Arthropod taxa in faeces of chicks of Black-tailed Godwits and Northern Lapwings in Germany and the Netherlands revealed by DNA metabarcoding

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Meadow-bird chicks are dependent on arthropods, many of which are undergoing serious declines. For many insectivorous bird species it is currently unknown which arthropod taxa are included in their diet and contribute to chick growth and survival. As prey taxa consumed by chicks are difficult to determine through visual observations, we used DNA metabarcoding to identify arthropod species in faecal samples of Black-tailed Godwit *Limosa limosa limosa* and Northern Lapwing *Vanellus vanellus* chicks. Faecal samples were collected from chicks in breeding populations on agricultural and restored grasslands in Germany and the Netherlands. We used frequency of occurrence (FOO, the number of samples in which a taxon was detected) and relative read abundance (RRA, percentage of DNA reads of a taxon present within a sample) to determine species presence in faeces. While FOO indicates how often taxa may be eaten, RRA is an approximation of ingested biomass, making these variables complementary in the interpretation of the results. We found 25 arthropod or mollusc orders in the faeces of each species, with 114 families and 366 identified species included in the faeces of Black-tailed Godwits and 112 families and 313 identified species in the faeces of Lapwing chicks. Diptera (flies and mosquitoes) had the highest FOO in both species and also the highest RRA. In both species the RRA of Coleoptera (beetles) was second highest, followed by Hymenoptera (sawflies, wasps, bees and ants) in Black-tailed Godwits and Stylommatophora (land snails and slugs) in Lapwings. Overall, in Lapwing, the RRA of soil-dwelling organisms was higher than in Black-tailed Godwits. Our detailed species lists are a first step towards elucidating the requirements of developing chicks within these specific habitats and will aid restoration of habitats providing arthropod food resources.

INTRODUCTION

Arthropods are the main food source for wader chicks and are critical for their growth and survival (Beintema & Visser 1989). However, the global decline in insect abundance and diversity (Hallmann *et al.* 2014, Wagner 2020), particularly in agricultural areas (Outhwaite *et al.* 2022), may be a key reason behind the declines in European meadow-birds (Schekkerman & Beintema 2007). Several studies have focused on the abundance of arthropods as a vital food resource for meadow-bird chicks (e.g. de Felici *et al.* 2019, Silva-Monteiro *et al.* 2022), as sufficient numbers of arthropods need to be available to sustain the required prey intake for chicks. For meadow-bird conservation it is imperative to know which taxonomic groups of insects and other arthropods conservation efforts need to focus on. A critical knowledge gap is therefore the composition of arthropod species that are currently consumed by chicks (but see Beintema *et al.* 1991).

Unfortunately, food items consumed by chicks are difficult to identify in the field as prey items are small, and chicks are prone to forage out of sight of observers. Identifying arthropod fragments from faecal samples under a microscope (e.g. Beintema *et al.* 1991) requires advanced taxonomic knowledge. Moreover, such analyses are complicated by the variable resistance of different arthropod parts to the digestive process (e.g. Green & Tyler 1989). Benefiting from decades of work to attach genetic sequences to (museum) specimens (Hebert *et al.* 2003), ecologists now can use these so-called DNA barcodes for species identification. Importantly, when scaled up to a technique called DNA metabarcoding, many species within a sample can be detected, which is particularly convenient for smaller-sized and soft-bodied organisms which are challenging or impossible to visually identify (Symondson 2002), making it a relatively time-efficient method. It is now a frequently utilised tool to identify multiple arthropod species present in ecosystems (Arribas *et al.* 2022) or in diets of insectivores (King *et al.* 2015, Alberdi *et al.* 2019). In addition, it has been successfully applied in determining the occurrence of arthropod prey species across multiple avian species (e.g. Jedlicka *et al.* 2013, Verkuil *et al.* 2022), including in waders (e.g. Wirta *et al.* 2015, Gerwing *et al.* 2016). Also, in a passerine species, it has been shown that DNA metabarcoding can be used to determine the relative biomass contributions of different arthropod orders and families in the diet (Verkuil *et al.* 2022).

Here we applied DNA metabarcoding to describe the species present in faeces of chicks of two meadow-bird species: Black-tailed Godwit of the nominate subspecies *Limosa limosa limosa* (hereafter godwit) and Northern Lapwing *Vanellus vanellus* (hereafter lapwing). Both species are declining and are focal species of conservation efforts (Thorup 2006, Franks *et al.* 2018). Godwit and lapwing chicks forage independently on arthropods after hatching (Beintema *et al.* 1991). Recently, work has started to determine if sufficient numbers of arthropods are available for godwit and lapwing chicks on their breeding grounds in the Netherlands and Germany (Hooijmeijer *et al.* 2024, Lagendijk *et al.* 2024). As a first step, we collected faecal samples of godwit and lapwing chicks in grasslands in Germany and the Netherlands during one season. We provide a taxon list detailing the most important arthropod families and species, and evaluate the relative importance of the detected taxa by comparing the frequency of occurrence with the estimated biomass contribution. This will elucidate which taxonomic groups (including species) are currently included in the diet of chicks of the two meadow-bird species and will support conservation management in targeting particular arthropod groups or taxa in important meadow breeding areas.
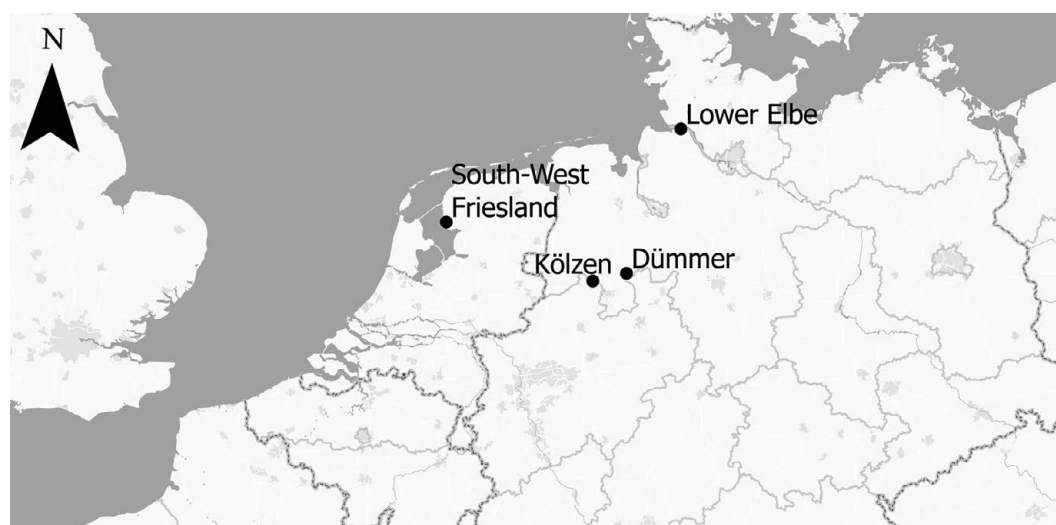

Fig. 1. Map with the four sampling areas in Germany and the Netherlands where the faecal samples from Black-tailed Godwit and Northern Lapwing chicks were collected in 2022.

METHODS

Faecal samples of godwit and lapwing chicks were collected in three areas in Germany, Dümmer (52°28'N, 8°19'E), Lower Elbe (53°51'N, 9°11'E) and Kölzen Neuenkirchen (hereafter Kölzen; 52°24'N 7°48'E), and one in the Netherlands, in south-west Friesland (hereafter SW Friesland; 52°55'N, 5°28'E; Fig. 1). Dümmer and Lower Elbe are fully protected grassland restoration areas, generally characterised by high groundwater levels, temporarily flooded grasslands, a complete ban on the use of insecticides, and low-intensity farming. Both Kölzen and SW Friesland are more conventionally used agricultural areas with a majority of intensely managed monoculture grassland, arable land, and a minority of herb-rich meadow-bird reserves (see Howison *et al.* 2018 for a description of the SW Friesland study area). All four study areas are important for meadow-breeding waders and have been included in larger studies of meadow-bird conservation (Kentie *et al.* 2015, Blüml & Krüger 2022, Hooijmeijer *et al.* 2024). Here we provide a species-specific description of chick diet during the 2022 breeding season (May–July), irrespective of area; to be able to draw any substantiated conclusions on differences among study areas requires higher sample sizes (one of our future research endeavours).

Samples were collected from chicks when they were caught to be individually marked for demographic research at 5–28 days old. Immediately after being caught, godwit and lapwing chicks were placed in a paper or cotton bag or on kitchen towel. These items were clean and either unused or machine washed to sterilise them. After a maximum of three minutes, chicks were removed from the container whether they had defecated or not, thereby ensuring a relatively short handling time. Any faecal material was collected in a sterile 2 mL vial with 96% ETOH as storage buffer, and stored in a fridge or freezer on the day of collection. After the field season, vials were stored temporarily at –20 °C and archived at –80 °C. In total, we processed 58 godwit and 49 lapwing faecal samples (i.e. Dümmer respectively 15 and 14 samples, Lower Elbe 11 and 22 samples, Kölzen one and 13, and SW Friesland 31 samples, godwits only).

DNA extraction was carried out using Invitrogen™ PureLink™ Microbiome DNA Purification Kit. For PCR amplification we used the modified invertebrate COI primers LCO1490_5T and HCO1777_15T in combination with Quantabio™ AccuStart II PCR ToughMix Reagent (see for detailed extraction and PCR protocols Verkuil *et al.* (2022)). Negative controls were included to check for any contamination during field sampling (i.e. from field researchers, their clothing and collection materials and additional collection surfaces), and during lab procedures. Field negative controls were taken by repeating the procedure of faecal collection without taking an actual faecal sample (minimum of one per sample area). We swabbed clothing when a faecal sample was collected from clothing to test for contami-

nation on clothing (every occurrence). In the lab negative controls were included during extraction (every 50th extraction) and PCR (every 36th PCR reaction) by repeating the extraction and PCR process without an actual sample or DNA material, respectively. Faecal samples and negative field controls were randomised prior DNA extraction.

PCR products of field samples and negative controls were sequenced by the Human Genetics lab of the Leiden University Medical Center with a MiSeq© (V3 kit). This generated DNA sequences, from which the unique sequences of 220 base pairs were filtered, called operational taxonomic units or OTUs using Usearch 9.2 (Edgar 2010; details in Verkuil *et al.* 2022). OTUs were assigned to taxa following Beentjes *et al.* (2019) and using the BOLD reference database (Ratnasingham & Hebert 2007). Species detected (using a cluster identity of 98%) were tested for occurrence in the Dutch Species Register with the taxon matcher tool (Beentjes *et al.* 2019). All species were manually checked for their occurrence in Germany; species not present in the Dutch Species Register were also manually checked (details in Tables S1). Then we tallied how many sequence read copies (hereafter reads) of each OTU were present in each sample (details in Verkuil *et al.* 2022). Relative number of reads of each prey taxon is indicative of the biomass of prey remnants in the faeces but is not a measure of the number of individual prey items ingested.

The average number of reads of each taxon was calculated for the negative controls and swabs, and subtracted from each field sample to account for potential pollution of the field sample. Samples with fewer than 1,000 reads were discarded from the data set (i.e. nine lapwing samples). This cut-off of 1,000 reads was based on visual assessments of the scatterplots of richness and Shannon index with sample read counts (Verkuil *et al.* 2022).

We used two measures to determine the relative importance of taxa detected in the faecal samples: (1) the relative read abundance (RRA), i.e. percentage of DNA reads present of a taxon per sample and (2) the frequency of occurrence (FOO), i.e. the number of times a taxon was encountered in the faecal samples. Assuming contaminants were sufficiently removed by subtracting reads found in negative controls, and since we only report the top taxa (see below), we applied a forgiving cut-off of a relative read abundance of 0.01% to obtain an exhaustive species list. All remaining species have been checked for occurrence in the study area (Suppl. 1) before determining FOO. RRA and FOO were calculated at order, family and species level. We then tabulated the most common eight orders, 15 families and 30 species for each meadow-bird species (Tables 2–5). FOO and RRA were visually compared for the most common families in godwits and lapwings, 15 and 17 respectively. Analyses were performed in R version 4.2.3 (R Core Team 2023) using the phyloseq-package (McMurdie & Holmes 2013).

RESULTS

Sequencing yields

Taxonomic assignment of the OTUs resulted in 3,429,818 (of a total of 3,920,165) reads being assigned to Animalia. Reads were deleted when assigned to Chordata (7.1% or 242,868 reads, mostly birds), Nematoda (5.3%, 183,193 reads), Rotifers (0.51%, 17,461 reads), and Annelida (0.03%, 917 reads). The remaining number of reads assigned to Arthropoda and Mollusca, and selected for further analysis, amounted to 2,985,325 reads.

Field and lab controls

The controls (taken in lab, field and from materials) contained 19,688 reads (0.5% of total), of which 3,286 reads were rejected as non-target reads. Overall, 11,478

Table 1. Occurrence (% of samples; FOO: frequency of occurrence) of detected orders within the diet of 58 Black-tailed Godwit and 40 Northern Lapwing chicks in Germany and the Netherlands in 2022.

Order	Common name	FOO (%) in Godwits	FOO (%) in Lapwings
Diptera	Flies	100	100
Coleoptera	Beetles	79.3	95
Hymenoptera	Sawflies, wasps, bees and ants	79.3	70
Lepidoptera	Butterflies and moths	74.1	55
Stylommatophora	Land snails and slugs	50	77.5
Hemiptera	True bugs	63.8	52.5
Hygrophila	Freshwater aquatic snails	24.1	35
Trombidiformes	Acariform mites	8.6	22.5
Isopoda	Woodlice and relatives	8.6	20
Sarcoptiformes	Acariform mites	17.2	5
Araneae	Spiders	8.6	12.5
Trichoptera	Caddisflies	10.3	5
Entomobryomorpha	Springtails	10.3	2.5
Odonata	Dragonflies, damselflies and allies	6.9	7.5
Ephemeroptera	Mayflies	8.6	2.5
Orthoptera	Grasshoppers, locusts, crickets and allies	5.2	7.5
Symphypleona	Springtails	5.2	7.5
Julida	Millipedes	0	10
Lithobiomorpha	Stone centipedes	0	10
Neuroptera	Lacewings, mantidflies, antlions and allies	3.5	5
Mecoptera	Scorpion flies	3.5	2.5
Polydesmida	Millipedes	0	7.5
Cyclopoida	Copepods	1.7	2.5
Mesostigmata	Parasitiform mites	1.7	2.5
Blattodea	Cockroaches	1.7	0
Megaloptera	Alderflies, dobsonflies and fishflies	1.7	0
Opiliones	Harvestmen	1.7	0
Poduromorpha	Springtails	0	2.5
Psocodea	Bark-, book- and parasitic lice	1.7	0

reads in the controls belonged to Arthropoda and Mollusca (0.4% of 2.99 million target reads), with 53 species present with 10 or more reads. The field controls had 7,034 reads (0.2%), lab controls had 3,381 reads (0.1%), and swabs of collection materials had 1,063 reads (0.04%). The most abundant contaminant was German Cockroach *Blattella germanica* with 2,160 reads in one field control and 578 reads in four lab controls, with 568 reads in one control. Besides the cockroach, only the field controls had six species represented with >200 reads (up to 1,232), whereas in lab and materials controls no other species recorded exceeded 200 reads (representing 0.6% of mean total read count (32,398) of all samples including controls, or 0.7% of mean read count (27,793) of Arthropoda and Mollusca in the faecal samples).

Overall taxonomic richness and species occurrence

We found a total of 29 orders (Table 1). Specifically, we detected 25 orders, 114 families and 366 species in the faeces of godwit chicks (58 samples), and 25 orders, 112 families and 313 species in the faeces of lapwing chicks (40 samples). Most species were known to occur in our study areas. From the Dutch samples 49 species were not present in the Dutch Species Register, but 26 species were confirmed to occur in the Netherlands using other sources (e.g. GBIF, observation.org; Tables S1). Of the 23

remaining species, 21 were confirmed to occur within Europe, and two were from genera occurring in the Netherlands. In total 23 species in the German samples have not been confirmed to occur in Germany. Of these, five species have not been recorded in Europe, one belonged to a genus observed in Europe, and the other five to families occurring in Europe. (Tables S1).

Common Arthropod orders and families

Diptera (flies, mosquitos and allies) was the most common order, present in all faecal samples (Fig. 2, Table 2). Amongst Diptera, the most important family was the Chironomidae (non-biting midges) which occurred in all faecal samples from godwit chicks. In lapwings, the most important families were Chironomidae and Tipulidae (crane flies), each occurring in 80% of the samples (Fig. 2, Table 3). The second most encountered order was Hymenoptera (sawflies, wasps, bees and ants) in godwits (79% of the samples), and Coleoptera (beetles) in both godwits (79% of the samples) and lapwings (95% of the samples). The third most encountered order was Lepidoptera (butterflies and moths) in godwits (74% of the samples). Stylommatophora (land snails and slugs) were common in both species (godwit 50% and lapwings 78% of the samples Table 2), however the relative read abundance of Stylom-

Table 2. Eight most represented (FOO: frequency of occurrence) orders included in the diet of 58 Black-tailed Godwit and 40 Northern Lapwing chicks in Germany and the Netherlands in 2022. Also shown is the mean relative read abundance (RRA), which is averaged over all samples within bird species.

| Bird species	Order	Common name	FOO (%)	RRA (%)
Godwit	Diptera	Flies	100	81.6
	Coleoptera	Beetles	79.3	4.8
	Hymenoptera	Sawflies, wasps, bees and ants	79.3	4.4
	Lepidoptera	Butterflies and moths	74.1	2.1
	Hemiptera	True bugs	63.8	2.6
	Stylommatophora	Land snails and slugs	50	1.6
	no identification	-	36.2	0.6
	Hygrophila	Freshwater aquatic snails	24.1	0.5
Lapwing	Diptera	Flies	100	55.6
	Coleoptera	Beetles	95	23.5
	Stylommatophora	Land snails and slugs	77.5	7.3
	Hymenoptera	Sawflies, wasps, bees and ants	70	2.5
	Lepidoptera	Butterflies and moths	55	2.4
	Hemiptera	True bugs	52.5	2.2
	no identification	-	42.5	1.7
	Hygrophila	Freshwater aquatic snails	35	1.5
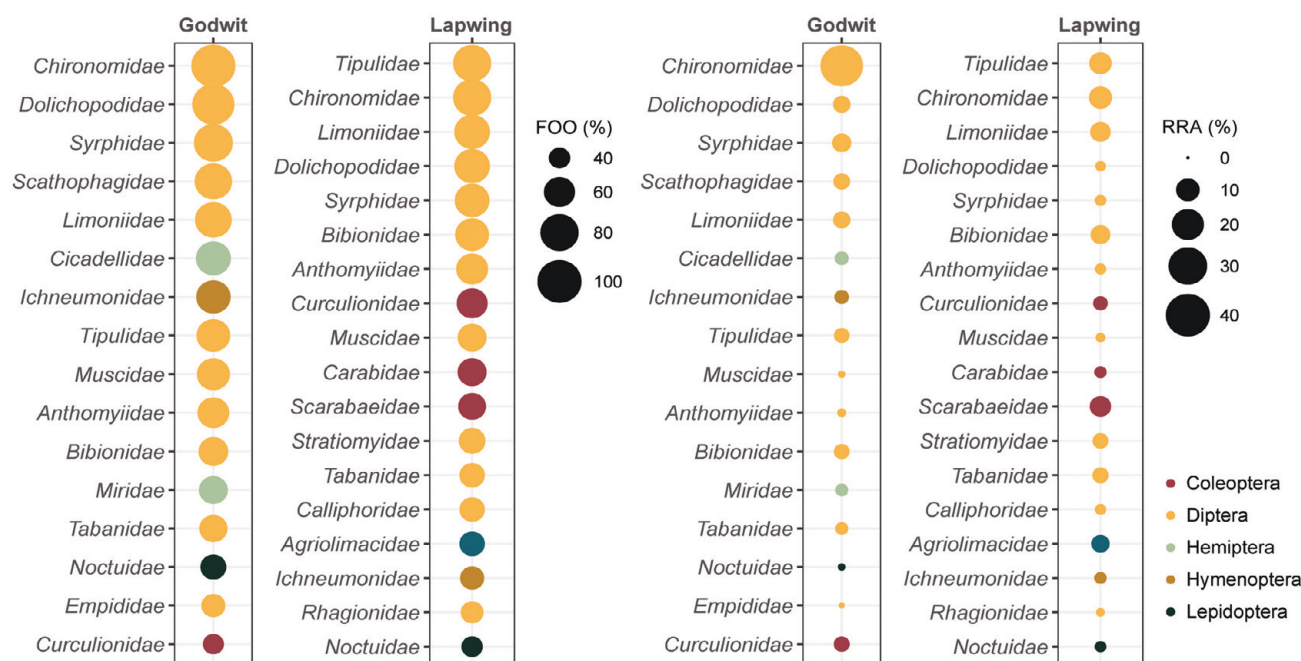
Fig. 2. The frequency of occurrence (FOO, % of samples) compared to the relative read abundance (RRA, average % of reads over all samples) of arthropod families detected with DNA metabarcoding in faecal samples of Black-tailed Godwit and Northern Lapwing chicks collected during the 2022 breeding season in Germany and the Netherlands. Shown are families occurring in >20 of 58 samples in godwits and in >15 of 40 samples in lapwings. Note the differences in scales between FOO and RRA.

matophora was much higher in lapwing (7.3 vs. 1.6%). Generally, the lapwing samples contained more epigaeic taxa than in the godwit samples (Tables 2 & 3).

Common Arthropod species

The most common species in the godwit samples (FOO range: 78–91%) were three non-biting midges *Chironomus annularius*, *C.* cf. *nudiventris*, *Glyptotendipes pallens*, *Dolichopus plumipes* (long-legged flies) and *Scathophaga stercoraria* (dung flies); Table 4). The three non-biting midges showed the greatest relative read abundance (average RRA range: 5.7–6.7%) in godwit samples (Table 4). In the lapwing samples, the most common species (FOO range: 45–68%) were *Dilophus febrilis* (march flies), *Tipula paludosa* (crane flies), *Dolichopus plumipes* (long-legged flies), and *Chloromyia formosa* (soldier flies). There were also many samples with unidentified Diptera (FOO: 63%; Table 5). However, the species which contributed most to the DNA reads (average RRA range: 4.9–7.7%) were *Liothorax plagiatus* (scarab beetles), *D. febrilis* (march flies), *Enochrus* species (water scavenger beetles) and *Deroceras reticulatum* (land slugs; Table 5).

Relative read abundance of commonly occurring families

In godwits, RRA of commonly occurring families (with a high FOO) ranged between 0.2 and 39.9% on average per sample, with Chironomidae dominating RRA. In lapwings, the RRA of the most commonly consumed families ranged between on average 0.8 to 9.7% per

sample (Fig. 2, Table 3). Generally, patterns of FOO and RRA were incongruent, and more so in lapwings (Fig. 2).

DISCUSSION

We present a list of taxa detected in faeces, and presumably, therefore present in the diet of godwit and lapwing chicks. In this single-year study with a limited number of samples, we detected a large number of taxa, suggesting a quite broad dietary spectrum. This could suggest that, in 2022, chicks could relatively easily find suitable prey items. Our finding that lapwings eat more soil-dwelling organisms while godwits eat more aquatic organisms confirms the known niche differentiation between the species within meadow areas (Beintema *et al.* 1991). This shows the potential for our approach for the next step: evaluating the feeding ecology of meadow-bird chicks across different years and sites in relation to agricultural management and progressing grassland restoration efforts while also assessing the role of Annelida with an additional primer set. In three of our study areas, a concurrent study on insect diversity and abundance is being undertaken providing an opportunity to elucidate whether meadow-bird chicks eat what is readily available, or select for particular food items (Hooijmeijer *et al.* 2024, Lagendijk *et al.* 2024). This is a crucial piece of the puzzle on how to conserve and protect the meadow-bird breeding populations through the promotion of favourable habitat for their prey, thus ensuring food security for chicks.

Table 3. Top 15 most represented (FOO: frequency of occurrence) families included in the diet of 58 Black-tailed Godwit and 40 Northern Lapwing chicks in Germany and the Netherlands in 2022. Also shown is the mean relative read abundance (RRA).

Bird species	Order	Family	Family of	FOO (%)	RRA (%)
Godwit	Diptera (flies)	Chironomidae	Non-biting midges	100	39.9
		Dolichopodidae	Long-legged flies	93.1	5.1
		Syrphidae	Hoverflies	82.8	6.4
		Scathophagidae	Dung flies	77.6	4.6
		Limoniidae	Crane flies	74.1	5.0
	Hymenoptera (sawflies, wasps, bees and ants)	Ichneumonidae	Ichneumonid wasps	69.0	3.2
	Diptera (flies)	Tipulidae	Crane flies	67.2	3.8
		Muscidae	House flies	65.5	0.5
		Anthomyiidae	Root maggot flies	62.1	0.9
		Bibionidae	March flies	56.9	3.9
	Hemiptera (true bugs)	Miridae	Plant bugs	55.2	2.4
	Diptera (flies)	Tabanidae	Horse and deer flies	53.5	2.5
		no identification	Unknown	48.3	2.1
	Lepidoptera (butterflies and moths)	Noctuidae	Owlet moths	48.3	0.5
	Diptera	Empididae	Dagger- and balloon flies	44.8	0.2
Lapwing	Diptera (flies)	Chironomidae	Non-biting midges	80	9.7
		Tipulidae	Crane flies	80	9.3
		Dolichopodidae	Long-legged flies	72.5	1.5
		Limoniidae	Crane flies	72.5	7.4
		Syrphidae	Hoverflies	70	1.8
		Bibionidae	March flies	67.5	6.8
		Anthomyiidae	Root maggot flies	62.5	1.7
		no identification	Unknown	62.5	0.8
	Coleoptera (beetles)	Curculionidae	True weevils	60	3.3
		Carabidae	Ground beetles	55	2.1
	Diptera (flies)	Muscidae	House flies	55	1.2
	Coleoptera (beetles)	Scarabaeidae	Scarab beetles	52.5	8.5
	Diptera (flies)	Stratiomyidae	Soldier flies	50	4.1
		Calliphoridae	Blow flies	47.5	1.7
		Tabanidae	Horse and deer flies	47.5	4.4
	Stylommatophora (land snails and slugs)	Agriolimacidae	Land slugs	47.5	5.6

Table 4. Top 30 species included in the diet of 58 Black-tailed Godwit chicks in Germany and the Netherlands in 2022, ordered by frequency of occurrence (FOO). Also shown is the mean relative read abundance (RRA).

Order	Family	Family of	Genus	Species	FOO (%)	RRA (%)
Diptera	Chironomidae	Non-biting midges	*Chironomus*	*Chironomus annularius*	91.4	6.7
	Dolichopodidae	Long-legged flies	*Dolichopus*	*Dolichopus plumipes*	89.7	3.8
	Chironomidae	Non-biting midges	*Chironomus*	*Chironomus* cf. *nudiventris*	79.3	6.7
			Glyptotendipes	*Glyptotendipes pallens*	77.6	5.7
	Scathophagidae	Dung flies	*Scathophaga*	*Scathophaga stercoraria*	77.6	4.4
	Chironomidae	Non-biting midges	*Chironomus*	*Chironomus plumosus*	74.1	5.5
			Chironomus	no identification	65.5	1.0
			Cryptochironomus	*Cryptochironomus supplicans*	60.3	2.2
			Chironomus	*Chironomus curabilis*	58.6	1.9
	Bibionidae	March flies	*Dilophus*	*Dilophus febrilis*	56.9	3.9
	Chironomidae	Non-biting midges	*Glyptotendipes*	*Glyptotendipes paripes*	56.9	2.7
	Chironomidae	Non-biting midges	*Procladius*	*Procladius ferrugineus*	55.2	1.7
	Chironomidae	Non-biting midges	*Benthalia*	*Benthalia sp. HM-2012*	50	0.5
			Chironomus	*Chironomus riparius*	50	0.5
	Dolichopodidae	Long-legged flies	*Dolichopus*	*Dolichopus brevipennis*	50	0.5
	Chironomidae	Non-biting midges	*Procladius*	*Procladius sp. 1ES*	48.3	0.2
	no identification	-	no identification	no identification	48.3	2.1
	Tabanidae	Horse and deer flies	*Haematopota*	no identification	46.6	2.0
	Tipulidae	Crane flies	*Nephrotoma*	*Nephrotoma appendiculata*	44.8	3.6
	Syrphidae	Hoverflies	*Melanostoma*	*Melanostoma mellinum*	43.1	1.2
	Limoniidae	Crane flies	*Erioconopa*	*Erioconopa trivialis*	41.4	2.3
	Syrphidae	Hover flies	*Platycheirus*	*Platycheirus quadratus*	39.7	2.5
	Dolichopodidae	Long-legged flies	*Dolichopus*	no identification	36.2	0.3
	Chironomidae	Non-biting midges	*Cryptochironomus*	no identification	34.5	1.0
	Anthomyiidae	Root maggot flies	*Delia*	*Delia platura*	32.8	0.1
	Limoniidae	Crane flies	*Molophilus*	no identification	32.8	0.4
no identification	no identification	-	no identification	no identification	32.8	0.1
Diptera	Syrphidae	Hover flies	*Eristalis*	*Eristalis arbustorum*	31.0	1.3
	Tipulidae	Crane flies	*Tipula*	no identification	31.0	0.1
Hymenoptera	Ichneumonidae	Ichneumonid wasps	no identification	no identification	31.0	0.3

To determine the relative importance of the detected taxa we used two variables: (1) FOO and (2) RRA, and have shown that FOO and RRA are not interchangeable (Fig. 2). Taxa that are generally important for chick growth will have a high FOO (i.e. occur in many samples) with a high RRA (i.e. were eaten in large amounts). FOO is an accurate measure but could overestimate the importance of prey items eaten in small quantities, and is particularly biased when the number of samples is small. Nevertheless, taxa with a high FOO but a low RRA, could still be crucial to growth, e.g. provide essential nutrients, while not contributing much to the biomass of the diet. RRA is ecologically an important measure because it is equivalent to the ingested biomass of a prey taxa (Deagle *et al.* 2019). For the primer set and protocols in this study, it was validated earlier that the number of DNA sequences at

Table 5. Top 30 species included in the diet of 40 Northern Lapwing chicks in Germany in 2022, ordered by frequency of occurrence (FOO). Also shown is the mean relative read abundance (RRA).

Order	Family	Family of	Genus	Species	FOO (%)	RRA (%)
Diptera (flies)	Bibionidae	March flies	*Dilophus*	*Dilophus febrilis*	67.5	6.8
	no identification	-	no identification	no identification	62.5	0.8
	Tipulidae	Crane flies	*Tipula*	*Tipula paludosa*	47.5	2.9
	Dolichopodidae	Long-legged flies	*Dolichopus*	*Dolichopus plumipes*	45	1.3
	Stratiomyidae	Soldier flies	*Chloromyia*	*Chloromyia formosa*	45	3.9
	Tipulidae	Crane flies	*Tipula*	no identification	45	0.7
No identification	no identification	-	no identification	no identification	42.5	1.7
Diptera (flies)	Chironomidae	Non-biting midges	*Chironomus*	*Chironomus annularius*	40	2.0
	Chironomidae	Non-biting midges	*Chironomus*	*Chironomus* cf. *nudiventris*	40	1.2
	Limoniidae	Crane flies	*Erioconopa*	*Erioconopa trivialis*	40	1.3
Coleoptera (beetles)	Scarabaeidae	Scarab beetles	*Liothorax*	*Liothorax plagiatus*	37.5	7.7
Diptera (flies)	Syrphidae	Hoverflies	*Episyrphus*	*Episyrphus balteatus*	37.5	0.9
Stylommatophora (land snails and slugs)	Agriolimacidae	Land slugs	*Deroceras*	*Deroceras reticulatum*	37.5	4.9
Diptera (flies)	Chironomidae	Non-biting midges	*Glyptotendipes*	*Glyptotendipes pallens*	35	0.4
	Lonchopteridae	Spear-winged flies	*Lonchoptera*	*Lonchoptera lutea*	32.5	0.1
	Tabanidae	Horse and deer flies	*Haematopota*	no identification	32.5	0.8
Hymenoptera (sawflies, wasps, bees and ants)	Ichneumonidae	Ichneumonid wasps	no identification	no identification	32.5	1.9
Diptera (flies)	Chironomidae	Non-biting midges	*Chironomus*	*Chironomus curabilis*	30	1.5
	Tipulidae	Crane flies	*Tipula*	*Tipula subcunctans*	30	0.7
	Ephydridae	Shore flies	*Hydrellia*	*Hydrellia griseola*	27.5	0.9
	Limoniidae	Crane flies	*Molophilus*	no identification	27.5	2.7
	Scathophagidae	Dung flies	*Scathophaga*	*Scathophaga stercoraria*	27.5	1.3
	Cecidomyiidae	Gall midges	no identification	no identification	25	0.2
	Chironomidae	Non-biting midges	*Chironomus*	no identification	25	1.3
	Keroplatidae	Fungus gnats	*Orfelia*	*Orfelia nemoralis*	25	0.7
Coleoptera (beetles)	Curculionidae	True weevils	*Tournotaris*	*Tournotaris bimaculata*	22.5	0.8
	Hydrophilidae	Water scavenger beetles	*Enochrus*	no identification	22.5	6.7
Diptera (flies)	Anthomyiidae	Root maggot flies	*Delia*	*Delia florilega*	22.5	0.1
	Calliphoridae	Blow flies	*Cynomya*	*Cynomya cadaverina*	22.5	0.2
	Chironomidae	Non-biting midges	*Chironomus*	*Chironomus riparius*	22.5	0.0
Hygrophila (freshwater aquatic snails)	Lymnaeidae	Pond snails	*Radix*	no identification	22.5	1.4
Stylommatophora (land snails and slugs)	Arionidae	Round back slugs	*Arion*	*Arion vulgaris* x *Arion ater*	22.5	1.3

order and family level do indeed reflect the amount of biomass (Verkuil *et al.* 2022). There are challenges associated with obtaining accurate RRA values, including species-specific DNA degradation, the specificity of PCR primers, and the amplification of rare species (Elbrecht & Leese 2015, Taberlet *et al.* 2018, Alberdi *et al.* 2019, Deagle *et al.* 2019). We used a low annealing temperature and multiple PCR reactions per sample, so that there are multiple PCR starting points, thereby optimizing the probability of retrieving accurate dietary species abundances (Verkuil *et al.* 2022).

DNA metabarcoding is an increasingly valuable method in ecological studies. Now that validation studies can verify these methods (see Elbrecht & Leese 2015, Verkuil *et al.* 2022), their application can go beyond taxa identification, i.e. to diet-habitat correlations, to gain broader insights in the ecological and energetic role of different prey types and habitats. However, the use of DNA metabarcoding techniques comes with a few caveats. Firstly, without supplementary field observations, the life stage at which insects were consumed by chicks is not known. Also, when using DNA metabarcoding to determine which species are part of an organisms' diet, the method will also find secondary prey (species which have been eaten by predatory species which in turn were predated upon by the host) and parasites (e.g. mites, gut parasites or parasitic wasps, e.g. Ichneumonidae infecting Lepidoptera larvae; Sheppard *et al.* 2005). In addition, some species are not yet in the reference sequences in the public databases. e.g. BOLD or GenBank (Ratnasingham & Hebert 2007, Benson *et al.* 2009), and 'no identifications' remain present. Lastly, the method is sensitive to contamination, which is especially difficult to avoid in field work settings, as the number of taxa and their read abundance in the field controls has demonstrated. It therefore remains important to take field controls when faeces are collected from surfaces such as clothing or hands that are not clean.

As the methods we applied follow from extensive validation tests (Verkuil *et al.* 2022) and are accompanied by in-depth ecological field-based knowledge, we expect this will significantly contribute to the question of which prey meadow-bird chicks select from the available arthropod diversity and abundance. While several studies have quantified insect or arthropod abundance (biomass) available as food for chicks, information on which species are important within the insectivorous diets in agricultural and grassland habitats are only just appearing (Cabodevilla *et al.* 2021 [farmland birds], this study).

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ONLINE SUPPLEMENTARY MATERIAL

Tables S1. Occurrence checklist for arthropod species detected in faeces of chicks of Black-tailed Godwits and Northern Lapwings in Germany and the Netherlands.

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