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Dataset of Grey plover (*Pluvialis squatarola*) diet composition on the SW Black Sea coast using DNA metabarcoding

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Abstract

Background

Wader populations have been declining globally due to widespread anthropogenic habitat degradation and loss. In this context, food quality and availability at migration stop-over sites is crucial for wader migration success.

We studied the diet composition and preferences of the Grey plover (*Pluvialis squatarola*) at a stop-over site on the Black Sea-Mediterranean Flyway - Pomorie Lake, SW Black Sea coast, Bulgaria - through DNA metabarcoding of faeces. The study aimed to determine the bird's feeding strategy, coastal habitat use and possible seasonal variations as a result of local prey availability.

New information

The dataset includes new data on Grey plover (*Pluvialis squatarola*) prey items on the south-western Black Sea coast, Bulgaria. The diet composition data provides valuable insights on coastal biodiversity in the area as "sampled" by this generalist invertivore and contributes to the knowledge of Grey plover prey choice and habitat use in a little-studied microtidal marine region.

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Keywords

shorebirds, Black-bellied plover, High Throughput Sequencing (HTS), Illumina MiSeq, coastal food webs, south-western Black Sea, environmental DNA, CO1

Introduction

Long-distance migration between wintering and breeding grounds is one of the most energetically demanding processes in the animal kingdom and is well known in many species of waders. Most migratory birds need to make several stop-overs to rest and refuel for the next stages of their migration (Schaub and Jenni 2000, Skagen 2006). High-quality stop-over sites, with sufficient resources and protection from disturbance, are essential for bird survival and migration success (Myers et al. 1987, McGowan et al. 2011). Coastal and inland wetlands are often preferred stop-over sites; however, in recent years, these habitats have been subjected to fragmentation, changes in hydrology and land use, organic and/or chemical pollution etc. due to human activities and climate change, leading to their deterioration at a global scale (Finlayson et al. 2018).

The Grey plover (*Pluvialis squatarola*) is a long-distance migratory wader which breeds in the Arctic and uses stop-overs at tropical, subtropical and temperate mudflats, beaches and coasts worldwide (Byrkjedal and Thompson 1998). On the Black Sea coast (part of the Black Sea-Mediterranean Flyway (BSMF)), it can be observed in spring, autumn and winter (Nankinov 1996, Michev and Profirov 2003). The species is protected under the Bulgarian Biodiversity Act and its populations are declining globally.

The Grey plover is a generalist feeder with a broad diet spectrum. In western Europe, its most common prey during the non-breeding season include polychaetes, small bivalves and crustaceans, with diet variations depending on location, habitat, season and sub-strate (Pienkowski 1982, Bocher et al. 2014, Poole et al. 2020). The Grey plover's diet on the BSMF is poorly studied (Prostov 1964, Chernichko 2015, Kirikova 2017, Khrokov 2019).

Wader diet is typically analysed by morphological taxonomic identification of the prey remains in stomach contents or faeces (Verkuil 1996) - a time-consuming process that requires specific taxonomic expertise. Many invertebrate prey species decompose quickly and/or lack hard indigestible parts; therefore, they tend to remain undetected or underestimated (Perez-Hurtado et al. 1997). Considering the generalist feeding strategy and the wide diet spectrum reported for Grey plover along its migratory routes, as well as the lack of data for the Black Sea coast, we used DNA metabarcoding of faecal samples to determine the bird's diet composition in the Black Sea coastal habitats. Environmental DNA (eDNA) is a non-invasive method of diet study (Pompanon et al. 2012) that is increasingly applied in ecological research (e.g. Stolz et al. (2023), Zurdo et al. (2023)). Provided that the reference molecular databases for the target species are sufficiently

populated with sequences from reliably identified specimens (Meiklejohn et al. 2019, Pentinsaari et al. 2020, Keck et al. 2023), the method can give unprecedented taxonomic resolution in prey identification and allow the detection of cryptic or decomposed prey and even of unexpected prey items (e.g. Gerwing et al. (2016), Novcic et al. (2016), McInnes et al. (2017), Heller (2020)). de Sousa et al. (2019) even introduced the term dietary DNA (dDNA) to complement eDNA for dietary purposes and local biodiversity description.

This paper presents the raw CO1 dataset of obtained sequences published and available in BOLD. The results are discussed and analysed in detail in Vassileva et al. (2024).

Sampling methods

Sampling description: Seventy-six Grey plover faecal samples were collected at Pomorie Lake (SW Black Sea coast) in October–November 2020 (n = 61) and in March 2021 (n = 15), preserved in 95% ethanol and sent to AIM - Advanced Identification Methods GmbH, Germany for DNA extraction and metabarcoding. Samples were collected immediately after bird defecation was observed in situ; to further ensure that they indeed belonged to Grey plovers, no attempt was made to block host DNA during amplification.

Molecular analyses and bioinformatics are described in detail in Vassileva et al. (2024).

Briefly, species identification of faecal organic material was performed using DNA metabarcoding following the protocol of Hausmann et al. (2020). Initially, two molecular markers were targeted: CO1 for animal and ITS2 - for plant identification. Subsequently, only CO1 results were retained for the analyses, since many fewer taxa with a low number of reads were detected by ITS2 in only a few samples, suggesting that the birds targeted animal prey exclusively. The current dataset includes only the CO1 sequences, but the ITS2 sequences have also been published and are available in GenBank (accession numbers PP060518-PP060554). For amplification of the CO1 target region, Illumina-ready primers derived from the primer pair by Leray et al. (2013) (dgHCO 5′-GGWACWGGWTGAACWGTWTAYCCYCC-3’ mlCOIntF 5′-TAAACTTCAGGGTGACCAAARAAYCA-3′) were used, with forward and reverse HTS primers equipped with complementary sites for the Illumina sequencing tails. High-throughput sequencing (HTS) was performed on an Illumina MiSeq. No attempt was made to block the amplification of the host DNA during PCR in order to confirm that the collected faeces indeed belonged to Grey plovers.

The obtained sequences were clustered into operational taxonomic units (OTUs), which were blasted against: (1) a custom database downloaded from GenBank (a local copy of the NCBI nucleotide database downloaded from ftp://ftp.ncbi.nlm.nih.gov/blast/db/) and (2) a custom database created from data downloaded from BOLD (www.boldsystems.org), including taxonomy and BIN information, using Geneious (v.10.2.5 - Biomatters, Auckland, New Zealand) and following the methods described in Morinière et al. (2016).

As an additional control measure, the OTUs were classified into taxa using the naive Bayesian classifier of the Ribosomal Database Project (RDP), trained on a curated CO1 dataset of arthropods and chordates (plus outgroups; see Porter and Hajibabaei (2018)). Further details on the bioinformatics procedures can be found in the supplements of Uhler et al. (2021).

Geographic coverage

Description: The faecal samples were collected on the sand stripe between Pomorie Lake (part of the Burgas Lakes complex, south-western Black Sea coast, Bulgaria) and the Black Sea. The study area is located on the Black Sea - Mediterranean Flyway.

Coordinates: 42.62154; 27.63367.

Taxonomic coverage

Taxa included:

Rank	Scientific Name
phylum	Annelida
phylum	Arthropoda
phylum	Ascomycota
phylum	Bacillariophyta
phylum	Basidiomycota
phylum	Chlorophyta
phylum	Choanozoa
phylum	Chordata
phylum	Cnidaria
phylum	Eukarya_unassigned
phylum	Evosea
phylum	Mollusca
phylum	Nematoda
phylum	Ochrophyta
phylum	Oomycota
phylum	Platyhelminthes
phylum	Porifera

phylum	Rhodophyta
phylum	Tardigrada
class	Clitellata
class	Polychaeta
class	Arachnida
class	Branchiopoda
class	Chilopoda
class	Collembola
class	Diplopoda
class	Hexanauplia
class	Insecta
class	Malacostraca
class	Dothideomycetes
class	Eurotiomycetes
class	Lecanoromycetes
class	Leotiomycetes
class	Saccharomycetes
class	Sordariomycetes
class	Bacillariophyceae
class	Coscinodiscophyceae
class	Agaricomycetes
class	Mamiellophyceae
class	Choanoflagellatea
class	Actinopterygii
class	Aves
class	Mammalia
class	Hydrozoa
class	Eumycetozoa
class	Bivalvia
class	Gastropoda
class	Chromadorea

class	Phaeophyceae
class	Monogenea
class	Trematoda
class	Demospongiae
class	Florideophyceae
class	Heterotardigrada
order	Haplotaxida
order	Opisthopora
order	Phyllodocida
order	Sabellida
order	Spionida
order	Araneae
order	Ixodida
order	Opiliones
order	Sarcoptiformes
order	Anomopoda
order	Lithobiomorpha
order	Entomobryomorpha
order	Julida
order	Cyclopoida
order	Blattodea
order	Coleoptera
order	Dermaptera
order	Diptera
order	Ephemeroptera
order	Hemiptera
order	Hymenoptera
order	Lepidoptera
order	Neuroptera
order	Orthoptera
order	Psocodea

order	Thysanoptera
order	Trichoptera
order	Amphipoda
order	Decapoda
order	Isopoda
order	Capnodiales
order	Cladosporiales
order	Eurotiales
order	Lecanorales
order	Saccharomycetales
order	Glomerellales
order	Bacillariales
order	Naviculales
order	Thalassiosirales
order	Agaricales
order	Mamiellales
order	Craspedida
order	Blenniiformes
order	Charadriiformes
order	Carnivora
order	Primates
order	Leptothecata
order	Siphonophorae
order	Physariida
order	Mytilida
order	Venerida
order	Littorinimorpha
order	Rhabditida
order	Fucales
order	Peronosporales
order	Plagiorchiida

order	Axinellida
order	Ceramiales
order	Corallinales
order	Echiniscoidea
family	Lumbricidae
family	Nereididae
family	Polynoidae
family	Sabellariidae
family	Spionidae
family	Araneidae
family	Lycosidae
family	Tetragnathidae
family	Ixodidae
family	Phalangiidae
family	Lithobiidae
family	Tomoceridae
family	Julidae
family	Ectobiidae
family	Cantharidae
family	Carabidae
family	Cerambycidae
family	Chrysomelidae
family	Cleridae
family	Coccinellidae
family	Curculionidae
family	Elateridae
family	Melyridae
family	Staphylinidae
family	Tenebrionidae
family	Forficulidae
family	Agromyzidae

family	Anthomyiidae
family	Asilidae
family	Bibionidae
family	Calliphoridae
family	Cecidomyiidae
family	Ceratopogonidae
family	Chironomidae
family	Chloropidae
family	Conopidae
family	Dolichopodidae
family	Drosophilidae
family	Ephydridae
family	Fanniidae
family	Heleomyzidae
family	Hybotidae
family	Limoniidae
family	Lonchaeidae
family	Lonchopteridae
family	Milichiidae
family	Muscidae
family	Mycetophilidae
family	Phoridae
family	Polleniidae
family	Psychodidae
family	Rhagionidae
family	Rhiniidae
family	Sarcophagidae
family	Scathophagidae
family	Sciaridae
family	Simuliidae
family	Sphaeroceridae

family	Syrphidae
family	Tachinidae
family	Tephritidae
family	Tipulidae
family	Baetidae
family	Acanthosomatidae
family	Aphididae
family	Aphrophoridae
family	Cicadellidae
family	Coreidae
family	Issidae
family	Pentatomidae
family	Psyllidae
family	Rhyparochromidae
family	Andrenidae
family	Apidae
family	Braconidae
family	Colletidae
family	Crabronidae
family	Diapriidae
family	Eulophidae
family	Formicidae
family	Halictidae
family	Ichneumonidae
family	Megachilidae
family	Perilampidae
family	Pompilidae
family	Pteromalidae
family	Tenthredinidae
family	Vespidae
family	Blastobasidae

family	Crambidae
family	Erebidae
family	Geometridae
family	Hepialidae
family	Incurvariidae
family	Lypusidae
family	Noctuidae
family	Nymphalidae
family	Oecophoridae
family	Pieridae
family	Pyralidae
family	Tortricidae
family	Mantispidae
family	Acrididae
family	Tettigoniidae
family	Psocidae
family	Stenopsocidae
family	Phlaeothripidae
family	Hydropsychidae
family	Alpheidae
family	Grapsidae
family	Polybiidae
family	Ischnomesidae
family	Cladosporiaceae
family	Aspergillaceae
family	Trichocomaceae
family	Ramalinaceae
family	Saccharomycetaceae
family	Bacillariaceae
family	Pinnulariaceae
family	Skeletonemataceae

family	Mamiellaceae
family	Salpingoecidae
family	Blenniidae
family	Charadriidae
family	Laridae
family	Canidae
family	Hominidae
family	Apolemiidae
family	Prayidae
family	Mytilidae
family	Mesodesmatidae
family	Littorinidae
family	Rhabditidae
family	Sargassaceae
family	Peronosporaceae
family	Callithamniaceae
family	Rhodomelaceae
family	Echiniscoididae

Temporal coverage

Data range: 2020-10-25 - 2021-3-27.

Notes: In the BOLD dataset, all sequences were assigned the date 2020-10-31 for simplicity, as they were usually present in multiple samples from different dates, but required a single entry in the database. This is reflected in the collection notes (fieldNotes).

Usage licence

Usage licence: Open Data Commons Attribution License

Data resources

Data package title: *Pluvialis squatarola* faeces metabarcoding - CO1

Resource link: http://dx.doi.org/10.5883/DS-PLUVS

Number of data sets: 1

Data set name: *Pluvialis squatarola* faeces metabarcoding - CO1 [BOLD_DATASET_CODES:DS-PLUVS]

Data format: Darwin Core

Description: The dataset contains 429 records (DNA sequences identified with CO1 from *P. squatarola* faeces), of which 244 records with BOLD Barcode Index Numbers (BINs) and 158 - with species assignment.

Arthropoda is the most represented group, constituting 78% of the records in the dataset. A total of 91% of all arthropod records (or 71% of all records) belong to Insecta, with Diptera, Hymenoptera and Lepidoptera the most represented groups. The other taxonomic groups have relatively minor shares (Fig. 1).

The dataset also includes metadata on the field methods (sampling method, geographic location, date), as well as the taxonomy (taxonomic identification methods, taxonomic classification) and the exact nucleotide sequence of each record.

Column label	Column description
materialSampleID	BOLD process ID - unique identifier for each record in BOLD.
recordNumber	BOLD sample ID - unique identifier for each record (sequence).
fieldNumber	BOLD field ID - unique field/sample identifier for each record (sequence), here identical to recordNumber.
occurrenceID	BOLD specimen ID - unique occurrence identifier for each record (sequence).
measurementMethod	BOLD Barcode Index Number (BIN) uniform resource identifier (URI) corresponding to the record (sequence).
institutionCode	Name of the institution having custody of the sequences.
kingdom	Full scientific name of the kingdom of the taxon to which the sequence is assigned.
phylum	Full scientific name of the phylum or division of the taxon to which the sequence is assigned.
class	Full scientific name of the class of the taxon to which the sequence is assigned.
order	Full scientific name of the order of the taxon to which the sequence is assigned.
family	Full scientific name of the family of the taxon to which the sequence is assigned.

subfamily	Full scientific name of the subfamily of the taxon to which the sequence is assigned.
tribe	Full scientific name of the tribe of the taxon to which the sequence is assigned.
genus	Full scientific name of the genus of the taxon to which the sequence is assigned.
scientificName	Full scientific name of the taxon to which the sequence is assigned.
scientificNameAuthorship	Authorship information for the scientific name (field scientificName) of the taxon to which the sequence is assigned.
identificationRemarks	Method used to assign a taxonomic identity to the sequence.
identifiedBy	Name of the person/people who assigned the taxon identification to the sequence.
typeStatus	Status of the specimen voucher in BOLD.
materialSample	Type of tissue/material sampled from which the DNA sequences were derived.
recordedBy	Name of the person/people who collected the original field samples.
eventDate	Field sample collection date.
fieldNotes	Additional notes related to the field sampling.
country/waterBody	Country in which the field sampling occurred.
countryCode	Standard two-letter code for the country in which the field sampling occurred.
stateProvince	Full name of the NUTS3 (Nomenclature of territorial units for statistics level 3) administrative region in which the field sampling occurred.
county	Full name of the municipality in which the field sampling occurred.
municipality	Full name of the Natura 2000 area in which the field sampling occurred.
verbatimLocality	Additional text description of the exact location of the collection site.
decimalLatitude & decimalLongitude	Geographic latitude and longitude of the sampling location in decimal degrees (WGS84, EPSG 4326).
coordinatePrecision	Coordinate precision of the field decimalLatitude & decimalLongitude.
georeferenceSources	Resources used to georeference the sampling location.
verbatimElevation	Original description of the elevation (altitude above sea level in metres) of the sampling location.
samplingProtocol	Sampling protocol used for the field sampling.
dynamicProperties	Exact nucleotide sequence corresponding to the record.
measurementDeterminedBy	Institution that uploaded and curated the dataset.
measurementDeterminedDate	Date of upload of the dataset to BOLD.

datasetName	Dataset unique identifier in BOLD.

Additional information

Some of the sequences are likely contamination (e.g. most of Chordata, soil fungi) and others are ecto- and endoparasites (e.g. Ixodida, Platyhelminthes, Nematoda). While these cannot be considered part of the Grey plover's diet and were, therefore, excluded from the published diet analyses (Vassileva et al. 2024), they are included in this dataset for the sake of completeness and biodiversity listing purposes. That said, a critical review and conservative ecological approaches should be applied to the raw metabarcoding data prior to their validation. A thorough verification against the local ecological communities is highly recommended in order to attribute them to the living systems.

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Author contributions

Liliana V. Vassileva: Writing – review and editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualisation. **Lyudmila Lozanova:** Writing – review and editing, Writing – original draft, Visualisation, Funding acquisition, Formal analysis, Data curation. **Martin P. Marinov:** Writing – review and editing, Writing – original draft, Visualisation, Funding acquisition, Formal analysis, Data curation. **Jérôme Morinière:** Writing – review and editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Boyko Neov:** Writing – review and editing, Writing – original draft, Funding acquisition, Formal analysis, Data curation. **Boris P. Nikolov:** Writing – review and editing, Supervision. **Nikolay Simov:** Writing – review and editing, Writing – original draft, Investigation. **Stefania Klayn:** Writing – review and editing, Writing – original draft, Investigation, Funding acquisition, Formal analysis.

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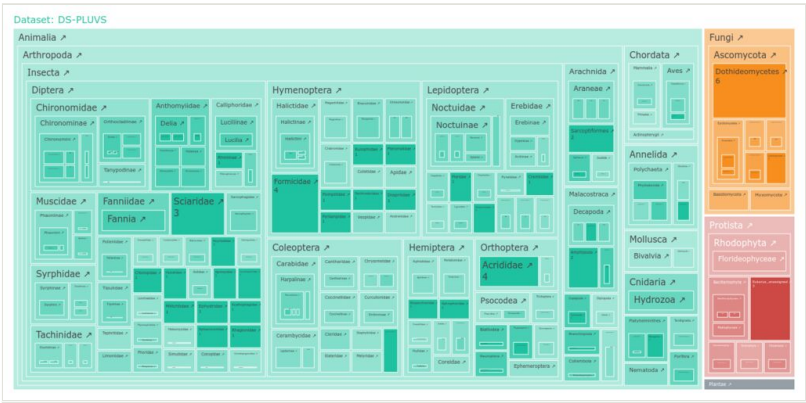

Figure 1.

Treemap of the taxonomic composition of sequences identified in the *P. squatarola* faecal samples. An interactive version of the plot can be found on the dataset's page in BOLD.