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Food sources of wintering piscivorous waterbirds in coastal waters: A triple stable isotope approach for the southeastern Baltic Sea

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ABSTRACT

This study uses a triple isotope approach ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$) to quantify the main food sources for wintering piscivorous waterbirds in the coastal zone of the southeastern Baltic Sea. Significant differences of $\delta^{15}N$ and $\delta^{34}S$ values among pelagic fishes, benthic fishes, and benthopelagic European smelt (*Osmerus eperlanus*) were detected, while $\delta^{13}C$ was similar among these sources. Using different combinations of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values in mixing models, we found that common guillemot (*Uria aalge*) and red-throated diver (*Gavia stellata*) mostly foraged on pelagic prey (50–70% and 51–56%, respectively), whereas great crested grebe (*Podiceps cristatus*) consumed benthic prey (48–53%). European smelt comprised a substantial proportion of the diet of studied birds (19–36%). A stable isotope approach can be recommended as a non-lethal method to study avian diets in the coastal waters of the Baltic Sea.

1. Introduction

Marine birds are experiencing a significant decline in numbers related to decreasing reproductive success and environmental challenges at wintering sites (Skov et al., 2011; Croxall et al., 2012). Piscivorous waterbirds are affected by food web alterations at all trophic levels through cascading effects in ecosystems, especially those with low biodiversity such as the Baltic Sea (Casini et al., 2006; Österblom et al., 2006; Kadin et al., 2012). On the other hand, dietary preferences are flexible and depend on the local availability of prey (Jakubas and Mioduszewska, 2005). It is important to find effective methods to identify prey species and foraging strategies of waterbirds in order to predict potential threats related to food resources.

The inaccessibility of wintering offshore habitats to researchers complicates trophic studies of waterbirds, and thus most direct methods, including visual observations of feeding behavior and analyses of feces or regurgitated food remains, are not applicable (Duffy and Jackson, 1986; Barrett et al., 2007). This results in rather limited availability of waterbird diet data. Previous dietary studies of waterbirds wintering in the southeastern Baltic Sea, including the Lithuanian coastal zone, have been performed by analyzing gut contents of bycaught specimens (Žydelis, 2002). At present, the policy of environmental and coastal fisheries targeting zero bycatch mortality warrants research on the applicability of alternative non-lethal methods to monitor the feeding habits of wintering waterbirds (Korpinen and Braege, 2013). One of the suitable techniques could be stable isotope analysis (SIA) of bird blood (Cherel et al., 2008; Mariano-Jelicich et al., 2008). Stable isotope values of blood carry integrated information about bird diet for more than two weeks (Vander Zanden et al., 2015), therefore, even samples from specimens with empty stomachs are relevant (Bearhop et al., 2002; Ogden et al., 2004).

Stable carbon ($^{13}C/^{12}C$, $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$, $\delta^{15}N$) isotope ratios are widely used to reconstruct diets and characterize trophic relationships in food webs (Boecklen et al., 2011). The $\delta^{13}C$ is used to determine consumer links to benthic or pelagic food chains, whereas $\delta^{15}N$ shows the trophic position in a food web (Post, 2002). Sulfur isotope composition ($^{34}S/^{32}S$, $\delta^{34}S$) might be useful to distinguish pelagic and benthic components in food webs (Connolly et al., 2004; Croisetière et al., 2009). Strong

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discrimination against heavy (^{34}S) isotope in sulfate-reducing bacteria of anoxic sediments leads to lower δ^{34}S values in infaunal benthic organisms than in organisms inhabiting oxic sediments or a water column (Fry and Chumchal, 2011; Karube et al., 2012; Proulx and Hare, 2014). This difference is expected to propagate to upper trophic levels and potentially help to differentiate benthic and pelagic fishes, as well as bird guilds exploiting these fish communities. The applicability of δ^{34}S has not been widely tested in food webs of the Baltic Sea (but see Mittermayr et al., 2014). Previous analysis of δ^{13}C and δ^{15}N in this system revealed that isotopic differentiation between the food web components might be complicated because of large variation in stable isotope values in the primary sources of organic matter, due to riverine discharge and nitrogen-fixing cyanobacteria blooms (Rolff, 2000; Rolff and Elmgren, 2000; Lesutienė et al., 2014). Using a triple stable isotope approach might make it possible to distinguish food sources of consumers in complex ecosystems where endpoints have not been identified unambiguously by δ^{13}C and δ^{15}N. To our knowledge, only few studies are performed using triple isotope approach on bird blood (e.g. Knoff et al., 2001; Yohannes et al., 2014).

In this study, we used SIA for the identification of the main food sources for wintering piscivorous waterbirds in the coastal zone of the southeastern Baltic Sea. We investigated the value of different combinations of δ^{13}C, δ^{15}N, and δ^{34}S to estimate proportions of pelagic fishes, benthic fishes, and benthopelagic European smelt (*Osmerus eperlanus*) in the diet of great crested grebe (*Podiceps cristatus*), red-throated diver (*Gavia stellata*), and common guillemot (*Uria aalge*).

2. Materials and methods

2.1. Ethics statement

Permits to capture birds were obtained from the Environmental Protection Agency of Lithuania (EPA: permit 2012 No. 7 and 2013 No. 1). All birds were eventually released. Fish specimens were provided by commercial fishermen. According to Lithuanian law, no other approval was required by the national animal welfare authorities to undertake this research.

2.2. Study area and species

The Baltic Sea is one of the most important wintering sites for waterbird populations within the western Palearctic area. The Lithuanian coastal zone serves as an important stopover area for internationally significant concentrations of wintering benthivorous ducks and piscivorous waterbirds (Durinck et al., 1994; Vaitkus, 1999). It is high-energy open coast influenced by freshwater flow from the hypertrophic Curonian Lagoon (Fig. 1).

In the Lithuanian coastal zone, abundance of wintering divers (black-throated diver *Gavia arctica* and red-throated diver; species ratio estimated as 1:10, respectively) was fluctuating from 40 to 900 individuals with a decreasing tendency during the last two decades. Abundance of wintering great crested grebe fluctuates from 500 to 3300 individuals; the total number is considered stable (Sorokaitė et al., 2007; Raudonikis and Sorokaitė, 2011). Common guillemot is a regular but not numerous wintering species in the coastal zone, though solitary individuals or small flocks are recorded frequently (Švažas, 1993; DENOFLIT, 2012). According to gut content analysis, divers and grebes mostly feed on smelt and pelagic fishes in the Lithuanian coastal waters (Žydelis, 2002), while guillemots are thought to feed on pelagic fishes (Käkelä et al., 2007). However, waterbird diet composition in the Baltic Sea has not been investigated in detail.

The coastal benthic fish community is mainly represented by European flounder *Platichthys flesus*, European plaice *Pleuronectes platessa*, and sand goby *Potamoschistus minutus* (Repečka, 2003, 2005). Shoaling Baltic herring *Clupea harengus* and Baltic sprat *Sprattus sprattus* together are the most dominant fishes in the pelagic habitat. Anadromous European smelt concentrates in this coastal area before spawning in rivers, and accounts for 90% of the total commercial fish catches during winter season. However, commercial catches of smelts have fluctuated annually from 18 to 280 t over the last two decades (data from Fisheries Service under the Ministry of Agriculture of the Republic of Lithuania, henceforth Fisheries Service). The abundance dynamic has been impacted by changes in smelt spawning success, which is attributed to climate-related hydrological factors and the status of predatory fish populations in the southeastern Baltic Sea (Švagždys, 2009).

2.3. Sampling and stable isotope analysis

Bird and fish samples for SIA were collected during the period between December 2012 and March 2013. The mean daily air temperatures during the study period ranged from −13.7 to 3.9 °C. The Curonian Lagoon was under ice cover from December 2012 to April 2013 (Lithuanian Hydrometeorological Service under the Ministry of Environment). Hydrological conditions confined the feeding habitat for waterbirds to the marine environment.

Waterbirds were captured 0.5–2 km from the shoreline using the night lighting technique (described in Whitworth et al., 1997). Blood samples from 19 birds were collected (Table 1). Blood (0.5–1 ml) was obtained from the medial metatarsal vein of live birds (methodology in Arora, 2010). The blood samples were stored frozen at −20 °C in cryogenic vials; before analysis they were freeze-dried for 48 h. Fishes were collected from the coastal fishery catches of gill nets and by trawling (Table 2). Fish samples were white dorsal muscle from one to three fish specimens (pooled to obtain three replicates for each taxonomic/size group). They were dried at 60 °C for 48 h and ground into a fine powder in an agate mortar. Fish and bird samples (0.5–0.7 mg for C and N; 1.2–2.0 mg for S) were weighed in tin capsules for SIA.

The carbon and nitrogen isotope composition in the samples were determined using a Thermo Scientific Delta V Advantage mass spectrometer coupled to a Flash EA 1112 elemental analyzer at the State Research Institute Center for Physical Sciences and Technology, Lithuania. Ratios of sulfur isotopes were determined using a SerCon elemental analyzer and custom cryofocusing system interfaced to a SerCon 20–22 IRMS (Sercon Ltd., Cheshire, UK) at the Stable Isotope Facility, University of California, Davis, CA, USA. Results of isotopic ratios were compared to conventional standards, i.e., Vienna Peedee Belemnite (VPDB), for carbon, atmospheric N_2 for nitrogen, and Vienna Canyon Diablo troilite (VCDT) for sulfur, defined as δ values: $\delta X = [(R_{sample}/R_{standard}) - 1)] \times 10^3$ (‰), where $X = {}^{13}C$, ^{15}N or ^{34}S, and $R = {}^{13}C/{}^{12}C$, $^{15}N/^{14}N$ or $^{34}S/^{32}S$. For calibration of reference gases CO_2 and N_2, the international standards from the International Atomic Energy Agency (Vienna) were used: IAEA-600 (Caffeine, $\delta^{13}C = -27.771 \pm 0.043‰_{VPDB}$) and NBS-22 (Oil $\delta^{13}C = -30.031 \pm 0.043‰_{VPDB}$) were used for ^{13}C and IAEA-600 (Caffeine, $\delta^{15}N = +1 \pm 0.2‰^{2}_{air\ N}$) for ^{15}N. Repeated analyses of homogeneous material yielded standard deviations of less than 0.08‰ for carbon and 0.2‰ for nitrogen.

For calibration of reference gases SO_2, three laboratory standards calibrated directly against IAEA-S-1 (Silver Sulfide, $\delta^{34}S = -0.30‰_{VCDT}$), IAEA-S-2 (Silver Sulfide, $\delta^{34}S = +22.7 \pm 0.2‰_{VCDT}$), and IAEA-S-3 (Silver Sulfide, $\delta^{34}S = -32.3 \pm 0.2‰_{VCDT}$) were used. Repeated analysis of three laboratory standards yielded standard deviations of less than 0.3‰. The long-term reproducibility of δ^{34}S measurements is ±0.4‰.