*), sublethal levels of the organochlorine insecticide mirex were directly linked to less intensive nest defense and long-term breeding success. However, making this conclusive association required research strategies that included long-term monitoring of colonies and blood biomarkers (Goutte et al., 2018).

Thus, the interplay of greater physiological costs from sublethal contaminant exposure may become critical to population processes during periods of negative energy balance, such as during unexpected closing of leads in arctic sea ice during spring that induce starvation (Fournier and Hines, 1994). Individual effects from contaminant and non-contaminant stressors on overall energy balance are difficult to separate. This pattern suggests that in long-lived sea ducks with high incubation constancy, subsidiary changes in energy balance due to sublethal stressors might impose mounting challenges that explain population declines.

4.5. Se:Hg ratios

Despite conclusions from early studies that Se and Hg accumulate in the liver in a 1:1 molar ratio in marine mammals, and that Se could thereby detoxify Hg (Koeman et al., 1973), our results neither support a particular molar ratio nor indicate detoxification of Hg. The high molar ratios of Se:Hg presented here (liver = 18:1, kidney = 20:1) likely represent higher Se availability (SI Fig. S1). Moreover, both elements had significant relationships with nutritional status. The magnitude of Se:Hg molar ratios in sea ducks and other marine vertebrates can vary tremendously. Among seabirds, Se accumulation is often much greater than that of Hg, with arithmetic means and standard deviations of Se:Hg at 26 ± 44 (liver) and 55 ± 65 (kidney) (Dietz et al., 2000). In other cases, the two elements lack any correlation as observed in livers of spectacled eiders wintering in the Bering Sea (Lovvorn et al., 2013).

4.6. Conclusion

In laboratory studies, R^2 values of 14 to 27% might constitute minor findings, but for wild, migratory populations, such correlations are important when considering the many uncontrolled variables affecting energy balance. For example, prominent habitat factors such as tidal exposure, water depth, predation risk, and diet diversity explained 5 to 19% of body mass in white-winged scoters across a region that includes our study area (Palm et al., 2013).

In summary, we show that a multivariate, permutational statistical approach can yield insights into the silent stresses imposed by contaminants that were not otherwise evident using univariate approaches. For example, PERMANOVA results showed that contaminant accumulation was largely context dependent, whereas parametric ANOVA indicated a distinction only in Cd, and only at only Padilla Bay. While linear regression potentially indicated stimulatory effects of Hg in two instances, Cd was not predictive of any single aspect of nutritional status according to univariate regression analyses. In contrast, DistLM indicated that accumulations of trace elements co-vary with nutritional status at all locations across winter. Recall that DistLM analyses determine how the nutritional status of each animal covaries according to their individual concentrations of the trace elements and evaluates the contaminant-health trends across the group (vectors in Fig. 5) — not with group averages of an element. While Cd accumulations were far below toxic thresholds, kidney Cd was an indicator of nutritional status in surf scoters at Penn Cove, and also for both species at Birch Bay. In contrast, even though *average* tissue Cd was higher in surf scoters from Padilla Bay, 19% of their nutritional status was explained by increasing burdens of liver Hg across the group. However, given the profound declines in nutritional status of surf scoters at Padilla Bay and Penn Cove, the magnitude of the combined effects of trace elements was lower than we would have predicted. Clearly investigations of other pollutants and non-contaminant stressors; their sublethal interactions; and broader suites of physiological indicators are needed.

We conclude that univariate, parametric statistics can have limited effectiveness in detecting the impacts of sublethal stressors by restricting evaluation of each pollutant in tissues to single physiological responses. In contrast, multivariate analyses indicated that even low levels of trace elements had substantial impacts on the overall nutritional status of scoters in Puget Sound, and that those effects were equivalent to repercussions of environmental factors and prey availability and quality (Palm et al., 2013). Our findings suggest that simultaneously considering combinations of contaminants relative to a suite of complex physiological responses can be an important tool for assessing how multiple stressors affect the energy needs of free-ranging animals and can contribute to world-wide declines in wildlife populations.

CRediT authorship contribution statement

Marjorie L Brooks: Conceptualization, Writing - Original draft preparation, Methodology, Statistical Modeling, Data curation, Writing - Original draft preparation, Supervision, Funding acquisition. **James R Lovvorn**: Conceptualization, Writing - Reviewing and Editing. **Jessica Hallman Behnke**: Investigation, Formal analysis. **Eric M Anderson**: Conceptualization, Methodology, Investigation, Writing - Reviewing and Editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Methods for trace element analyses in detail (SI 1); methods for statistical analyses in detail (SI 2); PERMANOVA results for sublethal responses in nutritional status (SI 3); table of trace element concentrations in kidney and liver tissues in female surf and white wing scoters collected in 2005 and 2006 (SI 4); table of mass, body composition, plasma metabolites, and stable isotope means and standard deviations (SD) in male scoters by subpopulation, collected within the Puget Sound, WA, in 2005 and 2006 (SI 5); table of levels of trace elements in this study compared to other wild populations and to toxic thresholds (SI 6);.table of PERMDISP main effects analyses for homogeneous dispersions of trace element levels (SI 7); table of PERMDISP pairwise analyses for homogeneous dispersions of trace element levels (SI 8); table of nutritional status comparisons with results of PERMANOVA main effects tests (SI 9); table of nutritional status comparison with results of *pairwise t-tests* in PERMANOVA (SI 10); table of PERMDISP main effects analyses for homogeneous dispersions of nutritional status (SI 11); table of PERMDISP pairwise analyses for homogeneous dispersions of nutritional status (SI 12); plot of Se:Hg ratios as molar concentrations that averaged 18.2:1 in liver and 19.8:1 in kidney (SI 13); plot of non-metric multidimensional scaling (MDS) of trace element levels in a) surf scoters at all three locations, and b) surf and white-winged scoters at Birch Bay. Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.144247.

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